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Review

# Molecular testing for advanced non-small cell lung cancer in Malaysia: Consensus statement from the College of Pathologists, Academy of Medicine Malaysia, the Malaysian Thoracic Society, and the Malaysian Oncological Society

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ABSTRACT

In the recent years, increased understanding of the molecular profiles of non-small cell lung cancer (NSCLC) has allowed for targeted treatment of actionable genetic mutations. The management of NSCLC now requires multiple molecular tests to guide the treatment strategy. In the light of this, there is a need to establish a molecular testing consensus statement for advanced NSCLC patients in Malaysia. This Malaysian consensus statement was developed by a panel of experts, chaired by a pathologist and composed of three other pathologists, four respiratory physicians and three oncologists. It reflects currently available scientific data and adaptations of recommendations from international guidelines to the local landscape. Expert recommendations on different aspects of molecular testing agreed upon by the panel are provided as structured discussions. These recommendations address the appropriate patients and samples to be tested, as well as when and how these tests should be performed. The algorithms for molecular testing in metastatic NSCLC, in the first line setting and upon disease progression beyond first line therapy, were developed.

## 1. Introduction

Lung cancer is the second most common cancer globally. However, it is the most common cause of cancer death. In 2015, there were 2 million incident cases of lung cancer and 1.7 million deaths worldwide [1].

In Malaysia, lung cancer is the third most common cancer, accounting for 10.2% of cancer cases. Most of the lung cancer cases in Malaysia are diagnosed at an advanced stage (stage III or stage IV). Between 2007–2011, 66.4% of male and 70.4% of female lung cancer patients were diagnosed at Stage IV [2]. In a study of lung cancer survival at a single referral hospital in Malaysia, all patients presented with either stage III or stage IV disease, and the overall median survival was only 18 weeks [3]. This makes lung cancer a lethal disease among Malaysians.

In recent years, there have been developments in our understanding of the heterogeneity of non-small cell lung cancer (NSCLC). NSCLC has evolved from being just histologically characterised to being molecularly profiled. Genetic alterations to the epidermal growth factor receptor (*EGFR*) is the most common actionable mutation in NSCLC [4].

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Treatment with EGFR tyrosine kinase inhibitors (TKIs) results in high response rates [5–7].

Numerous gene alterations can occur in NSCLC. Mutation testing has expanded to include new targets such as anaplastic lymphoma kinase (*ALK*) and ROS proto-oncogene 1 (*ROS1)* translocations, B-Raf proto-oncogene (*BRAF*) mutation, mesenchymal-epithelial transition (*MET*) amplification and mutation, RET proto-oncogene (*RET*) fusion, Erb-B2 receptor tyrosine kinase-2 (*ERBB2)* (Human epidermal growth factor receptor-2 (*HER2*)) mutation and Kirsten rat sarcoma 2 viral oncogene homologue (*KRAS*) mutation [8]. In addition, immunotherapy has emerged as part of standard of care for patients without driver mutation. Patients with tumours showing high programmed cell death ligand-1 (PD-L1) expression receiving immunotherapy are consistently shown to have better outcomes [8].

As such, the management of NSCLC now requires multiple molecular tests to guide the treatment strategy. There is now a need to establish a molecular testing consensus statement for advanced NSCLC patients in Malaysia. This Malaysian consensus statement reflects currently available scientific data and adaptations of recommendations from international guidelines to fit the local landscape.

## 2. Methods

The panel of experts was chaired by a pathologist and composed of three other pathologists, four respiratory physicians and three oncologists. The panel members were required to disclose any conflict of interest arising from the guideline's development and completion (listed in Appendix). This project was funded in full by the College of Pathologists, Academy of Medicine Malaysia.

The guidelines were drafted and reviewed by the panel members at three meetings, and continuously worked on through the shared document on an online word processor. It was drafted to consist of several main discussions, in which key questions and points were addressed by the assigned panel members. Expert recommendations agreed upon by the panel are provided for each of these questions. Relevant information, data and clinical practice recommendations from selected research articles and international guidelines were collected as references. Following the finalisation of the draft, the guidelines were submitted for international peer review by an independent review panel.

## 3. Outcomes

This consensus statement is organised into several main topics (Discussions 1–5) for easy comprehension. The recommendations are summarised in Table 1.

### 3.1. Discussion 1: To establish first line molecular testing in NSCLC patients in Malaysia

The treatment of patients with advanced NSCLC has changed dramatically over the past few years, due to our increased knowledge of the molecular basis of lung cancer (driver mutations and immune targets) and the drugs that affect these pathways, namely targeted agents and immunotherapy [8,9]. Therefore, it is important to test patients with newly-diagnosed advanced NSCLC for potential targetable molecular aberrations prior to the initiation of treatment [9,10]. This requires close coordination between the respiratory physician, interventional radiologist, pathologist and oncologist to ensure that the biopsy specimen is used judiciously to get all necessary information for optimal treatment decision-making [11].

When performing the initial biopsy to make a diagnosis of lung cancer, and during the initial diagnosis in pathology, it is important to save sufficient tissue for molecular testing [10,11].

All NSCLC patients with non-squamous histology and all never/light smokers (regardless of histology) should be tested for driver mutations (the more common abnormalities, i.e., *EGFR* activating mutations and *ALK* rearrangements). For patients with squamous histology, who have never smoked or have a light/remote history of smoking, other driver mutation testing may be considered [10].

**Table 1**
Summary of guideline recommendations.

| |
|---|
| Discussion 1: To Establish First Line Molecular Testing in NSCLC Patients in Malaysia |
| Recommendation 1.1: "Must-test" biomarkers, considered the standard of care for advanced lung adenocarcinoma patients, include *EGFR* mutation, *ALK* rearrangement and *ROS1* rearrangement [8]. |
| Recommendation 1.2: "Should-test" biomarkers are used to direct patients to clinical trials. These tests may be included as part of a larger testing panel performed for lung cancer patients [8]. |
| Recommendation 1.3: Beyond *EGFR*, *ALK* and *ROS1* mutation testing, multiplexed genetic sequencing panels (e.g. NGS) are preferred over multiple single gene tests. However, single gene tests are still acceptable. |
| Recommendation 1.4: Squamous cell carcinoma occurring in never/light smokers may be subjected to similar molecular testing. |
| Recommendation 1.5: Sequential testing is preferred. |
| Recommendation 1.6: Patient's specimen should be tested for *EGFR* mutation and *ALK* rearrangement at the time of diagnosis, or at disease recurrence or progression if not previously tested. |
| Recommendation 1.7: Core biopsy is the preferred sampling method. |
| Recommendation 1.8: Formalin- or alcohol-fixed and fresh tissue can be used for molecular analysis. |
| Recommendation 1.9: Any validated *EGFR* mutation test method may be used. |
| Recommendation 1.10: IHC is an equivalent alternative to FISH for ALK testing, provided the predictive performance of the assay in use is validated. |
| Recommendation 1.11: *EGFR*, *ALK*, *ROS1*, *BRAF*, *KRAS*, *MET*, *HER2*, *RET* and *NTRK1* gene alterations may be tested using a panel approach. |
| Recommendation 1.12: Biomarker testing results should be available within 10 working days of obtaining patient's specimen. |
| Recommendation 1.13: PD-L1 testing may be performed if driver mutations are not detected. |
| **Discussion 2: To Establish Mutations Testing Algorithm in NSCLC Patients Beyond First Line Therapy** |
| Recommendation 2.1: Molecular testing upon disease progression after first/second line therapy should take into account the previous molecular testing outcomes as well as therapies prescribed. |
| **Discussion 3: Immunotherapy and PD-L1 Testing** |
| Recommendation 3.1: PD-L1 IHC is recommended in all patients whose tumours are negative for common driver mutations (e.g. *EGFR, ALK, ROS1, BRAF* mutations). |
| Recommendation 3.2: FFPE tissue can be used for testing on a validated platform. |
| **Discussion 4: To Ensure the Delivery of High Quality Testing for Different Molecular Testing** |
| Recommendation 4.1: All laboratories conducting molecular testing for NSCLC must be accredited and participate in EQA to maintain accreditation. |

Abbreviations: ALK, anaplastic lymphoma kinase; BRAF, B-Raf proto-oncogene; EGFR, epidermal growth factor receptor; EQA, external quality assurance; FFPE, formalin-fixed and paraffin-embedded; FISH, fluorescence in situ hybridisation; HER2, human epidermal growth factor receptor-2; IHC, immunohistochemistry; KRAS, Kirsten rat sarcoma 2 viral oncogene homologue; MET, mesenchymal-epithelial transition factor; NGS, next-generation sequencing; NSCLC, non-small cell lung cancer; NTRK1, neurotrophic receptor tyrosine kinase 1; PD-L1, programmed cell death ligand-1; ROS1, ROS proto-oncogene 1; RET, RET proto-oncogene.

#### 3.1.1. What types of molecular testing should be performed?

Targeted therapies guided by molecular testing have become the standard of care for patients with lung cancer [13]. Driver mutations such as *EGFR* mutations, *ALK* and *ROS1* rearrangements act as predictive biomarkers for specific targeted therapy [13].

When next-generation sequencing (NGS) is performed, molecular testing for several other genes are also recommended—*BRAF*, *ERBB2* (*HER2*), *MET*, *RET*, and *KRAS* [8,12]. However, outside the context of a clinical trial, testing for these genes is not essential when only single gene tests are performed. BRAF testing may also be included as part of a

larger testing panel, performed either initially or when common driver mutations (i.e. *EGFR, ALK*, and *ROS1*) are not identified [8]. Other candidate biomarkers remain "investigational" and are not recommended for routine clinical use at this point in time [8]. The range of targets tested for is also determined by the availability of drugs against those targets.

PD-L1 expression should be tested before first-line treatment in patients with metastatic NSCLC without driver mutation [10].

#### 3.1.2. Which patients should undergo molecular testing?

Lung adenocarcinoma patients should not be excluded from molecular testing based on clinical characteristics alone [14]. Since the driver mutations are usually mutually exclusive, multiplex testing should be done for *EGFR* mutation and *ALK* rearrangement to select patients for EGFR-targeted therapy and ALK-targeted therapy. However, sequential testing may be more appropriate in the Malaysian setting, as the frequency of *EGFR* mutation is high in the Malaysian population (nearly 40% of all NSCLC cases [15]) and initial identification of this subset of patients would obviate unnecessary testing, although this practice impacts turnaround time. Currently there is no government subsidy and only partial industry support for molecular testing in Malaysia; this poses a financial burden to many patients. Parallel testing remains an option in special circumstances such as in full patient funding situations or if the clinical situation warrants a panel testing approach.

Besides *EGFR* mutation and *ALK* rearrangement, other targetable gene mutations (*BRAF*) and gene rearrangements (*ROS1*, *RET*, etc.) have low prevalence (approximately 1–5%) [16]. Currently, there is strong evidence to support ROS1 molecular testing in advanced-stage adenocarcinoma patients, irrespective of clinical characteristics [8].

EGFR, ALK and ROS1 testing is deemed appropriate for patients with adenocarcinoma, or for NSCLC patients in which an adenocarcinoma component cannot be excluded (such as in small biopsy samples), or for squamous cell carcinoma patients with a high possibility of *EGFR* mutation or *ALK* rearrangement (such as in patients with no smoking history and young age) [14,16–18].

#### 3.1.3. When should a patient's specimen be tested?

Patients presenting with metastatic disease (i.e. stage IV according to the 8th edition of TNM staging) should be tested for *EGFR* mutation and *ALK* rearrangement at the time of diagnosis. For patients diagnosed at an earlier stage at the time of initial presentation, and had not been previously tested, such testing should be performed at disease recurrence or progression [14]. Testing of patient with early-stage disease depends on the policy of each institution. Reflex testing is appropriate if agreed by the clinical care team in order to expedite the management of patient's specimen.

#### 3.1.4. What are the sample requirements for first line molecular testing?

Most of the specimens obtained from advanced NSCLC patients are tissue biopsies or cytological specimens. At presentation, less than 30% of NSCLCs are resectable [19]. The increasing number of new biomarkers for molecular testing is advantageous on small-biopsy specimens due to the limited amount of tissue available as well as tissue loss during repeated re-cutting of the paraffin blocks [19]. Specimens can be from the primary lung tumour or metastatic sites [14].

Different methods can be used to obtain tissue for diagnosis and molecular analysis. Among these are endoscopic biopsies, core-needle biopsy/cytology guided by endobronchial ultrasound (EBUS), endoscopic ultrasound (EUS), fine-needle aspiration (FNA), mediastinoscopy and thoracotomy [17]. Core biopsy of the tumour is the most preferred sample.

An expert group has recommended that [17]:

- A minimum of five endobronchial/transbronchial forceps biopsies should be taken. Five additional forceps biopsies or two cryobiopsies may be considered to obtain as much tissue as needed.
- At least four needle passes performed for every target lesion.
- For percutaneous core needle biopsy using an 18–20 G needle, three to six biopsies are preferred to ensure sufficient tissues are obtained.

Haematoxylin and eosin (H&E) stain is often adequate for tumour typing in most cases. If required, laboratories should prepare at least 10 or more unstained sections for diagnosis and classification of tumour as well as for molecular testing. Immunohistochemical testing should only be performed when there is doubt about tumour histogenesis. It should be limited to a minimum of two or three stains (TTF1, p40 and synaptophysin). Since small specimens may contain few tumour cells, limiting the use of ancillary immunohistochemistry (IHC) and clinical prioritisation of molecular testing is important. Pathologists should determine the integrity and adequacy of specimens obtained by assessing cancer cell percentage.

#### 3.1.5. How should specimens be processed for first line molecular testing?

The pre-fixation time, type of fixative used and fixation time all affect specimen quality [17]. Tissues may undergo significant biochemical changes within 10 min following sampling or resection. Either fresh or frozen, formalin-fixed or alcohol-fixed tissue may be used for molecular analysis. Tissue specimens are typically fixed with 10% neutral-buffered formalin in order to preserve tissue integrity. Heavy metal and acidic fixatives may cause DNA fragmentation; thus they are unsuitable for most molecular analyses. The use of harsh decalcifying solutions in the processing of bone biopsy samples may render such tissue unsuitable for molecular testing [14,17,19]. Alcohol-fixed cytology specimens may be used, provided that the staining protocol is adjusted and proper quality control and test validation is undertaken [20,21]. There is emerging evidence that formalin post-fixation (after alcohol) may offset any negative effects of alcohol [21,22].

Obtaining sufficient tissue may be challenging in certain circumstances. Alternatively, cytology samples (cell blocks and other cytologic preparations) may be used in place of tissue samples for molecular testing [14].

#### 3.1.6. How should EGFR testing be performed?

EGFR testing may be performed using any validated method. Methods for detecting mutations include direct sequencing, real-time polymerase chain reaction (PCR) and commercial kits. When selecting a particular method, pathologists should consider the pros and cons of each method, including the analytical sensitivity and turnaround time. The assays used or available in the laboratory should be able to detect mutations in specimens with as little as 10% cancer cells [14].

Clinical *EGFR* mutation testing should be able to detect all sensitising mutations with a frequency of at least 1% of mutated lung cancer cells. IHC tests for EGFR protein expression and EGFR copy number analysis (i.e., fluorescence in situ hybridisation (FISH) or chromogenic in situ hybridisation) are not recommended for selection of patients for EGFR TKI therapy [14].

#### 3.1.7. How should ALK testing be performed?

Among the methods for assessing gene rearrangement are FISH, reverse-transcriptase polymerase chain reaction (RT-PCR), IHC and NGS. Numerous studies have shown that a validated immunohistochemical analysis is an equivalent alternative to FISH for ALK testing because protein expression can serve as a surrogate marker of gene rearrangements [18,23,24]. Performing confirmatory FISH testing is optional.

Break apart FISH used to be the standard method to detect gene rearrangements. In addition to requiring specialised hardware, FISH also requires a level of technical expertise for accurate interpretation. The assay is adequately sensitive and can detect gene arrangements regardless of fusion partners.

RT-PCR cannot be recommended as an alternative to FISH for selecting patients for ALK inhibitor therapy [14]. NGS too cannot be recommended as an alternative to IHC or FISH for determination of *ALK* fusion.

At the current time, testing for secondary *ALK* mutations involved in acquired resistance to ALK TKI is not recommended [14].

*3.1.8. Should other genes be routinely tested in lung adenocarcinoma?*

The genetic mutations in NSCLC with FDA-approved therapies are *EGFR*, *ALK*, *ROS1* and *BRAF* mutations. Other mutations include *MET*, *HER2*, *RET*, and *NTRK*, all of which have their respective targeted therapies in clinical trials.

*ROS1* rearrangement is rare and mutually exclusive with other oncogenic driver mutations such as *EGFR* mutation and *ALK* rearrangement. The recommendation is to perform ROS1 testing after the tumour is tested negative for *EGFR* mutation and *ALK* rearrangement.

ROS1 IHC may be used to screen for *ROS1* mutations in lung adenocarcinoma patients. If the results are found positive, it should be confirmed by a molecular or cytogenetic method [8].

*BRAF* mutation testing is recommended as there is an approved targeted therapy for this driver mutation.

An expanded panel which includes ROS1, BRAF, KRAS, MET, HER2, RET and NTRK1 may be offered for lung cancer patients, provided adequate material is available [8]. This test should be considered in patients whose tumours have been tested negative for common driver mutations.

Beyond *EGFR*, *ALK* and *ROS1* mutation testing, multiplexed genetic sequencing panels are preferred over multiple single gene tests [8]. At the present time, however, NGS testing cannot be recommended as a first line approach for molecular profiling of NSCLC.

*3.1.9. How rapidly should test results be available?*

EGFR and ALK test results should be made available within 10 working days of obtaining patient's specimen.

*3.1.10. What is the testing algorithm for the first line molecular testing?*

The algorithms for molecular testing of non-squamous cell NSCLC and squamous cell carcinoma in the first line setting are shown in Figs. 1 and 2, respectively. "Light smoker" in Fig. 2 is defined as < 15 pack-years [25].

*3.1.11. What are the alternative methods of conducting molecular testing in NSCLC?*

Use of circulating cell-free plasma DNA (cfDNA) molecular methods for the primary diagnosis of lung adenocarcinoma is currently unsupported [26].

*3.2. Discussion 2: To establish mutations testing algorithm in NSCLC patients beyond first line therapy*

Molecular testing for patients who develop disease progression after first line and/or second line therapy should take into consideration the results of previous molecular testing and drug(s) prescribed:

*3.2.1. Recommended molecular testing when the biopsied sample was previously tested positive for a sensitising EGFR mutation and the first line therapy was an EGFR-TKI*

*3.2.1.1. Patients who were treated with first or second generation EGFR-TKI.* Nearly all patients who are on EGFR-TKI will eventually progress [27]. The average interval of progression-free survival (PFS) is about 9.2–14.7 months for patients treated with first and second generation TKIs [28].

Resistance to EGFR TKIs may occur via one of four types of mechanism: secondary *EGFR* mutation, activation of alternative pathways, phenotypic transformation and resistance to apoptosis [27,29]. Of these, secondary mutation is the most frequent mechanism—up to 60% of these patients may acquire T790 M mutation, which has been identified as an important cause of treatment failure [27]. This resistance mutation can be identified by either a PCR-based allele testing method or be part of a panel of more extensive testing, e.g. NGS.

If NGS is performed, certain mutations that are of clinical interest should be prioritised. These include *HER2* mutation, *MET* amplification, *MET* exon skipping, *RET* translocation, *BRAF* mutation, etc.—particularly if there is a clinical trial in which these patients can be enrolled [8].

A repeat tissue biopsy is preferred over liquid biopsy, as it may also help to identify small-cell transformation or epithelial mesenchymal transition [30,31].

If cfDNA from blood is used to detect a T790 M resistance mutation and this alteration is not detected on liquid biopsy, a tissue biopsy is recommended, as there is a 20–30% chance of missing T790 M mutation on liquid biopsy [32]. The presence of the original *EGFR* mutation in the repeat biopsy will serve as an indicator of sufficient circulating tumour DNA (ctDNA) obtained.

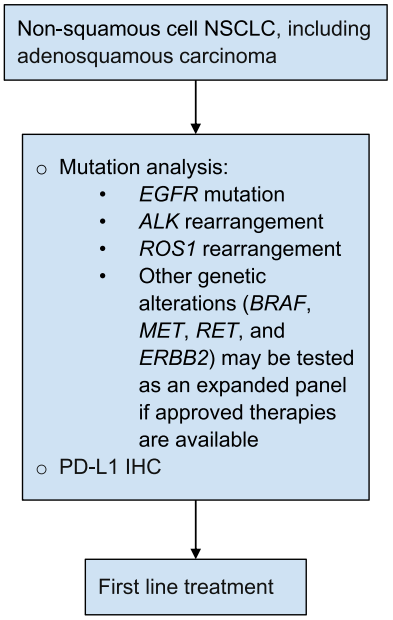


**Fig. 1.** Algorithm for mutational testing of non-squamous cell NSCLC in the first line setting. Abbreviations: ALK, anaplastic lymphoma kinase; BRAF, B-Raf proto-oncogene; EGFR, epidermal growth factor receptor; ERBB2, Erb-B2 receptor tyrosine kinase-2; IHC, immunohistochemistry; MET, mesenchymal-epithelial transition factor; NSCLC, non-small cell lung cancer; PD-L1, programmed cell death ligand-1; ROS1, ROS proto-oncogene 1; RET, RET proto-oncogene.

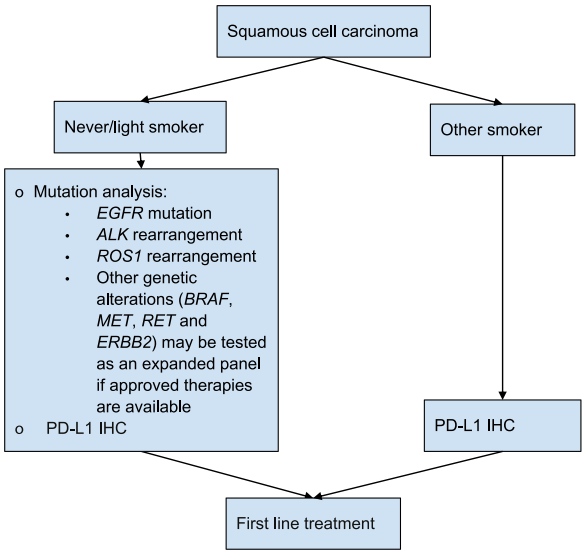


**Fig. 2.** Algorithm for mutational testing of squamous cell carcinoma in the first line setting. Light smoker is defined as < 15 pack-years [25]. Abbreviations: ALK, anaplastic lymphoma kinase; BRAF, B-Raf proto-oncogene; EGFR, epidermal growth factor receptor; ERBB2, Erb-B2 receptor tyrosine kinase-2; IHC, immunohistochemistry; MET, mesenchymal-epithelial transition factor; NSCLC, non-small cell lung cancer; PD-L1, programmed cell death ligand-1; ROS1, ROS proto-oncogene 1; RET, RET proto-oncogene.

**Table 2**
PD-L1 IHC assays according to drugs and diagnostic tests.

| Drug | PD-L1 diagnostic antibody clone | Type of diagnostic assay | PD-L1 binding domain | Platform | Second line criteria for PD-L1 positivity |
|---|---|---|---|---|---|
| Nivolumab (Bristol-Myers Squibb, New York City, NY) | 28-8 (rabbit) | Complementary | Extracellular | Link 48 Autostainer | ≥ 1% tumour cells |
| Pembrolizumab (Merck, Kenilworth, NJ) | 22C3 (mouse) | Companion | Extracellular | Link 48 Autostainer | ≥ 50% tumour cells |
| Atezolizumab (Genentech, San Francisco, CA/Roche, Basel, Switzerland) | SP142 (rabbit) | Complementary | Cytoplasmic | BenchMark ULTRA | Tumour cells and/or tumour infiltrating immune cells |
| Durvalumab (AstraZeneca, Cambridge, UK/ MedImmune, Gaithersburg, MD) | SP263 (rabbit) | Complementary | Extracellular | BenchMark | ≥ 25% tumour cells |
| Avelumab (Pfizer, New York City, NY/Merck Serono, Darmstadt, Germany) | 73-10 | Complementary | Unknown | Dako assay | ≥ 1% tumour cells |

Abbreviations: IHC, immunohistochemistry; PD-L1, programmed cell death ligand-1.
Adapted with permission from IASLC Atlas of PD-L1 Immunohistochemistry Testing in Lung Cancer. 2017 [20].

*3.2.1.2. Patients who were treated with third generation EGFR-TKI.* A repeat tissue biopsy is preferred to determine emergence of new resistance mutations or mechanisms.

In this context, a panel of more extensive NGS testing is recommended.

The testing of PD-L1 in these cases is considered optional—patients with *EGFR* mutations are less likely to respond to an immunotherapy [33].

*3.2.2. Recommended molecular testing when the first line therapy was an ALK-TKI and the biopsied sample was previously tested positive for ALK rearrangement*

*3.2.2.1. Patients who were treated with a first generation ALK-TKI.* A repeat biopsy or molecular testing may not be required. Patients may be switched to a second generation ALK-TKI.

*3.2.2.2. Patients who were treated with a second generation ALK-TKI.* A repeat biopsy is preferred and more comprehensive testing may be considered for research purposes and to determine reason for resistance.

Alternatively, patients may be treated with a third generation ALK-TKI.

*3.2.3. Recommended molecular testing when the first line therapy was a ROS1 inhibitor and the biopsied sample was previously tested positive for ROS1 fusion*

Repeat tissue biopsy and molecular testing may be performed for research purposes and to elucidate the mechanism of resistance.

*3.2.4. Recommended molecular testing when the first line therapy was chemotherapy and the biopsied sample was previously tested negative for EGFR, ALK, and ROS1 mutations*

The tumour may be tested for patient eligibility to receive immunotherapy, using approved and available biomarker assays, such as a validated PD-L1 IHC. This would apply if the drug to be used has been afforded companion diagnostic status and testing for PD-L1 is a mandatory requirement before treatment is commenced. However, such testing is not required for some currently approved drugs.

*3.2.5. Recommended molecular testing when the first line therapy was chemotherapy and no EGFR, ALK or ROS1 mutations testing performed*

These patients should be tested for *EGFR, ALK,* or *ROS1* mutations using either the archived tissue or repeat tissue biopsy.

PD-L1 testing may be considered if immunotherapy is contemplated.

### *3.3. Discussion 3: Immunotherapy and PD-L1 testing*

Immunotherapy has evolved and studies have shown their effectiveness in a subset of lung cancer patients [34,35]. This is based on the understanding that tumour antigens which are taken up by dendritic cells migrate to draining lymph nodes, where they are presented to T-cells which mature into cytotoxic T-cells that will kill the tumour. However, concurrent immunosuppression mechanisms exist in cytotoxic T-cells as well. For example, cytotoxic T-lymphocyte antigen-4 (CTLA-4) on the T-cell, competes with CD28 for binding of B7, the latter complex of CD28-B7 being co-stimulatory for maturation of the cytotoxic T-cell in the presence of tumour antigen [36]. Besides the immunosuppressive CTLA-4 pathway, many cancer cells express PD-L1 on their cellular surface. The interaction of PD-L1 with the programmed cell death protein-1 (PD-1) on T-lymphocytes negatively regulates the T-lymphocytes, and favours immunosuppression [20,36].

Tumour PD-L1 immunohistochemical expression is the most frequent predictor for anti-PD-1 and anti-PD-L1 immunotherapy [37–40]. Several harmonisation studies are underway to standardise PD-L1 testing and reporting [41–44]. The range of companion/complementary PD-L1 IHC which have been approved for its paired drug is shown in Table 2.

FFPE sections of biopsies or resected specimens and cell blocks of FNA aspirations or effusion specimens can be used for IHC testing of PD-L1. Liquid biopsies (utilising circulating tumour cells) are not recommended currently. However, there is interest in the assay of soluble PD-L1 in sera of patients [45,46].

For the interpretation of PD-L1 IHC, it is noteworthy that there are at least 5 different bioassays (Table 2) with different cut-off values for the tumour proportion score (TPS) that dictates treatment with its respective immunotherapy drug. TPS is normally defined as percentage of tumour cells demonstrating partial or complete membrane staining [47–49]. PD-L1 expression is usually heterogenous and most frequently seen at the tumour-stromal interface [50]. Sampling errors can therefore cause discordance when testing different areas of the tumour [51]. In a recent study, it has been suggested that testing on four biopsied fragments of the tumour can reduce sampling error [52]. The choice of PD-L1 IHC should, where possible, be based on its status as a companion diagnostic test, regulatory approval status, and evidence-based data from clinical trials or harmonisation studies such as the Blueprint study [41–43].

The pre-analytic conditions for tissue handling are set out in Table 3.

#### 3.3.1. PD-L1 tests: who and when?

High PD-L1 expression has been shown in the majority of trials involving anti–PD-1 and anti–PD-L1 therapies to be predictive of improved overall response rate and better overall survival [35,53,54]. This benefit appears to be incremental, whereby greater benefit is observed with increasing PD-L1 score [55,56]. PD-L1 testing should be considered for NSCLC patients with no druggable mutations.

#### 3.3.2. Can archival tissue be used to test for PD-L1?

PD-L1 testing may be performed on archival tissue using a validated protocol.

#### 3.3.3. What tissue is recommended for PD-L1 testing?

FFPE samples are recommended for PD-L1 testing. Alcohol-fixed cytology specimens can be used provided that staining protocol is adjusted, and proper quality control and test validation is undertaken [20,21].

### 3.4. Discussion 4: To ensure the delivery of high quality testing for different molecular tests: best practices for quality assurance of molecular testing

#### 3.4.1. Quality assurance systems in molecular testing for both tissue and liquid biopsy

Maintaining quality in every aspect of the molecular testing workflow of NSCLC is critical as accurate test results are a prerequisite before cancer therapy can commence [57]. This is especially important in the context of companion diagnostic tests, and therefore conformance with the specified protocols as per regulatory approvals must be adhered to [58–60].

Particular attention should be paid to the pre-analytical variables, which impact testing and are an important cause of test failure. All such parameters should be standardised and all tests should be accredited according to the International Organisation for Standardisation (ISO) 189:2012 [17].

Unaccredited research laboratories (with no otherwise equivalent recognition) should have their test results verified and reported by a laboratory holding such an accreditation or recognition [61–63].

All molecular testing results should be reported by accredited or otherwise recognised laboratories, consistent with national and international guidelines [59,64].

A quality assurance framework must be present for laboratories providing molecular genetic testing [63–65]. Local and international regulatory and professional bodies should, as appropriate, review whether these tests are performed as indicated.

Furthermore, all laboratories should state the analytical and clinical validity of all molecular diagnostic tests performed.

#### 3.4.2. Monitoring and maintaining quality of testing

Monitoring the accuracy of testing and reporting can be achieved by regular participation and successful performance in recognised international / external quality assurance (EQA) programs, or validated local quality assurance programs, if any such are available [65–67].

#### 3.4.3. Quality of result reporting

NSCLC histological subtypes should be reported as accurately as possible, and a diagnosis of “non-small cell carcinoma” should not exceed 10% of the histopathological reports issued. Optimal histological subtyping can be achieved by the judicious use of immunohistochemical stains, in most instances with as few as 2 markers (TTF-1 or Napsin A and p40 or p63). Pathologists should be constantly mindful of needless tissue wastage, which may compromise availability of tissue for further genetic testing [68–72].

It is also essential that molecular data should be interpreted and reported together with, and in the context of the tested sample [72].

The turnaround time for molecular testing of NSCLC should conform to good local and international practice guidelines, be issued in a timely manner and reported in a form, as described below.

All molecular testing reports should be issued using accepted terminology and nomenclature including, where appropriate, identification of reference sequences [64].

Choice of test methodology is an important determinant of sensitivity and specificity. This information must be made available, with

**Table 3**
Recommended pre-analytic conditions for IHC.

| Parameter | Recommendation |
|---|---|
| Cold ischaemia time | If possible, shorter than 30 minutes (not exceeding 1 hour). |
| Fixative | 10% neutral buffered formalin. Alcohol fixation is to be avoided. However, alcohol-fixed cytology specimens can be used provided that staining protocol is adjusted and proper quality control is undertaken [20,21]. There is emerging evidence that formalin post-fixation (after alcohol) may offset any negative effects of alcohol [21,22]. |
| Time of fixation (biopsy) | 6 to 48 hours. |
| Time of fixation (resection) | 24 to 48 hours. |
| Preparation | Paraffin-embedded sections, cut at a thickness of 3 to 5 µm. |
| Use of tissue section | If not used within days, sections should be stored in a closed box at 2-8 °C. It can be used for staining up to 2 months. |
| Storage time for FFPE blocks | Less than 3 years for PD-L1 IHC. |
| Storage conditions for FFPE blocks | Protected from light, heat, and humidity. |
| Storage time for tissue sections | Less than 2 months, particularly for testing with SP263 antibody. |
| Decalcification | EDTA, if necessary; avoid strong acids, e.g. nitric acid and hydrochloric acid. |

Abbreviations: EDTA, ethylene-diamine-tetra-acetic acid; FFPE, formalin-fixed and paraffin-embedded; IHC, immunohistochemistry; PD-L1, programmed cell death ligand-1.
Adapted with permission from IASLC Atlas of PD-L1 Immunohistochemistry Testing in Lung Cancer. 2017 [20].

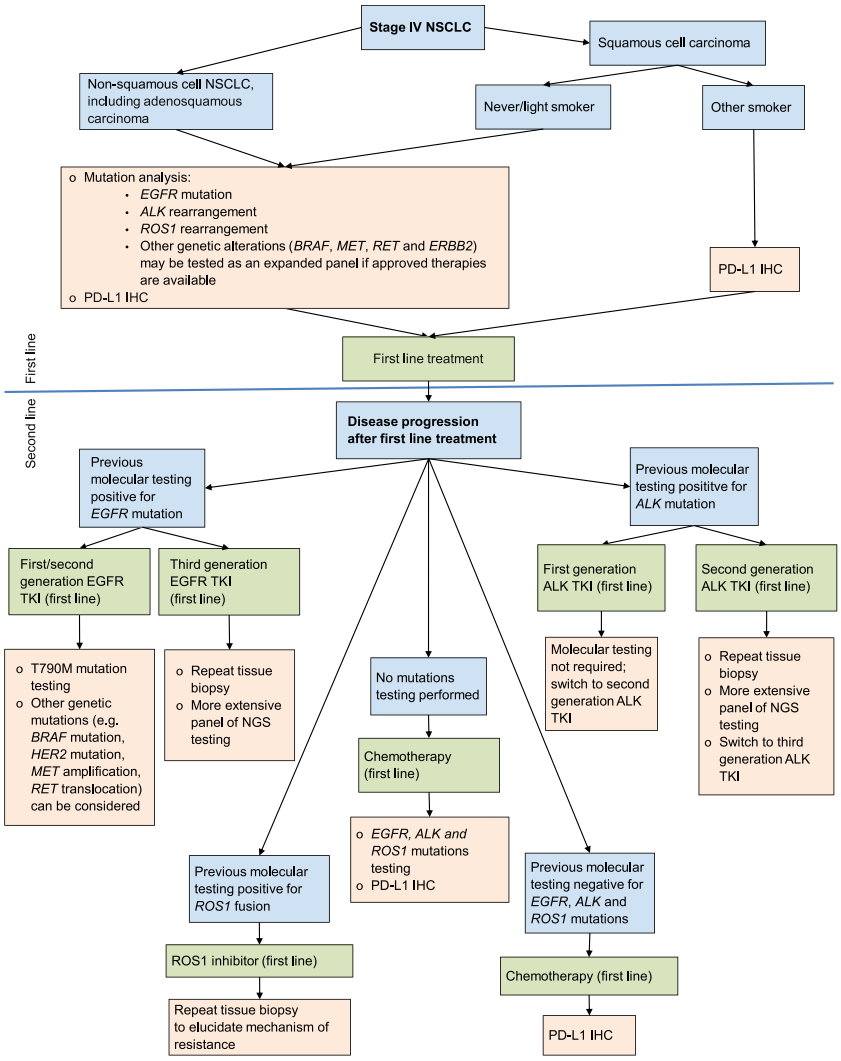


**Fig. 3.** Algorithm for molecular testing of metastatic NSCLC in the first line setting and upon disease progression beyond first line therapy. Light smoker is defined as < 15 pack-years [25]. Extensive NGS panel testing is recommended only if an approved drug is available or in clinical trial. Abbreviations: ALK, anaplastic lymphoma kinase; BRAF, B-Raf proto-oncogene; EGFR, epidermal growth factor receptor; ERBB2, Erb-B2 receptor tyrosine kinase-2; HER2, human epidermal growth factor receptor-2; IHC, immunohistochemistry; MET, mesenchymal-epithelial transition factor; NGS, next-generation sequencing; NSCLC, non-small cell lung cancer; PD-L1, programmed cell death ligand-1; ROS1, ROS proto-oncogene 1; RET, RET proto-oncogene; TKI: tyrosine kinase inhibitor.

adequate and unambiguous explanatory comments to allow the reader to assess the significance of the test outcome. There must be sufficient detail within the report to accurately describe the test findings, to allow interpretation so that appropriate therapy may be instituted. The relevance of the findings to potential therapeutic options should be mentioned.

In addition, all staff should be trained and receive continuous opportunities for further training and professional development [59,73].

### *3.5. Discussion 5: Development of testing recommendations in the era of genomic medicine*

Over the past decade, there has been a rapid pace of discoveries in the area of genomic medicine. There is an urgency to provide guidance in molecular testing for NSCLC, at times amidst the limitations of scientific literature. Under these circumstances, testing recommendations will need to be developed based on the current available data, with an integration of expert opinion, and a continuous update of these recommendations as more studies are published [14,59].

## 4. Conclusions

Progressive developments have been made in the treatment of advanced NSCLC. Increased understanding of the molecular profiles of NSCLC has allowed for targeted treatments of these actionable genetic mutations. Implementation of the appropriate treatment strategy according to type of molecular aberration is essential in the management of NSCLC. Thus, guidelines on molecular testing for advanced NSCLC suited to the Malaysian context was established. The algorithm for molecular testing of metastatic NSCLC in the first line setting and upon disease progression beyond first line therapy is illustrated in Fig. 3.

## Disclaimer

Content and recommendations in this consensus statement are based on available scientific data and clinical practice recommendations from international guidelines which have been adapted to the Malaysian landscape. Clinical judgement prevails in all decisions and should not replace individual responsibility.

## Disclosure

Disclosure of interests and activities by members of the expert panel are listed in the Appendix. This consensus statement was funded by the College of Pathologists, Academy of Medicine Malaysia.

## Declaration of Competing Interest

The authors disclose list of interest and activities in the Appendix.

## Appendix A

Disclosed interests and activities from August 2016 - July 2018.

| | Name | Interest / Activity type | Entity |
|---|---|---|---|
| 1 | Prof. Dr. Pathmanathan Rajadurai | Consulting / advisory fees | Roche, Pfizer, AstraZeneca, Merck Sharp & Dohme, Boehringer Ingelheim, Novartis |
| | | Speaker fees | Roche, Merck Sharp & Dohme, AstraZeneca, Boehringer Ingelheim, Pfizer |
| | | Position of influence | Editorial board, Pathology |
| | | Research funding | Roche, AstraZeneca |
| 2 | Prof. Dr. Cheah Phaik Leng | Position of influence | Editorial Board, Pathology<br>Associate Editor, Malaysian Journal of Pathology<br>Director of Genomic Medical Science @ University of Malaya Laboratory |
| | | Sponsorship of testing at Genomic Medical Science @ University of Malaya Laboratory | AstraZeneca, Roche, Merck Sharp & Dohme, Pfizer |
| 3 | Prof. Dr. How Soon Hin | Research grants | AstraZeneca, Boehringer Ingelheim, Merck Sharp & Dohme, Novartis, Pfizer, Merck |
| | | Honoraria and fees for lectures and advisory board meeting | AstraZeneca, Boehringer Ingelheim, Merck Sharp & Dohme, Novartis, Pfizer, Takeda |
| 4 | Prof. Dr. Liam Chong Kin | Research grants | AstraZeneca, Boehringer Ingelheim |
| | | Honoraria and fees for lectures and advisory board meeting | AstraZeneca, Boehringer Ingelheim, Merck Sharp & Dohme, Novartis, Pfizer, Takeda |
| 5 | Dr. Muhammad Azrif Ahmad Annuar | Honoraria and fees for lectures and advisory board meeting, CME sponsorship | AstraZeneca, Boehringer Ingelheim, Merck, Eli Lilly, Merck Sharp & Dohme, Eisai, Takeda, Sanofi Aventis, Roche, Pfizer, Novartis, Johnson & Johnson |
| 6 | Dr. Norhayati Omar | – | – |
| 7 | Dr. Noriah Othman | Speaker fee / honoraria | AstraZeneca, Novartis |
| 8 | Dr. Nurhayati Mohd Marzuki | Speaker fee / honoraria | AstraZeneca |
| | | Position of influence | Director of Institute of Respiratory Medicine, Kuala Lumpur Hospital |
| | | Advisory board | AstraZeneca, Boehringer Ingelheim, Pfizer, Roche |
| 9 | Assoc. Prof. Dr. Pang Yong Kek | Research grants | AstraZeneca, Boehringer Ingelheim, Merck Sharp & Dohme |
| | | Speaker fee / Honoraria | AstraZeneca, Boehringer Ingelheim, Novartis, Pfizer |
| | | Advisory board | Boehringer Ingelheim, Novartis |
| 10 | Dr. Ros Suzanna binti Ahmad Bustamam | Speaker fee / honoraria | Roche, Bayer |
| | | Advisory board | Roche |
| | | Position of influence | Head of Radiotherapy & Oncology Service, Ministry of Health, Malaysia |
| 11 | Assoc. Prof. Dr. Tho Lye Mun | Honoraria and fees for lectures and advisory board meeting, CME sponsorship | AstraZeneca, Boehringer Ingelheim, Merck, Eli Lilly, Merck Sharp & Dohme, Eisai, Takeda, Sanofi Aventis, Roche, Pfizer, Novartis, Astellas |

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