Precision medicine in NSCLC and pathology: how does ALK fit in the pathway?

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The evolution of personalised medicine in lung cancer has dramatically impacted diagnostic pathology. Current challenges centre on the growing demands placed on small tissue samples by molecular diagnostic techniques. In this review, expert recommendations are provided regarding successful identification of anaplastic lymphoma kinase (ALK)-rearranged non-small-cell lung cancer (NSCLC). Steps to correctly process and conserve tumour tissue during diagnostic testing are essential to ensure tissue availability. For example, storing extra tissue sections ready for molecular diagnostic steps allows faster testing and preserves tissue. Fluorescence in situ hybridisation (FISH) is commonly used to detect ALK rearrangements, with most laboratories favouring screening by immunohistochemistry followed by a confirmatory FISH assay. Reverse transcription–polymerase chain reaction can also identify ALK fusion gene mRNA transcripts but can be limited by the quality of RNA and the risk that rare fusion variants may not be captured. Next-generation sequencing (NGS) technology has recently provided an alternative method for detecting ALK rearrangements. While current experience is limited, NGS is set to become the most efficient approach as an increasing number of genetic abnormalities is required to be tested. Upfront, reflex testing for ALK gene rearrangement should become routine as ALK tyrosine kinase inhibitor therapy moves into the first-line setting. Guidelines recommend that EGFR and ALK tests are carried out in parallel on all confirmed and potential adenocarcinomas, and this is more efficient in terms of tissue usage and testing turnaround time for both of these actionable gene alterations. The practice of sequential testing is not recommended. Identification of ALK rearrangements is now essential for the diagnosis of NSCLC, underpinned by the benefits of ALK inhibitors. As scientific understanding and diagnostic technology develops, ALK testing will continue to be an evolving and challenging paradigm.

Key words: anaplastic lymphoma kinase, fluorescence in situ hybridisation, immunohistochemistry, next-generation sequencing, non-small-cell lung cancer

introduction

In this supplement edition of Annals of Oncology, many aspects of the identification and treatment of patients with anaplastic lymphoma kinase (ALK)-rearranged non-small-cell lung cancer (NSCLC) are considered. This paper discusses the complex diagnostic pathway that should be followed in many patients with NSCLC, in order to secure the most accurate and specific diagnosis, and testing of appropriate cases for actionable genetic alterations. The overall benefits of identifying and treating actionable driver mutations in NSCLC have been described [1] and much of this currently obtainable benefit can probably be attributed to targeting epidermal growth factor receptor (EGFR) mutations and ALK gene rearrangements.

The impact of precision or personalised medicine in lung cancer has resulted in considerable changes and challenges for diagnostic pathology. The simplistic division of lung cancer into small-cell and non-small-cell carcinoma is no longer sufficient for patient management. As well as more specific subtyping of NSCLC required for the selection of various therapies, NSCLC subtype currently drives the triage of cases for molecular testing. The core of this activity, mandated by international guidelines [2, 3], is the search for EGFR mutations and ALK gene rearrangements. This need for a more detailed and complex pathological diagnosis coincided with developments in endoscopic techniques and interventional radiology that, somewhat paradoxically, now deliver smaller tumour samples at a time when demand on tissue samples is growing. One of the major challenges for pathologists dealing with these samples is tissue management and conservation, to ensure that a specific diagnosis and appropriate molecular analysis can be completed [4].

diagnostic testing in NSCLC

Initial lung cancer tissue diagnosis is made on a wide range of sample types, from the primary tumour or accessible metastatic deposits (Table 1). Practice varies greatly, but in some countries, cytology samples provide the only source of diagnostic material in over 50% of patients, and only a minority of patients will have
the use of image-guided techniques [5, 6]. Provided the material is processed correctly and managed carefully, any biopsy or cytology sample can provide a complete diagnosis, including molecular testing, assuming that there is adequate representation of tumour cells [4].

Table 1. Sample types commonly used for lung cancer diagnosis

<table>
<thead>
<tr>
<th>Tissue biopsy samples</th>
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<tr>
<td>Endoscopic bronchial (and transbronchial) biopsy</td>
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<td>Percutaneous transthoracic core needle biopsy</td>
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<td>Pleural biopsy</td>
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<tr>
<td>Lymph node biopsy (mediastinal or cervical lymph nodes)</td>
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<td>Biopsy of liver, bone, adrenal, skin or other accessible metastases</td>
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Cytology-type samples

<table>
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<tr>
<th>Bronchial brushings and washings</th>
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<tr>
<td>Percutaneous transthoracic fine needle aspiration</td>
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<tr>
<td>Endobronchial ultrasound-guided (EBUS) transthoracic needle aspiration (primary or lymph nodes)</td>
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<tr>
<td>Trans-oesophageal ultrasound-guided (EUS) needle aspiration of lower mediastinal lymph nodes</td>
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<td>Pleural fluid</td>
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<tr>
<td>Lymph node aspirates</td>
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<tr>
<td>Needle aspirates of liver, bone, adrenal, skin or other accessible metastases</td>
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their primary tumour surgically resected. In general, lung cancer diagnostic samples are small, providing limited tumour material, but the chances of obtaining malignant tissue are increased by the use of image-guided techniques [5, 6]. Provided the material is processed correctly and managed carefully, any biopsy or cytology sample can provide a complete diagnosis, including molecular testing, assuming that there is adequate representation of tumour cells [4].

The standard approach to tissue fixation is to use 10% neutral buffered formalin, for at least 6 h, but no more than 48–72 h. Other fixatives are not recommended due to their possible adverse effect on immunohistochemistry (IHC) and molecular tests. Tissue is processed and embedded in paraffin wax to create the formalin-fixed, paraffin-embedded (FFPE) block, from which sections can be taken for all diagnostic and molecular tests. While many cytology-type samples are microscopically examined after smears or deposits of spun cells are stained on slides, a popular and extremely useful technique is the preparation of an artificial cell block from the cytology sample, which can then be processed and sectioned like a tissue sample. The profound influences of variance in these preparation and processing steps (so-called pre-analytics) on the outcome of all diagnostic procedures, including IHC and molecular testing, cannot be over emphasised, even if the effects are poorly understood and sometimes unpredictable [4].

Morphological examination of small biopsy and cytology samples should allow a specific subtype diagnosis of lung cancer in 60%–80% of cases [7]; a non-specific diagnosis of NSCLC-NOS is the best diagnosis possible by morphology alone in the remainder, especially in cytology-type samples. Only in these latter cases should limited IHC be used to predict the likely NSCLC subtype [8–11]. As few as two IHC stains (TTF1 positivity to predict adenocarcinoma, and p40 or p63 to predict squamous cell carcinoma) are all that are required in the vast majority of cases. Using more than two slides for this diagnostic step is wasteful and may compromise the possibility of molecular testing. Double IHC staining can easily allow four IHC markers to be tested on two slides. The recommended nomenclature for NSCLC cases subtyped by IHC provides the diagnosis of NSCLC and indicates the subtype favoured by the IHC staining (e.g. ‘NSCLC, probably adenocarcinoma’ when TTF1 is positive) [10].

In diagnostic samples where there is the possibility of lung cancer, determined by the biopsy site and clinical history provided, IHC and molecular testing can be anticipated and, when the FFPE block is first cut for the initial diagnosis, extra tissue sections may be cut and stored, ready for these additional diagnostic steps (Figure 1). This allows faster testing and saves precious tissue. Note that sections to be used for DNA or RNA extraction should be cut in a ‘molecularly sterile’ environment, something that is not normally feasible for the initial diagnostic block sectioning. Sections should not be stored for long periods if they are to be used for IHC or molecular testing, as antigens and nucleic acids rapidly deteriorate in tissue stored in this way. Storing cut sections in a sealed (dark) container at 4°C will slow this deterioration but, in general, it is much better to use sections within a few days of cutting [4].

Both EGFR mutation and ALK and ROS1 gene rearrangements are, for all practical purposes, found almost exclusively in non-squamous NSCLC, effectively adenocarcinomas. Tobacco carcinogenesis is not associated with the presence of any of these driver mutations; however, these molecular alterations may also be found in patients who smoke. These molecular drivers are associated with malignant transformation in the peripheral lung epithelium (the terminal respiratory unit or TRU) [12]. Thus, current recommendations suggest that these actionable alterations should be sought in all cases of definite, probable or possible adenocarcinoma, or where such a diagnosis cannot be reasonably excluded, and regardless of smoking habit, race or gender. Only in the exceptionally rare instance, at least in a Caucasian patient context, when a squamous cell carcinoma arises in a never or long-time ex-smoker, should squamous cell carcinomas be considered for testing [2, 3].

testing approaches for ALK rearrangements

Traditionally, the laboratory method most often used to detect a gene rearrangement is fluorescence in situ hybridisation (FISH). In the case of ALK in NSCLC, either inversions within chromosome 2 or translocation involving other chromosomes (see below) lead to the formation of an ALK fusion gene. FISH provides a visual demonstration of the rearrangement [13]. Alternative visualisation methods for in situ hybridisation (ISH) including chromogens or silver, which allow bright field microscopic examination, are being explored [14]. Next-generation sequencing (NGS) technology is also beginning to provide an alternative method for detecting fusion genes. Fusion gene DNA must be transcribed into abnormal mRNA sequences, and multiplex polymerase chain reaction (PCR) techniques have been developed to identify a range of possible ALK fusion gene mRNA transcripts as an alternative to FISH. Finally, to exert its oncogenic effects (described elsewhere in this supplement), the ALK fusion gene mRNA must be translated into protein containing the activated ALK tyrosine kinase moiety. ALK protein may be detected in tumour tissue sections using IHC. This sequence of events, and the testing opportunities each molecular phase provides, is illustrated in Figure 2 [13].
Figure 1. Tissue section preparation algorithm. There are several potential approaches to the problem of saving material. This algorithm represents one approach, developed and used by one of the authors over many years. All thoracic biopsy samples and cytology cell blocks are cut in this way, as well as other sample types if a history of possible metastatic lung cancer is given. For some cases, the extra sections will not be used; this is considered a reasonable trade for more rapid diagnosis when IHC or deeper levels are needed, and reduces the possibility of tissue exhaustion when cases are submitted for sectioning for DNA extraction. DL, deeper levels; FISH, fluorescence in situ hybridisation; H&E, haematoxylin and eosin; IHC, immunohistochemistry; NBF, neutral buffered formalin.

Figure 2. ALK gene rearrangement is associated with an alteration in DNA sequence, but in order to be biologically active, it needs to be transcribed into the mRNA fusion gene transcript, which in turn has to be translated into protein with an activated ALK tyrosine kinase. Consequently, several different testing opportunities are available. Panel C is adapted from Ou et al. [15], with permission from Elsevier. ALK, anaplastic lymphoma kinase; FISH, fluorescence in situ hybridisation; IHC, immunohistochemistry; PCR, polymerase chain reaction; TK, tyrosine kinase.
ALK fluorescence in situ hybridisation

FISH was the first ALK assay (Vysis ALK FISH Break-Apart Kit) approved by the US Food and Drug administration (FDA) as a companion diagnostic test for the drug crizotinib [16]. The main advantage of FISH is that it is not usually affected by the unavoidable variability of the pre-analytical phase in pathology laboratories worldwide. The technique works well on all types of samples, including decalcified tissues (with the appropriate protocol). Failure rates in recent series are around 4%–5% [17, 18]. The main disadvantage of FISH is that interpretation requires expertise and experience [13]. Therefore, most false-negative and false-positive results can be traced back to the post-analytical phase [19, 20]. In this regard, it is worth mentioning that an automated ALK FISH scanning algorithm (the BioView scoring system) has also received FDA clearance, and it is particularly useful for solving discrepancies or difficult FISH patterns (e.g. polisomies, monosomies, copy number gain, amplifications and duplications) [17, 21, 22].

Although in advanced ALK-positive tumours, the mean number of positive cells is 56%–70% [21, 23], and the percentage does not influence the benefit derived from ALK-targeted treatment [23], significant intratumour heterogeneity has been recently described in surgically resected ALK-rearranged lung adenocarcinomas [24]. Another interesting aspect to consider is that positive tumours may contain one pattern of positivity (break-apart or isolated red, Figure 3) or both simultaneously [17, 21, 23]. This should be taken into consideration because two independent groups have recently confirmed that some cases with the atypical variant of positivity (single red pattern) are indeed false positives, probably due to hybridisation failure of the green probe [19, 25]. This line of reasoning also suggests that at least some negative FISH cases where rearrangement was confirmed by a second method and a response to crizotinib was observed (i.e. exhibiting monosomies, polisomies, isolated green signals or doublets) could reflect problems with the hybridisation of the red probe [26–31].

Taken together, the intratumour heterogeneity and discrepant data support the idea of using a second assay in negative FISH cases with characteristic clinical or histological features, ALK IHC positivity or difficult hybridisation patterns.

ALK immunohistochemistry

IHC is a widely used technique in diagnostic pathology that allows the identification and tissue localisation of antigens (epitopes), usually of protein origin, within tissue sections. This technique has been successfully used to identify ALK protein in pathological tissue samples and cytological specimens (see below). ALK protein is physiologically expressed in peripheral neural tissues and in some neuroendocrine cells and tumours, but normal lung epithelium does not express detectable amounts of protein [32]. In ALK gene rearranged lung cancers, ALK protein is expressed at modest levels. Standard IHC techniques that might be used in anaplastic lymphoma diagnosis are too insensitive to detect ALK protein in lung cancer but, with the use of very sensitive IHC detection systems, ALK protein in rearranged lung cancers may be detected. The importance of the use of an adequately sensitive IHC detection method cannot be overemphasised [13].

Many studies have demonstrated a correlation between positive ALK IHC in NSCLC and a positive ALK break-apart FISH test in the same tumour [33–37]. Several anti-ALK primary antibody clones have been used in studies of lung cancer, including the ALK1 clone (DAKO, Glostrup, Denmark), 5A4 (Leica, Nussloch, Germany) and D5F3 (Ventana, AZ). Some studies demonstrated that the ALK1 clone may lack some sensitivity [33, 34, 38], but this may be related to inadequate detection. The majority of studies have used either the 5A4 or the D5F3 clone with highly sensitive detection systems; Figure 4 shows examples of positive staining with these clones. Reported correlation between positive ALK IHC and a positive ALK FISH test is somewhat variable, but in general, it is over 90% (supplementary Table S1, available at Annals of Oncology online). Some of this variability will depend on the definition of a positive ALK IHC test, and this is another reason to be aware of the detection system used with the primary antibody. Some detection systems with good amplification still provide a dynamic range of staining intensity, generally reported as +, ++ or ++++. There is then an issue regarding the level of staining that is regarded as ‘positive’, since the prevalence of a positive FISH test may be lower at lower staining levels [33, 39, 40]. Heterogeneity of staining may also be evident, especially

Figure 3. Lung adenocarcinoma hybridised with an ALK dual break-apart probe. (A) ALK-positive tumour showing the break-apart pattern, so-called ‘typical’ (split red and green signals, arrows). (B) ALK-positive tumour showing the isolated red signal pattern, so-called ‘atypical’ (arrows).
when staining intensity is lower, and while in most cases, a relatively homogeneous, even staining is seen, heterogeneity may be present in ~30% of the positive slides [31]. Although H-scoring is not recommended for scoring ALK IHC, this approach clearly demonstrated the heterogeneity and dynamic range of ALK IHC staining in NSCLC using the Leica 5A4 and Novolink combination [39]. The use of ultra-sensitive detection, such as those based upon tyramide amplification or Ventana’s Optiview system, banishes much of the dynamic range, but the scoring criteria are clone-dependent [21]. While this may not be desirable for some diagnostic IHC applications, for ALK IHC, the creation of a more binary test outcome is probably a good thing, provided a close relationship is maintained between IHC and FISH positivity, and thus response to tyrosine kinase inhibitors (TKIs).

The approval of the Ventana DS5-based companion diagnostic test by the FDA for ALK testing, together with the use of this IHC assay as the primary diagnostic modality in clinical trials of ceritinib and alectinib [41], the de facto acceptance of IHC as a predictive diagnostic by the European Medicines Agency (EMA) and the perception of ALK FISH testing as a more costly and time-consuming test have all promoted the use of ALK IHC as a primary diagnostic test rather than a screening tool (see the Testing algorithms section). If ALK IHC is to be used as the primary selector for ALK TKI therapy, users will need to be very clear regarding the predictive performance of the test in use, especially any definition of a ‘positive’ ALK IHC result, when the test employed demonstrates significant dynamic range of staining [33]. Generally speaking, an ALK IHC test result should be available more quickly than an ALK FISH or sequencing test (see below).

**ALK sequencing and fusion transcript analysis**

Although in the College of American Pathologists guidelines, reverse transcription–PCR (RT–PCR) is not recommended as an alternative to FISH, because of the high risk of failure and false negatives [3]; in recent years, a wide variety of methods, some of them commercially available, have been successfully applied to ALK rearrangement analysis in FFPE tissues [42–50]. Probably, the most popular approaches nowadays in most countries can be classified into three categories: (i) extract RNA and use a commercial RT–PCR kit; (ii) validate a targeted NGS assay; or (iii) customise a vendor solution or even develop an in-house panel usually with a hybridisation capture-based option [42, 44, 49–51]. Accordingly, the National Comprehensive Cancer Network NSCLC Guidelines Panel ‘strongly endorses broader molecular profiling’. However, centres with low throughput using large NGS panels may still depend on IHC or FISH for the timely detection of ALK rearrangements, because of the very demanding clinical scenario with young patients, aggressive tumours and few treatment options [52].

**testing algorithm (and relation to guidelines)**

The place of the ALK test within the lung cancer diagnostic algorithm depends on a number of factors. For the purposes of this discussion, drug availability is assumed. The dynamic driving testing will change as ALK TKI therapy moves into the first-line setting. Treatment in the second line is frequently used as a reason not to test for ALK gene rearrangement upfront, at the time of initial diagnosis.

**reflex or bespoke testing**

The question of ‘who orders the test’ is frequently asked. Bespoke testing ordered by the treating physician, or possibly even at the request of the patient, will mean that each test is required to inform a treatment decision, but the testing cycle is longer, may be at greater risk of failure through tissue exhaustion and ALK-positive patients will be identified later in the course of their management. Reflex testing, initiated by the pathologist when an appropriate NSCLC subtype diagnosis is made (see above), will mean that some tests may be irrelevant to a treatment decision, but many oncologists prefer to know their patients’ ALK status during first-line chemotherapy. Furthermore, given that current guidelines recommend that EGFR and ALK testing should be carried out on all possible, probable or definite adenocarcinomas [2, 3], it is much easier and more efficient, in terms of tissue usage and both laboratory scientist.
and pathologist time, to reflex test for ALK rearrangement at initial diagnosis. Individual multidisciplinary teams (MDTs) or tumour boards may select the approach best suited to their own working practices. When reflex testing is practiced, additional MDT-driven tests may still be carried out where the pathologist was unaware of patient circumstances, for example, a never-smoking status in a patient with squamous cell carcinoma.

**Screening by IHC**

Regardless of how and when the decision to test is taken, the next issue concerns methodology. The merits of several technical approaches have already been discussed above. Although screening by IHC followed by confirmation by FISH testing has been criticised by those who favour FISH testing or those who have concerns regarding the possibility of FISH positive, IHC-negative cases that may be missed by the IHC screening approach [53], most laboratories with extensive experience of ALK testing in NSCLC have favoured this approach. The majority of data support IHC screening as a robust, sensitive and specific approach unlikely to miss many patients who may benefit from ALK TKI therapy [33]. The true nature and significance of cases with discrepant ALK IHC and FISH test results (FISH−/IHC+ and FISH+/IHC−) is still not very clear. The reality is that if IHC and FISH are each carried out and scored according to recommendations and there are no technical, pre-analytical or tissue-quality issues to confound outcomes, both tests are robust. Consequently, IHC for screening, and ultimately primary testing, is favoured in terms of quicker turnaround times and cost. In reality, most discrepant FISH/IHC tests are probably due to technical issues rather than true biology, and both FISH and NGS testing are likely no less prone to this than IHC [54]. It may be worth bearing in mind that, as the protein is the oncogenic driver and the target of the drug, the presence of the protein may be paramount in determining response to ALK TKI therapy.

**Other approaches**

RNA-based multiplex PCR approaches are very sensitive and specific but may not be suitable in many instances due to access to appropriate technology and skills and availability of RNA of sufficient quality from routine FFPE tissues. There is some risk that the multiplex PCR panel in use might not cover rare fusion variants. This testing approach is advocated by some in instances where IHC and FISH test outcomes are discrepant [36].

Fusion gene panels are now available for use with NGS approaches that claim excellent results. Experience with this technology is limited but, as more genetic abnormalities are added to the testing ‘menu’ in NSCLC, NGS panels for mutations, fusion genes and gene copy numbers will become the most efficient approach to multiplex gene testing. It remains to be seen whether a fusion gene reported on NGS screening is reliably predictive of therapeutic response, or whether the fusion should be confirmed by another technique, such as IHC. The introduction of NGS will radically alter the testing algorithms for actionable genomic alterations in NSCLC.

Figure 5 shows a typical algorithm that incorporates ALK testing as part of the approach to testing cases of NSCLC. For most patients, the only genomic alterations for which there are approved drugs available are EGFR mutations and ALK gene rearrangements. Most health systems will not reimburse testing for non-approved drug targets and care must be taken in advocating a more expansive list of genes for testing, even though this can be shown to be beneficial for patients [1]; best practice recommendations should support the pursuit of research but cannot mandate it. Although the three most commonly tested genomic alterations in NSCLC (KRAS mutation, EGFR mutation and ALK rearrangement) are, for all practical purposes, mutually exclusive, the practice of sequential testing for the most common alteration first, followed by the next most common when the first test is negative (etc.), is not recommended since rarer alterations such as ALK fusion would take much longer to be discovered, and the risk of test failure is higher.

For many laboratories, the inclusion of a ROSI fusion gene test for adenocarcinoma patients is not routine since crizotinib is not routinely available for those rare patients with this rearrangement. Many laboratories will instead deploy ROSI testing when the other, more common molecular alterations (KRAS, EGFR and ALK) are negative in cases of advanced stage adenocarcinoma, and when the clinical situation (predominantly a never smoking history) or the histological characteristics (e.g. presence of signet-ring cells) indicate a higher chance of an oncogene-addicted tumour.

As NGS becomes more widely used, many of these caveats and selection procedures may become redundant. Selection of NSCLC tumours based upon their histology, and in some circumstances on the clinical features of the patient (smoking history, age, gender, ethnicity), could be replaced by a routine screen of all NSCLC cases for a large panel of molecular alterations, provided sufficient DNA/RNA is available for the multiple gene panel to be tested. Currently, most healthcare systems only have access to EGFR and ALK TKIs. Other alterations, such as KRAS, BRAF and HER2 mutations and ROSI fusion genes, are among the more common additional tests carried out. Any therapy introduced on the basis of these or other positive tests from NGS will often be through a clinical trial or an early access programme. NGS testing becomes relatively cost-efficient when replacing 5–6 standalone molecular tests but again, this will vary between laboratories. Furthermore, it is not yet clear whether some of the NGS-detected alterations will require a confirmatory step using an alternative methodology (IHC, FISH etc.). Oncologists will not be specifically asking for particular genetic tests to be carried out but will instead be presented, in the NGS data output, with a large amount of information, including molecular alterations that are of uncertain significance or that have no available therapeutic intervention, or both.

**Key factors for success in ALK testing**

Every laboratory, hospital and lung cancer MDT will have to plan their service according to the skills and resources available, and the demand and expectations placed upon it. There are many factors involved in providing not only ALK testing but a complete molecular testing service for lung cancer that is fit for purpose. Ultimately, the aim is to provide the best possible service for patients so that they might benefit, wherever possible and appropriate, from molecularly targeted therapy. The following are several key factors to consider:
Awareness of the need for testing in appropriate circumstances is essential. All steps should be taken, within the confines of patient safety, to maximise tumour tissue collection for diagnostic purposes. Tumour samples should be processed appropriately to facilitate all likely, or possible, diagnostic techniques that might be needed on that sample. Laboratory technicians, scientists and pathologists must ensure that initial diagnostic procedures do not exhaust the sample, rendering molecular testing impossible. Appropriate molecular diagnostic techniques should be carried out in the prescribed fashion; pathologists should understand the tests, their assessment and the meaning of test outcomes. Laboratories must participate and perform adequately in external quality assessment schemes for tests provided. Communication within the MDT and with other users of a molecular testing service is essential to ensure best outcomes.

**Conclusion**

The identification of patients with ALK-rearranged NSCLC is now a mandatory part of the diagnostic algorithm for patients with lung cancer. The benefits of treating these patients, in the appropriate clinical setting, with an ALK TKI have been well demonstrated and underpin this requirement. Testing methodology has evolved in a number of ways. In part, this reflects our increased knowledge of the relationship between the presence of the fusion gene and the expression of ALK protein in tumours. Developments in NGS technology have also changed the way in which ALK testing is carried out in some centres; this technology is likely to develop and become more widely used. With rising interest in molecular testing on blood samples, attempts have been made to identify ALK fusion genes in circulating tumour cells and in cell-free, circulating DNA, a practice that is likely to develop, although to what extent remains to be seen.

The almost inevitable development of resistance during ALK TKI therapy has raised both academic questions regarding the mechanism(s) of resistance at play in the patient and practical issues around how to detect those changes conferring resistance. The evolution of ALK gene mutations seems to be a frequent mechanism, as well as concurrent mutation in KRAS and some other genes. Histological transformation to pleomorphic (sarcomatoid) carcinoma has also been described. As new TKIs with activity against ALK gene mutations become available, the debate around the need for re-biopsy of the tumour at relapse and testing strategies for the identification of resistance mechanisms will intensify. It is clear that, for reasons of technology and
biology. ALK testing in NSCLC will continue to be an evolving and challenging paradigm for all concerned.

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references


