



## Impact of the College of American Pathologists, the International Association for the Study of Lung Cancer, and the Association for Molecular Pathology clinical practice guidelines for *EGFR* and *ALK* testing in lung cancer in Canada

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### ABSTRACT

This paper summarizes the practical impact of the College of American Pathologists, the International Association for the Study of Lung Cancer, and the Association for Molecular Pathology lung cancer biomarkers guidelines on the lung cancer approach in Canada, providing possible practical solutions for other similar health care systems in which scientific reality needs to be constantly balanced against economic reality.

### KEY WORDS

Lung cancer, College of American Pathologists, CAP, International Association for the Study of Lung Cancer, IASLC, Association for Molecular Pathology, AMP, guidelines

### 1. BACKGROUND

The field of lung cancer is undergoing a revolution<sup>1</sup>. There has never been a more exciting time to be part of a lung cancer care team. The explosion of molecular and genetic information on lung cancer has forever changed the understanding of, diagnostic approach to, and treatment pathways for lung cancer patients—and that change is just the beginning. Pathologists are increasingly called upon to provide not only a more detailed, accurate diagnosis but also an increasing number of prognostic and predictive analyses and interpretations.

With the fast pace of development and the demand for new and complex clinical tests, several aspects of testing have to be standardized, and guidelines have to be developed to align expectations, clinical practice, and performance. International guidelines can serve as the scientific backbone for testing, but the economic reality of each health care system plays an increasingly important role in the way medicine is practiced.

The present paper summarizes the practical impact of the College of American Pathologists (CAP),

the International Association for the Study of Lung Cancer (IASLC), and the Association for Molecular Pathology (AMP) lung cancer biomarkers guidelines<sup>2,3</sup> on the lung cancer diagnostic algorithm in Canada, providing practical potential solutions for other similar health care systems in which scientific reality has to be constantly balanced against economic reality.

### 2. IMPLEMENTATION

In November 2011, three major international organizations—CAP, IASLC, and AMP—together made draft recommendations for lung cancer biomarker guidelines available for public comment<sup>2</sup>. This important document was developed by a panel of experts chaired by Drs. Phil Cagle (CAP), Neal Lindeman (AMP), and Marc Ladanyi (IASLC) after a comprehensive literature review that selected 521 articles, published from January 2004 to February 2012, from electronic databases of publications. The final guidelines—recently published in the *Journal of Molecular Diagnostics*, *Journal of Thoracic Oncology*, and the *Archives of Pathology*—are structured in five sections:

1. When should testing be performed?
2. How should *EGFR* testing be performed?
3. How should *ALK* testing be performed?
4. Should other genes be routinely tested in lung adenocarcinoma?
5. How should molecular testing of lung adenocarcinomas be implemented and operationalized?

Quality assurance for biomarker testing in Canada, as in most parts of the world, is in its infancy, and guidelines such as those presented in the CAP–IASLC–AMP document are essential not only for the laboratories that perform the tests or are contemplating initiating a testing program, but also for tissue procurers (respirologists, radiologists, thoracic surgeons), pathologists handling the tissue for the initial assessment, and clinicians using the results.

There is general consensus in the lung cancer community that clinical characteristics (for example, age, sex, ethnicity, and smoking history) are not sufficiently sensitive to select patients for *EGFR* or *ALK* testing, which is the document's first recommendation<sup>4</sup>. Even with budgetary restrictions in place in a lab, such criteria should not be used to exclude from testing patients who might benefit from therapies targeted to *EGFR* or *ALK*. It is now evident that mutations may be found in all demographic subgroups<sup>5</sup>. It is important to remember that the first case of *ALK*-positive lung cancer was reported in a smoker<sup>6</sup>, that translocations have been detected in smokers and elderly patients<sup>7</sup>, and that *EGFR* mutations have been described in smokers of many different ethnic backgrounds. On the other hand, some patients who might otherwise not qualify for testing can be tested for *ALK* rearrangement. For example, very young patients (<40 years of age) can be tested, given that the median age of onset for *ALK*-positive lung cancer is approximately 10 years earlier than for *ALK*-negative cases, whose reported mean is in the mid-fifties<sup>8</sup>. The unusual cases of nonsmokers or light smokers with squamous cell carcinoma represent another clinical group that should be tested for *EGFR* and *ALK* as exceptions. In Canada, such patients are currently often triaged within the local lung tumour group, with further biomarker testing initiated as appropriate. By contrast, the type of lung cancer to be tested is a reasonable criterion for selecting patients for testing, because *EGFR* mutations and *ALK* rearrangements are most often detected in adenocarcinomas<sup>9</sup>.

In conjunction with clinical, radiologic, molecular, and surgical information, histologic profile criteria remain the foundation for classifying lung cancer subtypes. Standardized criteria for the classification of biopsy, cytology, and resection specimens are important both for research results and for directing patient care. The American Thoracic Society, IASLC, and the European Respiratory Society published an update for classification of the most common subtype of non-small-cell lung cancer (NSCLC), adenocarcinoma<sup>10</sup>. The intent of that collaborative document was to provide uniform terminology and diagnostic criteria, together with accompanying strategies to optimally manage tissue for molecular and immunohistochemical studies.

To summarize the CAP-IASLC-AMP recommendations for clinical practice, the histologic types of lung cancer that should be excluded from *EGFR* and *ALK* testing are neuroendocrine carcinomas (small-cell and large-cell neuroendocrine carcinomas) and pure squamous cell carcinomas<sup>2</sup>. In accordance with the 2011 guidelines from the U.S. National Comprehensive Cancer Network<sup>11</sup>, the use of "NSCLC not otherwise specified" should be minimized through the use of immunohistochemistry (IHC) for the subclassification of all carcinomas within that group. Biomarker testing should be performed on the most

recent specimen available, be it from a primary lesion or a metastatic one<sup>2</sup>. A single area from the tumour is sufficient for testing, and in patients with synchronous primaries, both tumours should be tested<sup>2</sup>.

Patients with advanced-stage lung cancer (stages III and IV) show superior outcomes with first-line targeted therapies, and studies are underway to determine the benefit of testing early-stage lung cancer patients at the time of diagnosis. In Canada, given the economic restrictions associated with testing, only advanced-stage lung cancer patients are tested for *EGFR* (and more recently for *ALK*) at the request of a clinician—most often a medical oncologist. Reflex testing is not performed in Canada because some patients may be considered only for palliative care because of their poor performance status or comorbidities, or they might not be candidates for tyrosine kinase inhibitor therapy for other clinical reasons, including poor performance status, poor renal or liver function, and so on.

*EGFR* testing is performed in Canada only as a predictive test and not for the sole purpose of being used as a favorable prognostic factor. Because many lung cancers are initially diagnosed in community hospitals, pathologists are often unaware of clinical stage at the time of the diagnosis, and therefore, in Canada, testing is not initiated by the pathologist. In some Canadian centres (the University Health Network, for instance), all specimens sent for *EGFR* testing undergo a review by a pulmonary pathologist, and further subclassification by IHC is performed as needed. In most other centralized laboratories, the specimens are assessed by a pathologist for tumour content and eligibility for molecular testing, but no formal review is performed because of a lack of resources for this extra step. For those institutions, the lack of secondary or tertiary review may slightly increase the number of "NSCLC not otherwise specified" results.

When multiple specimens are available, one of the most important decisions made is the choice of recent specimens to test. That decision is often made by the clinician (medical oncologist, respirologist, interventional radiologist, or surgeon). The requesting physician should be familiar with all parts of the pathology report and should understand that a successful *EGFR* testing outcome is not determined by sample type, but greatly depends on malignant cell content, DNA quality, and of course, sample size. The gross description, for example, specifies the fixative used and mentions whether decalcification was performed—factors that both can interfere with DNA quality. For cytology specimens, this area of the report specifies whether a cell block is available for cytology or whether only smears were performed. By becoming familiar with those terminologies, the clinician can accurately identify the best specimen for testing. In our Canadian experience, multidisciplinary education events are extremely helpful for

communicating such issues and often make a huge impact on the entire testing process. Understanding each subspecialty's limitations and issues can significantly improve tissue acquisition and processing. Strong communication with the local testing laboratory is often a plus. One of the CAP guidelines about monitoring states that "the percentage of specimens that are being rejected as inadequate for each specimen type" is to be actively calculated in all centralized laboratories in Canada and that feedback is to be provided to the lung tumour groups "to assist in obtaining the specimen type that is most likely to yield a diagnostic result."

The goal of the surgical pathologists reporting lung cancer cases is to acquire expertise in diagnosing lung cancer cell subtypes in small samples, with routine histology and with the conservative use of IHC and special stains<sup>12,13</sup>. An effort should be made to preserve the tissue in the block for further testing<sup>13</sup>. Pathologists also need to gain expertise in identifying and selecting appropriate tissues for molecular studies—which is often easier in theory than in practice.

The guidelines specify that pathologists should determine the adequacy of specimens for *EGFR* testing by assessing cancer-cell content and DNA quantity and quality. In Canada, DNA quality and quantity are assessed mainly by the cytogeneticist assigning the *EGFR* test. Knowledge of the molecular tests is required to select the best tumour area for testing (with the highest number of viable tumour nuclei, without necrosis or excessive hemorrhage, and so on). Quality assurance for this important initial step in molecular testing is vital.

From a medicolegal viewpoint, it is important that pathologists understand the clinical implications of certain diagnoses. A diagnosis of squamous cell carcinoma, for example, denies a patient several treatment options. The same is true when pathologists assess the tumour content of a specimen for molecular testing. Insufficient tumour tissue is the main reason for molecular test cancellations<sup>14</sup>. Tests should be cancelled upfront only by pathologists familiar with tissue requirements, the local testing algorithm, and the sensitivity and specificity of each test. In Canada, assessment of samples is performed in centralized laboratories and not by the originating hospitals who receive the initial request for testing. The direction from the CAP guidelines is that any specimen meeting the laboratory's requirements for tumour content, fixation, and quality as established during validation may be chosen for analysis.

The guideline recommendation for the turnaround time for *EGFR* and *ALK* testing is a maximum of 2 weeks (10 working days). Currently, most Canadian laboratories send their specimens to specialized laboratories for *EGFR* and *ALK* testing. The Canadian national clinical experience with respect to *EGFR* mutation testing is strong. Initially, it was performed for at least 12 months (from March 2010)

in only five laboratories throughout Canada<sup>15</sup> as part of AstraZeneca's Iressa Alliance Program. The extensive data from that program have been shared with all participating institutions, representing a great forum for scientific discussions and future research. The experiences of the busiest laboratories—the University Health Network in Toronto and the BC Cancer Agency Laboratory in Vancouver—which involved more than 2000 patients, were presented at the 2012 U.S. and Canadian Academy of Pathology annual meeting in Vancouver<sup>16,17</sup>. The turnaround time accomplished by those laboratories was an average of 11 working days from the ordering of the test (approximately 7 days for the intra-laboratory turnaround time). The biggest challenge was obtaining the tissue samples from the originating hospitals. The CAP recommendation is that "samples should be sent to an outside Pathology lab within 3 working days of receiving requests and to intramural molecular Pathology laboratories within 24 hours."

In Canada, compared with other countries, molecular testing is centralized to a large extent, and transferring tissue to the testing laboratory often increases the overall turnaround time. Archival tissue requests are an economic burden for all laboratories and have to be taken into account in the future budgeting for community hospital laboratories in particular. Requests for additional clinical testing, including molecular testing, requests for clinical trials, and requests for research projects are constantly sent to laboratories, often with tight time constraints. In Canada, the centralized laboratories worked together with community hospitals to assure that each hospital had a well-established process in place for handling archival tissue requests in a selective and timely manner. Education events were also organized for community pathologists to assure their understanding of the need for rapid testing in lung cancer patients and of the clinical impact of the testing. Additionally, pathologists were encouraged to identify blocks suitable for additional testing. This small detail, when available, proved to save clerical time at the originating hospitals and the central laboratory. It is also very helpful for the clinicians sending the test requests and the pathologists responsible for identifying the best available testing specimen in a timely manner.

The education events had a positive effect on preservation and delivery of tissue for molecular testing. However, as already mentioned, evaluation of tumour content should be performed by a pathologist from the lab where the test is performed. Minimum percentage requirements for tumour cellularity vary with the test methodology being used and are determined by each laboratory during validation of the test<sup>2</sup>. Test validation should include an assessment of the sample by a pathologist (critical for accurate testing) and an evaluation of any tumour enrichment procedures<sup>18</sup> being used (such as macro or microdissection, coring, scrolling, and so on). Unmodified Sanger sequencing

is no longer considered to have a sensitivity sufficient for *EGFR* testing. Methods with better sensitivity are encouraged, with 10% sensitivity being the recommended analytical goal. In other words, testing should be able to identify mutations in samples with as few as 10% cancer cells<sup>1,19</sup>. In addition to establishment of the analytic sensitivity of the *EGFR* testing method during validation, other CAP guidelines implemented in Canadian molecular reference laboratories included testing a low-positive control specimen (near the lower limit of the tumour content of specimens accepted by the laboratory) in each clinical assay run, and participation in *EGFR* quality assurance programs assuring that testing is “monitored in an ongoing fashion once clinical testing is initiated.”

One of the most challenging recommendations for Canadian laboratories is that “*EGFR* testing should capture all individual mutations reported in at least 1% of *EGFR*-mutant lung adenocarcinomas”<sup>2</sup>. In balancing the scientific and economic aspects of testing, all but 1 Canadian laboratory are providing testing only for the most common mutations: short in-frame exon 19 deletions and the L858R point mutation in exon 21<sup>1,15,20</sup>. Although other *EGFR* mutations may be missed as a result, this approach to testing was—and remains—the most practical given current economic reality. Clinical implementation of this recommendation in Canada will require additional funding for the validation and standardization of new tests that may not be as widely available as current testing procedures. As more experience with tyrosine kinase inhibitor therapies is gained, clinical interest in evaluating not only other *EGFR*-activating mutations<sup>21</sup> (for instance, in exon 18: E709, G719 mutations; in exon 19: all deletions and rare insertions; in exon 20: insertions, S768; in exon 21: L858R, L861Q, T854) but also resistance mutations will grow, and therefore implementation of new *EGFR* mutation testing methods is crucial and should be addressed immediately. However, as stated in the Disclaimer of the CAP clinical practice guideline, “adherence to any practice guideline or consensus statement is voluntary, with the ultimate determination regarding its application to be made by the physician in light of each patient’s individual circumstances and preferences.”

Finally, although the genes *T790M*, *D761Y*, *L747S*, and an insertion in exon 20 are associated with resistance to epidermal growth factor receptor (*EGFR*) tyrosine kinase inhibitors, this information is rarely used clinically at the present time, and therefore testing for resistance mutations is not clinically available in Canada<sup>22–27</sup>. As mentioned in numerous other publications, *EGFR* IHC and copy number analysis or *KRAS* mutation testing are not recommended for selection of *EGFR* tyrosine kinase inhibitor therapy<sup>10,19</sup>.

*ALK* rearrangements occur in approximately 2%–5% of NSCLCs and have been reported in up to 13% of selected populations, inclusive of patients with at least 2 of the following characteristics: female sex,

Asian ethnicity, never or light smoking history, and adenocarcinoma histology<sup>4,6,28</sup>. *ALK* rearrangements are mutually exclusive of *EGFR* and *KRAS* mutations<sup>29,30</sup>. Most *ALK*-positive lung cancers are adenocarcinomas, with certain histologic subtypes (solid, signet-ring cell) being more commonly described in Western populations but not in East Asian populations. However, the recommendation is that the same population and tumour types that are candidates for *EGFR* testing should be tested for *ALK* gene rearrangements<sup>2</sup>.

The identification of an effective therapy for *ALK*-positive NSCLC places great emphasis on rapid, accurate, and cost-effective identification of patients with this subtype of lung cancer<sup>31</sup>. Fluorescence *in situ* hybridization (FISH) is currently the standard method to detect *ALK* rearrangements and, because of its use in clinical trials, the only test correlated with clinical response<sup>32</sup>.

Although *EGFR* and *ALK* testing have many similarities related to sample type, fixation, and so on, *ALK* testing has several unique characteristics that require special attention and guidelines. Because cells in a FISH test are analyzed individually through a microscope, “tumour architecture and cytology are more critical than tumour percentage as determinants of adequacy for *ALK* testing”<sup>2</sup>, and therefore evaluation of each slide by a pathologist is essential. The recommended number of cells to be analyzed is 50, and the suggested cut-off for *ALK* positivity is 15%<sup>33</sup>. However, it is essential that laboratories validate their own clinically sensitive and specific cut-offs. “Validation samples should include all types of sample processing,” including cytology samples, but “validation of different tissues of origin that are processed identically is not necessary”<sup>2</sup>. Technical details related to the analytical sensitivity and specificity of *ALK* FISH are discussed in the CAP recommendations, and although those details are beyond the scope of the present publication, they are of critical importance for the laboratories interested in introducing this complex test.

Given those technical details, and the recommendation that *ALK* FISH slides should be interpreted by two independent scorers possessing specialized training in FISH analysis of solid tumours (with guidance from a pathologist with training or experience in FISH)<sup>2</sup>, the test should be performed exclusively by laboratories with proven proficiency in FISH testing. Special attention should also be given to the interpretative criteria for FISH assays of *ALK* rearrangements, which are not identical to those applied in other neoplasms, even if an identical FISH probe set is used.

In Canada, crizotinib is approved for *ALK*-positive advanced (not amenable to curative therapy) or metastatic NSCLC. Health Canada specifies that, using a validated *ALK* assay, assessment for *ALK*-positive advanced or metastatic NSCLC should be performed by laboratories with demonstrated proficiency in the specific technology being used<sup>1,2,10,19</sup>. The methodology

for *ALK* testing in Canada is therefore not restricted to the Vysis molecular FISH assay (Vysis LSI *ALK* Dual Color, Break Apart Rearrangement Probe; Abbott Laboratories, Abbott Park, IL, U.S.A.) approved by the U.S. Food and Drug Administration.

The implementation of *ALK* testing in Canada, which is currently in progress, involves 13 laboratories from five Canadian provinces. The Canadian *ALK* Project aims to optimize *ALK* testing across Canada and to compare two different methodologies available for *ALK* testing (IHC and FISH). Through this exercise, the Project hopes to provide laboratories with extensive working details about the selection of *ALK* antibodies, detection systems, cut-off values, interpretation, and so on. This national effort also supports the parallel development of a national quality assurance program for *ALK* IHC. Although funding for the testing and the work flow are to be further developed by each laboratory individually, the approach is creating a reliable and economically viable testing network, which allows for sample exchange for quality assurance and for the development of databases for future research.

Because FISH is relatively expensive and time-consuming, and because it requires advanced technical and professional expertise, it is not readily available as a routine method in pathology practice in most laboratories. In contrast, IHC is relatively inexpensive and faster, and perfectly adapted for routine practice by academic and most community hospitals.

Given the high incidence of lung cancer and the large number of specimens that have to be screened to identify *ALK*-positive patients, the Canadian *ALK* Project is assuring validation of the inexpensive methodology (IHC), which can then be used as a screening test. However, it is imperative that laboratories be familiar with the sensitivities and specificities of the various commercially available *ALK* antibodies and with the interpretation and reporting details<sup>34</sup>. The clinical practice guideline recommends that “a properly validated IHC method may be used as a screening modality, and that tumours which fail to demonstrate *ALK* immunoreactivity with a sensitive IHC method need not be tested for *ALK* rearrangement by FISH. Tumours that are positive for *ALK* IHC, whether weakly or strongly, should still be referred to FISH for confirmation of a rearrangement.” That recommendation accords strongly with the Canadian guidelines and practical experience. It also once again shows the importance of *ALK* IHC as a screening test for the identification of a low-incidence event (such as an *ALK* rearrangement) in the setting of a high-incidence diagnosis such as NSCLC.

### 3. DISCUSSION

To date, Canadian clinical laboratories are performing only *EGFR* and *ALK* testing as the standard of care for patients with NSCLC.

One of the strengths of the implementation of molecular testing in lung cancer in Canada was the national approach taken. Multidisciplinary teams worked together in a common effort to finding the most scientifically feasible solution for testing within the country's economic reality. The result was not only the validation and standardization of the tests nationally, but also the formation of a quality assurance and maintenance program and of a network of laboratories that assure data and specimen exchange, and local and national guideline development.

The testing algorithm is an important consideration in the Canadian health care system, because a well-developed and operationalized system can save money while maintaining a clinically acceptable turnaround time. With the addition of *ALK* testing as a standard-of-care test and the pressure to test for additional *EGFR* mutations, the community is facing the challenging reality of increasingly small tissue samples (characteristic for lung cancer patients). This important issue is currently a priority for Canadian lung cancer teams, who are aiming to modify the understanding of tissue procurers (respirologists, interventional radiologists, thoracic surgeons) and of pathologists when it comes to tissue processing<sup>19</sup>.

As detailed earlier, realization of the CAP-IASLC-AMP recommendations within the Canadian system while taking into account limitations of resources has much to offer patients with lung cancer. It is important that laboratories be integrated into the overall cancer care system and that pathologists be engaged in the implementation of all targeted therapies that depend on laboratory testing<sup>10,19,35</sup>. As a professional group, pathologists should be proactive in determining laboratory procedures for all such implementations, based on a balance between patient care and resource availability.

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### 5. CONFLICT OF INTEREST DISCLOSURES

DNI is a consultant for AstraZeneca, Pfizer, and Eli Lilly, and took part in Advisory Boards for all these companies.

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