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Standardizing biomarker testing for Canadian patients with advanced lung cancer

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ABSTRACT

Background The development and approval of both targeted and immune therapies for patients with advanced non-small cell lung cancer (NSCLC) has significantly improved patient survival rates and quality of life. Biomarker testing for patients newly diagnosed with NSCLC, as well as for patients progressing after treatment with epidermal growth factor receptor (*EGFR*) inhibitors, is the standard of care in Canada and many parts of the world.

Methods A group of thoracic oncology experts in the field of thoracic oncology met to describe the standard for biomarker testing for lung cancer in the Canadian context, focusing on evidence-based recommendations for standard-of-care testing for *EGFR*, anaplastic lymphoma kinase (*ALK*), *ROS1*, *BRAF V600* and programmed death-ligand (PD-L1) at the time of diagnosis of advanced disease and *EGFR T790M* upon progression. As well, additional exploratory molecules and targets are likely to impact future patient care, including *MET exon 14 skipping mutations and whole gene amplification*, *RET translocations*, *HER2 (ERBB2) mutations*, *NTRK*, *RAS (KRAS* and *NRAS)*, as well as *TP53*.

Results The standard of care must include the incorporation of testing for novel biomarkers as they become available, as it will be difficult for national guidelines to keep pace with technological advances in this area.

Conclusions Canadian patients with NSCLC should be treated equally; the minimum standard of care is defined in this paper.

Key Words Biomarker testing, lung cancer, *EGFR*, *ALK*, *ROS1*, *BRAF V600X*, *MET*, PD-L1, Canada

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INTRODUCTION

According to Canadian Cancer Statistics 2017[1], lung cancer is one of the most common malignancies, accounting for 14% of all newly diagnosed cancers in both genders. Tobacco consumption is still the most important risk factor for this disease. Incidence rates for lung cancer also differ across the country for the same reason[1]. Lung cancer remains the leading cause of cancer-related mortality, accounting for 26% of all cancer deaths in both genders in 2017.

Our understanding of lung cancer has advanced over the last decade. The development and approval of small-molecule tyrosine kinase inhibitors (TKIs) and immune therapies has significantly improved patient outcomes. A multitude of actionable gene alterations have already been identified in lung cancer[2]. The Lung Cancer Mutation Consortium found that two-thirds of non-small cell lung cancer (NSCLC) patients with adenocarcinomas (ADCs) have an oncogenic driver, and that when these patients receive the corresponding targeted agent, they will have improved survival and quality of life[3]. Thus, biomarker testing is essential to identify patients eligible for targeted therapy. Molecular testing is reserved for those mutations with evidence to support their characterization as predictive biomarkers indicative of therapeutic efficacy[4].

The purpose of this article is to articulate the standard-of-care molecular testing for advanced lung cancer in the Canadian context, focusing specifically on actionable driver mutations. Key pathology issues with sample selection and analytics are described elsewhere[5]. A key challenge in this area is the rapid change with respect to

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new targets and technologies, and recommendations need to accommodate new and emerging data. Suggestions for general improvements for molecular testing in the Canadian landscape will also be extended and discussed. This project was initiated by Lung Cancer Canada, Canada's only charitable organization solely focused on lung cancer.

METHODS

Process and Panel Composition

Lung Cancer Canada selected an Expert Committee from across Canada, on the basis of interest and expertise. The Expert Committee identified and reviewed lung cancer molecular testing guidelines, meta-analyses, and other relevant documents from the literature to determine which standards are appropriate for Canadian patients. During the review process, the Expert Committee discussed points of disagreement and reached consensus for testing recommendations suitable for the Canadian context.

This article describes biomarker testing for advanced NSCLC only. More specifically, only actionable mutations and immunotherapy will be discussed.

STANDARD-OF-CARE BIOMARKER TESTING IN CANADA: *EGFR*, *ALK*, *ROS1*, PD-L1 AND *EGFR T790M*

In the era of targeted and immune therapies, lung cancer diagnosis is based on a combination of histological, immunohistochemical, and molecular analysis[6]. Multidisciplinary collaboration should aim at, first, achieving precise histopathological subtyping and then biomarker testing, both in a timely manner. To achieve these goals, complete clinical information should be provided to pathologists on pathology request forms by clinicians to help limit the number of immunohistochemical stains needed to make the diagnosis with precise histopathological subtype in order to maximize the amount of residual tissue available for subsequent biomarker testing. In the same manner, pathologists should use a limited panel of diagnostic immunohistochemical markers (i.e., TTF-1 and p40) to resolve most cases. On small biopsies and cytology specimens, this allows for obtaining a diagnosis of ADC, squamous cell carcinoma (SQCC) or non-small cell carcinoma not otherwise specified (NSCC-NOS) in the vast majority of cases in a matter of days. In the past, only samples with ADC histology were sent for testing. Standard practice now consists of evaluating non-squamous histologies (i.e., ADC, NSCC-NOS, adenosquamous carcinoma [ASQC], and large cell carcinoma [LCC]) for targetable molecular alterations. Never-smokers with other histologies (i.e., SQCC and small cell lung carcinoma [SCLC]) should also be considered for testing.

Lung cancers have a very high number of point mutations, chromosomal rearrangements, and copy number changes compared with other tumours[7]. Genetic mutations and rearrangements can constitutively activate signal transduction pathways driving cell survival, cell proliferation, and metastasis. The ability to specifically and effectively inhibit driver mutations with targeted agents has led to clear and profound survival improvements for patients with lung cancer.

Genetic alterations can be found in all NSCLC histologies, including ADC, SQCC, ASQC and LCC, with various mutation rates and in current, former, and never-smokers. Although associations have been made between specific gene mutations and ethnic background, sex, age, and smoking history, none of these clinical characteristics are strong enough to enable patient selection[6]. Therefore, all patients with NSCLC ideally need to be tested for gene mutations regardless of clinical characteristics. Only genetic alterations with an associated targeted therapy are recommended as standard-of-care testing.

The following biomarkers should be considered as standard of care today for every patient diagnosed with advanced lung cancer across the country (Table I).

EGFR molecular testing at diagnosis

Recommendation 1

All patients with advanced non-squamous NSCLC as well as non-smokers with other histology (squamous and small cell carcinoma) need to be tested for the presence of *EGFR* mutations at diagnosis. Identifying both the common *EGFR* mutations and any individual mutations that are reported with a frequency of at least 1% of *EGFR*-mutated lung adenocarcinomas is standard of care.

The epidermal growth factor receptor (*EGFR*) gene encodes a receptor tyrosine kinase. Epidermal growth factor receptor mutations were the first targetable mutations to be discovered in lung cancer. They are present in approximately 20% of patients with NSCLC in Canada[8] and range from 35 to 51% in East Asia[9-12]. Two *EGFR* mutations in lung cancer are considered common: 90% of epidermal growth factor receptor mutations are either the exon 21 *L858R* point mutation or an exon 19 deletion (*del19)*[13]. Uncommon mutations which are present at low frequency, and which also sensitize tumours to *EGFR* TKIs include the exon 18 *G719X*, exon 20 *S768I,* and exon 21 *L861Q* point mutations[14-16]. Not all *EGFR* mutations confer sensitivity to *EGFR* TKIs. Exon 20 *T790M* mutation and deletions are almost invariably resistant to *EGFR* TKIs[17]. Gene amplifications and other types of mutations can be present; however these are not currently detected with standard testing.

Methods for detecting *EGFR* mutations include polymerase chain reaction (PCR)-based methods on either formalin-fixed, paraffin-embedded (FFPE) tissue or fresh, frozen, or alcohol-fixed specimens. Other tissue treatments (e.g., acidic or heavy metal fixatives, mordants, or decalcifying solutions) should be avoided in specimens destined for *EGFR* testing. Cytology samples are suitable for *EGFR* testing, with cell blocks being widely preferred over smears. A recent study compared the reliability of fine needle aspirations (cytology) and core needle biopsy specimens (histology specimens) for molecular testing using next generation sequencing (NGS). The study demonstrated that fine needle aspiration samples may provide better cellularity, higher tumour fraction, and superior sequencing metrics than core needle biopsy samples[18]. As technologies evolve, we can look forward to more efficient and less invasive methods, such as blood tests, to identify *EGFR* mutations. Newer NGS technologies, such as massively parallel sequencing, have changed the way laboratory

TABLE I Molecular testing targets for NSCLC in Canada

Gene/histology	Mutation type	Frequency	HC approved therapy
EGFR			
Non-squamous or squamous, non-smoking	Gene mutations: both common (*L858R* and *Del19*) and uncommon accounting for ≥1% frequency of mutations.	20%	First and second generation *EGFR* TKIs: erlotinib, gefitinib, afatinib
Acquired after progression on *EGFR* TKI	*T790M*	>50%	Osimertinib
ALK			
Non-squamous	Gene rearrangements	3–5%	Crizotinib
ROS1			
Non-squamous	Gene rearrangements	1–3%	Crizotinib (not yet HC approved)
BRAF V600X			
Non-squamous	Point mutations	3%	Trametinib/Dabrafenib
PD-L1			
Both squamous and non-squamous	Protein expression levels	Depends on expression level	Pembrolizumab
MET	Exon 14 skip or amplification of the whole gene	3–5% (Exon 14 skip)	Cabozantinib and Crizotinib[a]
RET	Gene rearrangements	1%	Cabozanitinib and Vandetanib[a]
ERBB2	*Exon 20 insertion*	1-2%	HER-2 targeted agents[a]
NTRK	Gene rearrangements	<1%	Entrectinib, LOXO-101, crizotinib[a]
RAS (KRAS and NRAS)		*KRAS* 20–30% *NRAS* <1%	—
TP53		50%	—

[a] Not yet approved by Health Canada
NSCLC = non-small cell lung cancer; HC = Health Canada; *EGFR* = epidermal growth factor receptor; ALK = anaplastic lymphoma kinase; ROS1 = UR2 sarcoma virus oncogene homolog 1; PD-L1 = programmed death receptor ligand 1;
Genes, mutations, and mutation frequency in NSCLC, as well as Health Canada approved therapies. Additional genes recommended for testing are included.

tumour molecular profiling is performed, and *EGFR* testing may be incorporated into larger panel-based testing. At this point in time, identification of the above-mentioned *EGFR* alterations is standard of care, but the technology used for testing should remain the choice of each testing laboratory, as long as acceptable performance metrics (e.g., limit of detection) are met.

A clear understanding of each molecular pathology report by treating clinicians is mandatory in this setting. Each laboratory should qualify the *EGFR* mutation status based on the testing method used. Epidermal growth factor receptor "no mutation detected" means that tumours were tested for one or more *EGFR* mutations and none were detected; this terminology is not always identical to *EGFR* "wild type," which implies testing for all known *EGFR* driver mutations by more comprehensive testing methods. For example, we recommend that if the testing only included sequencing of *EGFR* exons 19 and 21 (the location of the two most common *EGFR* mutations) and no mutation is detected, then the *EGFR* mutation status for that tumour is clearly specified as "wild type (or undetectable) at exons 19 and 21."

T790M is rarely (<5%) found in untreated *EGFR*-mutated tumours[19], generally occurs concurrently with other *EGFR*-sensitizing mutations, and has been found to be associated with decreased sensitivity to first- and second-generation *EGFR* TKIs[20]. The *T790M* mutation can also occur as a germline mutation, especially when it is identified without the sensitizing mutations.

Patients with *EGFR*-sensitizing mutations respond well to *EGFR* TKIs including erlotinib[21,22], gefitinib[23-26], and afatinib[27-29], which are the current Health Canada-approved first-line treatments for patients with confirmed *EGFR* mutations.

An algorithm for the current standard of care for molecular testing in the Canadian context is shown in Figure 1. As some of the common driver mutations in NSCLC are mutually exclusive, a more efficient (and complex) algorithm may eventually evolve.

EGFR molecular testing at progression

Recommendation 2

All patients with *EGFR*-sensitizing mutations who progress after being treated with first- and second-generation *EGFR* TKIs need to be tested for the *T790M* mutation if treatment with a third-generation TKI is being considered.

For patients who are treated with first- or second-generation *EGFR* TKIs, the median time to progression is 9 to 14 months[30,31]. More than half of the patients with a *EGFR*-sensitizing mutation who progress while being treated with *EGFR*-targeted TKIs will acquire a *T790M*

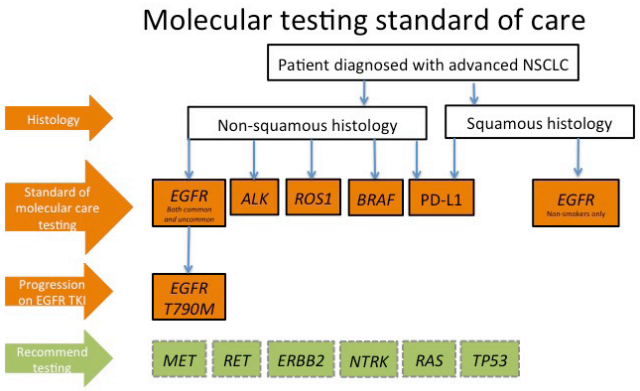

FIGURE 1 Molecular testing standard of care. Genes to be included in standard of care testing are orange, genes recommended for testing are shown in green. NSCLC = non-small cell lung cancer; *EGFR* = epidermal growth factor receptor; *ALK* = anaplastic lymphoma kinase; *ROS1* = UR2 sarcoma virus oncogene homolog 1; PD-L1 = programmed death receptor ligand 1.

mutation in exon 20, causing the tumour to become resistant to the initial TKI[32].

Testing methods include PCR-based sequencing methods for now. As an alternative to an invasive re-biopsy, plasma testing can be used to analyze mutations in the cell-free circulating tumour DNA (ctDNA)[33]. For those who test *T790M*-negative with a plasma assay, tissue testing (biopsy) is still required, as this may represent a false negative result or be explained by an alternative mechanism of resistance such as tumour type transformation into SCLC, which can only be diagnosed on a tissue biopsy[34].

Retesting at progression on first- or second-generation *EGFR* TKIs is standard of care, as third-generation TKIs are effective treatment for patients whose tumours harbour *EGFR T790M* mutations[35-37]. Patients with acquired resistance to first-line treatments and who develop acquired *T790M* mutations should be treated with osimertinib[35].

ALK molecular testing recommendations

Recommendation 3

All patients with advanced non-squamous NSCLC need to be tested for *ALK* rearrangements at diagnosis. At this time, there is no recommendation to test NSCLC patients for further mutations after progression on *ALK* inhibitors.

The anaplastic lymphoma kinase (*ALK*) gene encodes a receptor tyrosine kinase that is part of the insulin receptor family. Anaplastic lymphoma kinase is a hotspot for translocation events, and rearrangement occurs in 3 to 5% of NSCLCs[38-40]. The most common rearrangement results in a small inversion within the short arm of chromosome 2, involving the genes encoding for *ALK* (2p23) and *EML4* (2p21). Although *EML4* is the most common translocation partner found in NSCLC, more than 24 different translocation partners have been identified[38,41].

A network of pulmonary and molecular pathologists and cytogeneticists working in academic centres across Canada initiated the Canadian *ALK* (CALK) study to address the challenge of standardization and optimization of detection tests for *ALK*-positive NSCLC[42]. The CALK study demonstrated that immunohistochemistry (IHC) is an acceptable screening method to detect *ALK*-rearranged lung cancers. In 2015, a group of Canadian oncologists and pathologists created a consensus statement supporting the results of the CALK study. They highlighted the importance of *ALK* testing and treatment for patients with advanced, non-squamous NSCLC[43]. The consensus statement re-emphasized that positive *ALK* IHC is sufficient for obtaining access to *ALK* inhibitors, but cases with lower intensity staining (weak or equivocal, 1+ or 2+ in the four-tiered IHC approach) need additional validation with *ALK* fluorescence *in situ* hybridization (FISH). Anaplastic lymphoma kinase IHC requires high levels of reliability and ongoing quality assurance, and its implementation in pathology laboratories should follow strict validation standards. Hybrid capture-based NGS gene panels to assess multiple different types of clinically relevant genomic abnormalities in NSCLC are promising and feasible to detect targetable gene rearrangements in lung cancer, including *ALK*.

The results of several recent clinical trials confirm the efficacy and tolerability of crizotinib which is approved by Health Canada for treatment-naïve NSCLC patients with *ALK* rearrangements[44-46]. Crizotinib is a first-generation inhibitor of several surface membrane receptor tyrosine kinases including *ALK*. In addition to inhibiting *ALK*, crizotinib has efficacy against the c-ros oncogene 1 (*ROS1*) and the hepatocyte growth factor receptor (*HGFR*/c-Met)[47]. Ceritinib and alectinib are second-generation *ALK* inhibitors approved by Health Canada for patients with *ALK*-translocated NSCLC who have progressed or are intolerant to crizotinib. Alectinib will likely soon replace crizotinib in the first-line treatment of *ALK*-translocated NSCLC, based on the results of the ALEX trial, but is not approved by Health Canada for first-line use at the time of publication of this manuscript. Multiple other *ALK* inhibitors are soon to come forward and be approved.

Most patients with *ALK* rearrangements eventually acquire resistance to TKIs through a variety of molecular mechanisms, including secondary mutations in the *ALK* tyrosine kinase domain, *ALK* gene amplification, and activation of other signalling pathways[48,49]. Emerging information supports the preferential use of some of these *ALK* inhibitors according to the secondary mutation profile. As these data evolve there may be a role in the future for molecular testing after progression of an *ALK* inhibitor to determine optimal sequencing of therapies, but this cannot be recommended at this time.

ROS1 molecular testing recommendations

Recommendation 4

All patients with advanced non-squamous NSCLC need to be tested for *ROS1* rearrangements at diagnosis.

ROS1 (UR2 sarcoma virus oncogene homolog 1) is a receptor tyrosine kinase that is structurally related to *ALK*. *ROS1* rearrangements have been identified in 1 to 3% of lung adenocarcinoma[6,50]. *ROS1* is activated by translocation with other genes; one publication identifies up to 26 other fusion partners[51,52]. *ROS1* fusion proteins retain the *ROS1* kinase domain, which is constitutively activated and drives cell transformation.

A variety of techniques can be used to detect *ROS1* translocations, including FISH, IHC, NGS of RNA and DNA, and PCR[6]. Clinically, the presence of a *ROS1* rearrangement is detected by FISH, with a *ROS1* break apart probe. However, FISH testing is not able to discern which particular *ROS1* fusion is found in a clinical sample. The Canadian *ROS* (CROS) initiative, ongoing in 14 centres across Canada, is currently working to validate IHC testing for *ROS1* translocations in NSCLC tumour samples[53].

Although *ROS1*-rearranged tumours are sensitive to crizotinib to the same extent as *ALK*-rearranged tumours[47], this drug is not yet approved in Canada for use in patients with *ROS1*-rearranged NSCLC. Despite this, *ROS1* testing is still recommended, as the treating clinician may wish to access crizotinib through insurance or compassionate access while full regulatory approval is still pending.

BRAF V600 testing recommendations

Recommendation 5

All patients with advanced non-squamous nsclc need to be tested for the *BRAF V600* mutation at diagnosis.

BRAF is an oncogene encoding a RAS-regulated kinase. *BRAF* mutations are found in 3% of NSCLC[54], half of which are the exon 15 *V600X* mutation[55,56]. *BRAF* mutations in lung cancer also occur at other positions within the kinase domain, including G469A (39%) and D594G (11%)[54]. Mutations in the *BRAF* gene activate the kinase, leading to activation of the mitogen-activated protein kinase (MAPK) signalling cascade. *BRAF* mutations are usually detected using PCR-based methods including NGS from FFPE tissue.

Recent data have shown an overall response rate of 63% when a combination of *BRAF* inhibitor and *MEK* inhibitor, dabrafenib and trametinib, was used in patients with the *BRAF V600E*-mutated adenocarcinoma, which supports the therapeutic value of inhibiting this oncogene[57]. These promising data have recently led to Health Canada's approval of this combination in patients with tumours positive for a *BRAF V600* mutation after progression on a platinum doublet.

PD-L1 testing recommendations

Recommendation 6

All patients with advanced NSCLC need to be tested for PD-L1 expression at diagnosis.

Immune checkpoint inhibitors have fulfilled their promise for the therapy of lung cancer. The expression of immune checkpoint proteins is one mechanism for tumours to deactivate the normal host immune response and evade destruction[58]. Immune checkpoint inhibitors are efficacious in lung cancer and are targeted against a number of molecular targets. These include the inhibitory programmed death 1 (PD-1) receptor expressed on T cells, natural killer cells, and some B cells[58]. The two PD-1 ligands are programmed death receptor ligand 1 (PD-L1) and *PD-L2*, both of which are expressed in a wide range of effector cells, antigen-presenting cells, and T cells[58].

The use of PD-L1 as a predictive biomarker for use with PD-1/PD-L1 directed immune therapy agents is complicated. Programmed death-ligand 1 expression is heterogeneous and can be induced in response to a number of stimuli. Although tumour PD-L1 expression levels generally correlate to responses with immune therapy agents, some PD-L1 negative tumours still respond to these agents[59]. As well, each of the five therapeutic monoclonal antibodies has a different IHC-based companion or complementary biomarker test to measure the PD-L1 protein expression.

The C-22C3 Quality Validation Project is currently taking place in 19 sites across Canada, with the purpose of standardizing a non-kit based assay for PD-L1 expression using the 22C3 antibody on different immunostaining platforms.

Nivolumab and pembrolizumab are two anti-PD-1 antibodies that are approved after failure of conventional chemotherapy for advanced NSCLC in Canada. Nivolumab can be prescribed without biomarker testing for PD-L1 expression. Pembrolizumab is limited to patients with PD-L1-positive tumours. Pembrolizumab has also shown improved efficacy compared with platinum doublet in patients who are treatment-naïve with tumours expressing 50% PD-L1 in 50% of tumour cells or more and is approved by Health Canada. The Expert Committee feels that PD-L1 testing should be readily available at the time of diagnosis of both non-squamous and squamous NSCLC to allow for rapid initiation of pembrolizumab to eligible patients[60]. Most recently, in July 2017, Health Canada approved pembrolizumab in the first-line setting in advanced NSCLC.

Based on Health Canada drug approval recommendations, only pembrolizumab requires PD-L1 testing for treatment of platinum-refractory NSCLC. Therefore, at this time, PD-L1 testing using the method used in clinical trials (22C3 Pharma Dx) represents the most relevant testing approach and is used by most academic and private laboratories in Canada.

ADDITIONAL GENE MUTATIONS OF INTEREST IN NSCLC

The ongoing discovery of new driver mutations and corresponding therapies is changing the lung cancer molecular testing landscape. Molecular testing for *MET, RET, HER2 (ERBB2), NTRK, KRAS, NRAS, and TP53* is not required but recommended at this time for patients with lung cancer, especially if the gene mutation tests are included in panels or as part of a clinical trial.

MET mutations

The *MET* gene encodes the hepatocyte growth factor receptor (HGFR) tyrosine kinase. Binding of the hepatocyte growth factor ligand leads to dimerization of the receptor, phosphorylation of the kinase domain, and subsequent activation of downstream signalling pathways PI3K-AKT and RAS-MAP kinase. *MET* signalling can be increased through overexpression of HGF or HGFR proteins, decreased HGFR degradation, *MET* amplification, or by *MET* mutations such as kinase domain mutations or exon 14 splice-site skipping mutations, although not all of these signalling methods are affected by TKIs[6,61] The incidence of *MET* exon 14 alterations is 3 to 5% in NSCLC[62-65]. Methods to detect *MET* aberrations include NGS for exon 14 alterations and FISH and NGS for *MET* amplification. Multiple efficacious *MET* inhibitors exist, including cabozantinib and crizotinib[63,66,67].

RET mutations

The *RET* gene encodes a cell surface tyrosine kinase receptor. Similar to *ALK* and *ROS, RET* can be rearranged so that the intact RET receptor tyrosine kinase is fused to the 5' end of a partner gene. *RET* is rearranged in 1% of lung adenocarcinoma, and in approximately 16% of NSCLC tumours that lack other oncogenic drivers[6].

RET fusions were initially identified by RT-PCR (reverse transcription PCR), IHC, and next-generation sequencing. There is no current standard test for identification of *RET* fusions in patient samples, but FISH or targeted capture/NGS are potential methods. At this point, there are no approved therapies for *RET* in NSCLC, although multiple studies with *RET* inhibitors are underway[68-71].

Cabozantinib and vandetanib have demonstrated an overall response ranging from 18 to 53% but with relatively short progression-free survival (4.7 to 5.5 months), likely reflecting the aggressive nature of NSCLC harbouring this oncogene, especially when compared with the response of other driver mutations to TKIs[69,70].

HER2 (*ERBB2*) mutations

The *HER2* gene (*ERBB2*) encodes an *EGFR* family receptor tyrosine kinase. Gene mutations are mostly localized to exon 20, either in-frame insertions or point mutations. Unlike the case of breast cancer, actionable *HER2* mutations are present without amplification[72]. Mutations in *HER2* have been detected in 1 to 2% of NSCLC and also can be found in tumour biopsies of patients with mutant *EGFR* but *EGFR* TKI resistance[73-75]. Mutations for *HER2* can be detected using PCR or NGS. A number of different therapies are being tested in patients whose tumours harbour *HER2* mutations, and retrospective data suggest clinical benefit from *HER-2*-targeted agents[76].

NTRK mutations

Gene alterations in *NTRK 1/2/3*, encoding members of the nerve growth factor receptor or tropomyosin receptor kinase (TRK) family, have been observed in 1% or less of NSCLC. Most alterations consist of the TRK kinase domain fused with multiple partners. These rare tumours are mostly described in adenocarcinoma[77]. Specific TRK kinase inhibitors such as entrectinib and LOXO-101 are effective for *NTRK* mutated tumours[78], as are crizotinib and several other TKIs in development[79].

RAS mutations

KRAS is a membrane-bound intracellular GTPase. *KRAS* mutations occur in approximately 20 to 30% of non-squamous NSCLC[80,81], typically in exon 2, codon 12, 13, and 61[82,83]. There are no targeted therapies approved for patients with *KRAS* mutations. *NRAS* mutations are seen in less than 1% of tumours and associated with a decreased response to *EGFR* TKIs, but may respond to *MEK* inhibition[84].

KRAS and *NRAS* mutations are usually found in tumours wild type for *EGFR, ALK*, and other driver mutations, and *KRAS* biomarker testing could be incorporated into molecular testing algorithms to improve overall testing efficiency, as *KRAS*- or *NRAS*-positive lung adenocarcinomas are rarely associated with *ALK, ROS,* or other rare alterations.

TP53 mutations

TP53 mutations are common, present in approximately 50% of lung cancers, and are prognostic of poor outcomes[6,85]. Mutations in this gene deactivate the G1 cell-cycle checkpoint[6]. Dual *TP53*/*EGFR* mutations are associated with lower response rates and shorter progression-free survival when treated with *EGFR* TKI therapy[86]. Therapies that target *TP53* mutated lung cancers are being tested in clinical trials.

ADOPTION OF NOVEL AND APPROPRIATE TESTING TECHNIQUES AS THEY BECOME AVAILABLE

Each of the genetic and protein detection methods currently used for biomarker testing has various advantages and disadvantages. The specific tests, assays, equipment, and technology (e.g., single or multi-platform), vary from province to province, as well as from one centre to another. New and more cost-effective technologies are emerging that will be able to simultaneously identify more genomic abnormalities, improve sensitivity, require less DNA/RNA and potentially shorten turnaround times. The Expert Committee recommends that these novel and appropriate technologies be adopted as soon as they become available and are demonstrated to meet clinical performance requirements in a robust and reproducible manner. Quality control and quality assurance policies and procedures need to be established, as for all clinical laboratory analyses.

SUGGESTED IMPROVEMENTS FOR THE CANADIAN BIOMARKER TESTING LANDSCAPE

In Canada, there is significant provincial variability in access to, and coverage of, biomarker testing. The Expert Committee has suggested the following improvements to the Canadian biomarker testing landscape.

Reflex Testing for All Molecular Mutations at Diagnosis

Early and consistent access to molecular testing is of critical importance to the effective delivery of lung cancer therapy, as was emphasized in an earlier Lung Cancer Canada white paper[87]. Oncologists and treating physicians may not be aware of what biomarkers they should test for, what their labs can detect or what their options are if they want to screen for other markers. As a result, not all eligible lung cancer patients are tested for all mutations. Reflex testing is molecular testing that is initiated when the results of a biopsy indicate that lung cancer is present, regardless of cancer staging status. This process has been reported to reduce time to treatment in lung cancer patients[88,89]. In view of the fact that the five-year survival in early-stage lung cancer (localized tumour) is only about 56%[90], nearly half of these patients would die within five years and tumour progression would occur before the patients' death. Therefore, molecular testing should be encouraged even at an early stage of the disease. Reflex testing for *EGFR, ALK, ROS1, and BRAF* in all non-squamous NSCLC patients and PD-L1 in both non-squamous and squamous patients in all provinces would ensure that timely molecular testing results inform the most appropriate therapy selection. The

testing of *MET*, *RET*, *ERBB2*, *NTRK*, *RAS*, and *TP53* gene alternations is recommended but not required at this time.

Conserve Tissue to Maximize the Amount Available for Testing

A key challenge with lung cancer testing in general is the small size of tumour samples, given that about 75% of lung cancer patients present at an advanced stage and are not surgical candidates. Minimally invasive procedures such as fine-needle aspirations of metastatic or primary sites, bronchial washings, and brushings are still often used to procure tumour tissue in lung cancer patients. Multidisciplinary approaches to tissue procurement, clinical information provided by clinicians to pathologists on cytology and pathology request forms, and specimen handling in the laboratory and during signout, are key determinants to subsequent successful molecular testing. Tissue conservation is a key consideration in lung cancer molecular testing, especially as increasing numbers of markers are being analyzed. For the same reason, multiplexed genotyping is recommended. All patients with insufficient tissue for recommended biomarker testing need to be offered a repeat biopsy early in their disease course, and the value of a repeat biopsy needs to be communicated to the patient.

Consistent Access and Coverage of Biomarker Tests from One Province to Another

Significant provincial variability exists in the access to, and financial coverage of, biomarker testing in Canada. More standardized and sustainable funding across Canada will ensure that a patient's prognosis does not depend on his or her province of residence. We need to ensure that a biomarker test is approved at the same time the associated therapeutic agent becomes available. History has shown that some drugs are approved before funding of the associated biomarker test.

DISCUSSION

Testing Recommendations and Endorsement

The Expert Committee recommends reflex biomarker testing for all advanced NSCLC patients at diagnosis regardless of clinical characteristics. At minimum, biomarker testing for *EGFR* mutations (both common and uncommon), *ALK* and *ROS1* rearrangements, and *BRAF* mutation should be the standard of care for all Canadian patients with advanced NSCLC and selected patients with SQCC. PD-L1 testing is recommended for all patients with advanced non-squamous and squamous NSCLC. As of 2017, *EGFR* mutations are ideally screened as part of a multigene NGS panel, which should include other relevant driver mutations associated with an effective target therapy. For patients with an *EGFR*-sensitizing mutation who progress on first- or second-generation *EGFR* TKIS, testing for the *EGFR T790M* mutation is standard of care and therefore repeat *EGFR* mutation testing on progression is required. Plasma-based *T790M* mutation testing should be made available to reduce the number of tissue biopsies to be performed.

Many new driver mutations are being discovered, and additional targeted therapies are being developed and tested. Relevant aberrations on the horizon include the *MET* exon 14 skipping mutations, *MET* gene amplifications, *RET* translocations, *HER2 (ERBB2)* mutations, *NTRK, RAS* mutations, and *TP53*. These are currently recommended for testing.

As it will be difficult for national guidelines to keep pace with technological advances in this area, the standard of care will include the incorporation of additional biomarkers as new data become available.

Improvements are needed to change the biomarker testing landscape in Canada. Reflex testing for all clinically relevant genomic abnormalities and predictive biomarkers at the time of diagnosis of NSCLC will ensure that timely testing results guide the most appropriate therapy selection. Second, conserving lung tumour tissue is necessary to maximize the amount available for lung cancer biomarker testing, especially as increasing numbers of markers are being analyzed. More standardized and sustainable funding across Canada, which would ensure that lung cancer treatment and prognosis does not vary from one province to the next, is critical. Despite national testing recommendations, development of local testing algorithms through a multidisciplinary approach is strongly recommended.

The Expert Committee suggests a national oversight of molecular testing in Canada to ensure more uniform testing for all Canadians, from one centre, region, and province to the next. Lung Cancer Canada's white paper[87] called for "national policy standards and a sustainable public funding model for lung cancer molecular testing so that all patients across Canada are treated in a timely fashion, now and in the future." Although little has changed from a policy standpoint, molecular genetic testing is evolving steadily and having a bigger influence on patient management. Unfortunately, at present, there is no national Canadian body to provide formal oversight for standard-of-care genetic testing, although national organizations like the Canadian College of Medical Geneticists do set guidelines for testing and interpretation of genetic data. Decisions are thus made at the provincial level, with wide variation in implementation and funding of different tests. The Expert Committee encourages treating physicians and patient advocacy bodies to make sure provinces adhere to these guidelines for the standard of care.

An updated version of the evidence-based CAP/IASLC/AMP lung cancer molecular testing guideline will soon be published. While many of the CAP/IASLC/AMP recommendations align with those of the Expert Committee, not all are appropriate for the Canadian context. In that regard, we eagerly anticipate the outcomes of the CROS and 22C3 groups for guidance about *ROS1* and PD-L1 testing, similar to what the CALK provided for *ALK* testing in Canada.

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CONFLICT OF INTERESTS DISCLOSURE

We have read and understood Current Oncology's policy on conflicts of interest disclosure and declare the following interests: BM has received honoraria from Boehringer Ingelheim, Eli Lily, Pfizer, Roche, Merck, Bristol-Myers Squibb, Novartis, and AstraZeneca. She is in a consulting/advisory role with Boehringer Ingelheim, her institution has received research funding from Roche and Bayer and she has received travel/accommodations/expenses from Boehringer Ingelheim, AstraZeneca, Novartis, and Pfizer. NB has received consulting honoraria from Boehringer Ingelheim, Eli Lily, Pfizer, Roche, Merck, Bristol-Myers Squibb, Novartis, Sanofi, AstraZeneca and a research grant from Merck; PC has received honoraria from Boehringer Ingelheim, Eli Lily, Pfizer, Roche, Merck, Bristol-Myers Squibb, Novartis, AstraZeneca, research grants from Boehringer Ingelheim, Hoffmann La Roche, and AstraZeneca, and institutional educational grants from EMD Serono, Merck and Pfizer; CC has received consulting honoraria from Boehringer Ingelheim, Eli Lily, Pfizer, Roche, Merck, Bristol-Myers Squibb, Novartis, and AstraZeneca and research grants from Pfizer and Merck; RJ has received consulting honoraria from Amgen, AstraZeneca, Bristol Myers Squibb, Eli Lilly, EMD Serono, Merck, Novartis, Pfizer, and Roche and a research grant from Bristol Myers Squibb and Merck; SKR has received honoraria from Astra-Zeneca, Novartis, Pfizer, Roche, and Bristol-Myers Squibb and research grant funding from Pfizer, Astra-Zeneca, Novartis, and Bristol-Myers Squibb; MST has received honoraria from Pfizer, Ventana/Roche, Merck, AstraZeneca, and Bristol-Myers Squibb and research grants from Pfizer, Merck, and AstraZeneca; PWP has received honoraria from Boehringer Ingelheim, Merck, Astra Zeneca, Bristol-Myers Squibb, Lilly, and Novartis; ZX has received honoraria and grants from Pfizer, Roche, Merck, Boehringer Ingelheim, AstraZeneca, Bristol-Myers Squibb, Novartis, and Eli Lily; DNI has received honoraria from Boehringer Ingelheim, Eli Lily, Pfizer, Roche, Merck, Bristol-Myers Squibb, Novartis, and AstraZeneca.

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DISCLAIMER

The lung cancer biomarker testing landscape is inconsistent across Canada. With the rapid development of scientific knowledge, new evidence may emerge between the time information is developed and when it is published or read. The information addresses only the topics specifically identified therein and is not applicable to other interventions, diseases, or stages of diseases. This information does not mandate any particular course of medical care. In all cases, the selected course of action should be considered by the treating provider in the context of treating the individual patient.

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