Pathological diagnosis and molecular testing in non-small cell lung cancer: Belgian guidelines

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In recent years, the management of patients with non-small cell lung cancer has been modified thanks to the development of targeted therapies. The pathologist is now asked to give the most accurate possible diagnosis in association with theranostic information in order to provide the best therapeutic option. Different international societies have already underlined the importance of guidelines for managing samples of non-small cell lung cancer. These Belgian guidelines have the goal of adapting these international recommendations to the Belgian landscape.

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Introduction

Lung cancer remains one of the most commonly diagnosed malignancies worldwide and the leading cause of cancer-related death.[1] Until the last decade, non-small cell lung cancer (NSCLC) was considered a single disease, and systemic treatment of metastatic NSCLC was limited to platinum-based chemotherapy doublets, resulting in approximately 20% response rates and a median survival of eight months.[2] Only recently, histology (squamous versus non-squamous) has emerged as an important criterion for the management of patients with NSCLC.[3] In recent years, the oncology community has seen a paradigm shift in the molecular diagnosis and treatment of NSCLC, thanks to the identification of mutations within the epidermal growth factor receptor (EGFR) gene that confer sensitivity to EGFR tyrosine kinase inhibitors (TKI) (erlotinib, gefitinib, and afatinib), and rearrangements of the anaplastic lymphoma kinase gene (ALK) that confer sensitivity to ALK inhibitors (crizotinib and ceritinib). The use of TKI is now well established in clinical practice and molecular testing has become a standard of care.[4]

However, these increased needs during histological diagnosis (squamous cell carcinoma versus adenocarcinoma) and molecular pathology are associated with a decrease in sample size. The majority of patients with NSCLC are diagnosed at a late stage, rendering surgical resection impossible. Most diagnostic samples are obtained by other procedures, such as bronchial or transthoracic biopsies, leading to small samples with low tumour cell content. In addition, cytological specimens can be obtained, in particular of mediastinal lymph nodes, via endobronchial ultrasound (EBUS) or endoscopic oesophageal ultrasound (EUS). The pathologist

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is now facing a new challenge: optimising the use of available tumour tissue. The pathologist is asked to provide the most accurate possible diagnosis in association with theranostic information in order to provide the best therapeutic option. Pathologists have a crucial and responsible role in co-ordinating molecular testing, as analysis is performed on formalin-fixed, paraffin-embedded (FFPE) tissue. Their role includes tumour diagnosis, careful attention to fixation procedures to preserve tissue quality and selection of the most appropriate tumour block with evaluation of the percentage of tumour cells for molecular analysis.

Different international societies have already underlined the importance of guidelines for managing samples of NSCLC.[4,5] These Belgian guidelines have the goal of adapting these international recommendations to the Belgian landscape.

Sampling procedures

Different procedures to obtain tissue for diagnosis and molecular analysis are described, including (non-exhaustive list): endoscopic biopsies, core-needle biopsy / cytology guided by EBUS, EUS, fine-needle aspiration (FNA), mediastinoscopy and thoracotomy. Recent recommendations of a European expert group state that 1) at least five endobronchial/transbronchial forceps biopsies should be taken; to maximise the volume of tissue, an additional five forceps biopsies or two cryobiopsies could be considered, 2) at least four EBUS/endoscopic ultrasound needle aspiration passes per target lesion are recommended and 3) at least two percutaneous core needle biopsies using an 18–20 G needle should be taken; in order to maximize the volume of tissue, 3–6 core needle biopsies could be considered.[6]

It is also important to mention that accurate and relevant clinical information is needed in order to handle samples efficiently. The 'minimum' required clinical information should be 1) the sample site(s), 2) the fact that the clinical information suggests a primary tumour or a metastasis, 3) the exact question the pathologist was asked, 4) the relevant previous medical history and 5) the smoking history. This information is useful in helping the pathologist determine the diagnostic priorities, anticipate the use of immunohistochemistry (IHC) or molecular tests and avoid unnecessary use of tumour tissue.[7] For example, if a biopsy is obtained from a patient with an EGFR-mutated lung cancer who has experienced EGFR TKI resistance, the molecular approach will be different than in the situation were a biopsy is taken from a tumour in which the histological and molecular characteristics are not yet known.

Tissue handling

Pre-analytical parameters are crucial for molecular testing. These parameters include time to fixation, fixation time and type of fixative. The standardisation of such factors remains difficult. However, the use of standardised procedures should minimise too large variations of these parameters. The time to fixation should be as short as possible (at most one hour) in order to avoid degradation of proteins or nucleic acids.[4] The samples should be fixed using standard 10% neutral buffered formalin (4% formaldehyde).[4] Others fixators such as Bouin's or B5 as well as decalcifying solutions must be avoided because they lead to degradation of nucleic acids and are therefore not suitable for molecular testing.[4] In the same way, the use of eosin for enhancing visualisation of the tissue should be avoided because it hampered in situ hybridisation.

Fixation time is also a critical issue. Guidelines recommend that fixation time should be between 6-48 hours.[4] Formalin over-fixation should be avoided because over-fixation can damage DNA and introduce artificial mutations through excessive cross-linking or deamination. This is a general problem in molecular testing.[8,9] Cytology is considered to be a powerful diagnostic tool in the diagnosis of lung cancer. International guidelines recommend preparing cellblocks from cytology samples, including pleural fluids. Indeed, the tumour cell content of cellblocks is sometimes higher than in biopsies and cellblocks may also be used for immunohistochemical and molecular analyses.[10]

Pathological diagnosis

Terminology in non-resection and resection samples

NSCLC have to be classified into more specific types, such as adenocarcinoma (ADC) or squamous cell carcinoma (SCC), whenever possible, for several reasons: 1) ADC or NSCLC not otherwise specified (NOS) should be tested for EGFR mutations, 2) ADC histology is associated with an improved outcome with pemetrexed therapy in contrast to SCC histology and 3) potential life-threatening haemorrhage may occur in patients with SCC who receive bevacizumab.[5]

In 2011, for the first time, the International Association for the Study of Lung Cancer (IASLC), the American Thoracic Society (ATS) and the European Respiratory Society (ERS) proposed a new approach to lung cancer biopsies and cytological samples.[10] This proposed

terminology is now included in the new WHO classification published in 2015.[11] Pathologists should classify NSCLC into more specific types whenever possible and the term NSCLC-NOS should be limited. For this purpose, if needed, judicious use of IHC may be useful (see below). The term 'non SCC' should not be used by pathologists in diagnostic reports.[10,11] This term is used by clinicians to categorise patients with different histological types of lung cancer who can be treated in a similar manner.[10] It is also important to note that certain diagnoses (such as large cell carcinomas or ADC in situ) can be made only on resection specimens.[11]

When should immunohistochemistry be used?

The IASLC/ATS/ERS working group on lung ADC and the WHO recommends that immunohistochemical diagnostic work-up should be limited in order to spare tissue for molecular testing.[10,11] Clinical information is needed to exclude a possible metastasis. In the case of a primary lung tumour, the use of IHC is not indicated in morphologically obvious SCC or ADC. A much more common difficulty in small biopsies or cytological samples is classifying poorly differentiated tumours where clear differentiation is difficult or impossible to evaluate on H&E slides.[10] Judicious use of two IHC markers helps for cases with an indeterminate morphology: one ADC marker and one squamous marker seem to be sufficient.[4,10] Much effort has been focused on the development of IHC panels for the accurate histological subtyping of NSCLC. These panels have been continuously modified with the emergence of novel markers. Biomarkers associated with glandular differentiation are TTF1 and Napsin A; biomarkers associated with squamous differentiation are p63, p40 and CK5/6.[4,11] P40 has been reported to be the most specific and sensitive squamous marker.[11] The use of TTF1 and p40 has been recommended for the distinction between ADC and SCC (*Figure 1*).[4,11] In cases where the classification in ADC or SCC is not obvious on H&E slides and where an ADC marker (i.e. TTF-1) is positive, the tumour should be classified as 'NSCLC favour ADC' (regardless of the results of the squamous cell marker) and cases where a squamous cell marker is positive, with at least moderate to diffuse staining, and a ADC marker is negative should be classified as 'NSCLC favour SCC'.[10] NSCLC lacking definitive morphological and/or immunohistochemical evidence of squamous or glandular differentiation should be classified as NSCLC-NOS.[4] Use of routine IHC neuroendocrine markers is not recommended unless the morphology suggests a neuroendocrine differentiation.[10]

In the same way, the use of IHC for distinguishing a primitive from a metastatic lesion (such as cytokeratin 7 or 20) is not recommended, unless there is a clinical suspicion of metastasis. Multidisciplinary correlation is necessary if the possibility exists that the tumour is not a primary lung tumour.

Molecular testing

When is molecular testing indicated?

Molecular studies are recommended for all patients with at least an advanced stage or a recurrent disease and with at least one ADC component.[4,5] EGFR and ALK molecular testing should be used to select patients for targeted TKI therapy.[4] Even if mutations of EGFR have been more frequently detected in young non-smoking female patients, particularly in Asian patients, and ALK rearrangements have been more frequently detected in young non-smoking patients, clinical criteria are not sufficient to identify patients for molecular testing.[4,5]

For small samples (biopsies or cytology) where an ADC component cannot be completely excluded, molecular testing may be performed in samples even with squamous or small cell histology (possible mixed histology).[5] In this particular setting, clinical criteria such as young age, non-smoking history or ethnicity may be useful for selecting patients for molecular testing.[5] The European Society for Medical Oncology (ESMO) guidelines recommend that SCC from patients with minimal, remote or no smoking history should strongly be considered for molecular testing.[4]

According to international guidelines, EGFR and ALK testing should be ordered at the time of diagnosis for patients presenting with stage IV disease (according to the 7th edition of the TNM staging system) who are suitable for therapy, or at the time of recurrence or progression for patients with lower-stage disease who were not previously tested for EGFR and ALK. Molecular testing at the time of diagnosis for patients with lower stage disease is encouraged by international guidelines but the decision should be made locally in accordance with the oncology team.[5] One model, 'reflex (automatic) testing', argues that reflex testing by pathologists based on diagnosis and tissue availability within the pathology department can be more efficient.[12] The decision to implement reflex testing should be based on a business plan, taking into account the cost in time and money of specimen retrieval from pathology archives when the result becomes clinically necessary.[12] However, in the Belgian context,

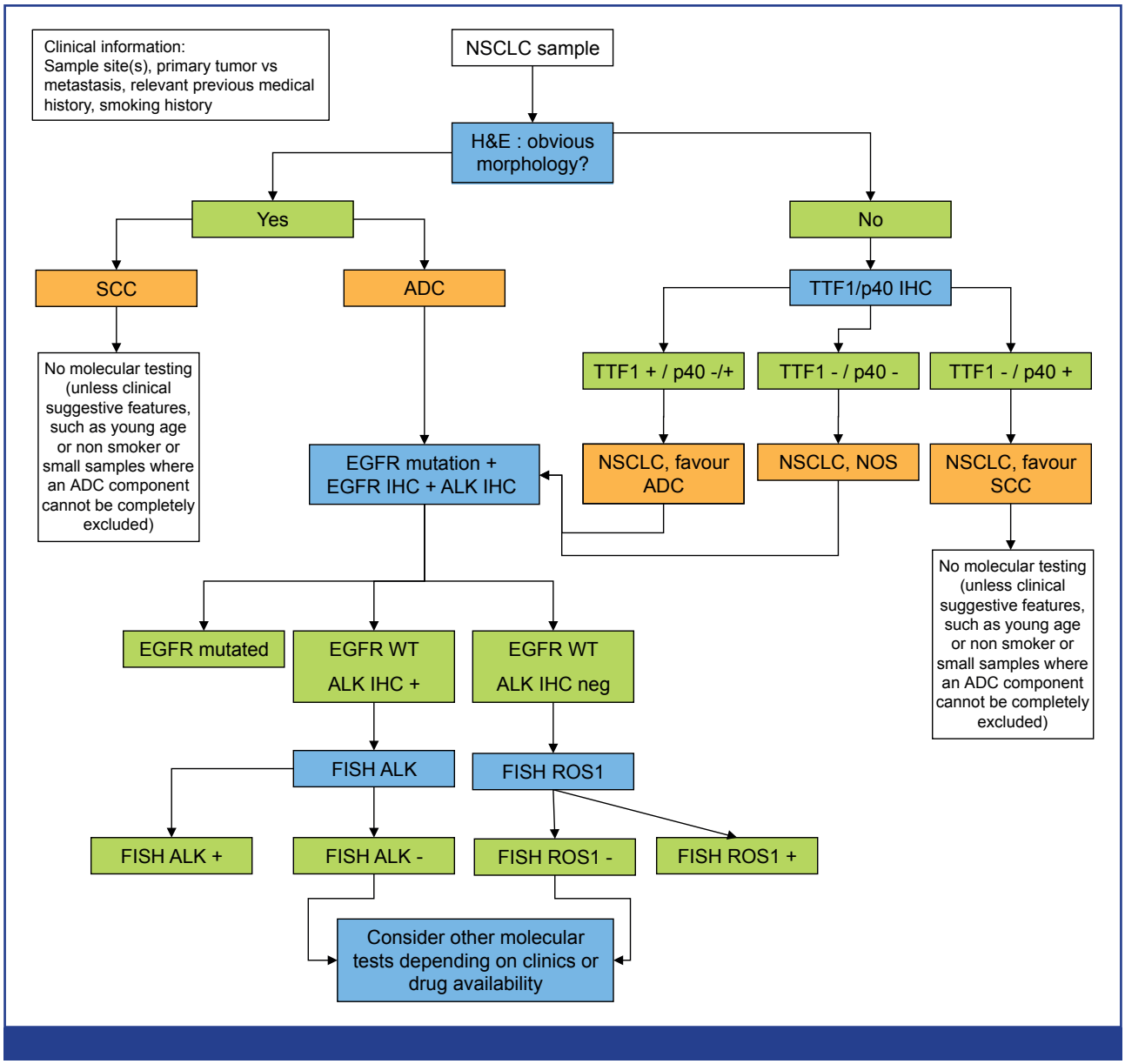

Figure 1. Algorithm for the optimisation of the management of NSCLC samples.

article 33bis specifies that the molecular test (in general) should be requested as part of a recognised cancer care program. In order to avoid delay in testing for patients who are candidates for TKI therapies, good communication between the clinical care team and the primary pathology laboratory is crucial. A procedure should be provided for the clinical care team to communicate to the pathologist if molecular testing is needed (by, for example, indicating on the request form that molecular testing is required – this avoids delay between the diagnostic procedure and the molecular testing).[5] In addition, the pathologist should alert the clinician as soon as possible if the quality of the sample is insufficient to perform further testing.

For patients treated with TKI and showing progression according to the RECIST criteria after an initial response, the question whether to retest and perform a new biopsy should be discussed. ESMO guidelines state that rebiopsy at disease progression may be considered.[13] In the same way, the National Comprehensive Cancer Network (NCCN) guidelines consider that prior to changing therapy, a biopsy is reasonable for determining the mechanism of resistance (*www.nccn.org*). The possibility of rebiopsy should be openly discussed within the multidisciplinary oncology committee (COM/MOC) and the decision should be taken on individual basis. Since more therapies are becoming available for treating resistance mechanisms, the work-

ing group feels that rebiopsy should be encouraged.

Role of the pathologist in the selection of the sample

Primary tumour or metastasis?

To guide initial treatment selection, molecular testing may be performed on either primary tumour or metastasis. Indeed, discordances in EGFR mutation status between primary tumour and metastasis seem to be rare.[5] Some of the discrepancies may reflect false negative results, for example, when analysing metastasis in lymph nodes, due to the admixture with lymphoid cells. Accordingly, the choice of which sample to test should be based on the sample characteristics, i.e., tumour content (in particular the percentage of tumour cells versus non-tumour cells) and preanalytic features.[5]

For patients with multiple, apparently separated primary lung ADC, testing each tumour may be considered. In contrast, according to the guidelines, testing of different areas in the same tumour is not recommended.[5]

Importance of the macrodissection

For DNA or RNA-based methods, the pathologist has to mark the tumour areas for macrodissection.[4] The ratio of the number of tumour cells to non-tumour cells (inflammatory cells, stromal cells, normal bronchial epithelial cells and endothelial cells) is an important factor that influences the reliability of mutational analysis. It must be as high as possible. Dissecting areas rich in tumour cells from surrounding normal tissue is the typical method for enriching a specimen in tumour DNA.[4]

The minimum requirements in tumour cellularity will depend on the methodology used for analysis. It is important for the pathologist to have a thorough understanding of the method used and – in particular- of the sensitivity of the test. The limit of detection (LOD) for each assay is critical to ensure testing is carried out appropriately; an LOD of 10% would theoretically require at least 20% of the cells in a sample to be tumour cells to ensure detection of a heterozygous mutation. A close working relationship between pathologists and molecular biologists is essential.[14] The pathologist should review all available tumour samples from the same patient in order to select the most suitable sample for molecular analyses.[4] Practically speaking, the selection of the sample should be made in function of the content of tumour cells, the percentage of tumour cells (related to non-tumour cells), the absence of necrosis and the preanalytical parameters. The test should be able to detect mutations in specimens with as few as 10% tumour cells.

For ALK and ROS1 FISH analysis, the pathologist should verify whether there is a minimum of 50 tumour cell nuclei.

Which tests are indicated?

As reported by the KCE, laboratories performing molecular tests for oncology should offer the full panel for a given tumour (e.g. at least EGFR, ALK and ROS1 for lung cancers) [Hulstaert F, Huybrechts M, Van Den Bruel A, et al. Molecular Diagnostics in Belgium. Health Technology Assessment (HTA). Brussels: Belgian Health Care Knowledge Centre (KCE); 2005 22/11/2005. KCE Reports 20 Available from: *https://kce.fgov.be/publication/report/molecular-diagnostics-in-belgium].*

In case of sequential testing, international guidelines recommend that tissues should be prioritised for EGFR and ALK testing.

EGFR

IHC

Although there is nowadays no evidence that immunohistochemical testing for total EGFR is a predictive marker for EGFR TKI sensitivity, Belgian law requires this testing for reimbursement of Erlotinib (Tarceva) in second or further lines of treatment. At least 10% of the tumour cells must show positive staining.

Mutated EGFR allele-specific antibodies for IHC are currently too insensitive to be used as a stand-alone assay and cannot be recommended.[4]

Mutational testing

Activating mutations in the tyrosine kinase domain of the EGFR gene are found in 10-16% of European patients with lung ADC.[4] Eighty to 90% of these mutations are either deletions in exon 19 or the L858R mutation in exon 21 of EGFR. However, other rarer mutations can be found in exons 18 to 21. This is why methods with a wide coverage of exons 18 to 21 are now strongly encouraged. These methods have to include the detection of the TKI resistance-associated mutations, such as T790M. There is a vast number of published methods for detection and identification of mutations.[14] However, no specific method (in contrast to ALK analysis) is recommended. The method needs to be accredited and validated by an external quality assurance program.[4]

ALK

ALK gene rearrangement is observed in 3-5% of patients with lung ADC.[4] ALK rearrangements are nearly always mutually exclusive with EGFR mutations. Rear-

rangements result in a fusion between part of the ALK gene and part of a partner gene, the most common being echinoderm microtubule-associated protein-like 4 (EML4). This leads to the production of a chimeric protein, which has constitutive ALK kinase activity. This kinase activity is responsible for uncontrolled growth and survival of cancer cells.[15]

IHC

ALK IHC may be considered a screening methodology for selecting specimens for ALK FISH testing.[4] ALK IHC is a procedure that is simple, quick, cheap, easily integrated into a diagnostic protocol and familiar to pathologists. However, challenges, such as choice of antibody, signal enhancement system and scoring system exist; this is why this immunohistochemical test must then be carefully validated.[15] Because protein ALK levels are relatively low, the IHC detection must be very sensitive.[4] Two different commercially available antibodies are considered as offering acceptable performance: the mouse monoclonal 5A4 clone (Novocastra, Leica), and the rabbit monoclonal D5F3 clone (Ventana).[16] The choice between these antibodies must be made by the pathologist. It is recommended to follow the instructions provided by the company or supplier when using these antibodies. Several studies have compared IHC to FISH (considered to be the gold standard) and indicated a wide range of accuracy, partly due to the antibody used.[17,18] Any case showing a positive immunohistochemical staining is eligible for FISH. It is not recommended to differentiate between low, intermediate or high immunohistochemical positivity, since FISH positive cases have been seen in each category. Belgian law requires ALK IHC prescreening, followed by FISH confirmation in case of positive IHC, for reimbursement of crizotinib (Xalkori).

FISH

Definitive assessment of ALK rearrangement is determined by FISH.[4] The majority of clinical studies examining ALK status have used FISH. However, cases have been reported of patients who were positive with IHC and negative with FISH responding to crizotinib.[17,19] In the USA, prescription of crizotinib is related to ALK status determined by the use of the Vysis ALK break apart FISH probe kit (Abbott Molecular Probes, Abbott Park, IL) or the Ventana ALK (D5F3) IHC assay. Belgian regulations require that ALK status be confirmed by FISH for reimbursement of crizotinib (*www.inami.fgov.be/ www.riziv.fgov.be).* Although other IVD-CE labelled kits are available in Europe, a recent study assessing external quality reports that the majority of laboratories use the Vysis ALK break apart FISH probe kit.[20]

For ALK FISH testing, 50 tumour cells nuclei are to be evaluated. Selection of tumour nuclei is important and not so easy on FISH slides and to this end, sufficient morphological knowledge for interpreting FISH slides is important. This underlines the necessity of a pathologist for FISH slide evaluation. Scientists who are experienced and well trained in solid tumour testing are competent in FISH slide enumeration. However, a pathologist with experience in FISH reading should be available for cases where there is any doubt about the location of the tumour cells.

Other techniques

RT-PCR is not recommended by international guidelines as an alternative to FISH.[5]

However, targeted next generation sequencing (NGS) tests have the potential to identify gene rearrangements. Some panels are already commercially available. Some studies reported patients who were negative by FISH and positive by NGS and responded to crizotinib.[17,19,21]

ROS1

ROS1 gene rearrangement is observed in ~2% of patients with lung ADC. Rearrangements result in a fusion between part of the ROS1 gene and part of a partner gene leading to the oncogenic transformation.[22] ROS1 rearrangements are mutually exclusive with EGFR mutations and ALK rearrangements.

The American Society of Clinical Oncology (ASCO) has recently updated its guidelines for treating advanced lung cancer.[23] These updated ASCO recommendations incorporate ROS1-guided options. ASCO now recommends crizotinib as a first-line option for patients with Stage IV NSCLC and ROS1 rearrangements.[23] However, there are currently no guidelines that specify when ROS1 rearrangement testing is indicated. NCCN guidelines propose considering ROS1 testing for metastatic patients with ADC, large cell or NSCLC NOS histology and with a negative test for EGFR and ALK, or for metastatic patients with SCC histology in never-smokers or small biopsy specimens and with a negative test for EGFR and ALK (*www.nccn.org).*

Clinical trials studying the response to crizotinib of patients with NSCLC and ROS1 rearrangement used FISH for ROS1 testing.[24] ROS1 IHC has been described; there are some discrepancies regarding its sensitivity.[25-27] However, more recent studies using the D4D6 antibody demonstrated good results and may be considered as a screening tool, as detailed for ALK.[25,27] Now ROS1 FISH using a break apart probe is considered as the best test for ROS1 testing, but as already mentioned for ALK, tar-

geted NGS is also a promising tool for gene rearrangement screening.

Other molecular markers

Different biomarkers for which potentially active agents are being evaluated, such as MET amplification, MET exon 14 skipping, PIK3CA or BRAF mutations, HER2 amplifications or mutations, have been proposed as valuable for managing patients with lung cancer. It is interesting to note that, for patients with metastatic disease, the NCCN NSCLC guidelines panel strongly endorses broader molecular profiling with the goal of identifying rare driver mutations for which effective drugs may be available, or to appropriately counsel patients regarding the availability of clinical trials (*www.NCCN.org*).

Targeted NGS technologies, by simultaneous sequencing of thousands of short DNA sequences in a massively parallel way, may offer a cost effective approach for detecting multiple mutations with a minimum amount of DNA.[4] Several studies have already validated the clinical use of targeted NGS in term of sensitivity, speed and cost.[28-32] Moreover, this technology, using gene panels, allows for screening of other potentially clinically actionable alterations in cancer-related genes. This technology is considered appropriate for testing mutations if it is validated by external quality assurance control, such as for all molecular methods.[4]

At the present moment, no consensus has emerged for PDL1 IHC about the choice of the antibody or the scoring method because different companies suggest different choices of antibodies and different scoring systems. In addition, given the fact that some negative IHC patients could response to the therapy, the working group has agreed that it is not possible to give general recommendations currently.

Turn-around-time

In the scope of 'on-demand' testing, a crucial item is the turn around time (TAT). Several definitions of TAT can be used: the time to issuing a final report from:

- The clinical request for molecular testing,
- The request of histological tissue from its source laboratory, or
- The receipt of the tissue block at the testing laboratory.

The last definition is the most commonly used.

International guidelines recommend that the results of molecular testing (EGFR and ALK) should be available within ten working days after receiving the sample in the testing laboratory.[5] Moreover, the expert consensus opinion is that laboratories should establish procedures to ensure that the samples are sent to the molecular laboratory within three working days of receiving the request.[5] However, in a recent paper on EGFR testing in Flanders, the median time for the local pathology labs to prepare and ship the tumour samples was between five and sixteen days in 37,5% of cases. However, once the local pathologists were made aware of the delays in processing EGFR-mutation requests and shipping tumour samples, corrective actions were taken by the local labs. This resulted in a marked improvement in the local processing times: all tumour samples were shipped within seven days.[33] Local pathologists should be aware of this problem and instruct their secretaries accordingly. In addition, when test results arrive, they must be urgently communicated to the physician in charge of the patient.

Reporting

Guidelines for reporting the results of molecular tests are based on the International Organisation for Standardisation (ISO) 15189:2012 requirements for medical laboratories. Molecular reports should include both results and clinical interpretation that are understandable by clinicians.[4] The protocols should contain the sample characteristics (including identity of the block used for molecular analysis) and the percentage of tumour cells. For sequencing assays, each gene and exon sequenced should be listed. For targeted mutation assays, each targeted mutation should be listed. The analytic sensitivity of the test (that is related to percentage of tumour cells in the extracted sample) should be stated clearly.[4] In this way, details of the specific mutation found and their relevance to the response to EGFR directed TKIs should be documented. It should be stressed that data on the clinical significance of rare mutations are frequently updated. It is the responsibility of the test coordinator to be aware of these data.

If a result is inconclusive, whether due to assay failure or to an insufficient specimen, or for another reason, the report should state why and suggest requirements for testing a different specimen that would be more likely to yield a successful result.

The same overall principles apply to ALK or ROS1 reports, with a few exceptions. For IHC, the methodology should be indicated, including the clone used. For FISH, the results section should also include the number of cells analysed, and the number and percentage of cells with gene rearrangement.

Key messages for clinical practice

1. NSCLC have to be classified into specific subtypes. Judicious use of two IHC markers (one ADC and one SCC markers) helps for cases with an indeterminate morphology on H&E sections.
2. Preanalytical parameters should be standardised and all tests should be accredited according to the International Organisation for Standardisation (ISO) 15189:2012.
3. For patients with an advanced stage or a recurrent disease, EGFR, ALK and ROS1 testing are recommended for lung cancer samples with at least one ADC component.
4. EGFR mutation testing: a wide coverage of exon 18-21 is encouraged, including detection of TKI resistance-associated mutations.
5. ALK and ROS1 testing: according to Belgian law, pre-screening with ALK IHC is mandatory, followed by FISH confirmation in the case of positive IHC. For the moment, ROS1 FISH testing is considered the gold standard.
6. The results of molecular testing should be available within ten working days after receiving the sample in the testing laboratory.
7. These guidelines will be updated reflecting changes in daily practice.

Quality

These molecular studies demand competence. The procedure needs to be standardised and performed in reference labs that are accredited according to the International Organisation for Standardisation (ISO) 15189:2012 and participate in internal and external quality controls as detailed in law article 33bis.[4] For successful patient treatment, it is of great importance that molecular test results are highly reliable and accurate. This is also true for IHC. A recent study about external quality assessment showed that the error rates for IHC were greater than those for FISH.[20] Advice on IHC protocols is provided on the NORDIQC website (*www.nordiqc.org).* The study of Tembuyser et al. shows an improvement of ALK testing after external quality assessment. This suggests that laboratories take into account the results and the comments of the external quality assessors to enhance their performance.[20] Participation in external quality assessment allows for rapid exposure of errors or deviations from the protocol. Evaluation of the prevalence of positive cases in comparison to the data reported in the literature and the expected frequency is another control method to detect deviation from protocol. The pathologists involved in molecular testing must be qualified according to Belgian law and accordingly recognised by RIZV/INAMI.

Conclusions

Accurate histologic subtyping of NSCLC is now required because the management of the patients depends on the histological diagnosis. In this field, the pathologist needs clinical data (the smoking history and to exclude the possibility of metastatic disease in the lung) and the use of IHC is recommended only in cases where the subtyping cannot be made on H&E sections. Molecular testing represents a paradigm shift in lung cancer treatment and is now a standard of care. However, the increase in the number of markers to test is associated with a decrease in the sample size. The pathologist is facing a new challenge: optimisation of available tumour tissue. In *Figure 1* a proposal for an algorithm for the optimisation of the management of NSCLC samples is depicted. The development of new-targeted therapies with predictive biomarkers renders this a rapidly changing field and these guidelines will very likely require updates reflecting changes in daily practice.

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