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Advances in early-stage lung cancer patients: from preanalytics to molecular analysis

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ABSTRACT

Lung Cancer (LC) remains one of the leading causes of cancer death worldwide. Molecular profiling of mandatory testing biomarkers significantly meliorates clinical outcome of advanced stage LC patients while intercepting early-stage lesions represents an unmet need. Recent advances in treatments for early-stage LC patients lay the basis for accelerating molecular analysis of actionable drivers before metastatic stages. In this scenario, liquid biopsy emerged as an innovative tool to guide clinical decision-making processes of early-stage LC patients. In addition, digital pathology is pioneering an integrative path to optimize morpho-molecular analysis. Undoubtedly, the lack of standardized preanalytical procedures significantly reduces the clinical availability of these approaches. This review aims to explore critical gaps affecting the clinical implementation of these approaches able to improve the clinical outcome of early-stage LC patients.

1. Introduction

In the era of genomic profiling, lung cancer (LC) has significantly shifted the clinical paradigm in accordance with a rapidly growing series of predictive biomarkers. Tissue samples remain gold standard to successfully perform molecular testing by electing advanced stage LC patients to target treatments, but several limitations dramatically affect availability of formalin fixed paraffin embedded (FFPE) diagnostic sample to genomic profile (Normanno et al., 2020). The advent of next generation sequencing strategies lay the basis for comprehensive investigation of clinically actionable biomarkers in NSCLC patients but preanalytical, analytical and postanalytical challenges remain (Ko et al., 2025). In addition, clinical benefit from anticancer agents has been seen in perioperative setting accelerating for early testing of actionable biomarkers. As a consequence, barriers in terms of handling procedures successfully administrating molecular data drastically impact early-stage LC patients (Huang et al., 2023). In this scenario, liquid biopsy (LBx) emerged as an integrative, easy to collect, dynamic tool supporting molecular test when tissue samples are not informative. In particular, LBx samples can longitudinally track evolutionary molecular landscape posing basis for novel clinical decision-making procedures in early-stage LC patients (Malapelle et al., 2021). The wide series of genomic data potentially administrating perioperative settings need computational strategies able to systematically harmonize records aligning with clinical stratification. Here, we sought to overview barriers handling biological samples, and administrating molecular data to optimize clinical administration of early-stage LC patients.

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2. Current status of molecular diagnostics for lung cancer

In recent years, molecular analysis of predictive biomarkers has significantly modified the clinical algorithm of LC identifying patients who could benefit from selective anticancer drugs (Riely et al., 2025). International societies CAP/AMP/IASCL approved a panel of "mandatory testing genes" in advanced stage non-small cell lung cancer patients (NSCLC) patients including *EGFR*, *ERBB2* (HER2) and *BRAF* hotspot mutations; *KRAS* p.G12C variant, MET exon 14 skipping alterations; *ALK*, *ROS1*, *RET* and NTRK1–3 aberrant rearrangements (Kalemkerian et al., 2018a, 2018b; Riely et al., 2025). Alongside genomic markers, PD-L1 evaluation by immunohistochemistry (IHC) acts as a key player electing LC patients to immune-checkpoint inhibitors (ICIs) (Herbst et al., 2016; Reck et al., 2016). Despite meliorating clinical outcomes, the identification of predictive biomarkers in 30–35% advanced stage LC patients without actionable drivers represents an unmet need (Mihaila et al., 2024). It has been ascertained that preventing metastases in localized LC patients can improve the survival rate in 60.0% of patients (Howington et al., 2013; Alduais et al., 2023). Low-dose computed tomography (LDCT) has been elected as the most promising tool to early detect LC patients but screening procedures are affected by several crucial points (limited access, higher risks of adverse events in healthy population, consistent technical costs, low likelihood of target population, heterogenous clinical criteria selecting populations to LDCT) (Zhao and Wu, 2015; Tao et al., 2024; Wu et al., 2016)). As a consequence, the identification of novel biomarkers able to optimize clinical management of early-stage LC patients has become a priority (Yuan et al., 2025). Novello *et al.* investigated excision repair cross complementation group 1 (*ERCC1*) and thymidylate synthase (*TS*) expression profile in resectable LC patients optimizing clinical administration of adjuvant chemotherapy (ITACA trial). Not surprisingly, not statistically significant benefit in terms of OS was achieved in LC patients under personalized adjuvant scheme compared with control group. (Novello et al., 2022)

Several trials posed challenges in shifting the "molecular model" in the administration of early-stage LC patients. Adjuvant chemotherapy is the standard of care in high-risk resectable LC patients but less than 50.0% of cases significantly benefit from conventional therapeutic schemes due to several limitations: higher disease recurrence rate, lower benefit in multiple metastatic sites, severe adverse events and heterogeneous response rate among patients (Król et al., 2023). In this scenario, the lack of actionable biomarkers to optimize clinical stratification of early-stage lung cancer is an opening challenge (Russo et al., 2025). Conversely, personalized treatments specifically targeting neoplastic cells can guide clinical decision algorithms in post operative LC patients (Huang et al., 2025). Remarkably, epidermal growth factor receptor (*EGFR*) predictive alterations have been investigated in the phase 3, double-blind trial (ADAURA) stratifying IB-IIIA LC patients to Osimertinib (Wu et al., 2018, Russo et al., 2026)). A statistically significant clinical benefit was identified in the osimertinib group achieving a 5-year overall survival of 85.0% (95% confidence interval [CI], 79–89) compared with 73.0% (95% CI, 66–78) of the control arm (Wu et al., 2018). In the same scenario, *ALK* aberrant rearrangements were investigated in ALINA trial (phase 3, open-label, randomized) administrating alectinib or intravenous platinum-based chemotherapy. Interestingly, target treatment highlighted 2 years disease free (DF) (93.8%) in comparison with chemotherapy group (63.0%). Shifting from advanced setting to early-stage, PD−1/PD-L1 blocking also revealed significantly higher DFS in stage IB to IIIA LC patients under PD−1/PD-L1 or CTLA4 inhibitors than placebo groups demonstrating the crucial role of predictive biomarkers in perioperative LC patients (Opalikhin et al., 2025). As conceivable, a diagnostically available tissue sample represents the "gold "standard" source to successfully approach PD-L1 staining and molecular profile of current biomarkers in early-stage LC patients (Zdrenka et al., 2024). Beyond conventional formalin fixed and paraffin embedded (FFPE) samples, cytological material may be diagnostic in this setting. In particular, rapid on-site evaluation (ROSE) of suspicious lesions plays a central role in guiding clinical algorithms of LC patients (Hong et al., 2025). Undoubtedly, FFPE samples and cytological specimens are affected by technical issues for successfully genotyping molecular biomarkers and/or staining immune markers. Firstly, preanalytical artifacts (depending on prolonged ischemia time, fixation procedures, storage conditions) may dramatically impact on the false positive rate; secondly, low neoplastic cell abundance in small biopsies or cytological samples can improve the false negative rate leaving behind patients who could benefit from personalized treatments (Navani et al., 2022). As a consequence, alternative sources of nucleic acids are under investigation. Liquid biopsy (LBx) emerged as a pioneering tool accelerating molecular analysis of clinically relevant biomarkers in routine practice (Borea and Reduzzi, 2025). Within the term LBxa plethora of biological fluids (peripheral blood, urine, peritoneal washing, cerebrospinal fluid, tears) are grouped showing heterogeneity in collecting samples for further analysis. Liquid sources are less invasive, dynamic, saving time tools integrating uninformative tissue samples and/or longitudinally tracking molecular drivers across samples from different collecting points (Malapelle et al., 2022). Given these advantages, circulating tumor DNA (ctDNA), accounting for 5.0–10.0% of circulating free DNA (cfDNA), may serve to modulate the de-escalation therapy in locally advanced LC patients under ICIs regimen (Gelmini et al., 2025). Recent trials highlighted that ctDNA negative LC patients do not benefit from adjuvant ICIs treatment, reducing overtreated LC patients (Sanderson et al., 2025). Moreover, minimal residual disease (MRD) evaluation of ctDNA traces in resectable LC patients demonstrated a significant decreasing rate after surgery (Denis, 2025). Moreover, tumor informed alterations in peripheral blood can augment clinical sensitivity of post-treatment analysis achieving 94.0% (Cheng et al., 2023). In addition, post-surgery ctDNA clearance correlates with a statistically significant clinical benefit in terms of DFS in LC patients receiving adjuvant chemotherapy (Chen et al., 2023; Fu et al., 2023). Considering the low abundance of ctDNA in peripheral blood, highly sensitive technical platforms capturing traces of tumor informed molecular alterations are fundamental. Of course, comprehensive genome profile able to specifically detect ctDNA alterations on a background of cfDNA fragments have been implemented (Zollinger et al., 2024).

As remarked, LBx samples host several analytes (cfDNA, ctDNA, miRNAs, CTCs) expanding potential application of biofluids in clinical practice (Pisapia et al., 2025). Integrating molecular targets (ctDNA, CTCs, epigenomic signatures), clinical outcomes of early-stage LC patients can be optimized thanks to a dedicated surveillance program tracking molecular drivers in post-surgery collection points (Shen et al., 2022). In this rapidly evolving scenario, opening challenges depending on preanalytical issues, analytical procedures and the lack of molecular biomarkers guiding clinical administration of early-stage LC patients are investigated (Fusco et al., 2025).

3. Clinical advances in early-stage LC patients

Early-stage non–small cell lung cancer (NSCLC) should not be regarded as a uniformly low-risk disease. A large systematic review and meta-analysis including over 60.000 surgically resected patients showed that recurrence-free survival (RFS) substantially declines with advancing stage, falling to approximately 50.0% in stage II and ~34% in stage III at 5 years, despite complete resection (Rajaram et al., 2024). These data reveal the limitations of a standalone surgical approach paving the way for novel curative-intent paradigms toward integrated neoadjuvant and perioperative systemic therapies, including (ICIs) alone or in combination with platinum-based chemotherapy and targeted agents (Listì et al., 2019; Viscardi et al., 2022; Barcellini et al., 2025; Zer et al., 2025).

Within this rapidly evolving clinical landscape, molecular biomarker testing has become a mandatory component of early-stage NSCLC management. In resectable stage IB–IIIA disease, *EGFR* testing is

essential, as adjuvant osimertinib has demonstrated significant DFS and overall survival (OS) benefits (Wu et al., 2020). While RT-PCR and its digital version (dPCR) offer rapid turnaround times and high analytical sensitivity, the scant reference range restricts detection to hotspot variants failing to detect uncommon sensitive or resistance-associated alterations to target drugs. Next-generation sequencing (NGS) is increasingly recommended as the upfront analytical strategy for *EGFR* testing in early-stage NSCLC, as it provides comprehensive coverage of exons 18–21, detects both common and uncommon mutations simultaneously covering multi-gene assessment while preserving tissue (Gristina et al., 2021; Penault-Llorca and Socinski, 2025). A double-blind, multicenter, randomized phase 3 trial (ARTS) evaluated clinical outcome in Asian *EGFR* mutant NSCLC patients under adjuvant aumolertinib (third generation inhibitor) targeting the most common *EGFR* mutations. Of note, DFS was significantly higher (95% CI 29·14 to not applicable) in aumolertinib group than control series of patients (19.42 months) also demonstrating a safety profile in the vast majority of patients (severe adverse events in a single case).

Similarly, *ALK* fusion testing is now required following evidence supporting adjuvant alectinib (Wu et al., 2024). Although fluorescence in situ hybridization (FISH) has been considered the reference standard, higher tissue requirement and challenging handling procedures significantly impact on the routine application. In contrast, ALKIHC provides high technical sensitivity and specificity supporting faster turnaround time (TAT) and lower tissue consumption. As a consequence, IHC is now widely accepted as a screening or standalone test whereas FISH is reserved for equivocal cases. When available, RNA-based NGS assays become the most insightful tools, comprehensively detecting *ALK* aberrant rearrangements and fusion partners in parallel with other actionable biomarkers (Penault-Llorca and Socinski, 2025).

Interestingly, ELEVATE trial demonstrated that ensartinib, approved to treat ALK-positive locally advanced/metastatic NSCLC patients,

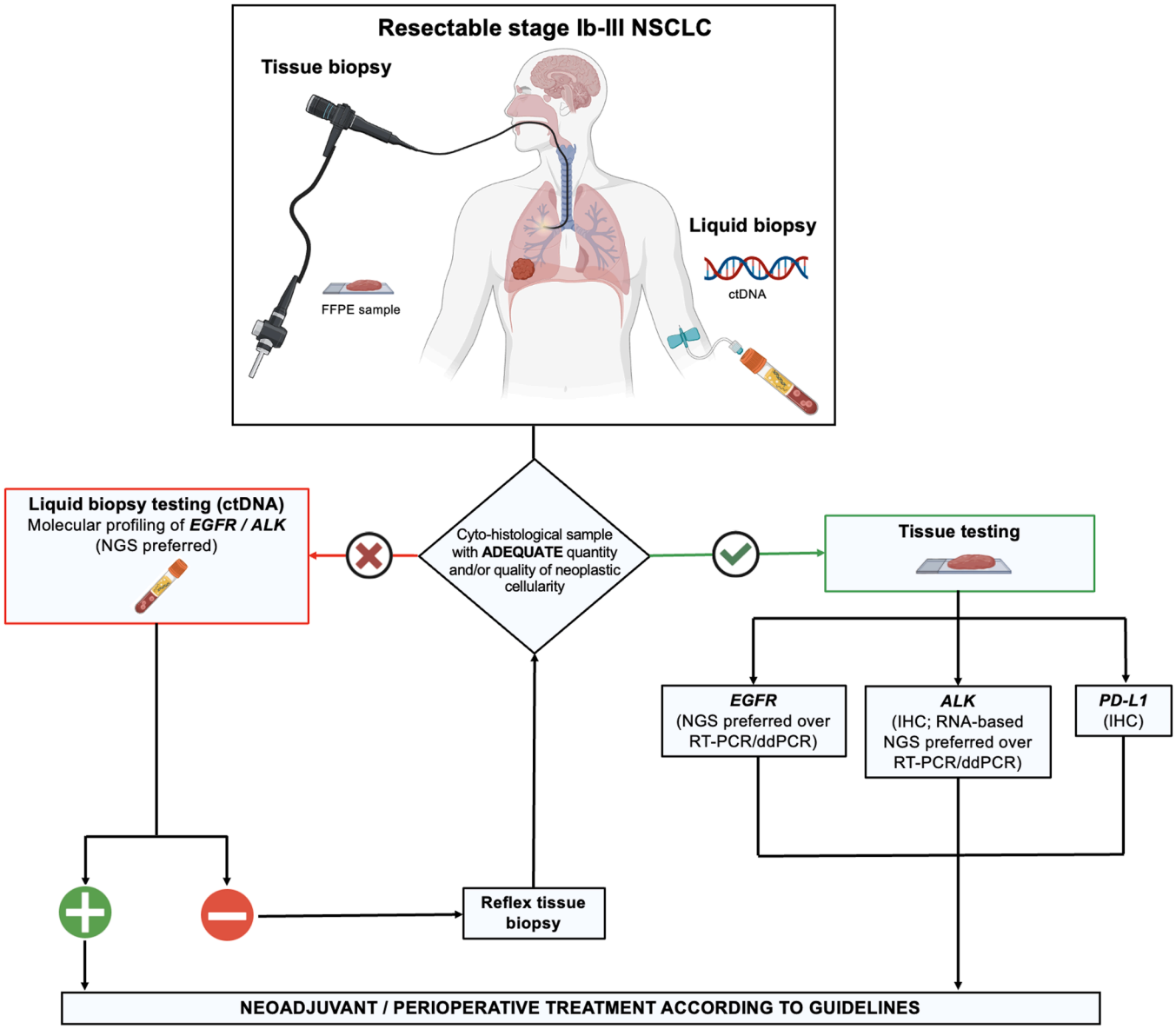

Fig. 1. Biomarker-driven algorithm for the management of resectable early-stage LC: tissue-based biomarker testing (including at least *EGFR*, *ALK*, and PD-L1) should represent the first and mandatory step whenever adequate tumor tissue is available. When tissue is unavailable, insufficient, or not suitable for molecular analysis, liquid biopsy–based testing may be employed as an alternative approach for molecular profiling. *Abbreviations*: ALK (anaplastic lymphoma kinase); ctDNA (circulating tumor DNA); ddPCR (droplet digital polymerase chain reaction); EGFR (epidermal growth factor receptor); FFPE (formalin-fixed paraffin-embedded); IHC (immunohistochemistry); NGS (next-generation sequencing); NSCLC (non–small cell lung cancer); PD-L1 (programmed death-ligand 1); RT-PCR (real-time polymerase chain reaction).

yielded a 2- year DFS of 93.7% and 91.5% in stage I and II-III NSCLC patients, respectively, experiencing high grade adverse events in 5.0% of cases (Yuan et al., 2023).

Tsuboi *et al.* designed an on-going, double-blind, phase III trial (LIBRETTO−432) assessing selpercatinib in early-stage REarranged during Trasfection (*RET)* rearranged NSCLC patients to consolidate the clinical role of drugs targeting *RET* aberrant rearrangements in perioperative NSCLC patients (Tsuboi et al., 2022). PD-L1 expression remains exclusively IHC-based and is indispensable for guiding neoadjuvant, perioperative, or selected adjuvant immunotherapy strategies in stage II–III disease (Zer et al., 2025). (Fig. 1) Conversely, anti CTLA−4 highlighted promising effect in perioperative setting correlating durable response and administrable grade 1/2 toxic effects in a small cohort of NSCLC patients, but controversial data emerged due to severe toxicities combining CTLA4 inhibitors with other ICIs. (Carthon et al., 2010). Of note, ICIs plus adjuvant chemotherapy have been consolidated in several trials sustaining the significant clinical benefit of combined strategies in comparison with single agent treatments. (Immunotherapy for Early-Stage Non–Small Cell Lung Cancer: A Practical Guide of Current Controversies) In particular, AEGEAN trial revealed a complete response in NSCLC patients under durvalumab plus chemotherapy group ((17.2% vs. 4.3%) (Oya and Tanaka, 2025). Despite advances in tissue-based testing, risk stratification conventionally relying on TNM stage, histology, and tumor size remains insufficient to capture biological heterogeneity and accurately identify patients at risk of relapse. This unmet need has catalyzed interest in MRD assessment using ctDNA from torrent blood (Gristina et al., 2023). Undoubtedly, MRD evaluation is affected by opening challenges derived from the low abundance of ctDNA in biological fluids, the lack of driver alterations. Having these issues, two main strategies have emerged: tumor-informed assays, which are highly sensitive track patient-specific mutations from tumor tissues, and tumor-agnostic assays, which rely on fixed genomic or epigenomic panels applied directly to plasma, providing greater logistical simplicity (Martínez-Castedo et al., 2025). Remarkably, tumor informed strategies depend on molecular drivers longitudinally tracked using highly sensitive technologies whereas tumor agnostic approaches can detect traces of ctDNA without any specific pattern. If the former are highly sensitive technologies requiring extensive TAT to identify molecular drivers on tissue samples, the latter are saving time approaches affected by moderate clinical sensitivity. (Camblor et al., 2026) Together, these approaches represent a natural transition toward biology-driven risk stratification in early-stage NSCLC.

Through ctDNA-based MRD assessment, LB is increasingly redefining risk stratification and treatment monitoring in the adjuvant and neoadjuvant management of early-stage NSCLC. In the adjuvant setting, the ADAURA MRD analysis demonstrated that ctDNA detection preceded radiologic recurrence by a median of 4.7 months in resected *EGFR*-mutated stage IB–IIIA NSCLC (Herbst et al., 2025). At 36 months, DFS/MRD event-free rates were 86.0% with osimertinib versus 36% with placebo (hazard raio [HR]0.23) revealing that most MRD events occur after treatment discontinuation. These findings support the central role of ctDNA as an early marker of molecular relapses and a potential tool to guide post-treatment surveillance or therapy continuation. Consistently, the ADMIN trial, focusing on uncommon *EGFR* mutations, highlighted that adjuvant osimertinib reduced ctDNA levels from 23.3% at baseline to 13.9% in 12 weeks, supporting the need for LB even in molecularly heterogeneous populations (Liu et al., 2025). In *EGFR/ALK* wild-type cases, ctDNA also plays a strong prognostic role. In the IMpower010 trial, post-operative ctDNA positivity (∼21% of patients) showed shorter DFS, while chemotherapy-induced ctDNA clearance occurred in 62.0% of ctDNA-positive patients and correlated with better outcomes (Wakelee et al., 2024). Importantly, adjuvant atezolizumab improved DFS irrespectively of ctDNA status, indicating that ctDNA serves as a prognostic tool rather than predictive biomarker in this setting, while remaining valuable for longitudinal disease monitoring. In the neoadjuvant context, ctDNA dynamics have shown stringent associations with pathological response and long-term survival. In the NeoADAURA trial, baseline MRD negativity was prognostic for improved event-free survival (EFS) (HR0.24), and osimertinib-based regimens achieved pre-surgical MRD clearance rates of approximately 83–84%, comparing to 58.0% with chemotherapy alone, strongly correlating with major pathological response (Blakely et al., 2025). In the CheckMate 816 trial (40454642), presurgical ctDNA clearance markedly improved 5-year OS (∼75% versus ∼53% in non-cleared patients; HR 0.38–0.39) and with higher pathological complete response (pCR) rates, particularly in the nivolumab plus chemotherapy arm (Forde et al., 2025). Complementary evidence from LCMC3 showed that undetectable post-surgical ctDNA after neoadjuvant atezolizumab alone was associated with superior DFS (HR 0.29), while ctDNA clearance predicted both improved DFS (HR 0.25) and OS (HR 0.26), independently of disease stage (Owen et al., 2024).

These observations in the adjuvant and neoadjuvant settings lay the basis for biological and clinical rationale in perioperative strategies, where longitudinal molecular monitoring becomes central to treatment personalization. In the perioperative management of early-stage NSCLC, LB acts as a key player to refine risk stratification and predict long-term outcomes beyond conventional pathological and clinical parameters. Across multiple perioperative chemo-immunotherapy trials in resectable NSCLC, baseline ctDNA level consistently reflects tumor burden impacting on the prognosis, while ctDNA clearance during treatment serves as a robust tool of therapeutic efficacy. In the non-randomized phase II NADIM trial, baseline ctDNA levels prognostically measured mutant allelic fraction of clinically relevant alterations (sumMAF) demonstrated that patients harboring low pretreatment ctDNA fraction remarkably achieved superior 5-year EFS and OS. In addition, ctDNA clearance after neoadjuvant chemo-immunotherapy emerged as a dominant biomarker translating into approximately 85% 5-year EFS and 92% 5-year OS, compared with ∼61% and ∼59%, respectively, in patients where ctDNA levels are detectable (Provencio et al., 2024). Notably, ctDNA clearance retained prognostic value even in patients without pCR, outperforming PD-L1 expression and tumor mutational burden (TMB) as a long-term predictor. These findings were reinforced in the randomized NADIM II trial, where higher baseline ctDNA independently predicted poorer EFS and OS, while neoadjuvant nivolumab plus chemotherapy increased pre-surgical ctDNA fraction (67% versus 44% with chemotherapy alone), supporting ctDNA as a quantitative, dynamic biomarker complementary to TNM staging. Evidence from phase III perioperative trials further reinforces the clinical relevance of LBx (Provencio et al., 2023).

In the CheckMate 77 T trial, patients with presurgical ctDNA clearance after neoadjuvant nivolumab achieved a significantly higher EFS highlighting ctDNA as an early molecular readout of treatment benefit beyond pCR (Cascone et al., 2025). In exploratory analysis of CheckMate 77 T trial median EFS was not calculated in N2 subgroup under nivolumab and 17.0 months in control cases. In addition, 1-year event-free survival of 71.0% was assessed in experimental group demonstrating the pivotal role of nivolumab integrating neoadjuvant chemotherapy in LC patients. (Provencio et al., 2026)) In the AEGEAN trial, post-surgical ctDNA-defined MRD identified a small but extremely high-risk subgroup, with MRD-positive patients experiencing dramatically inferior outcomes (12-month DFS 14.3% vs 89.3% in MRD-negative patients; HR >14 across arms), indicating failure of molecular eradication despite surgery and systemic therapy (Reck et al., 2025). Exploratory analyses further demonstrated that combining on treatment ctDNA dynamics with radiomic features enhanced prediction of pCR and EFS, underscoring the potential of integrated, minimally invasive biomarker strategies (Heymach et al., 2025).

Overall, emerging evidence indicates that ctDNA analysis shows a partial analysis of the molecular landscape of early-stage NSCLC, supporting the need for integrated, multiomic strategies. Beyond ctDNA, the perioperative detection of clustered circulating tumor cells (CTCs) identifies a biologically aggressive MRD-positive subgroup with a

markedly increased risk of recurrence and a clear, selective benefit from adjuvant chemotherapy, even in clinically early-stage disease (Sawabata et al., 2024). In parallel, tumor-agnostic cfDNA methylation profiling has demonstrated the ability to detect MRD at extremely low tumor fractions with high specificity and strong postoperative prognostic value, offering a scalable alternative when tumor-informed approaches are not feasible (Yang et al., 2025). Together, these findings highlight that MRD is a multidimensional biological state, encompassing genomic, epigenomic, and cellular components. Future perioperative management of early-stage NSCLC will likely rely on multiomic MRD frameworks that integrate ctDNA mutations, methylation signatures, and CTC phenotypes to optimize risk stratification, personalize adjuvant therapy, and avoid overtreatment in biologically cured patients. (Table 1)

4. From surgery to sample management in the era of genomic profile

The management of early-stage NSCLC has been significantly transformed by the integration of molecular profiling into clinical decision-making (Jeon et al., 2025). Although surgical resection remains the primary curative approach, resected tissues become central players to capture molecular markers guiding clinical strategies (Hassan, 2025). In early-stage NSCLC, where tissue samples are often limited and re-biopsy are rare, the adequacy of diagnostic routine sample (in terms of neoplastic cell abundance, artifacts fixation from preanalytical procedures) handling from surgery toward molecular analysis is a critical factor that influences both diagnostic accuracy and therapeutic eligibility (Liam et al., 2020).

Early-stage NSCLC poses distinct challenges in tissue collection and management in advanced stages of the disease (Navani et al., 2022). Neoplastic lesions identified by lung cancer screening programs or incidental imaging are often small, subsolid and minimally invasive. As a result, these cases frequently require sub-lobar resections, such as wedge resections or segmentectomies (Sienel et al., 2008). Although these surgical techniques preserve lung function, they often result in limited tissue volumes, thereby increasing the risk of insufficient material for comprehensive histological and molecular analyses (Grover et al., 2024).

Furthermore, early-stage tumours exhibit significant intratumoral heterogeneity at both morphological and molecular levels (Zuo et al., 2025). It has been demonstrated that areas of the same lesion can show distinct histological and/or molecular features significantly impacting on the accuracy of diagnostic procedures (Passaro et al., 2020). As a result, sampling bias can lead to under-representation of clinically relevant driver alterations, especially when only small tumour areas are available for morphological evaluation (Litchfield et al., 2020). This challenge is stressed by the clinical unmet need to adequately handle scant diagnostic tissue samples for multiple diagnostic applications, including histopathological classification, tumor grading, staging, IHC, and comprehensive molecular profiling (Lazzari et al., 2020).

As remarked, the surgeon plays a critical role in this process. Ensuring correct specimen orientation, maintaining prompt communication with the pathology team, and preventing mechanical or thermal damage are essential for preserving tissue integrity and accelerating personalized adjuvant treatment strategies.

The pre-analytical procedures have been considered as the main source of variability and potential errors in molecular testing. Pre-analytical factors significantly impact on nucleic acid yield and fragmentation, thereby affecting the success rate of molecular testing and the identification of actionable alterations (Marchiò et al., 2025).

Cold ischemia time, spanning from the tissue devascularization and fixation, is crucial for preserving the integrity of DNA, RNA, and proteins. Prolonged cold ischemia significantly accelerates nucleic acid degradation and changes in gene expression profiles. Current guidelines recommend minimizing cold ischemia time, ideally carrying out this step between 30 and 60 min whenever possible (Bass et al., 2014).

Fixation parameters are another key determinant of sample adequacy for further processing procedures. Neutral buffered formalin at a concentration of 10% is the standard fixative for lung cancer specimens. Both under-fixation and over-fixation can compromise molecular analyses. In particular, excessive fixation times may cause cross-linking among proteins drastically impacting on nucleic acid purification and improving sequencing artifacts. International guidelines recommend fixation times between 6 and 24 h, depending on specimen size (Hewitt et al., 2008).

Neoplastic cell abundance is another critical factor in early-stage NSCLC. A minimum proportion of neoplastic cells is needed to achieve consistent results adopting sensitive NGS assays, particularly for detecting low-frequency variants (Dufraing et al., 2018). Pathologist-guided macrodissection or microdissection is frequently

Table 1
Key studies evaluating ctDNA-based liquid biopsy for MRD assessment in early-stage NSCLC.

Study (Trial ID)	Setting	Population	ctDNA MRD-assessment	Clinical findings
ADAURA (NCT02511106)	Adjuvant (osimertinib)	Resected stage IB–IIIA, EGFR-mutated NSCLC	Tumor-informed	Median ctDNA detection of recurrence before imaging (4.7 mo); 36-mo DFS/MRD-EFS (86% vs 36%, HR 0.23)
ADMIN (NCT05546866)	Adjuvant (osimertinib)	Resected NSCLC with uncommon *EGFR* mutations	Tumor-informed	ctDNA clearance (from 23.3% at baseline to 13.9% at 12 weeks)
IMpower010 (NCT02486718)	Adjuvant (atezolizumab post-PBC)	Resected IB-IIIA, *EGFR/ALK* wild-type NSCLC	Tumor-informed	ctDNA post-PBC clearance (62% of patients) associated with improved DFS
NeoADAURA (NCT04351555)	Neoadjuvant (osimertinib)	Resectable II-IIIB, *EGFR*-mutated NSCLC	Tumor-informed	Presurgical ctDNA clearance ~83–84% vs 58% with PBC
CheckMate 816 (NCT02998528)	Neoadjuvant (nivolumab + PBC)	Resectable IB-IIIA *EGFR/ALK* wild-type NSCLC	Tumor-informed	Presurgical ctDNA clearance associated with improved 5-year OS (~75% vs ~53%)
LCMC3 (NCT02927301)	Neoadjuvant (atezolizumab)	Resectable IB-IIIA NSCLC *EGFR/ALK* wild-type	Tumor-informed	Post-surgical ctDNA negativity associated with improved DFS (HR 0.29) and OS (HR 0.26)
NADIM (NCT03081689)	Perioperative (PBC +nivolumab)	Stage III resectable NSCLC *EGFR/ALK* wild-type	Tumor-agnostic	Low baseline ctDNA and clearance associated with improved 5-year EFS (~85%) and OS (~92%)
NADIM II (NCT03838159)	Perioperative (PBC + nivolumab)	Stage III resectable NSCLC *EGFR/ALK* wild-type	Tumor-agnostic	Baseline ctDNA prognostic and related to tumor size; Presurgical ctDNA negativity 67% vs 44% with PBC
CheckMate 77 T (NCT04025879)	Perioperative (PBC + nivolumab)	Resectable IIA-IIIB NSCLC *EGFR/ALK* wild-type	ND	Presurgical clearance associated with improved EFS regardless of pCR
AEGEAN (NCT03800134)	Perioperative (PBC + durvalumab)	Resectable IIA-IIIB NSCLC *EGFR/ALK* wild-type	Tumor-informed	Post-surgical MRD positivity identified a very high-risk subgroup (12-month DFS 14.3%)

Abbreviations: ALK, anaplastic lymphoma kinase; ctDNA, circulating tumor DNA; DFS, disease-free survival; EFS, event-free survival; EGFR, epidermal growth factor receptor; HR, hazard ratio; mo, months; MRD, minimal residual disease; ND, not determined; NGS, next-generation sequencing; NSCLC, non-small cell lung cancer; OS, overall survival; pCR, pathological complete response; PBC, platinum-based chemotherapy

required to enrich tumour content and improve analytical performance (Wisner et al., 2022). Inadequate tumour purity may lead to false-negative results, which could prevent patients from receiving efficient target drugs or ICIs treatments (Goswami et al., 2016; Haider et al., 2020).

To fill this gap, international societies including the College of American Pathologists (CAP), Italian Society of Anatomic Pathology and Diagnostic Cytopathology (SIAPeC), American Society of Clinical Oncology (ASCO), Association for Molecular Pathology (AMP), and European Society for Medical Oncology (ESMO) recommended handling procedures to standardize pre-analytics and quality control check. However, compliance with these guidelines varies among institutions, highlighting the need for structured workflows and ongoing education (Marchiò et al., 2025; Kalemkerian et al., 2018a, 2018b).

Liquid biopsy has emerged as a minimally invasive method for molecular profiling in lung cancer, demonstrating the potential to capture tumor heterogeneity across spatial and temporal dimensions (Jahani et al., 2025). Nevertheless, its clinical utility in early-stage NSCLC is still being actively investigated. A major limitation of liquid biopsy in early-stage disease is the low concentration of ctDNA, which often falls below the detection threshold of current assays. Consequently, sensitivity is substantially lower than in advanced-stage disease, and negative plasma test results do not exclude the detection of actionable genomic alterations (Rolfo et al., 2018).

Despite these limitations, liquid biopsy demonstrates potential in specific clinical scenarios. The detection of ctDNA after surgical resection has been proposed as a marker of minimal residual disease (MRD), and several studies have shown a strong association between postoperative ctDNA positivity and increased risk of recurrence (Chaudhuri et al., 2017). In this context, liquid biopsy may serve as a dynamic biomarker to improve risk stratification and guide adjuvant treatment decisions.

Pre-analytical variables are also critical for the success of LBx. Factors such as blood collection tube, plasma separation, storage conditions, and DNA purification methods can affect ctDNA yield and assay performance. Standardization of these procedures is essential to ensure reproducibility and clinical validity to implement LBx in diagnostic routine scenarios (Merker et al., 2018; Marchiò et al., 2025).

Currently, tissue biopsy remains the gold standard for molecular characterization in early-stage NSCLC. Given the impressive series of clinical trials supporting the pivotal role of molecular analysis in neoadjuvant/perioperative setting, harmonized preanalytical procedures of diagnostic samples are mandatory to shift molecular paradigm from advanced to early-stage NSCLC patients. Particularly, small biopsies suffer from the lack of optimized management consistently leaving behind patients from target treatments (Roy-Chowdhuri et al., 2025). Liquid biopsy should be considered a complementary approach, particularly for MRD assessment and longitudinal monitoring.

5. Digitalizing the path: the new role of artificial intelligence in early-stage LC patients

The application of artificial intelligence (AI) tools can significantly support pathologists in the assessment of patients with early-stage lung cancer (Viswanathan et al., 2022). Practical examples include the automated detection and classification of adenocarcinoma growth patterns, which are useful for tumor grading, as well as AI-assisted detection of lymph node metastases for staging purposes (Pham et al., 2019; Wei et al., 2019). These tools can identify even small metastatic foci while reducing slide review turnaround times for pathologists. Recently, a fully quantitative clinical-grade by automatized AI tool for pattern classification and quantification (PATQUANT) has been proposed to integrate conventional lung adenocarcinoma grading based on morphological analysis of growth patterns, demonstrating excellent prognostic performance that outperforms traditional grading methods (Wang et al., 2025). One of the most pressing challenges in lung cancer remains the quantification of tumor cellularity fraction (TCF) for molecular testing. Although TCF assessment is a critical step in ensuring the adequacy of dissected material for downstream genomic analyses, several issues (time-consuming, inter-observer variability, and lacks strict guidelines) persist (Smits et al., 2014). In routine practice, morphological evaluation is often approached at low magnification with a panoramic tumor view (Frei et al., 2023). To address these limitations, AI-based tools have been developed to reduce TAT and variability, demonstrating results comparable to pathologist consensus and ground truth, as well as robustness across centers in the hands of different skilled pathologists (L'Imperio et al., 2025a, 2025b). Notably, these tools may also influence molecular results accelerating for CNVs detection, hardly to capture also adopting NGS systems. Although AI can alleviate several routine diagnostic tasks, one of the most impactful areas is the interpretation PD-L1 and tumor proportion score (TPS) in non–small cell lung cancer (NSCLC). In recent years, several algorithms have been developed to assist in PD-L1 evaluation, showing strong concordance between pathologist- and AI-derived TPS, as well as good prediction of tumor response and progression-free survival in clinical validation trials (Kim et al., 2024). While supervised AI approaches currently represent the standard for predictive IHC interpretation, less human-dependent methods are emerging demonstrating consistent results in patient stratification. The recently developed quantitative continuous score (QCS) enables automatized tumor detection and subcellular compartment identification (cell membrane versus cytoplasm) based on training with pathologist annotations (Garassino et al., 2024). Using this framework, the brown chromogen diaminobenzidine (DAB) is quantitatively measured and computationally processed to define optimized cutoffs for stratifying patient response to specific therapies (Reis-Filho et al., 2024). Promising results have been reported for HER2 assessment in breast cancer and PD-L1 evaluation in lung cancer, where QCS outperformed pathologists in stratifying overall survival following immunotherapy (Schmidt et al., 2021; Kapil et al., 2024; Robbins et al., 2025). Furthermore, data from the TROPION-PanTumor01 trial evaluating datopotamab deruxtecan (Dato-DXd), an antibody–drug conjugate, in NSCLC demonstrated the superiority of QCS over conventional H-score assessment measuring TROP2 expression (Garassino et al., 2024). These findings suggest the need of fully computational pipelines for predictive biomarker evaluation. Importantly, the impact of AI in lung pathology extends beyond H&E interpretation and IHC scoring to the detection of subtle tissue and tumor features associated with molecular alterations. Compared with other oncologic domains, where AI-based prediction of molecular alterations from H&E slides achieved strong performance early on, such as microsatellite instability in colorectal cancer, molecular profiling in endometrial carcinoma or homologous recombination deficiency in breast cancer, progress in lung cancer has been slower (Beretta et al., 2022; Echle et al., 2022; Fremond et al., 2023; Bergstrom et al., 2024). Early studies demonstrated promising results, with area under the curve (AUC) values ranging from 0.73 to 0.86 for predicting *EGFR*, *SATB1*, *STK11*, and *TP53* mutations and pilot studies reporting reasonable performance for *ALK* rearrangement prediction (AUC 0.73) (Coudray et al., 2018; Terada et al., 2022). More recently, advances in AI methodologies, including the development of foundation models and large-language vision models, have substantially accelerated progress in molecular prediction for lung cancer. Notably, some models can now detect *EGFR* mutations with high confidence and significantly faster TAT compared with conventional NGS platforms and rapid RT-PCR systems accelerating for the best therapeutic option while awaiting definitive molecular results (Waissengrin et al., 2023). This progress is exemplified by the real-world deployment of a fine-tuned pathology foundation model, which achieved an AUC of 0.89 detecting *EGFR* mutations and could potentially reduce the need for rapid molecular testing in 43.0% of cases, while shortening turnaround time to 44 min compared with 2–3 weeks for NGS (Campanella et al., 2025). Beyond molecular prediction, AI analysis of lung cancer H&E slides has also demonstrated the ability to predict tumor prognosis and evolution

independently of established clinicopathologic prognostic factors (Kludt et al., 2024). Data on PD-L1 assessment adopting AI algorithms successfully revealed a comparable analytical performance with n = 24 experienced pathologists also meliorating data interpretation of SP142 assay which posed limitations due to variability in scoring system. (Hirsch et al., 2017a, 2017b) Finally, the integration of molecularly informed multimodal AI models holds significant promise for improving both predictive and prognostic stratification in lung cancer (Chen et al., 2023), paving the way toward a new era of precision pathology (Vaidya et al., 2025). In addition, neoadjuvant therapy can significantly benefit from AI algorithm. PathR simultaneously measuring major pathologic response (MPR) and complete pathologic response on diagnostic slides may guide clinical decisions of neoadjuvant approaches optimizing inspection of clinic-pathological data. Ishikawa, 2024).

6. Conclusions

Within this evolving landscape, the surgical specimen must be recognized as a non-renewable resource with value extending beyond just histological diagnosis. Investing in standardized pre-analytical workflows, multidisciplinary collaboration, and technological innovation is a prerequisite for delivering personalized care to patients with early-stage NSCLC (Marchiò et al., 2025). As shown by the rapidly increasing series of predictive biomarkers in early-stage LC patients, sequential testing approaches (based on the conventional singleplex technologies) are affected by several limitations (high TAT, lack of tissue material to completely profile genomic markers) dramatically impacting on the selection of the best-in-class option for cancer care (Fusco et al., 2025). Therefore, NGS technologies, able to simultaneously cover all the actionable genomic hallmarks, become crucial in optimizing clinical administration of LC early-stage LC patients saving cases who could risk being left behind. In particular, CGPs can consistently detect less common actionable alterations (exon 20 insertions, aberrant rearrangements) uncovered by single plex strategies stratifying LC patients to the best neoadjuvant approach. (Viteri et al., 2023) On demand request by clinicians of molecular analysis should shift toward the comprehensive analysis of each clinically relevant biomarkers (genomic and proteomic) at baseline to better manage clinical patients (Gristina et al., 2021; Penault-Llorca and Socinski, 2025).

Moreover, comprehensive NGS profile highlighted lower turnaround time (TAT) accessing to molecular results within 3.6 weeks than sequential testing approaches (6 weeks). In addition, simultaneous analysis of clinically relevant biomarkers can save $1597.23/patient in comparison with singleplex Penault-Llorca and Socinski, (2025).

NGS systems enable detection of less common actionable alterations saving perioperative NSCLC patients from not efficient neoadjuvant ICIs. In detail, treatment failed in higher proportion of NSCLC patients driven by actionable alterations (HR: 5.51, 95%CI: 1.68–18.1). (Zhou et al., 2024)

The growing pitfalls of molecular diagnostics in early-stage NSCLC highlight the need for integrated and standardized diagnostic pathways. The integration of surgical expertise, pathology, molecular biology, and digital technologies is essential to fully realize the potential of genomic profiling in early-stage NSCLC. As targeted adjuvant therapies and immunotherapies continue to expand, inadequate sample handling will have increasingly significant consequences, including missed therapeutic opportunities and suboptimal patient outcomes. In this scenario, LBx emerged as reliable tool dynamically monitoring tumour burden across longitudinal collection points. Interestingly, preliminary data suggest that ctDNA negative patients can significantly benefit from de-escalation treatments saving early-stage NSCLC patients from toxic events. Further investigations will be proven to consolidate this approach in perspective real world cases (Chen et al., 2025). Fragmented approaches, where surgery, pathology, and molecular testing operate independently, are inadequate to address the requirements of precision oncology. Implementing standard operating procedures (SOPs) across disciplines represents a crucial step toward harmonization (Herbst et al., 2025). These SOPs should address specimen handling in the operating room, fixation and processing protocols in pathology laboratories, and quality metrics for molecular testing. In particular, computational pathology has been radically shifting the clinical paradigm of early-stage LC patients posing challenges to successfully implement this model in clinical practice. First, rigorous standardization of the pre-analytical phase is essential to avoid artifacts such as tissue folds or out-of-focus regions that may impair algorithmic performance. Additionally, these approaches rely on IHC-stained whole-slide images, harmonized procedures in terms of antibody clones, staining platforms, and detection systems are paramount to consistently collecting molecular data across institutions accelerating equitable access to cancer care. Another key challenge is the challenging interpretation system of deep learning models, which function as “black boxes” whose internal computations cannot be independently verified by pathologists. Finally, successful clinical integration of AI algorithms depends on the appropriate reimbursement frameworks, stakeholder engagement, and the resolution of ethical and regulatory issues, including liability and potential algorithmic bias (Pham et al., 2019; Wei et al., 2019).

Multidisciplinary Tumor Boards (MTBs) are central to this framework, as they facilitate coordinated decision-making and ensure that molecular testing strategies are aligned with clinical objectives (Hirsch et al., 2017a, 2017b; Thai et al., 2021). Centralizing high-complexity molecular testing within specialized laboratories can further enhance quality and cost-effectiveness, as long as efficient networks are established for timely sample transfer and reporting. Within this evolving landscape, the surgical specimen must be recognized as a non-renewable resource with value extending beyond just histological diagnosis. Investing in standardized pre-analytical workflows, multidisciplinary collaboration, and technological innovation is a prerequisite for delivering personalized care to patients with early-stage NSCLC.

Critical review

In the rapidly evolving era of precision medicine, molecular testing of predictive biomarkers has revolutionized the clinical management of LC patients. It has been consolidated the pivotal role of cancer profiling in advanced stage LC patients, but emerging aspects are moving molecular investigation forward early-stage LC patients. Clinical trials highlighted that early-stage LC patients may benefit from target inhibitors if *EGFR* sensitive alterations or *ALK* rearrangements are detected in tissue samples. Noteworthy, the lack of standardized pre analytical procedures administrating biological samples, of harmonized analytical strategies covering activating mutations and of data interpreting procedures can significantly impact on the clinical procedures of early-stage LC patients. Here, we warranted to overview the most challenging procedures in diagnostic routine stage of early-stage LC patients stressing the key role of multidisciplinary team to successfully administrate the increasing series of biological and clinical data to clinically manage early-stage LC patients.

CRediT authorship contribution statement

F.P.: Conceptualization; Study design; Data acquisition, analysis & interpretation; Draft writing & review. V.G.: Conceptualization; Study design; Data acquisition, analysis & interpretation; Draft writing & review. TD.BR.: Conceptualization; Study design; Data acquisition, analysis & interpretation; Draft writing & review. V.LI.: Conceptualization; Study design; Data acquisition, analysis & interpretation; Draft writing & review. A.M.: Conceptualization; Study design; Data acquisition, analysis & interpretation; Draft writing & review. G.R.: Data acquisition & analysis; Draft writing. C.S.: Data acquisition & analysis; Draft writing. R.B.: Data acquisition & analysis; Draft writing. D.C.: Data acquisition & analysis; Draft writing & review. A.R.: Conceptualization; Data analysis & interpretation; Draft review. G.T.: Conceptualization;

Data analysis & interpretation; Draft review. U.M.: Conceptualization; Study design; Data interpretation; Draft review. All authors had full access to all study data and took responsibility for their integrity and for the accuracy of the data analysis. All authors read and approved the final manuscript.

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- Author 2 received honoraria as consultant and/or speaker bureau from Daichii-Sankyo, Astrazeneca, MSD, Roche, Takeda, Novartis, BMS, Regeneron, Pfizer, Johnson&Johnson outside the submitted work.
- Author 4 received honoraria as consultant and/or speaker bureau from Eli Lilly, Roche, Novartis unrelated to the current work.
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Declarations

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