Challenges in NSCLC molecular testing
Barriers to implementation
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Over the past 4 years, the oncology community has seen a paradigm shift in the molecular diagnosis and treatment of lung cancer. Yet integrating this new knowledge into routine clinical practice in Canada has been a major challenge. Lung cancer remains the leading cause of cancer-related mortality in both Canadian women and men, with a 5-year survival rate of only 18%.2,3 Biomarker-directed cancer-related mortality in both Canadian women and men, a major challenge. Lung cancer remains the leading cause of cancer therapy may significantly improve treatment outcomes, including response, quality of life and progression-free survival, and in some cases may even improve overall survival. Canadian consensus guidelines on biomarker testing in lung cancer have been published, and multiple personalized treatment options in advanced lung cancer, based on biomarker testing, are now funded in provinces across Canada. But most patients are still not getting the molecular pathology testing that would permit them to receive personalized lung cancer therapy. Several barriers continue to hinder the application of this knowledge, including lack of integration of biomarker testing into routine pathology practice, lack of knowledge dissemination to involved specialties (beyond medical oncology and academic pathology), absence of specific or sufficient funding for biomarker testing, and insufficient tumour samples for testing. All of these culminate in Canada’s current failure to routinely offer the “right drug to the right person at the right time” in lung cancer.

Recent studies in patients with metastatic non-small cell lung cancer (NSCLC) have demonstrated that response to systemic treatment depends on pathologic subtype and the presence of specific alterations within the NSCLC genome.5 It is of pressing clinical importance to determine the histopathologic and molecular features of NSCLC tumours in order to guide appropriate treatment options for patients.6 Mutations within the epidermal growth factor receptor gene (EGFR) and presence of the echinoderm microtubule-associated protein-like 4-anaplastic lymphoma kinase (EML4-ALK) fusion gene are key therapeutic targets in NSCLC.7 Randomized clinical trials demonstrate substantial clinical benefits for metastatic NSCLC patients with EGFR mutations EML4-ALK gene rearrangements treated with EGFR tyrosine kinase inhibitors (TKIs), and inhibition of ALK with crizotinib in those with EML4-ALK gene rearrangements.8-14 Molecular testing of tumour samples from patients with NSCLC is now recommended by oncology societies worldwide.4,15-17

CHALLENGE #1: SUFFICIENT TISSUE ACQUISITION

The majority of advanced lung cancer patients are diagnosed based on minimally invasive sampling of primary lesion, such as percutaneous needle biopsy or transbronchial needle aspiration biopsy (TBNA). Two-thirds of Ontario’s lung cancer patients are diagnosed with fine needle aspirates (FNA), often too small for molecular and pathologic typing.18,19 International multidisciplinary guidance has been published for handling small lung cancer samples and biomarker testing, but this knowledge may not yet have spread beyond larger academic centres.20

A Canadian EGFR testing program was established for advanced lung cancer patients in 2010 to facilitate molecular testing.20,21 While 88% of tests were successful, 12% of tests could not proceed because samples never arrived at the test centre or were insufficient. The diagnosis of lung cancer using small samples is common, and often these samples contain too few tumour cells to perform reliable molecular testing.22,23

The processing and quality of tissue is also critical. For example, cytology specimens can be used for molecular testing when appropriately processed, including generation of a cell block. As few as 100 cells may be sufficient to detect EGFR mutations, and as few as 50 to detect the presence of ALK gene rearrangement.24 Tumour cellularity is also important, and appears to be the most significant factor for test success regardless of whether a cytology or pathology specimen is used.25 Non-malignant tissue within specimens can lead to decreased accuracy of molecular testing, particularly when tests are based on nucleic acids extracted from the whole sample as a source.

Solid tumours are heterogeneous and intratumoural diversity can also influence the outcome of molecular and genetic testing.26 While FNA is one of the most common diagnostic procedures in patients with NSCLC, the question remains whether such small biopsy specimens are representative of the tumour as a whole.27 Another potential challenge is heterogeneity between the primary tumour and metastatic lesion.28,29 Emerging evidence from other solid tumours suggests that repeat biopsy should be performed at the new onset of metastatic disease, whenever safe and feasible, to ensure the most accurate molecular profile.30 In lung cancer, this may be most important upon development of resistance. In both EGFR-mutated and ALK-rearranged NSCLC, emergence of additional resistance mutations has been demonstrated, for example T790M in EGFR-mutated lung cancer.31,32 But given the frailty of the advanced NSCLC population and

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potential delays and risks of repeated invasive procedures, a blood-based assay would be ideal for serial genomic profiling in advanced NSCLC patients. Multiple assays are under development to including the detection of circulating tumour cells with actionable mutations.

**CHALLENGE #2: INTEGRATING MOLECULAR TESTING INTO ROUTINE PATHOLOGY FOR NSCLC**

Based on the initial experience with the Canadian *EGFR* testing program, it was estimated that only 38% of potentially eligible patients had testing initiated in 2010/2011.\(^{21}\) Initiation of testing and waiting for results have both proven problematic in the Canadian setting. While there has been some uptake of reflex testing by academic lung pathologists, most molecular testing is initiated by medical oncologists at the time of treatment decision-making in advanced cancer patients. In the Canadian experience, *EGFR* testing results were reported after a median of 18 days (standard deviation 9.7 days), which included 7 days to reach the testing laboratory and 11 for the actual testing. This treatment delay of nearly 3 weeks can be devastating for many advanced lung cancer patients, who may quickly deteriorate.

In addition to Canadian consensus guidelines, provincial groups including CancerCare Ontario are developing additional guidance for pathologists in processing lung cancer samples for molecular testing. Molecular profiles of squamous cell and non-squamous cell lung cancers differ markedly.\(^{32}\) Both *EGFR* — sensitizing mutations and *EML4-ALK* gene rearrangements are found predominantly in lung adenocarcinoma, thus appropriate initial classification of NSCLC into adenocarcinoma is crucial for further molecular diagnostic procedures. Unfortunately, up to 40% of NSCLC in Canada is not reported as a pathologic subtype, but as “not otherwise specified” (NOS).\(^{18,19,33}\) This may be reduced to less than 15% with routine use of immunohistochemistry (IHC) as part of lung cancer diagnosis. Greater standardization of pathology diagnostic procedures in NSCLC will improve the quality and quantity of molecular and pathologic information available for Canadian lung cancer patients, and our ability to personalize treatment.

**CHALLENGE #3: CHOICE OF MOLECULAR TEST**

In most Canadian centres where molecular testing for NSCLC is performed, molecular tests are done serially rather than simultaneously or using multiplexing. Each testing method will have advantages and disadvantages. For example, there are at least 16 different methods to test for the presence of *EGFR* mutations, all having specific methodology, logistic requirements and different costs,\(^{4}\) using either direct sequencing- or polymerase chain reaction (PCR)-based methods. Some of the tests can detect the presence of mutation in the sample when only 0.1% of deoxynucleobase acid (DNA) is mutated, while other methods require 10-20% of DNA to be mutated for the test to be positive. While some tests, such as direct sequencing, can detect any mutation in the analyzed DNA, other methods (allele-specific methods) detect only certain mutations, although often at lower levels of mutation frequency.\(^{4}\) In the Canadian testing program, most laboratories use detection methods that are more sensitive to low levels of mutant tumour cells than direct sequencing.\(^{29}\) However, these may miss rarer sensitizing mutations.

To detect *EML4-ALK* rearrangement, the gold standard remains detection by fluorescence in-situ hybridization (FISH), which is labour-intensive and costly. Screening with IHC is ongoing at centres across Canada with FISH confirmation of positive cases (Tsao et al. unpublished data). Similar to other FISH testing, this is conducted at specialized cytogenetic laboratories and will require referral to larger centres, with the accompanying challenges of shipping tissue and transit times.

To save the time required for serial tests, many centres are examining multiplex testing. While current costs of multiplexing are greater than serial mutation testing, the rapidly falling cost of technology will likely make multiplex testing more feasible in future. Platforms such as Sequenom or SNAPSHOT allow for detection of multiple genomic abnormalities with sequencing. Newer platforms such as FoundationOne also incorporate detection of gene rearrangements (e.g. *EML4-ALK*) and changes in gene copy number. While some platforms take longer to yield results, others can provide results within 2 weeks (e.g. FoundationOne). Volume of tissue required and cost are limiting factors at present, but these factors are rapidly evolving.

**CHALLENGE #4: CAN WE AFFORD IT?**

Getting the right drug to the right person at the right time is a valuable and worthwhile advance in oncology. Routine molecular profiling and pathologic subtyping in lung cancer will yield two major effects. The first is identifying those who will not benefit from a specific treatment, for example pemetrexed in patients with squamous histology. This avoids potentially costly and toxic treatment that will not be of major value. The second effect is to identify those who may benefit from additional specific therapy, such as ALK inhibitor in those with ALK-rearranged NSCLC. While the first effect may save money, the second will likely come at an incremental cost, although with the potential for substantive incremental benefit. Another example is the use of *EGFR* TKI therapy in advanced NSCLC patients with *EGFR*-mutant NSCLC. In the first-line setting, this yields to significantly greater incremental quality-adjusted survival but at modestly greater cost.\(^{51}\) In patients with *EGFR*-mutant NSCLC previously treated with chemotherapy, erlotinib has a slightly higher cost-effectiveness ratio than treatment of those with *EGFR* wild-type tumour, but is clearly of major clinical benefit.\(^{36}\)

Use of simple techniques like IHC is relatively inexpensive, at less than $100 per case. Use of FISH testing is more expensive, in the $350 to $500 range, while multiplex sequencing is costlier yet, in the $1000 to $2000 range.\(^{46}\) Next-generation sequencing costs are still higher, up to $5000–$10,000 to sequence the cancer genome, but these prices are rapidly falling.\(^{47}\)

Cost drivers to consider include the frequency of the individual biomarkers, relating directly to the number needed to screen, use of a multiplex versus a serial testing approach, methodology used (e.g. IHC versus FISH), commercialization of tests with licensing of companion diagnostics linked to use of the novel therapeutics, and the need for repeat biopsy.
MOVING FORWARD
Better outcomes, less toxicity and greater value in treatment of advanced NSCLC are goals that Canadian patients, clinicians and society all desire. To achieve better tissue availability for our patients, we must ensure greater knowledge transfer with our interventional radiology, respirology and surgical colleagues, as well as pathologist and other oncology specialists. The desire for minimally invasive testing must be balanced with requirements for tissue sufficient to yield the necessary molecular diagnostic information. Greater understanding of tissue volumes and quality required, as well as greater awareness of the role of tissue processing to ensure successful molecular testing, must be promoted at all levels of the lung cancer diagnostic process. Dedicated public funding will allow pathologists to promote greater access to and standardization of molecular testing, and integration into the routine diagnosis of lung cancer. This integration will help deliver more timely results for targeted therapy decisions, making personalized lung cancer therapy a reality for more Canadian patients.

Economic evaluations of personalized treatment need to recognize the dramatic impact of novel therapies on small subgroups of patients, and the effects of biomarker frequency on cost effectiveness. Policy-makers need to understand that, unlike drug costs, the costs of technology and testing are rapidly falling. Funding assessments should incorporate a recognition of the value of testing to plan treatment for the individual, and also the value of genomic information to a population. Policy-makers, clinicians, pathologists and scientists must collaborate to ensure that Canadians have access to state-of-the-art diagnostics and treatments that truly improve outcome in advanced lung cancer.

References
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