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Molecular testing in stage I–III non-small cell lung cancer: Approaches and challenges

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ABSTRACT

Precision medicine in non-small cell lung cancer (NSCLC) is a rapidly evolving area, with the development of targeted therapies for advanced disease and concomitant molecular testing to inform clinical decision-making. In contrast, routine molecular testing in stage I–III disease has not been required, where standard of care comprises surgery with or without adjuvant or neoadjuvant chemotherapy, or concurrent chemoradiotherapy for unresectable stage III disease, without the integration of targeted therapy. However, the phase 3 ADAURA trial has recently shown that the epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor (TKI), osimertinib, reduces the risk of disease recurrence by 80% versus placebo in the adjuvant setting for patients with stage IB–IIIA *EGFR* mutation-positive NSCLC following complete tumor resection with or without adjuvant chemotherapy, according to physician and patient choice. Treatment with adjuvant osimertinib requires selection of patients based on the presence of an EGFR-TKI sensitizing mutation. Other targeted agents are currently being evaluated in the adjuvant and neoadjuvant settings. Approval of at least some of these other agents is highly likely in the coming years, bringing with it in parallel, a requirement for comprehensive molecular testing for stage I–III disease. In this review, we consider the implications of integrating molecular testing into practice when managing patients with stage I–III non-squamous NSCLC. We discuss best practices, approaches and challenges from pathology, surgical and oncology perspectives.

Abbreviations: ALK, anaplastic lymphoma kinase; AMP, Association for Molecular Pathology; CAP, College of American Pathologists; CI, confidence interval; CRT, chemoradiotherapy; DFS, disease-free survival; EGFR, epidermal growth factor receptor; *EGFR*m, *EGFR* mutation-positive; EMA, European Medicines Agency; Ex19del, exon 19 deletion; FDA, Federal Drug Administration; FFPE, formalin-fixed, paraffin-embedded; HR, hazard ratio; IASLC, International Association for the Study of Lung Cancer; MDT, multidisciplinary team; NCCN®, National Comprehensive Cancer Network®; NGS, next generation sequencing; NSCLC, non-small cell lung cancer; ORR, objective response rate; OS, overall survival; PD-L1, programmed cell death ligand-1; QALY, quality-adjusted life year; RT, radiotherapy; TKI, tyrosine kinase inhibitor.

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1. Introduction

Treatment of advanced non-small cell lung cancer (NSCLC) has been transformed in recent years with the advent of kinase inhibitors targeting genetic alterations in oncogenic drivers, including epidermal growth factor receptor (*EGFR*), anaplastic lymphoma kinase (*ALK*), *ROS1*, *BRAF*, and others. Targeted treatments have made biomarker testing an essential requirement to ensure that patients with actionable genetic alterations receive personalized treatment. The most recent guidelines recommend that patients with advanced NSCLC of the appropriate histological subtypes undergo molecular testing for specific genetic alterations in *EGFR*, *ALK, ROS1*, *BRAF*, *NTRK1/2/3*, *MET* exon 14 skipping, *RET* with further testing to be considered for emerging biomarkers such as *HER2* [1–3]. The overwhelming majority of NSCLCs with genetic alterations in oncogenic drivers are adenocarcinomas [4]. Therefore, guidelines recommend molecular testing for those patients with advanced NSCLC who have an adenocarcinoma component to their tumors (i.e. non-squamous NSCLC) [5–6]. In patients without adenocarcinoma histology, clinical features such as younger age and no history of tobacco exposure may indicate a higher likelihood of alterations in oncogenic drivers; molecular testing in these patients would therefore be appropriate [5]. Despite the strong rationale for molecular testing in the advanced NSCLC population, surveys of real-world practice suggest that implementation rates are variable [7–13]. In a recent large global survey by the International Association for the Study of Lung Cancer (IASLC), 89% of respondents requested molecular testing for lung cancer with adenocarcinoma histology, though the majority believed that < 50% of patients with lung cancer (type unspecified) in their country received molecular testing [11]. Testing rates for EGFR-tyrosine kinase inhibitor (EGFR-TKI) sensitizing mutations, the first actionable mutation to be established for NSCLC, have increased over time, but typically remain below 80% [9–10,12].

The situation is somewhat different in stage I–III NSCLC, where the standard of care is surgery with or without adjuvant or neoadjuvant chemotherapy (adjuvant preferred over neoadjuvant), or concurrent chemoradiotherapy (CRT) for unresectable stage III disease. Reflecting this practice, the need for molecular testing in stage I–III disease is less widely recognized by current guidelines, although those most recently updated including National Comprehensive Cancer Network® (NCCN®), Canadian, and Chinese/Asian guidelines recommend testing for at least *EGFR* mutations (Table 1).

Addictive oncogenic driver alterations, such as *EGFR* mutations, are truncal, and are therefore present throughout a tumor's life cycle, including in the earliest stages [14–15]. A meta-analysis of 115,815 patients with NSCLC found that the prevalence of *EGFR* mutation-positive (*EGFR*m) disease in stage I–III NSCLC was comparable to that in stage IV [15]. These findings support the rationale for exploring the role of EGFR-TKIs in the management of stage I–III NSCLC, and a number of studies have evaluated these agents in adjuvant and neoadjuvant settings (Table 2). Based on the results of the ADAURA study [16], osimertinib has been approved by the FDA for use as adjuvant treatment in patients with resected Ex19del or L858R *EGFR*m NSCLC [17] and by the EMA for use as adjuvant treatment in patients with completely resected stage IB–IIIA Ex19del or L858R *EGFR*m NSCLC [18]. Multiple adjuvant and neoadjuvant clinical trials are ongoing with other targeted agents (Table 3). It is likely that some of these agents will be approved for use in stage I–IIIA disease, with the parallel requirement for molecular testing.

In this review we consider the implications of integrating molecular testing, in particular *EGFR* testing, into practice when managing patients with stage I–III NSCLC with consideration for adjuvant molecular targeted therapies. Such testing is currently not routine, and we discuss proposed best practices, approaches and challenges from pathology,

Table 1
Current guideline recommendations for molecular testing and treatment with targeted agents in stage I–IIIA NSCLC.

Guideline [reference]	Disease stage	Molecular testing	Adjuvant treatment with targeted agents
National Comprehensive Cancer Network® (NCCN®), 2021 [1]	IB–IIIA	Test for *EGFR* mutation on diagnostic biopsy or surgical resection sample	Osimertinib for patients with completely resected stage IIB–IIIA or high-risk stage IB–IIA *EGFR*m NSCLC previously treated with adjuvant CT or unable to receive platinum-based CT
CAP/IASLC/AMP, 2018 [5]	I–IIIA	Insufficient data for evidence-based recommendation. Each institution to set own policy, weighing up benefits of testing all patients against costs of doing so.	Not included
ASCO, 2017 [74]	I–IIIA	Not included	Insufficient data to justify routine use of EGFR-TKIs in patients with *EGFR*m tumors
Canadian consensus recommendations, 2020 [67]	All/any	Comprehensive reflex biomarker testing, including *EGFR*, recommended for all patients diagnosed with non-squamous NSCLC regardless of disease stage; to be initiated by pathologist at time of initial diagnosis	Not included
ESMO, 2017 [4]	IB–III	Not included	Currently no role for targeted agents in adjuvant setting outside clinical trials
Asian Thoracic Oncology Research Group, 2020 [75]	III	Molecular testing at least for *EGFR* sensitizing mutations encouraged to facilitate discussion of optimal management of stage III disease with patients	The role of routine adjuvant EGFR-TKI under debate
Chinese guidelines for diagnosis and treatment of primary lung cancer, 2019 [76]	II–IIIA	*EGFR* mutation testing recommended for patients with stage II–IIIA (N1/N2 positive) non-squamous NSCLC	For *EGFR*m stage III-N2 NSCLC: adjuvant EGFR-TKI treatment +/- postoperative RT
Indian consensus guidelines, 2019 [77]	Early/any	All patients with adenocarcinoma. An individualized approach is recommended. Biomarker testing (genetic alterations in *EGFR*, *ALK*, *ROS1*, also PD-L1 expression) can be done at early stages of disease, where surgical intervention is preferred and adequate tissue biopsy is obtained.	Not included
Society for Translational Medicine consensus, 2019 [78]	IB–IIIA	Routine detection of *EGFR* mutations in surgically resected non-squamous NSCLC	An option for *EGFR*m NSCLC: (1) Stage II–IIIA, especially if at high risk of recurrence and poor expected tolerance to CT (2) High-risk stage IB

AMP, Association for Molecular Pathology; ASCO, American Society of Clinical Oncology; CAP, College of American Pathologists; CT, chemotherapy; EGFR, epidermal growth factor receptor; *EGFR*m, epidermal growth factor receptor mutation positive; ESMO, European Society for Medical Oncology; IASLC, International Association for the Study of Lung Cancer; NCCN, National Comprehensive Cancer Network; NSCLC, non-small cell lung cancer; PD-L1, programmed cell death ligand-1; RT, radiotherapy; TKI, tyrosine kinase inhibitor.

surgical and oncology perspectives. This narrative review was based on virtual meetings and online discussions between the authors to drive its structure and content. A search of the literature was conducted for adjuvant and neoadjuvant treatments for patients with stage I–III NSCLC to ensure comprehensive and relevant coverage of this disease setting. Articles were reviewed and selected based on their relevance to the scope of the review.

2. Rationale for testing in stage I–III disease

Although patients with stage I–III NSCLC are potentially curable [4], many patients with resected disease still relapse following surgical resection [19], and adjuvant treatments have considerable scope to reduce recurrence rates and thereby improve survival. Despite the survival advantage afforded by adjuvant chemotherapy in patients with resected NSCLC, the gains are modest, amounting to a 5-year absolute benefit of approximately 4–5%, and coming at a price of sometimes substantial toxicity [20–21]. New treatment strategies are needed, and targeting oncogenic drivers is one option.

As in the setting of advanced NSCLC, the earliest phase 3 trials evaluating adjuvant EGFR-TKIs in unselected patients failed to show any benefit in disease-free survival (DFS) or overall survival (OS) [22,23]. Of note, the RADIANT trial of erlotinib had a trend for improved DFS versus placebo in the *EGFR*m subgroup [23], and subsequent trials in *EGFR*m resected NSCLC have shown that adjuvant EGFR-TKI treatment improved DFS versus placebo or cisplatin-based chemotherapy (Table 2) [16,24–26].

Whether EGFR-TKIs can confer an OS benefit in an appropriately selected population is currently unclear; the phase 3 ADJUVANT/CTONG1104 study in *EGFR*m stage II–IIIA disease showed significant improvement in DFS with gefitinib versus chemotherapy [26], but not in OS [24]. The phase 3 ADAURA trial in *EGFR*m completely resected stage IB–IIIA disease, with or without standard adjuvant chemotherapy, was unblinded early due to an efficacy benefit; DFS significantly favored osimertinib versus placebo (hazard ratio [HR] 0.20; 99.12% confidence interval [CI], 0.14–0.30; $P < 0.001$) [16]. It remains to be seen whether this significant early DFS benefit with osimertinib translates into an OS benefit.

Evaluation of EGFR-TKIs in the neoadjuvant setting has lagged behind the adjuvant setting, and mostly relatively small, single arm studies have been published (Table 2). In a recent systematic review and pooled analysis of neoadjuvant erlotinib or gefitinib in 124 patients, the pooled objective response rate (ORR) was 59% and 80% underwent surgical resection [27]. While neoadjuvant EGFR-TKI treatment appeared feasible, outcomes were not remarkable but may be improved with more potent third-generation EGFR-TKIs such as osimertinib; consequently, results of the ongoing phase 3 NeoADAURA trial (NCT04351555) will be of interest (Table 3).

For unresectable stage III NSCLC, consolidation immunotherapy with the PD-L1 inhibitor, durvalumab, is recommended after definitive concurrent CRT based on the results of the PACIFIC trial [1,28–29]. However, only a minority of patients with *EGFR*m disease were included in PACIFIC [29]. Meanwhile, preliminary evidence suggests that the addition of EGFR-TKIs to either CRT or radiotherapy (RT) may be beneficial in patients with *EGFR*m unresectable stage III disease [30,31], while unselected patients showed no benefit [32]. Outcomes from the ongoing phase 3 LAURA trial (NCT03521154) will confirm whether there is a benefit to adding the EGFR-TKI osimertinib as maintenance therapy following CRT in this population.

Taken together, currently available data suggest that it is essential to test for *EGFR* mutations across stage I–III disease to ensure patients receive the most appropriate treatment to optimize clinical outcomes. Patients in unselected populations do not appear to respond to EGFR-TKIs [22,23,32], so testing is needed to identify patients with *EGFR* mutations, who will benefit. The pragmatic approach when the clear majority of patients present with advanced disease (stage IIIB–IV) would be to implement the molecular testing plan currently followed in advanced disease for all patients - that is, to test all patients with appropriate non-squamous NSCLC histology for *EGFR* mutations at a minimum. Disease stage may be unclear to pathologists at the time of diagnosis and patients may be assumed to have advanced disease until proven otherwise. Furthermore, a proportion of patients with stage I–IIIA disease will develop distant recurrence despite complete surgical resection [33,34]. Clinical staging information is often not available when the diagnostic biopsy is tested; therefore, implementing molecular testing in a stage-agnostic manner would be efficient.

Use of molecular testing in stage I–III disease not only informs adjuvant therapy but provides the opportunity to bank information in anticipation of disease relapse after patients have finished treatment. If molecular testing results are already available at disease recurrence, time and money spent requesting, obtaining, and testing a specimen would be saved, allowing the next treatment decision to be made earlier. However, in some cases, repeat testing may be needed to help chose an appropriate treatment. For example, for an apparent or suspected second primary tumor, mutation testing would be indicated and may help confirm an independent primary tumor if different driver mutations are identified. Also, in patients with disease recurrence on targeted treatment, drug resistance through alternative molecular mechanisms is likely, which would only be identified by further testing.

Molecular testing of patients with stage I–III disease may also be useful in some other circumstances, such as patients with multiple lung nodules which may represent either synchronous stage I–III lesions (potentially driven by different molecular events) or metastatic disease, with consequential impact on appropriate treatment strategy [35]. Historically, these cases have been assessed using the Martini and Melamed criteria [36], which may not always be accurate; genomic classification offers considerably more certainty [35,37].

3. Testing materials and methodology

3.1. Sample type and minimum material requirements

As for patients with advanced NSCLC, *EGFR* testing in stage I–III NSCLC would be appropriate primarily in patients with histology with an adenocarcinoma component [5,6]. An exception to this general principal might be minimally invasive adenocarcinoma or adenocarcinoma in situ, as these tumors are generally cured with complete surgical resection [4] and are not candidates for adjuvant therapy.

Collecting sufficient tumor tissue at the time of diagnostic biopsy may be challenging for some stage I–III tumors located in difficult to access areas that preclude successful biopsy; however, these tumors are often surgically resectable. Thus, if biopsy is not feasible or if testing fails due to insufficient sample, in those with resectable disease there is another opportunity for testing following surgery - an advantage that those with unresectable or metastatic disease do not have. Should molecular testing be required before neoadjuvant treatment, then this would depend on having obtained a successful biopsy with sufficient tissue for analysis before starting treatment. Molecular testing of resected tumors after neoadjuvant treatment has yet to be established but may be challenging if there has been a significant pathological response.

Surgical resections of stage I–IIIA disease have the advantage of larger amounts of tissue, which is useful if the initial biopsy is inadequate for molecular testing, however this tissue must be handled correctly to prevent DNA degradation and subsequent test failure or compromised test results [38]. Cold ischemia time can be minimized to the recommended 1 h or less by prompt transfer of tissue to the laboratory where formalin fixation can be performed; if this is impossible, it should be immersed in formalin and/or kept refrigerated at a temperature of 2–8 °C and processed as early as possible the next day [38,39]. This latter scenario should be seen as a last resort and is in no way a substitute for fixation in controlled conditions in the laboratory.

Table 2
Primary analyses from clinical trials of adjuvant and neoadjuvant EGFR-TKI in patients with stage I–IIIA, resectable *EGFR*m NSCLC.

Trial	Patient population	Study design	Primary outcome	Key secondary outcomes
Adjuvant EGFR-TKI				
RADIANT NCT00373425 [23]	Stage IB–IIIA completely resected, expressing EGFR protein or with *EGFR* amplification, ± standard adjuvant CT before randomization (N = 73)	Phase 3, randomized (2:1), double-blind; erlotinib vs placebo for 2 years	For erlotinib vs placebo: Median DFS 50.5 vs 48.2 mo; HR 0.90 (95% CI, 0.74–1.10; P = 0.324)	In *EGFR*m subgroup (n = 161), erlotinib vs placebo: Median DFS 46.4 vs 28.5 mo; HR 0.61 (95% CI, 0.38–0.98; P = 0.039 [NS][a]) OS data immature
ADJUVANT/ CTONG1104 NCT01405079 [24,26]	Stage II–IIIA completely resected, *EGFR*m[b] (N = 222)	Phase 3, randomized (1:1), open-label; gefitinib for 2 years vs CT (cisplatin plus vinorelbine for 4 cycles)	For gefitinib vs CT: Median DFS 28.7 vs 18.0 mo; HR 0.60 (95% CI, 0.42–0.87; P = 0.0054)	For gefitinib vs CT: Median OS 75.5 vs 62.8 mo; HR 0.92 (95% CI, 0.62–1.36; P = 0.674) 3-yr DFS 40% vs 33% 5-yr DFS 23% vs 23%
ADAURA NCT02511106 [16]	Stage IB–IIIA completely resected, *EGFR*m,[b] ± standard adjuvant CT before randomization (N = 682)	Phase 3, randomized (1:1), double-blind; osimertinib vs placebo for 3 years	For osimertinib vs placebo, stage II–IIIA disease (n = 470): 24-mo DFS 90% vs 44%; HR 0.17 (99.06% CI, 0.11–0.26; P < 0.001)	For osimertinib vs placebo, overall population: 24-mo DFS 89% vs 52%; HR 0.20 (99.12% CI, 0.14–0.30; P < 0.001) OS data immature
EVAN NCT01683175 [25]	Stage IIIA completely resected, EGFRm[b] (N = 102)	Phase 2, randomized (1:1), open-label; erlotinib for 2 years vs CT (cisplatin plus vinorelbine for 4 cycles)	For erlotinib vs CT: 2-year DFS 81% vs 45%; RR 1.82 (95% CI, 1.19–2.78; P = 0.0054)	Median DFS 42.4 vs 21.0 mo; HR 0.27 (95% CI 0.14–0.53; P < 0.0001) Median OS not reached at data cutoff. HR 0.17 (95% CI 0.05–0.58; P = 0.0013)
SELECT NCT00567359 [79]	Stage IA–IIIA resected, *EGFR*m[b,c] and standard adjuvant CT ± RT (N = 100)	Phase 2, open-label; erlotinib for 2 years	2-year DFS 88% (96% stage I, 78% stage II, 91% stage III)	5-year DFS 56% Median DFS & OS NR (at median FU 5.2 years) 5-year OS 86%
Neoadjuvant EGFR-TKI				
NCT01833572 [80]	Stage II–IIIA resectable *EGFR*m[b] NSCLC (N = 33)	Phase 2, open-label; gefitinib for 42 days prior to surgery	ORR 55%	MPR 24% Median DFS 33.5 mo Median OS NR
NCT01217619 [81]	Stage IIIA–N2 *EGFR*m NSCLC, deemed resectable after neoadjuvant treatment (N = 19)	Phase 2, open label; erlotinib for 56 days prior to surgery	Radical resection rate 68%	ORR 42% Median DFS 10.3 mo PFS 11.2 mo OS 51.6 mo
EMERGING-CTONG1103 NCT01407822 [82]	Stage IIIA–N2, potentially resectable *EGFR*m[d] NSCLC (N = 72)	Phase 2, randomized (1:1), open-label; erlotinib (42 days) or CT (gemcitabine / cisplatin; 2 cycles) prior to surgery; post-operative erlotinib (12 mo) or CT (2 cycles)	For erlotinib vs CT: ORR 54% vs 34%; OR 2.26 (95% CI 17.7%–50.8%; P = 0.092)	For erlotinib vs CT: Downstaging rate 11% vs 3% Complete resection rate 73% vs 63% pCR 0% vs 0% Median PFS 21.5 mo vs 11.4 mo; HR 0.39 (95% CI 0.23–0.67; P < 0.001) Median OS 45.8 mo vs 39.2 mo; HR 0.77 (95% CI 0.41–1.45; P = 0.417)
NCT00600587 [83]	Stage III-N2, resectable NSCLC (N = 24)	Phase 2, non-randomized (1:1), open-label; erlotinib for 42 days or CT (gemcitabine / carboplatin; 3 cycles) prior to surgery based on *EGFR* status before surgery	For *EGFR*m (erlotinib) vs *EGFR* wt (CT): Response rate 7/12 (58%) vs 3/12 (25%) (P = 0.18)	For *EGFR*m (erlotinib) vs *EGFR* wt (CT): Median PFS 6.9 mo vs 9.0 mo Median OS 14.5 mo vs 28.1 mo
[84]	Stage I–II completely resectable NSCLC[e] with *EGFR* testing (N = 50)	Phase 2, open-label; gefitinib for ≥21 days prior to surgery; 2 years post-operative gefitinib in responders	For *EGFR*m vs no *EGFR*m: Pre-surgical ORR (≥25% decrease in bidirectional measurements): 17/21 vs 4/21 (P = 0.0001)	Adjuvant gefitinib vs no adjuvant gefitinib: 2-year DFS 95% vs 78% *EGFR*m vs no *EGFR*m: 2-year DFS 90% vs 75%
[85]	Stage I resectable NSCLC (N = 36)	Phase 2, open-label; gefitinib for ≥28 days prior to surgery; 2 years post-operative gefitinib in responders after CT, if indicated	Tumor response by RECIST: PR 4/35 (11%), PD 3/35 (9%)	Strongest predictor of response was *EGFR*m

CI, confidence interval; CT, chemotherapy; DFS, disease-free survival; EGFR, epidermal growth factor receptor; *EGFR*m, epidermal growth factor receptor mutation positive; FU, follow-up; HR, hazard ratio; mo, month(s); MPR, major pathologic response (≤10% viable tumor); NR, not reached; NS, not significant; NSCLC, non-small cell lung cancer; ORR, objective response rate; OS, overall survival; pCR, pathological complete response; PD, progressive disease; PFS, progression-free survival; PR, partial response; RR, relative risk; RT, radiotherapy; TKI, tyrosine kinase inhibitor.

[a] Due to hierarchical testing procedure.

[b] *EGFR*m defined as exon 19 deletion or exon 21 L858R.

[c] Other *EGFR* mutations accepted on a case-by-case basis.

[d] *EGFR*m defined as mutations in exon 19 or 21.

[e] Patients had to be never smokers, history of smoking ≤15 pack-years, and/or adenocarcinoma tumors containing bronchioalveolar features.

Table 3
Ongoing[a] key phase 2 and phase 3 clinical trials of adjuvant, maintenance and neoadjuvant targeted therapies in stage I–IIIA resectable or stage III unresectable NSCLC.

Targeted therapy	Trial identification	Patient population (estimated N)	Study design	Primary outcome	Estimated primary completion date
Adjuvant EGFR-TKI, resectable					
Afatinib	NCT01746251	Stage I–III resected *EGFR*m (N = 92)	Phase 2, randomized, open-label; afatinib for 3 mo vs 2 years	RFS	Nov 2020
Almonertinib	NCT04687241	Stage II–IIIB resected *EGFR*m[b] (N = 192)	Phase 3, randomized, double-blind; almonertinib vs placebo ± post-operative CT	DFS	Jan 2026
Almonertinib	APEX NCT04762459	Stage II–IIIA resected *EGFR*m[b] (N = 606)	Phase 3, randomized, open-label; almonertinib ± CT vs CT	DFS	May 2026
Erlotinib	ALCHEMIST NCT02193282	Stage IB ≥ 4 cm–IIIA, completely resected *EGFR*m[b] (N = 450)	Phase 3, randomized; blinded erlotinib vs placebo, open-label erlotinib vs observation; ± post-operative CT/CRT	OS	Nov 2021
Icotinib	CORIN NCT02264210	Stage IB completely resected *EGFR*m[c,d] (N = 128)	Phase 2, randomized, open-label; icotonib vs observation	OS	Dec 2025
Icotinib	EVIDENCE NCT02448797	Stage II–IIIA resected *EGFR*m[c] (N = 320)	Phase 3, randomized, open-label, icotinib vs CT	DFS	Dec 2020
Icotinib	ICTAN NCT01996098	Stage II–IIIA resected *EGFR*m[c] (N = 318)	Phase 3, randomized, open-label, CT followed by icotinib for 6 mo or 12 mo vs CT	DFS	Jan 2020
Icotinib	ICWIP NCT02125240	Stage II–IIIA resected *EGFR*m[c] (N = 124)	Phase 3, randomized, double-blind, placebo-controlled; post-operative CT	DFS	Dec 2018
Neoadjuvant EGFR-TKI, resectable and potentially resectable					
Afatinib	Neoafa NCT04470076	Stage IIA–IIIB resectable *EGFR*m (N = 30)	Phase 2, open-label; afatinib + CT prior to surgery; post-operative afatinib	MPR ORR	Dec 2021
Afatinib	ASCENT NCT01553942	Stage IIIA *EGFR*m; candidate for CRT and considered for resection (N = 30)	Phase 2; afatinib followed by concurrent CRT prior to surgery; ± post-operative CT and afatinib	ORR	Dec 2020
Erlotinib	NCT01470716	Stage II–IIIA operable *EGFR*m[c] (N = 26)	Phase 2, open-label; erlotinib prior to surgery	PFS	Dec 2021
Icotinib	NCT03749213	Stage IIIA-N2 potentially resectable *EGFR*m[b] (N = 36)	Phase 2, open-label; icotinib prior to surgery; post-operative icotinib	ORR	Feb 2022
Icotinib	NCT02820116	Stage IIIA–IIIB resectable *EGFR*m[b] (N = 67)	Phase 2, open-label icotinib prior to surgery ± post-operative icotinib	Complete resection rate	Apr 2023
Osimertinib	NeoADAURA NCT04351555	Stage II–IIIB N2 resectable *EGFR*m[b] (N = 328)	Phase 3, randomized; osimertinib + CT vs placebo + CT (double-blind) vs open-label osimertinib monotherapy prior to surgery; post-operative optimal care (including osimertinib) ± CT	MPR	Mar 2024
Adjuvant ALK inhibitor, resectable					
Alectinib	ALINA NCT03456076	Stage IB–IIA resected *ALK*-positive (N = 255)	Phase 3, randomized, open-label; alectinib vs CT	DFS	June 2023
Neoadjuvant ALK inhibitor, resectable					
Alectinib	ALNEO EUDRACT number 2020–003432-25	Stage IIIA resectable *ALK*-positive (T4N0–1) (N = 33)	Phase 2, open-label; neoadjuvant and adjuvant alectinib	MPR	Mar 2026[e]
Adjuvant multiple targeted therapies, resectable					
Erlotinib / crizotinib / nivolumab / pembrolizumab	ALCHEMIST screening NCT02194738	Stage IB ≥ 4 cm–IIIA resected/resectable *ALK*-positive and/or *EGFR*m (N = 8300)	Platform study	Clinical genotyping to facilitate accrual; feasibility assessment of research grade FFPE tissue collection for CCG analysis	Sep 2026
Neoadjuvant / adjuvant multiple targeted therapies, resectable					
Alectinib / entrectinib / vemurafenib / cobimetinib / pralsetinib	NAUTIKA1 NCT04302025	Stage IIA–IIIA and select IIIB (T3N2) resectable with *ALK*, *ROS1*, *NTRK1/2/3*, *BRAF*, or *RET* molecular alterations (N = 60)	Phase 2, open-label; neoadjuvant targeted therapy and adjuvant targeted therapy after CT	MPR	Aug 2028

(*continued on next page*)

Table 3 (*continued*)

Targeted therapy	Trial identification	Patient population (estimated N)	Study design	Primary outcome	Estimated primary completion date
Neoadjuvant EGFR-TKI, unresectable					
Gefitinib	NEGOTIATE NCT02347839	Stage III (IIIA-bulky N2, IIIB) unresectable *EGFR*m[c] (N = 37)	Phase 2, open-label; neoadjuvant and adjuvant gefitinib	Resectability rate	Jan 2020
Maintenance EGFR-TKI, unresectable					
Osimertinib	LAURA NCT03521154	Stage III unresectable *EGFR*m[b] with no progression after CRT (N = 200)	Phase 3, randomized, double-blind, placebo-controlled; CRT followed by maintenance osimertinib	PFS	Jan 2023
Almonertinib	ADVANCE ChiCTR2000040590	Stage III unresectable *EGFR*m[b] (N = 254)	Phase 3, randomized, open-label; almonertinib + RT vs concurrent CRT	PFS	Dec 2024

ALK, anaplastic lymphoma kinase; BRAF, v-raf murine sarcoma viral oncogene homolog B1; CCG, Center for Cancer Genomics; CT, chemotherapy; DFS, disease-free survival; *EGFR*m, epidermal growth factor receptor mutation positive; EGFR-TKI, epidermal growth factor receptor tyrosine kinase inhibitor; FFPE, formalin fixed, paraffin embedded; MPR, major pathological response; NA, not available; NTRK, neurotrophic tyrosine receptor kinase; ORR, objective response rate; OS, overall survival; PFS, progression-free survival; RET, rearranged during transfection; ROS, ROS protooncogene 1 receptor tyrosine kinase; RFS, recurrence-free survival.

[a] Includes trials that are not yet recruiting and recruiting, as well as those that are active but not recruiting and which have not yet reported results. Trials listed by clinicaltrials.gov as past their completion date, with status not verified in more than 2 years, have not been included.

[b] EGFR-TKI sensitizing mutation (exon 19 deletion or exon 21 L858R).

[c] EGFR-TKI sensitizing mutation (any exon 19 or 21 mutation).

[d] Note that inclusion criteria specify patients with completely resected pathological confirmed stage IIA–IIIA NSCLC, whereas completely resected stage IB NSCLC is used elsewhere in the clinicaltrials.gov record.

[e] Based on starting of enrolment of March 2021 and projected maximum duration of study of 5 years.

Adequate fixation in large surgical specimens can be problematic and varies across institutions. The recommended fixation time is 6–48 h, with an optimal range of 8–18 h for larger surgical specimens [6,38]. Over-fixation can compromise DNA quality due to degradation, fragmentation and sequence alteration [38]. However, under-fixation will also lead to poor quality DNA and RNA, poor histology, and compromised immunohistochemistry. The standard recommended fixative is 10% pH neutral phosphate-buffered formalin [38]; mercury-containing or acidic fixatives should be avoided as they are damaging to DNA [6]. Also, a distinction needs to be made between inferior fixation achieved by simple immersion of a large resected lung specimen in fixative, versus infusion of fixative into a lung by per-bronchial instillation or injection [39]. The latter will achieve superior fixation of the tumor. There is the potential need for wider discussion and education to improve this practice in some centers.

Tumor heterogeneity is not generally an issue for *EGFR* testing at initial diagnosis [40], and thus testing of multiple areas within a single tumor is not recommended [6,41]. However, specific cases may demand particular care in selecting a representative part of the resected tumor for molecular testing, e.g. avoiding areas with high stromal or inflammatory cell content [5].

Cytology samples are another common source of DNA for molecular testing. The methods used to collect cells and process samples vary widely between institutions [42]. FDA-approved *EGFR* companion diagnostic tests for tissue (as opposed to plasma) specify DNA from formalin-fixed, paraffin-embedded (FFPE) tumor tissue [43], however, current guidelines state that any cytology sample with adequate tumor cellularity and preservation may be tested [5].

Cytology samples are commonly adequate with respect to tumor content and quality. Despite the potential challenges associated with cytology samples, a study of 2293 samples comparing *EGFR* testing in histology and cytology specimens found no significant difference in the failure rate, mutation rate or mutation type [44]. Indeed, a systematic review of 4495 cytology samples found that *EGFR* test success rates ranged from 80% to 100% [45].

Regardless of sample type or disease stage, the suggested minimum number of cells required for successful molecular testing is 100–400 tumor cells, although the risk of artefactual mutations may increase in formalin-fixed specimens with <300 cells [41]. Perhaps of greater pertinence, current guidelines specify that the analytic methods used must be able to detect mutation in a sample with a tumor cell content as low as 20% [5]. The pathologist's quality control of block selection for molecular profiling and deciding which method to use for enriching tumor cellularity are therefore of great importance. Marking and then scraping sections from larger tumor areas within a FFPE block is the more common practice, and is useful in maximizing separation of tumor and non-tumor tissues when they are admixed. Microdissection is another option to increase the tumor cell content of the sample [6]. Taking cores from a tissue block may also be used, but is less well controlled and the resulting core is more difficult to dewax than scrapings.

3.2. Role of plasma-based testing

There is much interest in the use of liquid biopsy for genotyping in advanced NSCLC, with obvious potential advantages related to convenience, the non-invasive nature of sampling, and a typically faster turnaround time [46,47]. Although some companion diagnostics using plasma have been approved based on data in advanced disease, there is not enough evidence to recommend their routine use in stage I–III molecular detection or genotyping.

Furthermore, one of the issues with liquid biopsy is that patients with earlier stage disease have lower disease burden than those with advanced disease, increasing the likelihood that plasma would contain insufficient circulating tumor DNA for analysis [48–50]. If tests become more sensitive in the future, while retaining specificity, then genotyping may be an option [51].

Potential future roles for plasma testing are in lung cancer screening programs, assessing pre- and post-surgery prognosis and, at greater sensitivities, for monitoring minimal residual disease after surgery or after adjuvant treatment to gain insights into response and recurrence [49,50,52–54].

3.3. Biomarkers

Currently, *EGFR* mutation is the only biomarker which needs to be

tested for to inform the appropriate adjuvant treatment decision for a patient with resectable NSCLC.

However, we would advocate the introduction of comprehensive testing for stage I–III disease. This strategy would not only help guide selection of adjuvant treatment (in anticipation of approval of other treatments in this setting), but might also allow access to adjuvant or neoadjuvant clinical trials, and would support later treatment decisions in the event of recurrent disease (assuming those biomarker data are clinically relevant).

Comprehensive testing should ideally include all targetable genetic alterations with approved therapies in the advanced setting (i.e., *EGFR*, *ALK, ROS1*, *BRAF*, *NTRK1/2/3*, *MET* exon 14 skipping, and *RET*) [1–3], as compatible with local reimbursement guidelines. Therapies directed at *HER2* mutations are also emerging, and testing for *KRAS* mutations would be beneficial [1–3]. The FDA recently approved a therapy directed at *KRAS* G12C mutations [55], and several more inhibitors of KRAS-regulated pathways and specific inhibitors against *KRAS* G12C mutations are in development [56,57].

3.4. Single gene versus NGS testing

Current treatment guidelines for advanced disease emphasize the use of appropriate validated methods for molecular testing, subject to external quality assurance [2,3,5]. The same approach should be used with stage I–III disease.

The choice of whether to analyze biomarkers using single gene testing or next generation sequencing (NGS) will vary between institutions based on a number of factors including cost, local reimbursement rules (which may impact on affordability where patients fund their own tests), approved treatments (dictating the need for specific molecular tests), the specific needs for an individual patient and required turnaround time, tissue quantity available, and local laboratory preference or capability. To date, most NGS testing is found in North America and European countries and is relatively uncommon in many Asian countries [58,59].

Compared with advanced NSCLC, there is less urgency for molecular testing results in stage I–IIIA versus stage IIIB-IV disease. The exception would be when neoadjuvant treatment is being considered, however, there are currently no approved biomarker-driven neoadjuvant therapies. As treatment options expand, and new therapies emerge in the neoadjuvant space, it will become increasingly important to test patients with NSCLC as soon as a diagnosis is made. Thus, the slower turnaround time for NGS compared with single gene testing is not generally a problem in the adjuvant setting. However, considerations regarding cost and/or reimbursement would be needed if using NGS at diagnosis and then at recurrence (if required), versus using a single gene test.

4. Testing strategy

4.1. Multidisciplinary team (MDT) approach

Treatment modalities for stage I–IIIA NSCLC are heterogeneous requiring cooperation between many specialties. A MDT approach involving all those involved in a patient's treatment is key to establishing the need for molecular testing, developing any regional or institutional algorithm, and coordinating molecular testing [60,61]. Communication between specialties is vital to avoid delays and reduce unnecessary procedures and pathology tests, and is also helpful to communicate the adequacy of the sample, status of molecular testing, and the testing results to all specialists involved in the care of an individual patient. This is especially helpful for clinicians in community hospitals, where patients may not receive their cancer care at a single institution. The MDT may also discuss patient staging once all the data are assembled, often after biopsy and diagnosis; clinical staging investigations may only be triggered by a positive tissue diagnosis.

4.2. Role of reflex testing

Reflex molecular testing occurs when a molecular testing is automatically ordered for specific, agreed biomarkers as soon as an appropriate diagnosis of NSCLC is made (for example, on diagnosis of non-squamous NSCLC, *EGFR* mutation testing is ordered by the pathologist), without referral back to the oncologist.

Reflex testing is advantageous for several reasons. Firstly, and of greatest importance, the probability of testing being missed for a particular patient is reduced. Retrospective real-world studies have shown an increase in the proportion or absolute number of patients with non-squamous NSCLC being tested for *EGFR* or *ALK* per center after implementation of reflex testing by pathologists (irrespective of clinical stage), compared with testing ordered by medical oncologists [62–64]. Other observed benefits include significant reductions in turnaround time and time to optimal first-line systemic treatment based on biomarker status, perhaps related to a significant increase in the proportion of patients with known biomarker status at first medical oncology consultation [63–65]. One study found that the overall mutation detection rate increased significantly after the introduction of reflex testing, possibly associated with the more comprehensive panel screened, compared with selection of single genes for mutation analysis [65]. Detection rates may also increase because clinicians are not preselecting patients for testing based on clinical characteristics. From the pathologist's point of view, reflex testing while a case is 'active' and being dealt with, is more efficient than removing the relevant block from storage and revisiting the case at a later date, and avoids loss of material from repeated re-cutting of sample blocks.

Less direct but relevant benefits of reflex testing, based on 3 years' experience in over 1800 patients across all stages of lung adenocarcinoma [66], included the ability to select patients for prospective clinical trials of mutation-specific adjuvant therapy. Reflex testing also allowed retrospective studies related to patient outcomes, including the prognostic significance of specific biomarkers and the impact of targeted adjuvant treatment.

For patients undergoing surgical resection, the pros and cons of reflex biopsy testing versus waiting for the potentially better surgical specimen need to be considered. In those cancer centers conducting clinical trials, advantages of biopsy testing are the selection of patients for neoadjuvant trials of targeted therapy, and biobanking of tumor samples with specific genotypes. Where pathologists are not aware of patient stage, conducting molecular testing on biopsies regardless of stage has the benefit that testing can be completed without the delay of staging, that is within the same turnaround time as for advanced stage disease. However, molecular testing of resection specimens should be conducted if pre-resection biopsy is not possible or reflex testing of the biopsy specimen failed because of insufficient biopsy material.

4.3. Implementing reflex testing

When molecular results were only required to inform treatment decisions in patients with advanced disease, there were still advantages to reflex testing at stages I–IIIA of disease, although at a slightly increased financial cost. With the FDA and EMA approval of adjuvant osimertinib for patients with *EGFR*m (ex19del/L858R) resected NSCLC based on the results of the ADAURA study [16–18], molecular results are required to inform treatment decisions in patients with stage IB–IIIA disease as well as advanced disease, so that reflex testing on either the diagnostic biopsy or the resection specimen should be in place.

The latest CAP/IASLC/AMP guidelines advise that reflex testing for lung tumor samples by pathologist is reasonable, but that implementing such testing should be an institutional decision, made after open discussion between pathologists and oncology teams in order to develop an optimal strategy [5]. We recommend that wider collaboration within an institution is essential to determine local best practice. Implementation of reflex testing will require the development of local guidelines with

input from all stakeholders including not only the MDT, but also laboratory funders and the administrative function within an institution, to ensure that all perspectives including costs and any local, regional or national reimbursement issues are captured. For example, reimbursement of NGS by Medicare in the US requires that diagnostic NGS tests are ordered by the treating physician/practitioner. Among other things, the guideline should specify the type of biopsy specimens acceptable for analysis (many laboratories accept only FFPE tissue blocks), and refer to guidelines for testing advanced disease for detailed instructions on handling specimens, the minimum tumor content required, biomarkers to be tested, and in which situations reflex testing should be performed on biopsies. For example, will reflex testing be restricted based on tumor stage; should all biopsies be tested or only those where no resection is planned? Reflex testing of resections might be performed if there is inadequate biopsy tissue, or might be routinely preferred. As best practice for any molecular testing, specific instructions should be in place about making the molecular analysis report accessible to the treating physician, including the medical oncologist, whether by incorporating results in the anatomic pathology report, adding them to the electronic health records, or faxing or e-mailing to the treating physician. Electronic health records have the advantage of being accessible to all physicians or surgeons, at least within a single institution. The pathologist should be responsible for communicating not only the analysis results (including a statement about the adequacy of tissue) but also any problems or delays with testing.

Once any regional or institutional guidelines have been agreed, these should be communicated to all members of the MDT, including the criteria for reflex testing. When planning the best way to update the team, multidisciplinary collaboration is likely to benefit the design and delivery of any educational meetings and materials.

4.3.1. Cost and reimbursement in reflex testing

The main difficulty in adopting reflex testing is likely to be cost and the reimbursement of those costs by public-funded healthcare systems, particularly if reimbursement is predicated on documented disease stage. Consensus recommendations from a Canadian expert multidisciplinary working group state that all patients with non-squamous NSCLC, regardless of stage, should undergo comprehensive reflex biomarker testing at diagnosis using targeted NGS; however, the group recognized the lack of standard funding for such testing and recommended that provincial reimbursement bodies support comprehensive rather than single gene testing [67]. Author perspectives on our own countries are provided. In the US, reflex testing for FDA-approved therapy can be justified and should be eligible for reimbursement. In Japan, reflex testing for approved therapy can be justified and is eligible for reimbursement. In Australia reimbursement is available for molecular testing for *EGFR*, *ALK* and *ROS1* regardless of stage. Many UK institutions practice reflex testing in a public-funded health system, recognizing the overall benefits of a faster and more efficient testing approach, and the hidden additional manpower costs incurred through less efficient bespoke testing strategies. In Switzerland, reflex testing is the preferred mode in many institutions, often irrespective of clinical stage, despite no standard national procedure. In Portugal, reflex testing is only used for PD-L1, with other molecular tests ordered as required by oncologists in patients with advanced disease. In Singapore the practice of reflex testing is variable; the institution with the largest patient volume in the country currently performs routine reflex testing for non-squamous NSCLC using a NGS panel based on the results of a cost-effectiveness assessment [68]. Readers are urged to consult the national, regional, and local guidelines for reimbursement for their own countries.

4.4. Proposed molecular testing algorithm

With the proviso that any algorithm implemented would need to account for regional and institutional variations in procedure, a proposed testing algorithm for stage I–III NSCLC is shown in Fig. 1. A preferred flow shown in bold is based on reflex testing of the diagnostic biopsy or, if this is inadequate, of any surgical specimen. An alternative, pragmatic approach mentioned above would be to implement this testing algorithm for all patients. Clinical staging investigations based on imaging and clinical examination may not be triggered until after a positive diagnostic tissue test, while pathological staging is based on analysis of the biopsy and any surgical specimen. In practice, the final sequence adopted by an institution may depend on reimbursement, and whether staging information is required to trigger molecular testing.

5. Barriers

The chief barriers to molecular testing in stage I–IIIA NSCLC are widely established clinical utility, education and reimbursement. A further barrier may be the lack of inclusion in many current, although not all, treatment guidelines, although these will likely change with updated versions (Table 1). However, it should be noted that these treatment guidelines were published before the results of the ADAURA trial were available, so there was no precedent for targeted treatments in the adjuvant setting.

Clinical need is likely to drive practice; therefore, it is critical to familiarize all members of the MDT with the clinical evidence supporting the role of EGFR-TKIs and other targeted treatments in stage I–III disease. Continuing education will be needed as further clinical data become available.

Demonstrating the cost-effectiveness of any new treatment is increasingly an important facet in changing practice; once both clinical need and cost-effectiveness have been established, reimbursement is more likely to follow but will vary by country. In Australia, analysis of the cost-effectiveness of *EGFR* testing for non-squamous lung cancer of any stage found that the majority of patients will eventually relapse resulting in no unnecessary EGFR testing and a small but favorable advantage in cost/QALY could be gained by testing at diagnosis; this analysis resulted in reimbursement for *EGFR* testing of any stage lung adenocarcinoma [69]. In contrast, a smaller, retrospective, single-center cost analysis of patients with stage I–IV NSCLC tested at diagnosis in 2012 did not support *EGFR* and *ALK* testing of all stages at diagnosis. For patients with stage I–II disease, cost of testing outweighed the clinical impact: progression was observed in 8/47 stage I–II patients, and most with progressive disease had a rebiopsy [70]. Limited data are available on this issue; there is a need for more publications to provide a more complete understanding.

6. Conclusions

Comprehensive molecular testing in stage I–III non-squamous disease is set to become a necessity in the not-so-distant future. With increasing numbers of clinical trials completed and ongoing for targeted therapies in the adjuvant and neoadjuvant setting, a clear clinical need is emerging. Biomarker testing strategy must match these clinical needs, evolving in parallel with the emergence of any new adjuvant or neoadjuvant molecularly targeted agents.

The results of the ADAURA trial have been practice changing for the management of *EGFR*m resectable NSCLC. Thus, while *EGFR* is the only biomarker which currently needs routine testing to inform appropriate adjuvant treatment selection for patients with resectable NSCLC, more comprehensive stage I–III molecular testing offers the advantage of 'banked' molecular data facilitating rapid decision making in the event of recurrent disease, as well as access to trials of novel targeted agents in the neoadjuvant and adjuvant setting. Indeed, a 'test all stages' strategy may be more pragmatic, especially when it facilitates reflex, stage-agnostic testing. The exact list of molecular markers and the stage required will likely change over time, meaning that pathologists will need to be responsive to clinical needs.

Reflex molecular testing of either biopsy or resection specimen is strongly preferred over on-demand testing and should be incorporated

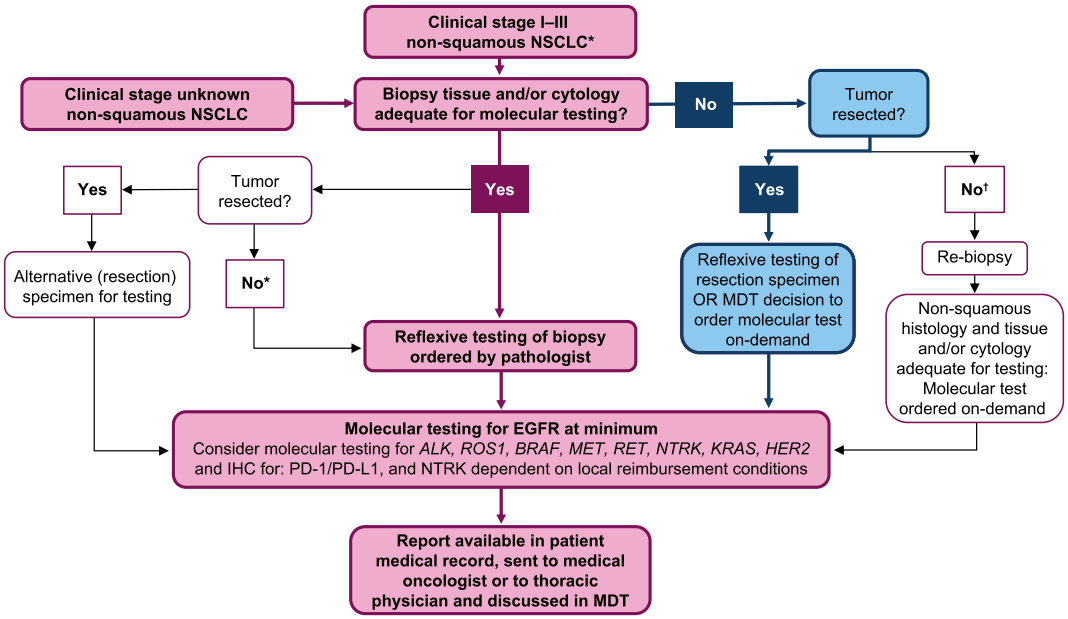

Fig. 1. Proposed algorithm for molecular testing in patients with stage I–III NSCLC (resectable and unresectable). Recommended pathways are shown in bold in shaded boxes. *Patients with squamous cell carcinoma who have clinical characteristics associated with high probability of an oncogenic driver, such as never or minimal smokers and young age, may be included. †Patient not suitable for surgery (unresectable tumor or patient medically inoperable) or declines surgery. EGFR, epidermal growth factor receptor; MDT, multidisciplinary team; NSCLC, non-small cell lung cancer.

in all appropriate guidelines in the context of testing for stage I–III disease. Advantages include an increase in the number of patients identified with actionable genetic alterations, faster turnaround times and shorter time to treatment. MDT collaboration is essential for establishment of reflex pathways.

Adjuvant or neoadjuvant immunotherapy is also being explored in stage I–III NSCLC; patients harboring genetic alterations in oncogenic drivers such as *EGFR* or *ALK* are generally excluded. These genetic alterations appear to predict poor response to immunotherapy in the advanced setting [71–73], so further study is required to determine if this holds true in patients with stage I–III disease, with the consequent need to identify patients with *EGFR*m. Testing for PD-L1 may therefore also be beneficial at stage I–III as approvals are anticipated for immunotherapies in the adjuvant setting. As more options become available for adjuvant treatment following resection, the need to ensure results are received for all tests becomes increasingly important, especially given that current data suggest that immunotherapy may not be as effective in patients with alterations in *EGFR* and *ALK*.

Molecular testing of stage I–III resected NSCLC might also have a potential role in risk stratification for disease relapse. Some mutations associated with poor prognosis have been identified in advanced disease, but data in stage I–III disease is currently limited and further studies are needed.

Molecular testing is set to become increasingly important across all stages of NSCLC. Stage I–III molecular testing may well be adopted into clinical practice for assessing risk prognosis pre- and post-surgery, and monitoring of recurrence or minimal residual disease, as research into these areas gathers pace. Targeted agents and immunotherapy have the potential to revolutionize the management of stage I–III NSCLC as they have transformed the treatment landscape for advanced disease. With clinical use established in that setting, molecular testing has become accepted and essential to guide treatment choice; we suggest that a similar pattern will soon follow for stage I–III disease.

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CRediT authorship contribution statement

Charu Aggarwal: Conceptualization, Formal analysis, Investigation, Methodology, Writing - original draft, Writing - review & editing. **Lukas Bubendorf:** Conceptualization, Data curation, Methodology, Writing - review & editing. **Wendy A. Cooper:** Conceptualization, Investigation, Methodology, Visualization, Writing - original draft, Writing - review & editing. **Peter Illei:** Conceptualization, Writing - review & editing. **Paula Borralho Nunes:** Conceptualization, Writing - review & editing. **Boon-Hean Ong:** Conceptualization, Writing - review & editing. **Ming-Sound Tsao:** Conceptualization, Data curation, Methodology, Writing - review & editing. **Yasushi Yatabe:** Formal analysis, Investigation, Methodology, Writing - review & editing. **Keith M. Kerr:** Conceptualization, Data curation, Methodology, Writing - review & editing.

Declaration of Competing Interest

Charu Aggarwal reports financial conflicts of interest for advisory council/committees from AstraZeneca, BMS, Celgene, Bluprint, Daiichi-Sankyo, Merck and Roche. Lukas Bubendorf reports financial conflicts of interest of advisory council/committees from Amgen, AstraZeneca, Bayer, Eli Lilly, Johnson & Johnson, Merck Sharpe Dohme, and Takeda; honoraria from Amgen, AstraZeneca, and Bayer; and grants or funds from MSD and Thermo Fisher. Wendy A. Cooper reports non-financial conflicts of interest of advisory boards for Amgen, AstraZeneca and Takeda. Peter Illei reports honoraria for advisory board from AstraZeneca. Paula Borralho Nunes reports financial conflicts of interest for advisory council/committees from AstraZeneca, MSD and Roche; and consulting fees from AstraZeneca, Merck Sharpe Dohme and Roche. Boon-Hean Ong reports honoraria from AstraZeneca, Johnson & Johnson, and Stryker; and travel funding from Broncus, Johnson & Johnson, Medtronic, Stryker. Ming-Sound Tsao reports honoraria from Amgen, AstraZeneca, Bayer, BMS and research grants for his institution from AstraZeneca and Bayer, unrelated to this work. Yasushi Yatabe reports honoraria from Agilent/Dako, Amgen, ArcherDx, AstraZeneca, Chugai Pharmaceutical, MSD, Novartis, Pfizer, Roche/Ventana, and Thermo

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