LUNG CANCER PATHOLOGY
Changing paradigm and priorities
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ABSTRACT
The last decade has witnessed the dramatic coming of age of targeted therapy in lung cancer. Several new targeted drugs have been approved but only for use in a subgroup of patients whose tumours harbour the genetic/molecular aberrations targeted by these drugs. In addition, several drugs are used only in lung cancer patients with specific histologic subtypes, as the latter may define efficacy or risk of toxicity. These recent developments have required pathologists to provide a more precise diagnosis of the tumour type, as well as additional molecular data on the presence or absence of the targetable molecular aberrations. Thus, pathology practice is not just diagnostic for cancer, but has to provide information on predictive biomarkers to guide drug selection. As more and more drugs and treatment options will be based on the molecular characteristics of the tumour, pathology practice must also embrace and adopt the new advances being achieved in molecular medicine and targeted therapy. The ability to meet the challenges presented by the changing paradigm in cancer treatment will define the future role of pathologists and pathology departments.

INTRODUCTION
Lung cancer is the leading cause of cancer-related death worldwide among males, and the second leading cause among females. In 2008, it accounted for 13% (1.6 million) of all cancer deaths worldwide. Despite recent data showing decreasing incidence in developing countries and notable advancements in its early detection and treatment, lung cancer is still projected to be the sixth leading cause of death in 2030, advancing from the ninth position in 2002. In North America and Canada, the mortality rate of lung cancer is greater than the next 3 most common cancers combined: colorectal, breast and prostate cancer. Currently as many as half of new lung cancer patients are former smokers who had quit for more than 10 years. This is due to significant lag time between carcinogenic initiation and development of invasive cancers, such that the benefits of smoking cessation take approximately 20 years to realize. Importantly, lung cancers of never-smokers demonstrate distinct clinical features and associations when compared to those of smokers, accounting for at least 20% of lung cancer cases worldwide and being more common among females. Thus, lung cancer will remain a major health burden worldwide for many years to come.

Currently, the overall 5-year survival rate for all lung cancer patients is approximately 15%. This poor overall rate reflects largely the fact that around two-thirds of patients are initially diagnosed with advanced stage disease with very little possibility of cure by surgery. The treatment of lung cancer patients is based primarily on the histologic diagnosis of the tumour and the stage of the disease. Two major histologic types with different biology and therapeutic responses are recognized: small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC). SCLC is a highly aggressive cancer with almost all patients presenting with advanced loco-regional or systemic metastases at diagnosis. Therefore, SCLC patients are rarely treated by surgery but mainly by chemotherapy, to which it is highly sensitive. Yet, despite the high initial response rate, very few SCLC patients are curable and their median survival time is approximately 18 months.

In contrast, NSCLC is a very heterogeneous disease with approximately 80–85% being represented by adenocarcinoma (ADC) and squamous cell carcinoma (SCC). Importantly, about one-third of NSCLC patients present with tumours that are still localized to the lung or immediate local lymph nodes, and thus can potentially be cured by complete surgical resection.
However, even for patients who are initially treated by surgery, there is a 30–60% risk of death from tumour recurrence. Clinical trials conducted in North America, Europe and Japan during the last 2 decades have demonstrated that for a subgroup of patients, especially Stage II patients, adjuvant chemotherapy is effective in significantly increasing survival. Currently, there is an intensive effort to identify additional molecular biomarkers that could further stratify patients at high risk of developing postsurgical metastatic recurrences, and thus who are most likely to benefit from adjuvant chemotherapy. Equally important is to identify patients who have inherently good-prognostic tumours, are at low risk for developing metastasis, and thus could be spared from the toxicity of adjuvant chemotherapy.

RECENT DEVELOPMENTS IN LUNG CANCER PATHOLOGY

For advanced NSCLC patients, the last 5–6 years have witnessed the availability of several novel drugs, some having been developed to target molecules/pathways that are crucial for the growth and survival of cancer cells. Most importantly, these targeted therapies have demonstrated safety or effectiveness in specific subgroups of patients, thus requiring selection of patients suitable for the use of these agents (Table 1).5,6 The fact that these therapy-related biomarkers include both tumour histologic typing and molecular aberrations highlights the importance of greater precision in histologic classification of the tumours, and the necessity of acquiring and preserving tissues for molecular testing to be performed after the initial histology-based diagnosis and classification. Furthermore, new efforts or strategies need to be developed for ensuring that minimal biopsy materials (e.g. from fine needle aspiration biopsy) are processed such that sufficient material would be available not only for the diagnosis of malignancy, but also for biomarker testing.

Consistent with this concept, a recently published report developed by multidisciplinary experts representing the International Association for the Study of Lung Cancer (IASLC), the American Thoracic Society (ATS) and the European Respiratory Society (ERS) provided some “good pathology practice” guidelines on diagnostic pathways for the handling and diagnosis of small or minimal biopsy specimens, including the panel of immunohistochemical (IHC) markers that are recommended for more accurate histologic typing of lung carcinomas.7 An algorithm for determining the type of specimens that should be subjected to molecular testing has also been proposed,7 as certain tumour types appear to demonstrate greater association with specific molecular aberrations.8,9

ROLE OF PATHOLOGISTS IN THE AGE OF PERSONALIZED MEDICINE

With the new developments described above, pathology has assumed even greater responsibilities in the diagnostic and therapeutic pathways of patient care. Aside from its traditional role in diagnostic pathology to identify and classify cancer and/or cancer cells based on histology and cytology, pathologic reports also need to include predictive pathology elements that are essential for selecting specific treatments for the patients. Importantly, such responsibility goes beyond deciding what markers to assay for or molecular tests to perform, and also includes many aspects of good laboratory practice and scientific knowledge associated with the performance of these tests. These include ensuring that: 1) adequate specimens are obtained during the biopsy procedure for both diagnostic and “predictive” molecular testing.

Table 1. New therapies for advanced NSCLC patients with their selection markers

<table>
<thead>
<tr>
<th>Drug</th>
<th>Treatment setting</th>
<th>Patient cohort</th>
<th>Selection marker</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gefitinib/erlotinib</td>
<td>first line</td>
<td>ADC</td>
<td>EGFR mutation*</td>
</tr>
<tr>
<td>Erlotinib</td>
<td>≥ second line or</td>
<td>NSCLC</td>
<td>none</td>
</tr>
<tr>
<td></td>
<td>maintenance</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cetuximab</td>
<td>first line with chemotherapy</td>
<td>NSCLC</td>
<td>none (potentially EGFR protein level by IHC)</td>
</tr>
<tr>
<td>Bevacizumab</td>
<td>first line with chemotherapy</td>
<td>non-squamous NSCLC</td>
<td>non-squamous histology</td>
</tr>
<tr>
<td>Pemetrexed</td>
<td>all</td>
<td>NSCLC</td>
<td>non-squamous histology</td>
</tr>
<tr>
<td>Crizotinib</td>
<td>all</td>
<td>NSCLC (ADC)</td>
<td>rearranged ALK gene</td>
</tr>
</tbody>
</table>

ADC=adenocarcinoma; ALK=anaplastic lymphoma kinase; EGFR=epidermal growth factor receptor; IHC=immunohistochemistry; NSCLC=non-small cell lung carcinoma
*Improvement in progression-free survival

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testing; 2) samples are appropriately processed such that molecular assays will not be compromised; 3) appropriate samples (e.g. paraffin block) with sufficient tumour cellularity are selected for molecular testing; 4) the areas of the tumour with greatest tumour cellularity are identified and chosen for molecular testing (e.g. sequencing or fluorescent in situ hybridization [FISH] analyses; and 5) molecular test results are integrated and correlated with the histopathologic diagnoses.

As genetic aberrations may occur in specific tumour types and tissue may be limited to perform multiple independent tests, the histologic evaluation may be used to streamline ordering of molecular tests, in order to conserve tissue and shorten the time for reporting the diagnostic results. As an example, mutations on the epidermal growth factor receptor (EGFR) gene are known to occur predominantly in ADC (~40–60% among East Asians and 15–20% among Caucasian lung cancer patients) and have been found much less frequently (2–4%) in SCC. Thus, for economic and practical reasons, EGFR testing is commonly limited to non-squamous NSCLC or ADC samples only. However, ADC is a highly heterogeneous tumour with multiple differentiation lineages. It is known that ~80% of lung ADC also stain positive for the nuclear marker thyroid transcription factor 1 (TTF-1), while SCC very rarely stains for TTF-1. Furthermore, mucinous ADC is mostly TTF-1 negative and is associated more commonly with the presence of v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog (KRAS) mutation.

It is also known that, except in very rare cases, the occurrence of EGFR and KRAS mutations are mutually exclusive. Therefore, while current practice tends to use histology (non-squamous) as the first criterion for ordering EGFR mutation testing, emerging evidence suggests that perhaps TTF-1 positivity could potentially be a better selection marker for testing. While TTF-1 is an IHC test that is routinely and extensively used in histopathology practice at relatively low cost, DNA sequence analysis used in EGFR mutation testing takes more time to complete and costs at least ten times more. Pending further confirmation of the low frequency of EGFR mutation being present in TTF-1 negative tumours, the adoption of TTF-1 as a selection marker could potentially reduce EGFR mutation testing volume by 20–30%. A similar algorithm could potentially be applied to the testing of anaplastic cell lymphoma kinase (ALK) gene rearrangement, as this genetic aberration has been reported to have a high association with a specific but rare histologic type of ADC with “signet ring” appearing tumour cells. Therefore, by integrating histopathology into a molecular-marker testing algorithm, pathologists have the opportunity to develop diagnostic algorithms that could make molecular testing more cost-effective, resulting in shortened turnaround times and increased efficiency for the molecular testing laboratory.

CHALLENGES FOR PATHOLOGISTS AND PATHOLOGY DEPARTMENTS

In the current era of greater societal expectation for better and more effective healthcare in the face of limited healthcare budgets and continuing increases in the costs of laboratory procedures and tests, pathologists face significant challenges to deliver their professional mandate. This means not only greater precision in their diagnoses but also greater information content necessary for the delivery of personalized medicine. Pathology reports will require molecular information about the tumour that is necessary for oncologists to tailor treatment with the most effective and/or best tolerated therapies. As the amount of biopsy tissues may be limited, especially in advanced cancer patients, strategic institu-
tional protocols with multidisciplinary inputs should be developed to prioritize the marker studies that are required for the initial accurate diagnosis, to ensure tumour samples are available for necessary molecular testing, and conserve samples for future tests that may as yet be identified and developed.

The field of molecular oncology, targeted therapy and predictive biomarkers is rapidly evolving. Every year we are witnessing reports on positive clinical trials involving new targeted therapies in patients whose tumours harbour specific molecular aberrations. The responsibility for the development and rapid clinical implementation of assays for these predictive markers falls within the professional responsibilities of pathologists, who increasingly need to keep abreast of new advances in the molecular pathology of cancers, especially those relevant to novel therapeutics being tested in clinical trials. This requires greater participation of pathologists in multidisciplinary rounds that allow them to interact closely with their clinical oncologist colleagues. Working closely with interventional radiologist colleagues to obtain adequate first biopsy specimens may also spare patients the need for repeat biopsies, thus reducing morbidity and cost, and at the same time shortening the time for treating physicians to obtain all diagnostic information necessary to make treatment decisions. Last but not least, pathologists should continue to strive to make their diagnostic algorithm more efficient and cost-effective by exploiting the association between specific histomorphologic and IHC features of tumours with molecular markers, as discussed above.

COSTS OF MOLECULAR DIAGNOSTICS AND PREDICTIVE PATHOLOGY
Current histopathology and cytopathology involve routine dye-based staining as well as IHC. The low material cost and high throughput of these techniques render their unit cost relatively low. When it comes to molecular assays such as mutation analysis by polymerase chain reaction (PCR) and sequencing or by FISH, the cost of the reagents and low throughput may make each test appear to be “expensive” (in the hundreds of dollars per test). As more and more markers become available, development of multiplex testing will be required due to the limited amount of biopsy materials available for testing. However, it is important to note that predictive marker testing tends to be a one-time cost, as the molecular marker relevant for treatment is either present or absent in the tumour. This is in contrast to imaging studies or blood tests, which tend to be performed multiple times during the course of patient treatment or care to track clinical progression.

The real cost of a test to a healthcare system should be measured based on the frequency of positive test results. For each EGFR mutation-positive patient identified, the overall cost of testing would be approximately 5 times the unit cost of the test, as only ~15–20% of non-squamous NSCLC patients tested would be positive. For targeted drugs with lower target prevalence, e.g. ALK gene rearrangement that is reported in only ~5% of lung ADC patients, the effective cost of the test by FISH would be substantially higher, at 20 times the unit cost. Fortunately, antibodies specific to mutant EGFR or fusion (aberrant) ALK proteins have been developed.17,18 Once their sensitivity and specificity have been determined and validated, their application in routine histopathology laboratory may further streamline the molecular testing algorithm.

PERSONAL PERSPECTIVE AND CONCLUDING REMARKS
Pathology has traditionally been the medical science discipline that focuses on recognizing pathologic changes occurring in diseases and on understanding the mechanism of disease development. Pathologists have been accustomed to adopting new technologies to enhance their diagnostic accuracy, such as electron microscopy and IHC. However, pathologists are currently facing a new challenge in continuing this traditional role in the age of molecular medicine, targeted therapy and personalized medicine. To meet these challenges, pathologists must continue to equip themselves with new knowledge and expertise in molecular biology and pathology, their clinical impacts, and technologies to detect and measure clinically relevant molecular changes. The challenge is unprecedented, as in all areas the
advances are very rapid and exponential. More resources need to be devoted to continuing education for the pathology community on advances in targeted therapies and biomarkers, which may enhance the accuracy of their histologic or cytologic diagnoses and provide critical information for patient treatment. In the conflicting world of less tissue for greater information, pathologists need to develop protocols for processing biopsy samples and ordering tests, with clear priorities to provide the most diagnostic content, lowest turnaround time and greatest cost-effectiveness. Rapid advances in functional imaging and nanotechnologies will further accelerate the development of new methods for diagnosis using minimal or noninvasive approaches. While morphologic diagnoses currently remain, in most instances, the accepted “gold standard,” this may shift as one day the accuracy and reliability of these new technologies may potentially supersede the relative subjectivity of microscopic morphologic assessment. As pathologists and laboratory physicians have the unique responsibility to issue diagnostic test results for clinical use, the ability of pathologists to continue their mandate rests with their ability to adapt, prioritize and adopt the rapid advances that are occurring in the field of molecular medicine.

REFERENCES

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