

Molecular testing in non-small cell lung cancer: A consensus recommendation

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ABSTRACT

Introduction: Lung cancer remains an important cause of cancer-related mortality in Singapore, with a greater proportion of non-smokers diagnosed with non-small cell lung cancer (NSCLC) in the past 2 decades. The higher prevalence of targetable genomic alterations in lung cancer diagnosed in Singapore compared with countries in the West, as well as the expanding therapeutic landscape for NSCLC in the era of precision medicine, are both factors that underscore the importance of efficient and effective molecular profiling.

Method: This article provides consensus recommendations for biomarker testing for early-stage to advanced NSCLC. These recommendations are made from a multidisciplinary group of lung cancer experts in Singapore with the aim of improving patient care and long-term outcomes.

Results: The recommendations address the considerations in both the advanced and early-stage settings, and take into account challenges in the implementation of biomarker testing as well as the limitations of available data. Biomarker testing for both tumour tissue and liquid biopsy are discussed.

Conclusion: This consensus statement discusses the approaches and challenges of integrating molecular testing into clinical practice for patients with early- to late-stage NSCLC, and provides practical recommendations for biomarker testing for NSCLC patients in Singapore.

Ann Acad Med Singap 2023;52:364-73

Keywords: non-small cell lung cancer, biomarkers, adenocarcinoma, liquid biopsy, molecular testing

INTRODUCTION

Lung cancer is a leading cause of cancer mortality worldwide, with an estimated 2.21 million new cases and 1.80 million deaths in 2020.¹ In Singapore, lung cancer is the third most frequent cancer in men and women, and accounts for the highest and third highest number of cancer deaths among men and women, respectively.²

In the last 2 decades, therapies that target sensitising driver mutations such as epidermal growth factor receptor (*EGFR*) and anaplastic lymphoma kinase (*ALK*) tyrosine kinase inhibitors (TKIs) have transformed the treatment landscape for lung cancer, with the list of targetable driver mutations and therapies still expanding today. Immune checkpoint inhibitors (ICI) have also significantly improved survival outcomes in patients with

non-small cell lung cancer (NSCLC) through invigorating the adaptive immune system to eliminate cancer cells.

With this backdrop, these guidelines aim to provide Singapore medical practitioners who diagnose and treat NSCLC patients with evidence-based, standardised pathways for molecular testing at diagnosis and progression. It is hoped that this would (1) streamline the workflow between cancer diagnosis, molecular testing, and treatment, and (2) enable practitioners to offer patients the most effective available treatment.

METHOD

The guideline development group included practitioners from Singapore's public and private sectors who are involved in the diagnosis and management of NSCLC

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CLINICAL IMPACT

What is New

- To our knowledge, this is the first consensus recommendation developed in Singapore for biomarker testing in early to advanced NSCLC.

Clinical Implications

- Patients with advanced, non-squamous NSCLC should have upfront testing done for at least *EGFR* mutation, *ALK* rearrangement and PD-L1.
- Plasma ctDNA testing should not be used in lieu of tissue diagnosis unless sampling is inadequate or unfeasible.
- Tumour biopsy is preferred in *EGFR* TKI resistance. Plasma ctDNA testing is an acceptable alternative.
- PD-L1 testing should be considered in stage I–III NSCLC, as well as *EGFR* testing in non-squamous histologies.

patients, namely, respiratory physicians, cardiothoracic surgeons, medical and radiation oncologists, and pathologists. A list of proposed statements was developed and each statement was deliberated upon at a consensus meeting organised by the Lung Cancer Consortium Singapore and held virtually on 11 February 2022. For each consensus recommendation statement, criteria for levels of evidence and grade of recommendation were adapted from European Society for Medical Oncology (ESMO) Standard Operating Procedures for Clinical Practice Guidelines³ and collectively determined by the authors in the context of an Asian clinical landscape.

Tissue and liquid biopsy considerations

Tissue biopsy sample type and minimum material requirements

Methods of obtaining tumour material for microscopic evaluation and biomarker testing include transthoracic needle biopsy, bronchoscopy, thoracoscopy, mediastinoscopy and surgery. Minimally invasive sampling methods are favoured as they carry lower risks of complications; therefore, many tissue specimens are small samples that contain limited tumour material. Formalin-fixed, paraffin-embedded material resulting from routine laboratory processing is commonly used for diagnosis and for biomarker testing including immunohistochemistry (IHC), fluorescence in-situ

hybridisation (FISH), and molecular (including next-generation sequencing, or NGS) panels.

Prior to molecular testing, tissue specimens are evaluated for tumour cellularity (TC), defined by tumour cells as a percentage of total number of nucleated cells, with minimum TC cut-offs depending on the assay. The College of American Pathologists, International Association for the Study of Lung Cancer, and Association for Molecular Pathology (CAP/IASLC/AMP) 2018 guidelines recommend use of assays that can detect molecular alterations in specimens with as little as 20% TC.⁴ For programmed death-ligand 1 (PD-L1) IHC and FISH testing, minimum numbers of tumour cells are considered instead.^{5–7} Other technical considerations, including fixative, duration of fixation, and age of the paraffin block or sections, also come into play.^{4,8}

Plasma-based testing methodology and limitations

The role of liquid biopsy using plasma circulating tumour DNA (ctDNA) is an area of considerable interest, as it enables biomarker testing with less risk and a faster turnaround time compared to tissue biopsy. Limitations include false negative results due to low tumour cell content as a proportion of the total cellularity, absence of shedding, and the short half-life of ctDNA.⁹

Challenges and barriers to biomarker testing

Use of targeted therapies for NSCLC is contingent on the accurate identification of driver mutations through molecular testing. Engagement with key stakeholders in Singapore revealed various challenges when adopting NGS panel testing, including costs to patients, long turnaround times of 10 working days on average delaying commencement of therapy, lack of local guidelines, insufficient education of both patients and the clinical community, and lack of conclusive cost-benefit studies.¹⁰ Cost was the most cited concern, as NGS panel testing ranges approximately SGD1500–5000 (USD1130–3760). Given the cap of SGD600 (USD450) per year that can be offset by the national medical savings scheme (i.e. MediSave) for cancer diagnostics, a significant out-of-pocket cost remains for most patients with newly diagnosed NSCLC. It is hoped that this guideline will pave the way for affordable reflex testing pathways that streamline turnaround times.

Treatment-naïve, advanced NSCLC

Current and emerging biomarkers for NSCLC

NSCLC is characterised by molecularly defined subsets, several of which are therapeutically tractable. The identification of activating mutations in the tyrosine kinase domain of the *EGFR* gene with associated tumour

response to *EGFR*-directed targeted therapy^{11,12} led to a paradigm shift in NSCLC treatment. Since then, the discovery of many other oncogenic alterations has followed, several with approved targeted therapies, including ROS proto-oncogene 1 (*ROS1*), Kirsten rat sarcoma viral oncogene homolog (*KRAS*) G12C, B-Raf proto-oncogene (*BRAF*) V600E, rearranged during transfection (*RET*), hepatocyte growth factor receptor (*MET*) exon 14 (*MET*ex14) skipping, Erb-B2 receptor tyrosine kinase 2 (*ERBB2*) and neurotrophic tyrosine receptor kinase (*NTRK*).

There is consensus across international guidelines such as the ESMO¹³ and National Comprehensive Cancer Network (NCCN)¹⁴ that molecular subtyping is imperative for therapeutic decision-making and should be performed prior to treatment initiation, where possible. Prevalence of oncogenic driver, access to both testing platforms and suitable treatments are factors for consideration. Both ESMO and NCCN recommend systematic testing of *EGFR*, *ALK*, *ROS1*, *BRAF* and *NTRK* in advanced NSCLC as these have approved first-line targeted therapies. PD-L1 expression with tumour proportion score (TPS) should also be evaluated for advanced NSCLC, as the level of PD-L1 expression is an important factor that influences the choice of front-line treatment in NSCLC without driver mutations.^{15,16}

Additional molecular testing for *KRAS*, *MET*ex14 skipping mutation, *ERBB2* and *RET* are recommended by the NCCN and IASLC given recent approvals. Emerging biomarkers such as *MET* amplifications and *NRG1* rearrangements are also of increasing interest with novel therapies in development, though these are not routinely performed in clinical practice. The role of tumour mutational burden (TMB) in predicting response to immune checkpoint inhibition is also unclear, with inconsistent results in terms of survival^{17,18} and thus is not currently incorporated into routine practice.

Multiplex versus single testing strategies

Given the expanding list of therapeutically relevant biomarkers and approved targeted therapies, molecular profiling at diagnosis is paramount. While single biomarker assays such as polymerase chain reaction, Sanger sequencing, IHC and FISH have traditionally been implemented due to accessibility and cost, multiplexed NGS assays are now increasingly utilised in practice to evaluate multiple targetable alterations in small tissue biopsy specimens with excellent sensitivity and specificity.^{19,20} Regardless, the optimal approach in an Asian population with a high prevalence of *EGFR*-mutated NSCLC remains unclear from a cost-

effectiveness perspective, with multiplex and sequential testing both showing merit in different healthcare systems.²¹⁻²⁴ Adding to the complexity is the lack of established gold standard methods of detection for emerging biomarkers, the adoption of panel-based NGS assays as companion diagnostics for many recently approved targeted drugs, as well as the practical issues of accessibility to testing platform and cost of therapy.

In an Asian population like Singapore's, where there is a high prevalence of targetable alterations, upfront molecular testing for at least *EGFR* mutation, *ALK* rearrangement and PD-L1 should be done on tissue biopsy or cytology material for advanced stage non-squamous NSCLC; and results should be available prior to treatment initiation. Where there is access to relevant targeted therapies, multiplex testing including *ROS1*, *KRAS* G12C, *BRAF* V600E, *MET*ex14 skipping, *RET*, *ERBB2* and *NTRK* should also be considered where available.

Practical considerations in molecular testing

Turnaround time is an important consideration in the implementation of molecular testing as it could lead to delays in initiation of optimal therapy. While molecular testing should ideally be conducted at the same institution where histological diagnosis is made to optimise turnaround time, feasibility of on-site testing is influenced by adequate caseload and assay complexity, and thus may not be available at every centre. The Asian Expert Consensus Recommendations on Biomarker Testing in Metastatic and Nonmetastatic NSCLC recommend a turnaround time of 2 weeks or less from time of sample receipt to result reporting.²⁵

Another strategy to optimise turnaround time is reflex testing, where molecular testing is initiated by pathologists upon histological diagnosis of non-squamous NSCLC. This has been shown to optimise tissue utilisation and improve profiling rates²⁶ over on-demand testing, which is ordered by the treating physician after review of histology results. Importantly, reflex testing is contingent on pathologist access to relevant clinical information as overall costs could increase if molecular profiling is ordered inappropriately, underscoring the need for good communication in a multidisciplinary team and adequate patient counselling at point of biopsy when feasible.

Role of plasma-based tests in treatment-naïve, advanced NSCLC

Tumour tissue genotyping and plasma ctDNA analysis are both valuable tools in the diagnostic work up of advanced NSCLC. There has been increasing uptake of the latter given the growing list targetable molecular

alterations and the inherent issues of tissue biopsies including yield and attrition.^{27,28} Notably, studies have demonstrated ctDNA testing to generally have very high specificity, but significantly compromised sensitivity, with approximately 30% false negative rate depending on platform used.¹⁴ The decision to adopt either testing strategy, be it concurrently or sequentially, should be individualised to the clinical context, methodologies available, and expected results.²⁹ In particular, the use of ctDNA testing may be considered in specific clinical scenarios, most notably if the patient is medically unsuitable for invasive tissue sampling, or if there is insufficient material for molecular testing following the pathologic confirmation of a NSCLC diagnosis.

Recommendations for molecular testing in treatment-naïve, advanced NSCLC (Fig. 1)

- All patients with advanced, non-squamous NSCLC should have upfront testing done for at least *EGFR* mutation, *ALK* rearrangement and PD-L1 done on tissue biopsy or cytology material. This should also be performed for non-smokers with advanced squamous NSCLC [I, A].
- Upfront or sequential multiplex testing including *ROS1*, *KRAS* G12C, *BRAF* V600E, *MET*_{ex14} skipping, *RET*, *ERBB2* and *NTRK* should also be considered where available, especially if there is access to relevant targeted therapies [II, A].

- Molecular profiling results should be available prior to treatment initiation where possible [V, A].
- Sequential single gene testing strategies are not preferred [II, A].
- Reflex molecular testing is preferred on histological diagnosis of advanced non-squamous NSCLC [V, C].
- Plasma ctDNA testing should not be used in lieu of a histological tissue diagnosis of NSCLC [V, C]. Plasma ctDNA can be considered for the genotyping of newly diagnosed advanced NSCLC where tissue sampling is not feasible or insufficient for molecular analysis [V, C].
- Tumour mutational burden (TMB) does not have an established role in routine clinical practice [II, C].

Advanced *EGFR*-mutated NSCLC with disease progression on tyrosine kinase inhibitor

EGFR tyrosine kinase inhibitors (TKIs) are the standard first line treatment for metastatic NSCLC harbouring sensitising *EGFR* mutations—most commonly exon 19 deletion and exon 21 L858R mutations. However, patients invariably develop resistance to *EGFR* TKIs, through secondary mutations in *EGFR*, phenotypic transformation, or the activation of alternative pathways.³⁰

After the first- or second-generation TKI treatments, 50% of patients develop *EGFR* exon 20 T790M mutations, while other resistance mechanisms include

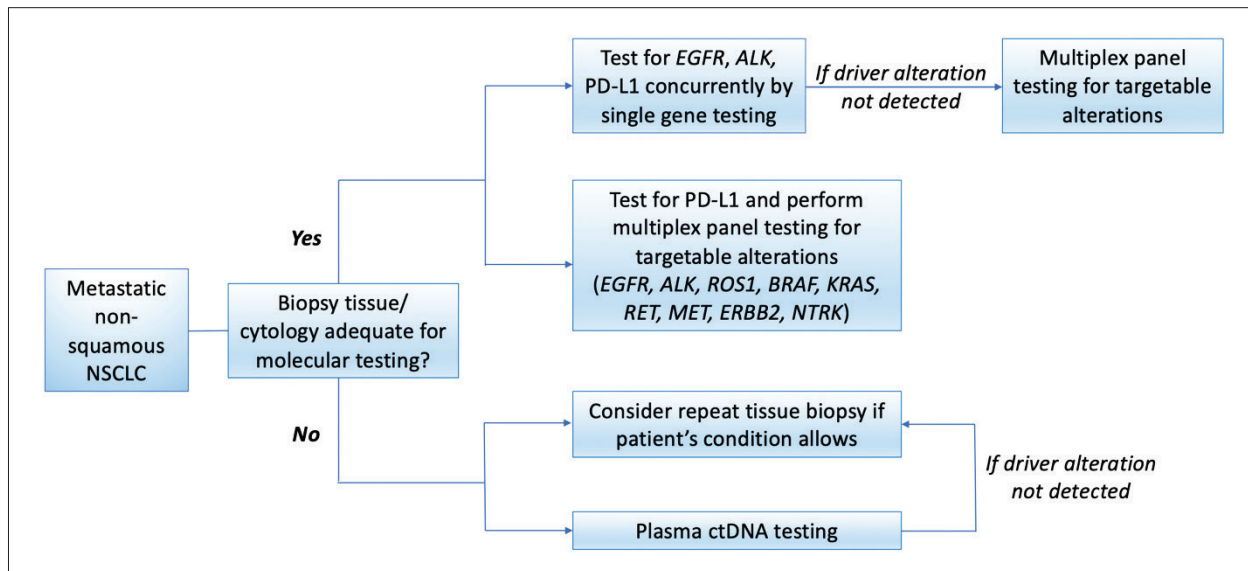


Fig. 1. Biomarker testing recommendations for metastatic non-squamous non-small cell lung cancer (NSCLC).

ALK: anaplastic lymphoma kinase; BRAF: B-Raf proto-oncogene; ctDNA: circulating tumour DNA; EGFR: epidermal growth factor receptor; ERBB2: Erb-B2 receptor tyrosine kinase 2; KRAS: Kirsten rat sarcoma viral oncogene homolog; MET: hepatocyte growth factor receptor; NTRK: neurotrophic tyrosine receptor kinase; PD-L1: programmed death-ligand 1; RET: rearranged during transfection; ROS1: ROS proto-oncogene 1

HER2 amplification (10–15%), small cell transformation (10%), *MET* amplification (5%), *KRAS* or *BRAF* mutations (approximately 1% each).³¹ The mechanism of resistance has treatment implications, particularly in that patients harbouring T790M mutations benefit from treatment from third-generation *EGFR* TKI osimertinib, based on the results of the AURA3 study.³² Recent years have seen the approval of multiple targeted agents against off-target resistance mutations, with many others in early phases of development.

On progression on first- or second-generation *EGFR* TKI, T790M testing should be done as a minimum, in view of the high proportion of T790M resistance mutations and the availability of effective agents. With regard to modality of T790M testing, tissue biopsy remains the gold standard, allowing concurrent evaluation for histologic transformation to small cell. However, this is not always possible due to risks or feasibility of obtaining tissue. ctDNA testing for plasma *EGFR* analysis of T790M is a reasonable alternative, with a positive predictive value of 0.85 and a negative predictive value of 0.60 compared to tissue testing.³³ Studies have shown that patients who are negative for tissue T790M but positive for plasma ctDNA T790M were confirmed to be T790M positive by NGS and had clinical benefit from osimertinib.³⁴ Based on this, in patients who are positive for plasma T790M, it would not be necessary to pursue a tissue biopsy as they may be treated with osimertinib; in patients who are negative for plasma T790M, we would recommend further tissue biopsy to confirm T790M status and rule out false negatives.

NGS can also be considered, either sequentially following a negative T790M result, or as an alternative to single gene testing. Use of upfront NGS allows optimal tissue utilisation, and the opportunity to pick up alternative driver mutations, but is limited by cost and turnaround time.

The profile of resistance mechanisms to third-generation *EGFR* TKI osimertinib differs from first- and second-generation *EGFR* TKIs, and also depends on whether osimertinib is given in the first or second line. Notably, T790M mutations are absent in cases of treatment failure on osimertinib.³⁵ Again, up to 15% demonstrate transformation, and there are various targetable mutations that occur. While these targetable mutations may have available therapies, they tend to happen at a relatively low frequency of <5%. Tissue biopsy for histopathology and NGS can be considered to identify subsequent treatment options. In addition to histological transformation, *MET* amplification and alternate drivers (e.g. *RET* re-arrangement), have

been described. However, in the majority of cases, mechanisms of resistance remain unknown and this remains an unmet medical need subject to ongoing research efforts.

Recommendations for molecular testing in advanced, *EGFR* TKI-resistant NSCLC

- Tumour biopsy is preferred for detection of *EGFR* T790M in the resistance setting. Plasma ctDNA testing is an acceptable alternative approach; however a negative result should be followed by tissue testing for *EGFR* T790M, as well as alternative mechanisms of resistance when feasible [I, A].
- In patients who develop disease progression on third-generation *EGFR* TKI, tissue biopsy for histopathology and multiplex testing can be considered [III, C].

Unresectable stage III NSCLC

Stage III NSCLC is an intrinsically heterogeneous disease, with distinct molecular and phenotypic sub-types. In unresectable disease, radiotherapy (RT) is the curative local therapy and treatment intensification with concurrent platinum-based doublet chemotherapy is the standard of care.³⁶ The introduction of immune checkpoint inhibitors (ICI) to concurrent chemoradiotherapy (CRT) represented a paradigm shift in the management of stage III disease. In the phase III PACIFIC trial, the addition of 1 year of consolidation durvalumab therapy resulted in a significant improvement in overall survival, which was sustained at long-term follow-up, with an estimated 5-year overall survival rate of 42.9% for durvalumab versus 33.4% for placebo.³⁷

In spite of these improved outcomes, there are concerns regarding the applicability of these results to real-world clinical practice. In a patient population commonly associated with high incidence of comorbidities, multi-modality CRT with ICI is difficult to tolerate. In a Dutch multi-centre study of 855 eligible patients, only 52% received curative-intent, multi-modality therapy and only 57% patients who underwent CRT received ICI. Main predictors for not receiving ICI included age ³70, diabetes and ³grade 3 dysphagia post-CRT.³⁸ This highlights the need for biomarkers to identify patients at risk of relapse, who are most responsive to ICI and will benefit most from treatment intensification.

PD-L1 status is a potential biomarker being considered in the unresectable stage III setting. In a post-hoc exploratory analysis of the PACIFIC study performed at the request of the European Medicines Agency

(EMA), patients with PD-L1 status $\geq 25\%$ benefited more from durvalumab (HR 0.53, 95% CI 0.33–0.85) compared to patients with PD-L1: 1 to 24% (HR 0.69, 95% CI 0.43–1.10) and $<1\%$ (HR 1.05, 95% CI 0.69–1.62).³⁹ As such, durvalumab was restricted for patients with PD-L1 $\geq 1\%$, a decision that was disputed by experts in the field, citing issues with the unplanned nature of the analysis, assessment of PD-L1 status on pre-CRT samples and only available in 63% of patients, as well as the over-performance of the control arm in the PD-L1 $<1\%$ sub-group compared to the intention-to-treat placebo arm.⁴⁰ Subsequent trial designs are now incorporating PD-L1 status as a stratification factor and this will shed more light on its utility as a biomarker.⁴¹ While awaiting these results, PD-L1 testing in patients with adequate tissue obtained at diagnosis may be considered to facilitate counselling with patients for consolidation durvalumab.

In the PACIFIC study, *EGFR*-mutated patients represented only 6% of all patients. Post-hoc exploratory analysis of this subgroup demonstrated similar survival outcomes (HR for PFS and OS 0.91 and 1.02, respectively) and wide 95% confidence intervals owing to the small numbers.⁴² *ALK* rearrangements were also not accounted for in this study. Retrospective analyses of oncogene-addicted stage III unresectable NSCLC have also similarly demonstrated limited activity of durvalumab consolidation.^{43,44} Moreover, a lack of efficacy had also been demonstrated with ICI in patients with *EGFR* mutations and *ALK* rearrangements in the advanced setting, generating uncertainty regarding its clinical benefit post-CRT.^{45,46} Given that there is insufficient data to exclude these patients from receiving adjuvant durvalumab, molecular testing for *EGFR* mutations and *ALK* rearrangement may be considered at diagnosis for facilitate clinical decision making.

Molecularly directed approaches are being explored in unresectable stage III NSCLC. The randomised phase III LAURA study is assessing the efficacy of maintenance third-generation *EGFR* TKI osimertinib post-CRT and is expected to read out in 2023.⁴⁷ Given the superior disease-free survival seen with osimertinib in early-stage completely resected *EGFR* mutated disease,⁴⁸ LAURA with a similar design and primary endpoint is expected to have a positive outcome as well. This could potentially lend further support to testing for sensitising *EGFR* mutations. Furthermore, given the high relapse rates following curative treatment for stage III disease, challenges with performing molecular testing on archival tissue, high prevalence of targetable genetic alterations in our local context as well as cost effectiveness of performing panel-based testing, there is merit in

considering reflex testing for PD-L1 expression, *EGFR* mutation and *ALK* rearrangements for non-squamous histologies as well as for other targetable genetic alterations with approved therapies in the advanced setting.^{21,49}

Recommendations for molecular testing in unresectable stage III NSCLC (Fig. 2)

- Molecular testing for *EGFR* mutation may be considered at diagnosis on tissue specimen for unresectable stage III non-squamous NSCLC [II, C].
- PD-L1 testing on tissue specimen at diagnosis for unresectable stage III NSCLC may be considered [II, C].
- Multiplex testing to include all targetable genetic alterations with approved therapies (such as *ALK*, *ROS1*, *KRAS* G12C, *BRAF* V600E, *MET* exon 14 skipping, *RET*, *ERBB2*, *NTRK*) in advanced NSCLC is beneficial and may be considered [V, C].
- Reflex testing at diagnosis for PD-L1 expression and *EGFR* mutation for non-squamous NSCLC may be considered [V, C].

Resected stage I–III NSCLC

EGFR mutation testing in early-stage NSCLC

Approximately 20% of lung cancer in Singapore is diagnosed in the early stages (stages I–II),⁵⁰ where treatment comprises surgical resection and mediastinal node dissection. Stage III lung cancer accounts for approximately 14% of lung cancer in Singapore, where surgical resection may be performed in patients with single station N2 disease as part of multimodality management.⁵⁰ A significant proportion of patients with stage I–III NSCLC eventually recur despite curative intent surgery,⁵¹ and thus adjuvant strategies continue to be evaluated with the aim of improving outcomes. The following studies highlight the role of *EGFR* biomarker testing in the selection of suitable patients for adjuvant *EGFR* TKI.

The phase III randomised ADAURA trial⁴⁸ enrolled a total of 682 patients with *EGFR* mutation-positive (Ex19del or L858R), completely resected stage IB–IIIA NSCLC to receive 3 years of osimertinib versus placebo following recovering from surgery and standard adjuvant chemotherapy, if given. HR for the primary outcome measure of disease-free survival (DFS) in patients with stage II and IIIA disease was 0.23 (95% CI 0.18, 0.30; 242/470 events; 51% maturity),⁵² and the 3-year DFS rate was 84% with osimertinib versus 34% with placebo. Additionally, fewer patients in the osimertinib arm experienced central nervous system (CNS) recurrences,

with a CNS DFS HR of 0.24 (95% CI 0.14, 0.42; 63/470 events).⁵²

The CTONG 1104 trial⁵³—a randomised phase III trial in patients with *EGFR* mutation-positive completely resected stage II–IIIA (N1–N2) NSCLC—compared adjuvant gefitinib versus standard doublet chemotherapy with DFS as the primary endpoint. Although DFS benefit was lost at 5 years (DFS at 3 and 5 years were 40.3% and 23.4% for gefitinib, versus 33.2% and 23.7% for chemotherapy), the overall survival of 75.5m in completely resected N1–N2 NSCLC was the best in this group of patients.⁵⁴

The ongoing Neo-ADAURA trial⁵⁵ is a phase III randomised study assessing neoadjuvant osimertinib as monotherapy or in combination with chemotherapy in patients with resectable stage II–IIIB *EGFR*-mutated NSCLC, versus standard of care chemotherapy alone, followed by surgery and adjuvant treatment as per investigator's choice, including osimertinib for up to 3 years. Patients are selected for *EGFR* mutation status and it is expected that this trial will further support *EGFR* biomarker testing in early non-squamous NSCLC.

PDL1 testing in early-stage NSCLC

The phase III Impower010 trial⁵⁶ assessed the role of adjuvant atezolizumab versus best supportive care (BSC) in 882 completely resected stage IB–IIIA NSCLC patients after surgery and cisplatin-based chemotherapy. A significant improvement in DFS was demonstrated for the atezolizumab arm (median DFS 42 versus 35 months; HR 0.79, 95% CI 0.64–0.96). Notably, the greatest magnitude of DFS benefit was observed in stage II–IIIA patients with PD-L1-expressing tumour cells $\geq 50\%$ (median DFS not evaluable versus 35.7 months; HR 0.43, 95% CI 0.27–0.68), leading to local regulatory approval for this subgroup.

The KEYNOTE 091 study⁵⁷ randomised 1177 participants with stage IB–IIIA NSCLC after complete surgical resection with or without adjuvant chemotherapy to receive either pembrolizumab or placebo for 1 year. An improvement in the primary endpoint DFS with pembrolizumab in all-comers regardless of PD-L1 expression was demonstrated (median 53.6 vs 42.0 mo; HR 0.76; 95% CI 0.63–0.91; $P=0.0014$), but there was a non-significant trend towards improvement in those with PD-L1 $\geq 50\%$. Overall survival results are immature and regulatory approvals are still pending.

In the neoadjuvant setting, the CheckMate 816 trial⁵⁸ randomised patients with stage IB–IIIA resectable NSCLC to received platinum-based chemotherapy with or without nivolumab, followed by resection. The

median event-free survival was 31.6 months (95% CI, 30.2 to not reached) with nivolumab plus chemotherapy and 20.8 months (95% CI, 14.0–26.7) with chemotherapy alone (HR for disease progression, disease recurrence, or death, 0.63; 97.38% CI, 0.43–0.91; $P=0.005$). This benefit was demonstrated across disease stages, histologies, tumour mutational burden and PD-L1 expression levels, but notably a greater event-free survival benefit was observed in patients with positive PD-L1 expression.

Adjuvant and neoadjuvant immunotherapy strategies continue to be investigated in the setting of resected NSCLC, and these studies highlight the utility and limitations of PD-L1 as a biomarker in the selection of treatment approaches. Nonetheless, given recent regulatory approvals, PD-L1 expression testing should be performed to facilitate clinical decision making in stage IB–IIIA NSCLC.

Testing for other biomarkers

Other biomarker such as *ALK*, *ROS1*, *KRAS* G12C, *BRAF* V600E, *MET*ex14 skipping, *RET*, *ERBB2* and *NRTK* are targetable mutations that are often tested for in advanced non-squamous NSCLC. Where available and feasible, extended testing for biomarkers, e.g. *ALK*, should be considered as emerging adjuvant or neoadjuvant studies exclude these subpopulations of oncogene-driven cancers. The ongoing ALINA trial⁵⁹ compares alectinib versus chemotherapy as adjuvant treatment for patients with resected stage IB–IIIA *ALK*+ NSCLC. There is no data yet available from ALINA, or any studies to guide the use of adjuvant targeted therapies for the other mutations in the early-stage setting.

Recommendations for molecular testing in resected stage I–III NSCLC (Fig. 2)

PD-L1 expression testing on tissue specimen should be performed for stage IB–IIIA NSCLC [I, A], as well as at least *EGFR* mutation testing for non-squamous histologies [I, A].

CONCLUSION

Comprehensive molecular testing is of increasing importance given the myriad of approved targeted therapies, as well as the numerous ongoing clinical trials that evaluate new therapies for specific genomic alterations in NSCLC. Importantly, biomarker testing strategies must evolve in parallel with emerging data both in the advanced and early-stage settings. This consensus aims to provide a framework for molecular testing in early and advanced NSCLC, tailored to the

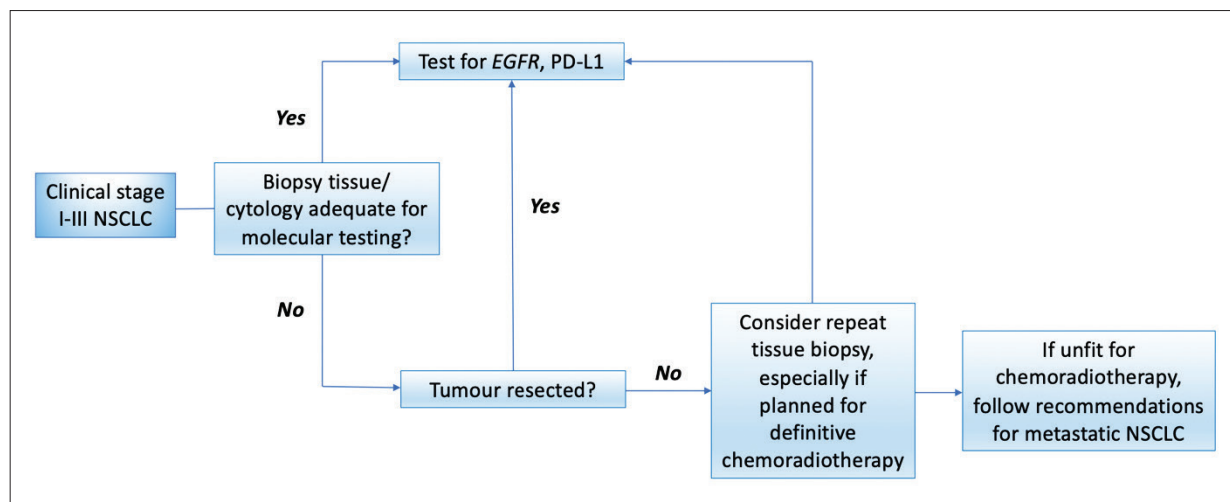


Fig. 2. Biomarker testing recommendations for clinical stage I–III non-small cell lung cancer (NSCLC). EGFR: epidermal growth factor receptor; PD-L1, programmed death-ligand 1

Singapore context where the presence of genomic alterations in NSCLC is high, with the aim of timely diagnosis and better patient outcomes.

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