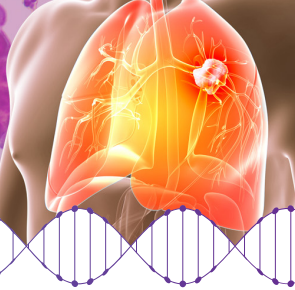
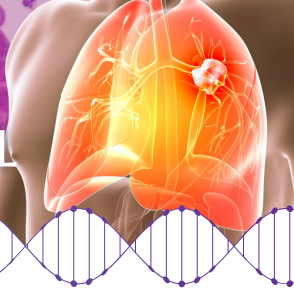
Consensus Statement on Molecular Testing for Advanced Non-Small Cell Lung Cancer in Malaysia

2019 Edition

CONTENTS



MEMBERS OF THE EXPERT PANEL



CHAIRPERSON

Professor Dr. Pathmanathan Rajadurai

Senior Consultant Pathologist
Subang Jaya Medical Centre, Selangor

Professor of Pathology
Monash University Malaysia

MEMBERS (alphabetical order)

Professor Dr. Cheah Phaik Leng

Senior Consultant Pathologist
University Malaya Medical Centre, Kuala Lumpur

Professor Dr. How Soon Hin

Consultant General Physician and Respiratory Physician
International Islamic University Malaysia, Pahang

Professor Dr. Liam Chong Kin

Senior Consultant Respiratory Physician
University Malaya Medical Centre, Kuala Lumpur

Dr. Muhammad Azrif Ahmad Annuar

Consultant Clinical Oncologist
Prince Court Medical Centre, Kuala Lumpur

Dr. Norhayati Omar

Consultant Histopathologist
Ministry of Health, Malaysia

Dr. Noriah Othman

Consultant Thoracic Pathologist
Ministry of Health, Malaysia

Dr. Nurhayati Mohd Marzuki

Consultant Respiratory Physician
Institute of Respiratory Medicine
Ministry of Health, Malaysia

Associate Professor Dr. Pang Yong Kek

Senior Consultant Respiratory Physician
University Malaya Medical Centre, Kuala Lumpur

Dr. Ros Suzanna binti Ahmad Bustamam

Head of Radiotherapy and Oncology Service
Ministry of Health, Malaysia

Dr. Tho Lye Mun

Consultant Clinical Oncologist
Beacon Hospital, Petaling Jaya

TERMS OF REFERENCE

Target audience

This consensus statement has been developed for the reference of healthcare professionals involved in the care of patients with advanced non-small cell lung cancer such as pathologists, general and chest physicians, oncologists, radiologists, general and cardiothoracic surgeons.

Disclosure

Disclosure of interests and activities by members of the expert panel are found in the Appendix.

Source of funding

This consensus statement was made possible by the College of Pathologists, Academy of Medicine Malaysia.

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 local landscape. Clinical judgement prevails in all decisions and should not replace individual responsibility.

Copyright ownership

MESSAGE FROM THE MASTER OF THE ACADEMY OF MEDICINE MALAYSIA

For many years, the treatment of advanced non-small cell lung cancer (NSCLC) was with chemotherapy. The general outlook was poor with low response rates. The management of advanced NSCLC has been transformed by the discovery of somatic gene alterations in patients leading to the activation of oncogenes. For this subgroup of advanced NSCLC patients, the treatment paradigm has evolved from non-specific curative approaches, to the use of therapeutic agents and immune checkpoint inhibitors targeting particular actionable genetic mutations.

Biomarker testing prior to the initiation of therapy has become imperative in order to identify these groups of tumours and indicate the most appropriate therapy which would confer benefit to the patient. This would require coordination between members of multiple disciplines, i.e., respiratory physicians, interventional radiologists, pathologists and oncologists. However, molecular testing performed would depend greatly on the local availability of the tests and treatments to be administered.

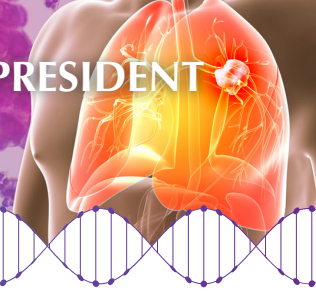
The "Consensus Statement on Molecular Testing for Advanced NSCLC in Malaysia" was developed to address this rapid progress in lung cancer care. It provides recommendations on the management of advanced NSCLC as well as molecular testing algorithms suited to the local practice.

I would like to congratulate Professor R Pathmanathan for taking the lead and the members of the expert panel for taking time off their busy schedule to deliberate and formulate the consensus documents. I am certain this consensus statement will be useful in guiding treatment decisions in NSCLC patients.

With kind regards,

Prof. Dr. Rosmawati Mohamed
Master, Academy of Medicine of Malaysia

MESSAGE FROM THE PRESIDENT OF THE COLLEGE OF PATHOLOGISTS



Lung cancer is one of the leading causes of cancer deaths in Malaysia and worldwide. According to the Malaysian Study on Cancer Survival (MyScan 2018), the lowest survival was cancer of the lung, trachea and bronchus with a 5-year survival rate of 11% and a median survival time of 6.8 months. The treatment of lung cancer depends on the type and stage of lung cancer presented. One of the latest developments in lung cancer treatment is the introduction of targeted therapy, which has been pivotal in the improvement of survival rates in advanced lung cancer patients. To ensure that optimal treatment is afforded to lung cancer patients, doctors must keep constantly updated with current developments in lung cancer medicine.

The Consensus Statement on Molecular Testing Guidelines for Advanced Non-Small Cell Lung Cancer (NSCLC) in Malaysia arose from a year-long collaboration between doctors of various specialties, who had generously contributed their time and focus towards the fruition of this project. It was created based on clinical practice recommendations from international guidelines, which have been localised to fit the Malaysian practice.

The contents of the consensus statement were put together to assist doctors in making apt treatment decisions for their advanced NSCLC patients. It is partitioned into several main sections, which include molecular testing recommendations in the first line and subsequent line settings, immunotherapy, as well as quality assurance in molecular testing. I consider the consensus statement a must-read for doctors with advanced stage NSCLC patients under their care.

Emeritus Prof. Dr. Cheong Soon Keng, FASc
President of the College of Pathologists, Academy of Medicine Malaysia

MESSAGE FROM THE PRESIDENT OF THE MALAYSIAN THORACIC SOCIETY

In recent years, major strides have been made in the diagnosis and management of patients with non-small cell lung cancer. Quite often, finding a specific molecular or bio-marker will influence how these patients are managed.

However, determining the type and sequence of tests to perform in certain clinical scenarios may be an intricate matter for some clinicians, especially in resource-limited environment.

This guideline is drafted by a group of experts from diverse professional backgrounds. Its objective is set to provide some helpful insight to healthcare providers who are seeking additional guidance from the local experts.

While observing limitations posed by constraints in Malaysia, great efforts have been made to ensure that all recommendations conform with the latest evidence in literatures.

On behalf of the Malaysian Thoracic Society, I would like to thank Prof. Pathmanathan and College of Pathologists for the kind invitation to collaborate on this project which resulted in the product of this meaningful guideline.

Yours sincerely,

Associate Prof. Dr. Pang Yong Kek
President of the Malaysian Thoracic Society

MESSAGE FROM THE PRESIDENT OF THE MALAYSIAN ONCOLOGICAL SOCIETY

Congratulations to the expert panel, ably led by Prof. Pathmanathan Rajadurai, and the College of Pathologists of the Academy of Medicine Malaysia on the completion of the Molecular Testing Guidelines for Advanced Non-Small Cell Lung Cancer in Malaysia. This effort is a great example of the collaborative teamwork between pathologists, oncologists and respiratory physicians required in the treatment of lung cancer.

Lung cancer is the third most common cancer in Malaysia and the majority of cases are diagnosed in the advanced stage. The treatment of advanced lung cancer requires the judicious use of chemotherapy, targeted therapy, radiotherapy, immunotherapy and palliative care in optimising outcomes for Malaysian patients. Clinical trials have shown that targeted therapy can be of great benefit to patients, whose cancers harbour actionable mutations, allowing them to lead good quality lives.

These molecular testing guidelines have been formulated to take into account resources available to doctors practising in Malaysia to decide whether targeted therapy is the most appropriate option. The guidelines are designed as a series of questions that the doctor may face in choosing which tests to perform and when to do them in the newly diagnosed and previously treated patients with lung cancer.

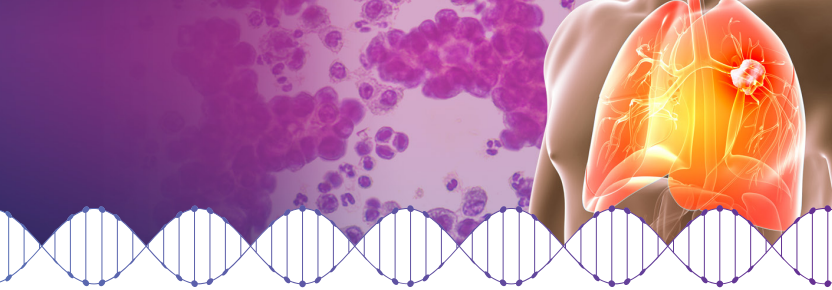
I believe that these guidelines will be useful to pathologists, respiratory physicians and oncologists in the diagnosis and management of lung cancer patients in Malaysia.

Dr. Muhammad Azrif Ahmad Annuar
Honorary President of the Malaysian Oncological Society, 2018-2021

ABBREVIATIONS

The following abbreviations are used in the text.

ALK	Anaplastic lymphoma kinase (gene)
BRAF	B-Raf proto-oncogene (gene)
cfDNA	Cell-free plasma DNA
ctDNA	Circulating tumour DNA
CTLA-4	Cytotoxic T-lymphocyte antigen-4
DNA	Deoxyribonucleic acid
EBUS	Endobronchial ultrasound
EDTA	Ethylene-diamine-tetra-acetic acid
EGFR	Epidermal growth factor receptor (gene)
EQA	External quality assurance
ERBB2	Erb-B2 receptor tyrosine kinase-2 (gene)
EUS	Endoscopic ultrasound
FDA	Food and Drug Administration
FFPE	Formalin-fixed and paraffin-embedded
FISH	Fluorescence in situ hybridisation
FNA	Fine-needle aspiration
H&E	Haematoxylin and eosin
HER2	Human epidermal growth factor receptor-2 (gene)
IHC	Immunohistochemistry
ISO	International Organisation for Standardization
KRAS	Kirsten rat sarcoma 2 viral oncogene homologue (gene)



MET	Mesenchymal-epithelial transition factor (gene)
NGS	Next-generation sequencing
NSCLC	Non-small cell lung cancer
NTRK1	Neurotrophic tyrosine receptor kinase 1 (gene)
PCR	Polymerase chain reaction
PD-1	Programmed cell death protein-1
PD-L1	Programmed cell death ligand-1
PFS	Progression-free survival
RET	RET proto-oncogene (gene)
ROS1	ROS proto-oncogene 1 (gene)
RT-PCR	Reverse-transcriptase polymerase chain reaction
TBL	Tracheal, bronchus and lung
TKI	Tyrosine kinase inhibitor
TNM	Tumour, nodes, metastasis
TTF1	Thyroid transcription factor-1
TPS	Tumour proportion score

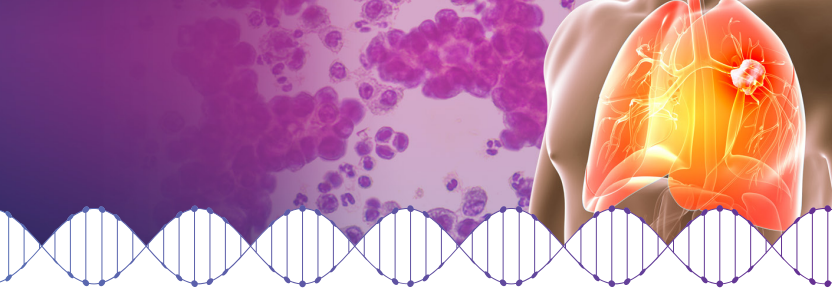
BACKGROUND

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 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] 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 mutations. Patients with tumours



showing high programmed cell death ligand-1 (PD-L1) expression receiving immunotherapy have 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 therefore a need to establish a molecular testing consensus statement for advanced NSCLC patients in Malaysia.

OBJECTIVE

The objective of this consensus statement is to establish evidence-based molecular testing recommendations for advanced NSCLC in Malaysia.

These recommendations will address the appropriate patients and samples to be tested, as well as the when and how these tests should be performed.

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 immune checkpoint inhibitors.[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.[11,12]

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, driver mutation testing may be considered.[10]

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,* and *RET.*[8,12] However, outside the context of a clinical trial, testing for these genes is not essential when only single gene tests are performed.[8] 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. 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]
(Refer to Discussion 3: Immunotherapy and PD-L1 Testing)

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 only partial government subsidy and 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. 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.

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

Most of the clinical samples from patients with advanced NSCLC available for molecular testing 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 sectioning 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.

A European 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 for molecular testing by assessing cancer cell percentage.

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 minutes following sampling or resection. Either fresh or frozen tissue, 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 and 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]

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]

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]

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 as a 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, and RET 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.

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.

10. What is the testing algorithm for 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 Figures 1 and 2, respectively. "Light smoker" in Fig. 2 is defined as < 15 pack-years.[25]

FIGURE 1. Algorithm for mutational testing of non-squamous cell NSCLC in the first line setting.

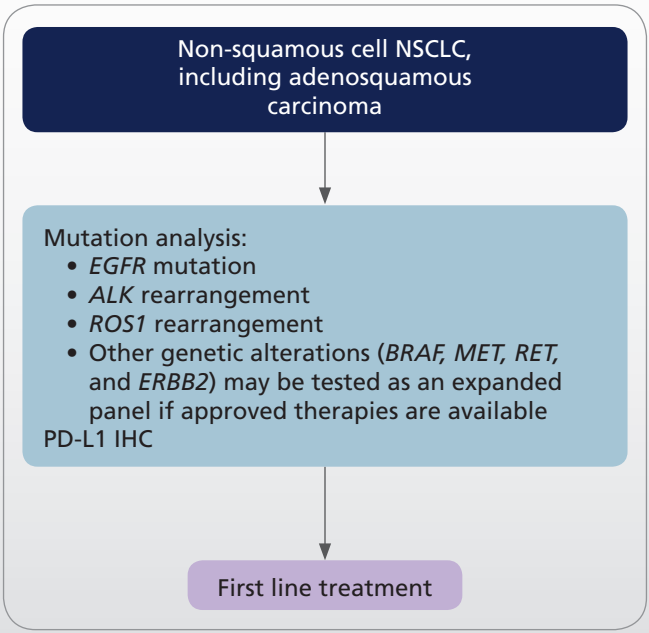
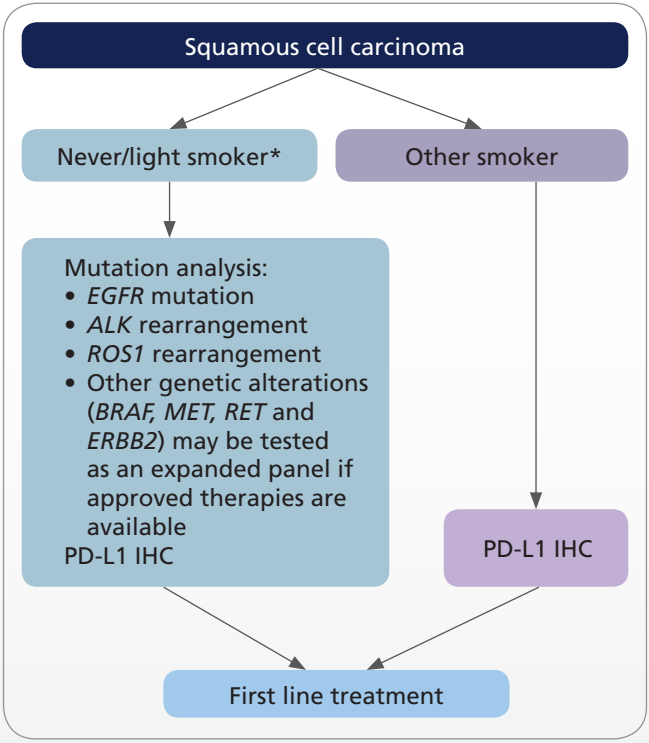


FIGURE 2. Algorithm for mutational testing of squamous cell carcinoma in the first line setting.



*Light smoker is defined as < 15 pack-years.[25]

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]

RECOMMENDATIONS

- "Must-test" biomarkers, which are standard-of-care for all advanced lung cancer patients with an adenocarcinoma component who are being considered for an approved targeted therapy, include *EGFR* mutation, *ALK* rearrangement, *ROS1* rearrangement and *BRAF* mutation.[8]
- "Should-test" biomarkers can be used to direct patients to clinical trials. These should be included in any large sequencing panel performed for lung cancer patients.[8]
- Multiplexed genetic sequencing panels (e.g. NGS) are preferred over multiple single-gene tests to identify other treatment options beyond *EGFR, ALK,* and *ROS1*. However, single gene assays are still acceptable.
- Squamous cell carcinoma occurring in never/light smokers may be subjected to similar molecular testing.
- Sequential testing is preferred.
- Patient's specimen should be tested at the time of diagnosis, recurrence or disease progression.
- Core biopsy is the preferred sampling method.
- Formalin- or alcohol-fixed and fresh tissue can be used for molecular analysis.
- Any validated *EGFR* mutation test method may be used.
- IHC is an equivalent alternative to FISH for ALK testing, provided the predictive performance of the assay in use is validated.
- *EGFR, ALK, ROS1, BRAF, KRAS, MET, HER2, RET* and *NTRK1* gene alterations may be tested using a panel approach.
- Biomarker testing results should be available within 10 working days of receiving the specimen.
- 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

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:

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.

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 T790M 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 14 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 T790M resistance mutation and this alteration is not detected on liquid biopsy, a repeat tissue biopsy is recommended, as there is a 20-30% chance of missing T790M 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.

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]

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

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.

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.

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 possible mechanism of resistance.

Recommended molecular testing when the first line therapy was a 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 all currently approved drugs.

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.

RECOMMENDATION

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

PD-L1 IHC in NSCLC

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 1.

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]

Table 1: 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 forPD-L1 positivity
Nivolumab (Bristol-Myers Squibb)	28-8 (rabbit)	Complementary	Extracellular	Link 48 Autostainer	≥1% tumour cells
Pembrolizumab (Merck)	22C3 (mouse)	Companion	Extracellular	Link 48 Autostainer	≥ 50% tumour cells
Atezolizumab (Genentech/Roche)	SP142 (rabbit)	Complementary	Cytoplasmic	BenchMark ULTRA	Tumour cells and/or tumour infiltrating immune cells
Durvalumab (AstraZeneca/ MedImmune)	SP263 (rabbit)	Complementary	Extracellular	BenchMark	≥ 25% tumour cells
Avelumab (Pfizer/Merck Serono)	73-10	Complementary	Unknown	Dako assay	≥ 1% tumour cells

Adapted from IASLC Atlas of PD-L1 IHC Testing in Lung Cancer. 2017.[20]

For the interpretation of PD-L1 IHC, it is noteworthy that there are at least 5 different bioassays (Table 1) with different cut-off values for the tumour proportion score (TPS) that dictates treatment with its respective immunomodulatory drug. TPS is normally defined as percentage of viable 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 2.

Table 2: 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 staining protocol is adjusted and proper quality control is undertaken.[32,49] There is emerging evidence that formalin post-fixation (after alcohol) may offset any negative effects of alcohol.[49,50]
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.

Table 2, continued.

Parameter	Recommendation
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

Adapted from IASLC Atlas of PD-L1 IHC Testing in Lung Cancer. 2017.[20]

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.[33,53,54] This benefit appears to be incremental, whereby greater benefit is observed with increasing PD-L1 scores.[55,56] PD-L1 testing should be considered for NSCLC patients with no druggable mutations.

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. 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]

RECOMMENDATIONS

- PD-L1 IHC is recommended in all patients whose tumours are negative for *EGFR* and *ALK* driver mutations.
- FFPE tissue can be used for testing on a validated platform.

DISCUSSION 4:
TO ENSURE THE DELIVERY OF HIGH QUALITY TESTING FOR DIFFERENT MOLECULAR TESTS

BEST PRACTICES FOR QUALITY ASSURANCE OF MOLECULAR TESTING

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 Organization for Standardisation (ISO) 15189:2012.[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 genetic 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.

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. 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 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]

RECOMMENDATION

All laboratories conducting molecular testing for NSCLC must be accredited and participate in EQA to maintain accreditation.

DISCUSSION 5:
DEVELOPMENT OF TESTING RECOMMENDATIONS IN THE ERA OF GENOMIC MEDICINE

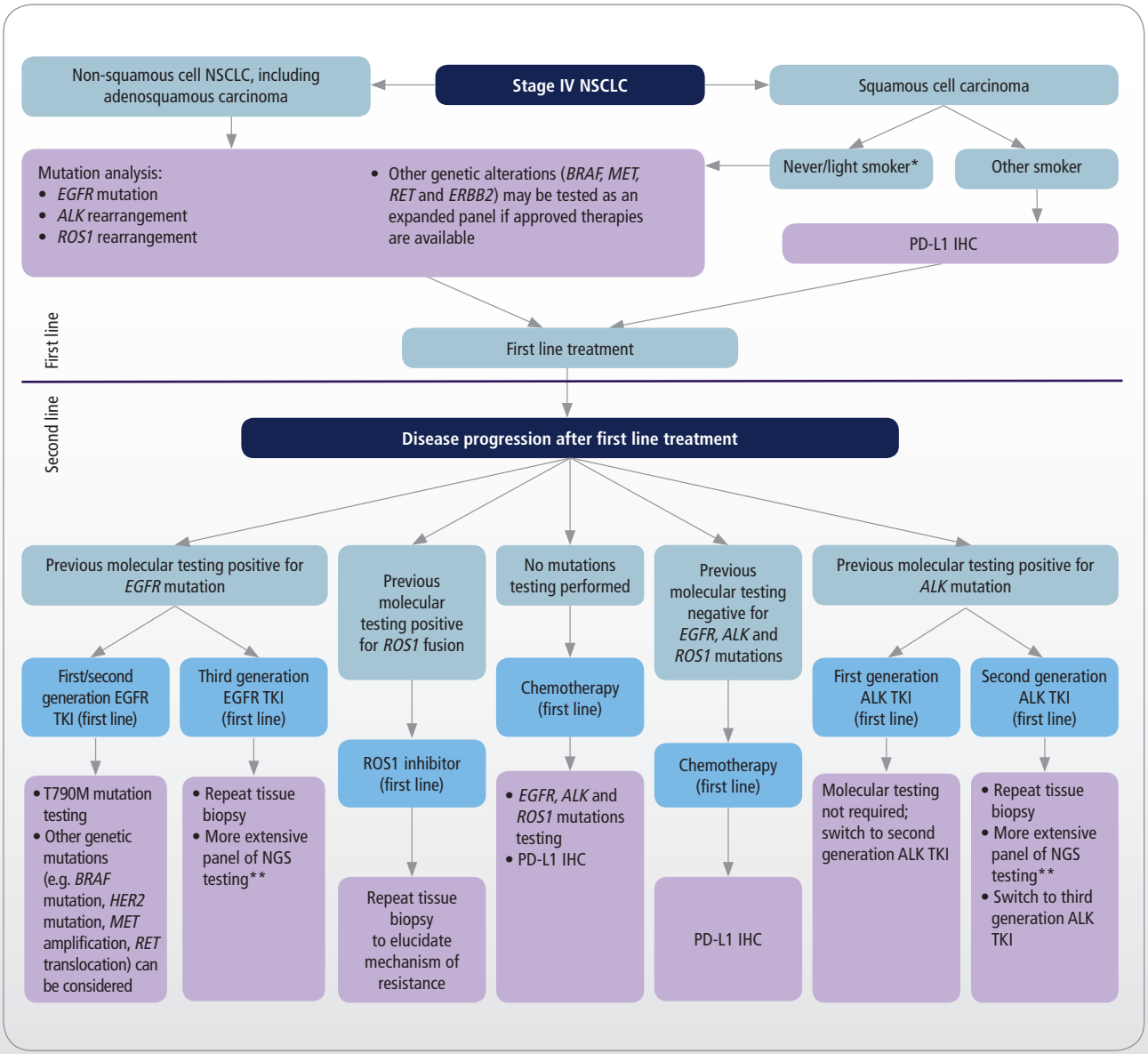
Over the past decade, there has been a rapid pace of discoveries in the area of genomic medicine. There is an urgency to provide guidance in molecular testing for NSCLC, at times amidst the limitations of scientific literature. Under these circumstances, testing recommendations will need to be developed 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]

CONCLUSION:

What is the algorithm for molecular testing in metastatic NSCLC patients?

The algorithms for molecular testing in metastatic NSCLC patients is shown in Figure 3.

FIGURE 3. Algorithm for molecular testing of metastatic NSCLC in the first line setting and upon disease progression beyond first line therapy.

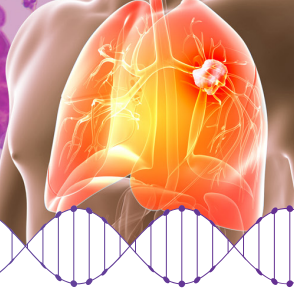


*Light smoker is defined as < 15 pack-years.[25]
** Testing is recommended only if an approved drug is available or in clinical trial.

ACKNOWLEDGEMENTS

This consensus statement was funded by the College of Pathologists, Academy of Medicine Malaysia. We would also like to acknowledge MediConnexions Consulting Pte Ltd for their editing assistance.

REFERENCES



1. Global Burden of Disease Cancer Collaboration. Global, regional, and national cancer incidence, mortality, years of life lost, years lived with disability, and disability-adjusted life-years for 32 cancer groups, 1990 to 2015: a systematic analysis for the Global Burden of Disease Study. *JAMA Oncol.* 2017;3(4):524-48.
2. National Cancer Institute, Ministry of Health Malaysia. Malaysian National Cancer Registry Report 2007-2011. Available at https://www.crc.gov.my/wp-content/uploads/documents/report/MNCRRrepor2007-2011.pdf. Accessed on 12 April 2018.
3. How SH, Ng TH, Kuan YC, Jamaluddin AR, Fauzi AR. Survival of lung cancer patients in a resource limited country. *Asia Pac J Clin Oncol.* 2015;11(3):221-27.
4. Jorge SEDC, Kobayashi SS, Costa DB. Epidermal growth factor receptor (EGFR) mutations in lung cancer: preclinical and clinical data. *Braz J Med Biol Res.* 2014;47(11):929-39.
5. Mok TS, Wu YL, Thongprasert S, *et al.* Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. *N Engl J Med.* 2009; 361: 947-57.
6. Rosell R, Carcereny E, Gervais R, *et al.* Erlotinib versus standard chemotherapy as first-line treatment for European patients with advanced EGFR mutation-positive non-small-cell lung cancer (EURTAC): a multicentre, open-label, randomised phase 3 trial. *Lancet Oncol.* 2012; 13: 239-46.
7. Sequist LV, Yang JC, Yamamoto N, *et al.* Phase III study of afatinib or cisplatin plus pemetrexed in patients with metastatic lung adenocarcinoma with EGFR mutations. *J Clin Oncol.* 2013; 31:3327-34.
8. Lindeman NI, Cagle PT, Aisner DL, *et al.* Updated molecular testing guideline for the selection of lung cancer patients for treatment with targeted tyrosine kinase inhibitors: guideline from the College of American Pathologists, the International Association for the Study of Lung Cancer, and the Association for Molecular Pathology. *J Thorac Oncol.* 2018;13(3):323-58.
9. Tan DS, Yom SS, Tsao MS, *et al.* The international association for the study of lung cancer consensus statement on optimizing management of EGFR mutation-positive non-small cell lung cancer: status in 2016. *J Thorac Oncol.* 2016;11(7):946-63.
10. Ettinger DS, Wood DE, Aisner DL, *et al.* Non–small cell lung cancer, version 5.2017. NCCN clinical practice guidelines in oncology. *J Natl Compr Canc Netw.* 2017;15(4):504-35.
11. Tsao MS, Hirsch FR, Yatabe Y. IASLC atlas of ALK and ROS1 testing in lung cancer. North Fort Myers, FL: Editorial Rx Press; 2016.
12. Mok TS, Carbone DP, Hirsch FR. IASLC atlas of EGFR testing in lung cancer. North Fort Myers, FL: Editorial Rx Press; 2017.
13. Li T, Kung HJ, Mack PC, Gandara DR. Genotyping and genomic profiling of non-small-cell lung cancer: implications for current and future therapies. *J Clin Oncol.* 2013; 31:1039-49.
14. Lindeman NI, Cagle PT, Beasley MB, *et al.* Molecular testing guideline for selection of lung cancer patients for EGFR and ALK tyrosine kinase inhibitors: guideline from the College of American Pathologists, International Association for the Study of Lung Cancer, and Association for Molecular Pathology. *J Mol Diagn.* 2013;15(4):415-53.
15. Liam CK, Wahid MI, Rajadurai P, Cheah YK, Ng TS. Epidermal growth factor receptor mutations in lung adenocarcinoma in Malaysian patients. *J Thorac Oncol*. 2013;8(6):766-72.
16. Shim HS, Choi YL, Kim L, *et al.* Molecular testing of lung cancers. *J Pathol Transl Med.* 2017;51(3):242-54.
17. Pauwels P, Remmelink M, Hoton D, *et al.* Pathological diagnosis and molecular testing in non-small cell lung cancer: Belgian guidelines. *Belg J Med Oncol.* 2016;10(4):123-31.

18. Kim H, Shim HS, Kim L, *et al.* Guideline recommendations for testing of ALK gene rearrangement in lung cancer: a proposal of the Korean Cardiopulmonary Pathology Study Group. *Korean J Pathol.* 2014;48(1):1-9.

19. Kim L, Tsao MS. Tumour tissue sampling for lung cancer management in the era of personalised therapy: what is good enough for molecular testing? *Eur Respir J.* 2014;44(4):1011-22.

20. Tsao MS, Kerr KM, Dacic S, Yatabe Y, Hirsch FR. IASLC Atlas of PD-L1 immunohistochemistry testing in lung cancer. North Fort Myers, FL: *Editorial Rx Press*; 2017.

21. Wang H, Agulnik J, Kasymjanova G, *et al.* Cytology cell blocks are suitable for immunohistochemical testing for PD-L1 in lung cancer. *Ann Oncol.* 2018;29(6):1417-22.

22. Zhou F, Moreira AL. Lung carcinoma predictive biomarker testing by immunoperoxidase stains in cytology and small biopsy specimens: advantages and limitations. *Arch Pathol Lab Me.* 2016;140(12):1331-7.

23. Paik JH, Choe G, Kim H, *et al.* Screening of anaplastic lymphoma kinase rearrangement by immunohistochemistry in non-small cell lung cancer: correlation with fluorescence in situ hybridization. *J Thorac Oncol.* 2011;6(3):466-72.

24. Cha YJ, Lee JS, Kim HR, *et al.* Screening of ROS1 rearrangements in lung adenocarcinoma by immunohistochemistry and comparison with ALK rearrangements. *PLoS One* 2014;9(7):e103333.

25. Planchard D, Popat S, Kerr K, *et al.* Metastatic non-small cell lung cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol.* 2018;29(Supplement_4):iv192-237.

26. Merker JD, Oxnard GR, Compton C, *et al.* Circulating tumor DNA analysis in patients with cancer: American Society of Clinical Oncology and College of American Pathologists joint review. *J Clin Oncol.* 2018;36(16):1631-41.

27. Yu HA, Arcila ME, Rekhtman N, *et al.* Analysis of tumor specimens at the time of acquired resistance to EGFR TKI therapy in 155 patients with EGFR mutant lung cancers. *Clin Cancer Res.* 2013;19(8):2240-7.

28. Wu YL, Cheng Y, Zhou X, *et al.* Dacomitinib versus gefitinib as first-line treatment for patients with EGFR-mutation-positive non-small-cell lung cancer (ARCHER 1050): a randomised, open-label, phase 3 trial. *Lancet Oncol.* 2017;18(11):1454-66.

29. Yamaoka T, Kusumoto S, Ando K, Ohba M, Ohmori T. Receptor tyrosine kinase-targeted cancer therapy. *Int J Mol Sci.* 2018;19(11):3491.

30. Cabanero M, Sangha R, Sheffield BS, *et al.* Management of EGFR-mutated non-small-cell lung cancer: practical implications from a clinical and pathology perspective. *Curr Oncol.* 2017;24(2):111-9.

31. John T, Bowden JJ, Clarke S, *et al.* Australian recommendations for EGFR T790M testing in advanced non–small cell lung cancer. *Asia Pac J Clin Oncol.* 2017;13(4):296-303.

32. Sundaresan TK, Sequist LV, Heymach JV, *et al.* Detection of T790M, the acquired resistance EGFR mutation, by tumor biopsy versus noninvasive blood-based analyses. *Clin Cancer Res.* 2016;22(5):1103-10.

33. Yu S, Liu D, Shen B, Shi M, Feng J. Immunotherapy strategy of EGFR mutant lung cancer. *Am J Cancer Res.* 2018;8(10):2106-15.

34. Borghaei H, Paz-Ares L, Horn L, *et al.* Nivolumab versus docetaxel in advanced nonsquamous non-small-cell lung cancer. *N Engl J Med.* 2015;373(17):1627-39.

35. Herbst RS, Baas P, Kim DW, *et al.* Pembrolizumab versus docetaxel for previously treated, PD-L1-positive, advanced non-small-cell lung cancer (KEYNOTE-010): a randomised controlled trial. *Lancet.* 2016;387(10027):1540-50.

36. Chen YM. Immune checkpoint inhibitors for nonsmall cell lung cancer treatment. *J Chin Med Assoc.* 2017;80(1):7-14.

37. Herbst RS, Soria JC, Kowanetz M, *et al.* Predictive correlates of response to the anti-PD-L1 antibody MPDL3280A in cancer patients. *Nature* 2014;515(7528):563-7.

38. Kerr KM, Tsao MS, Nicholson AG, *et al.* Programmed death-ligand 1 immunohistochemistry in lung cancer: in what state is this art? *J Thorac Oncol.* 2015;10(7):985-9.

39. Kerr KM, Hirsch FR. Programmed death ligand-1 immunohistochemistry: friend or foe? *Arch Pathol Lab Med.* 2016;140(4):326-31.

40. Cree IA, Booton R, Cane P, *et al.* PD L1 testing for lung cancer in the UK: recognizing the challenges for implementation. *Histopathology* 2016;69(2):177-86.

41. Hirsch FR, McElhinny A, Stanforth D, ***et al.*** PD-L1 immunohistochemistry assays for lung cancer: results from phase 1 of the Blueprint PD-L1 IHC assay comparison project. *J Thorac Oncol.* 2017;12(2):208-22.

42. Tsao MS, Kerr KM, Kockx M, ***et al.*** PD-L1 immunohistochemistry comparability study in real-life clinical samples: results of Blueprint phase 2 project. *J Thorac Oncol.* 2018;13(9):1302-1311.

43. Kerr K, Tsao M, Yatabe Y, *et al.* Phase 2B of Blueprint PD-L1 immunohistochemistry assay comparability study. *J Thorac Oncol.* 2018;13(10):S325.

44. Ratcliffe MJ, Sharpe A, Midha A, *et al.* Agreement between programmed cell death ligand-1 diagnostic assays across multiple protein expression cutoffs in non–small cell lung cancer. *Clin Cancer Res.* 2017;23(14):3585-91.

45. Chen Y, Wang Q, Shi B, *et al.* Development of a sandwich ELISA for evaluating soluble PD-L1 (CD274) in human sera of different ages as well as supernatants of PD-L1+ cell lines. *Cytokine* 2011;56(2):231-8.

46. Okuma Y, Hosomi Y, Nakahara Y, Watanabe K, Sagawa Y, Homma S. High plasma levels of soluble programmed cell death ligand 1 are prognostic for reduced survival in advanced lung cancer. *Lung Cancer* 2017;104:1-6.

47. Dolled-Filhart M, Roach C, Toland G, *et al.* Development of a companion diagnostic for pembrolizumab in non-small cell lung cancer using immunohistochemistry for programmed death ligand-1. *Arch Pathol Lab Med.* 2016;140(11):1243-9.

48. Phillips T, Simmons P, Inzunza HD, *et al.* Development of an automated PD-L1 immunohistochemistry (IHC) assay for non-small cell lung cancer. *Appl Immunohistochem Mol Morphol.* 2015;23(8):541-9.

49. Rangachari D, VanderLaan PA, Shea M, *et al.* Correlation between classic driver oncogene mutations in EGFR, ALK, or ROS1 and 22C3–PD-L1 ≥ 50% expression in lung adenocarcinoma. *J Thorac Oncol.* 2017;12(5):878-83.

50. McLaughlin J, Han G, Schalper KA, *et al.* Quantitative assessment of the heterogeneity of PD-L1 expression in non-small-cell lung cancer. *JAMA Oncol.* 2016;2(1):46-54.

51. Liu D, Wang S, Bindeman W. Clinical applications of PD-L1 bioassays for cancer immunotherapy. *J Hematol Oncol.* 2017;10(1):110.

52. Munari E, Zamboni G, Marconi M, *et al.* PD-L1 expression heterogeneity in non-small cell lung cancer: evaluation of small biopsies reliability. *Oncotarget* 2017;8(52):90123.

53. Garon EB, Rizvi NA, Hui R, *et al.* Pembrolizumab for the treatment of non-small-cell lung cancer. *New Engl J Med.* 2015;372:2018–28.

54. Fehrenbacher L, Spira A, Ballinger M, *et al.* Atezolizumab versus docetaxel for patients with previously treated non-small-cell lung cancer (POPLAR): a multicentre, open-label, phase 2 randomised controlled trial. *Lancet.* 2016;387(10030):1837-46.

55. Herbst RS, Baas P, Kim DW, *et al.* Factors associated with better overall survival (OS) in patients with previously treated, PD-L1–expressing, advanced NSCLC: multivariate analysis of KEYNOTE-010. *J Clin Oncol.* 2017;35(15_suppl):9090.

56. Leighl NB, Hellmann MD, Hui R, *et al.* KEYNOTE-001: 3-year overall survival for patients with advanced NSCLC treated with pembrolizumab. *J Clin Oncol.* 2017;35(15_suppl):9011.

57. Ellison G, Zhu G, Moulis A, Dearden S, Speake G, McCormack R. EGFR mutation testing in lung cancer: a review of available methods and their use for analysis of tumour tissue and cytology samples. *J Clin Pathol.* 2013;66(2):79-89.

58. Ravine D, Suthers G. Quality standards and samples in genetic testing. *J Clin Pathol.* 2012;65(5):389-93.

59. Laboratory Accreditation Scheme of Malaysia. SC 2.1 – specific criteria for accreditation in the field of medical molecular testing. Available at https://www.jsm.gov.my/documents/10180/329080/SC+2.1+Specific+Criteria+for+Accreditation+in+the+Field+of+Medical+Molecular+Testing%2C%20Issue+1%2C%2014+November+2017.pdf/3fbe0e3f-8bb2-4fda-b29f-00352c4ad902. Accessed on 21 August 2018.

60. Rainen L, Arbique JC, Asthana D, *et al.* Collection, transport, preparation, and storage of specimens for molecular methods; approved guideline. Wayne, Pennsylvania, USA: Clinical and Laboratory Standards Institute (CLSI); 2005.

61. McGovern MM, Benach MO, Wallenstein S, *et al.* Quality assurance in molecular genetic testing laboratories. *JAMA.* 1999;281:835–40.

62. Mattocks CJ, Morris MA, Matthijs G, *et al* . A standardized framework for the validation and verification of clinical molecular genetic tests. *Eur J Hum Genet.* 2010;18:1276–88.

63. Ferreira-Gonzalez A, Emmadi R, Day SP, *et al.* Revisiting oversight and regulation of molecular-based laboratory-developed tests: a position statement of the Association for Molecular Pathology. *J Mol Diagn.* 2014;16:3–6.

64. Richards S, Aziz N, Bale S, *et al.* Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med.* 2015;17(5):405-24.

65. Dequeker E, Ramsden S, Grody WW, *et al.* Quality control in molecular genetic testing. *Nat Rev Genet.* 2001;2:717–23.

66. van Krieken JH, Normanno N, Blackhall F, *et al.* Guideline on the requirements of external quality assessment programs in molecular pathology. *Virchows Arch.* 2013;462:27–37.

67. van Krieken JH, Siebers AG, Normanno N, *et al.* Quality assurance for molecular pathology group: European consensus conference for external quality assessment in molecular pathology. *Ann Oncol.* 2013;24:1958–63.

68. Kerr KM, Loo PS, Nicolson MC. Pathology and personalized medicine in lung cancer. *Lung Cancer Manage.* 2013; 2: 35–46.

69. Thunnissen E, Kerr KM, Herth FJ, *et al.* The challenge of NSCLC diagnosis and predictive analysis on small samples: practical approach of a working group. *Lung Cancer* 2012;76:1–18.

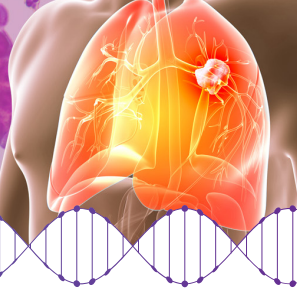
70. Cardarella S, Johnson BE. The impact of genomic changes on treatment of lung cancer. *Am J Respir Crit Care Med.* 2013;188:770–5.

71. Cagle PT, Allen TC, Dacic S, *et al.* Revolution in lung cancer: new challenges for the surgical pathologist. Arch Pathol Lab Med. 2011;135:110–6.

72. Pirker R, Herth FJF, Kerr KM, *et al.* Consensus for EGFR mutation testing in non small cell lung cancer: results from a European workshop. *J Thorac Oncol.* 2010;5:1706–13.

73. Cree IA, Deans Z, Ligtenberg MJL, *et al.* Guidance for laboratories performing molecular pathology for cancer patients. *J Clin Pathol.* 2014: jclinpath-2014.

APPENDIX



Disclosed interests and activities from August 2016 - July 2018.

	Name	Interest / Activity type	Entity
1	Professor 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	Professor Dr. Cheah Phaik Leng	Position of influence	Editorial Board, Pathology Associate Editor, Malaysian Journal of Pathology 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

	Name	Interest / Activity type	Entity
3	Professor 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	Professor 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

	Name	Interest / Activity type	Entity
8.	Dr. Nurhayati Mohd Marzuki	Speaker fee / honoraria	AstraZeneca
		Position of influence	Director of Institute of Respiratory Medicine, Hospital Kuala Lumpur
		Advisory board	AstraZeneca, Boehringer Ingelheim, Pfizer, Roche
9.	Associate Professor 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.	Associate Professor 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


COLLEGE OF PATHOLOGISTS


PERSATUAN TORASIK MALAYSIA


ONKOLOGI
PERSATUAN ONKOLOGI MALAYSIA
MALAYSIAN ONCOLOGICAL SOCIETY