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Current Uses and Pitfalls of Liquid Biopsy in NSCLC

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Introduction

Liquid biopsy has emerged as an important tool in the diagnosis and management of lung and other cancers. Various analytes and analytical methods have been studied, including genomic testing by next-generation sequencing (NGS) and non-NGS approaches, including those examining methylation or DNA fragment size. Liquid biopsy, especially from plasma or blood, has several advantages over percutaneous or endoscopic tissue biopsy. It is less invasive, can be used serially for monitoring, and better reflects tumoural heterogeneity across metastatic sites, as opposed to a single area of the biopsied tumour. Herein, we highlight the current uses of liquid biopsy using circulating tumour DNA (ctDNA) analysis in routine clinical practice and potential pitfalls.

Liquid Biopsy for Initial Tumour Genotyping in Advanced NSCLC

The International Association for the Study of Lung Cancer (IASLC), National Comprehensive Cancer Network (NCCN), European Society for Medical Oncology (ESMO), and American Society of Clinical Oncology (ASCO) recommend using validated and sensitive plasma ctDNA assays in routine clinical practice to ensure timely complete genotyping for patients with advanced non-small cell lung cancer (NSCLC) and other tumour types.[1-4] Complete tumour genotyping, in addition to pathologic subtype and programmed cell death ligand 1 (PD-L1) assessment, is essential for optimal treatment selection in advanced NSCLC and other advanced cancers. Based on genotyping and PD-L1 immunohistochemistry results, therapeutic options range from matched targeted therapy for patients with actionable alterations in their tumours to immunotherapy or chemo-immunotherapy for those without alterations or incomplete genotyping results.

Although tissue NGS is considered the gold standard, performing NGS on liquid biopsy plasma ctDNA samples has been shown to be non-inferior to tissue NGS. Additionally, it can significantly improve the rate of complete genotyping, meaning that a higher percentage of genomic alterations can be identified and characterized using liquid biopsy. Plasma NGS also has a quick turnaround time leading to faster available results.[5-7] Plasma and tissue NGS results are highly concordant, and resulting treatment choices have similar outcomes, whether alterations are detected in plasma or tissue.[6-9] Furthermore, both assessments have minimal risk of false positive results with validated assays. Thus, if an actionable alteration is identified in plasma before tissue results are available, clinicians should use the plasma results to start treatment.[10] As liquid biopsies have lower sensitivity than tissue testing, especially for detecting translocations and copy number variants (e.g. amplification), clinicians should consult tissue NGS results to determine the treatment approach if no actionable alteration is identified in plasma.

IASLC, NCCN, and ESMO guidelines recommend multiple approaches for the integration of liquid biopsy into routine care for patients with advanced NSCLC (**Figure 1**).[1-3] A sequential approach, ordering liquid biopsy after failure of tissue testing to obtain complete genotyping, can prevent repeat biopsy if there is insufficient tissue for genotyping. Complementary or concurrent plasma ctDNA testing improves the rate of complete genotyping and accelerates the time to results.[5-7, 9-12] For example, adding plasma testing to routine tissue testing increased the number of patients detected with targetable alterations in tumour by 15% compared to tissue NGS alone.[7] The concurrent approach of testing both plasma and tissue upfront is recommended by the NCCN and ESMO, particularly for patients with treatment-naïve advanced NSCLC.[2,3] Finally, a "plasma-first approach" has been used when insufficient or no tissue is available for NGS. Liquid biopsy before diagnosis in patients with suspected advanced lung cancer has been shown

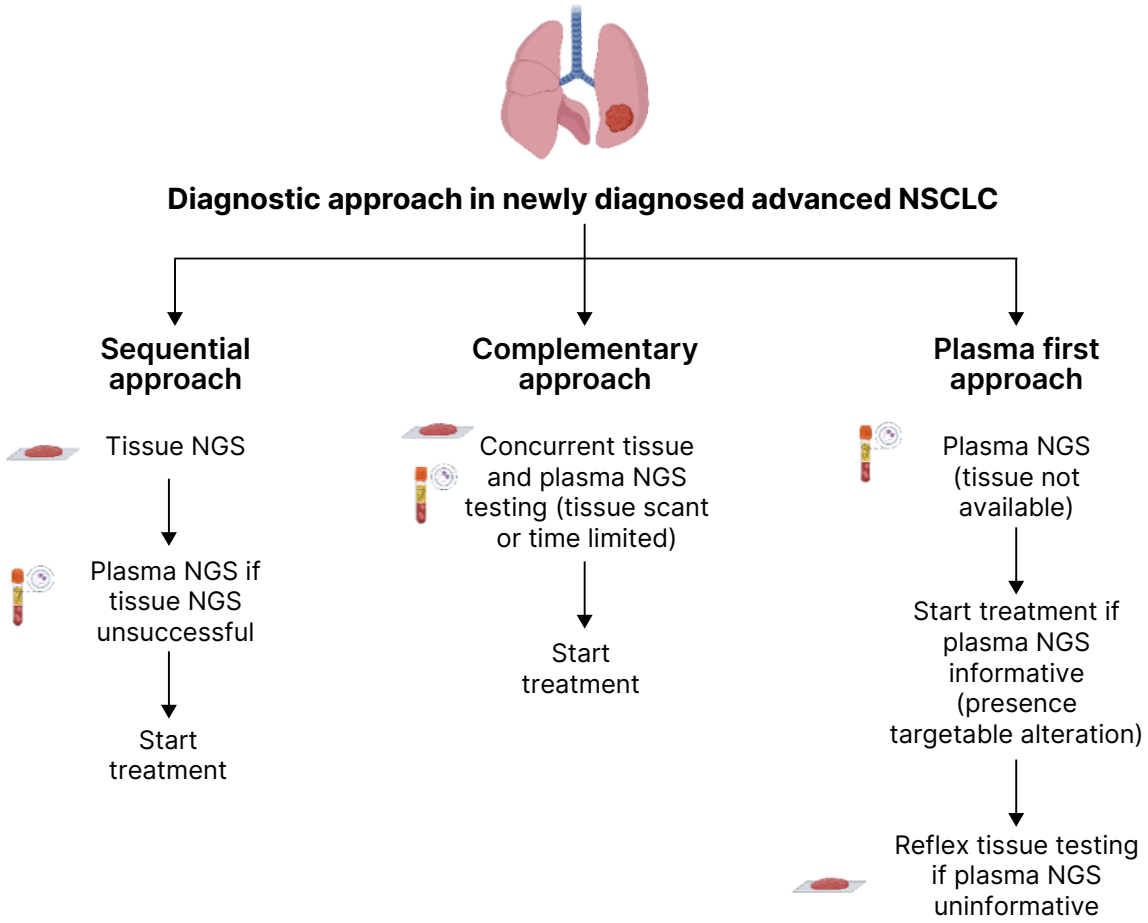

Figure 1. Liquid biopsy approaches for patients with newly diagnosed advanced NSCLC; *created with BioRender.com.*

Abbreviations: NGS: next-generation sequencing; **NSCLC:** non-small cell lung cancer.

to significantly accelerate time to treatment by approximately 35–45% across multiple studies.[9-12]

Liquid Biopsy to Detect Molecular Resistance

Liquid biopsy, specifically plasma ctDNA NGS testing, can be used to detect genomic mechanisms of resistance (MOR) after lung cancer progression on targeted therapy. As tumours evolve, novel genetic alterations and subclonal populations can emerge. Liquid biopsy provides a more comprehensive representation of tumour heterogeneity than single-site tumour tissue biopsies and can prevent repeat tumour biopsy if the plasma result is informative.[13] Initial testing for the epidermal growth factor receptor (EGFR) mutation *EGFR* T790M with liquid biopsy after treatment with first- or second-generation tyrosine kinase inhibitors (TKIs) (e.g. gefitinib, afatinib) is recommended by international guidelines to identify patients that may benefit from third-generation TKIs (e.g. osimertinib).[1-4] Studies have shown that up to 60% of patients may be spared from repeat tumour biopsy using a plasma-first approach.[14]

With the recent shift to the use of third-generation TKIs as initial treatment, molecular resistance to treatment has become more complex.[15] However, both on-target and off-target molecular bypass pathways (e.g. C797S or G724 mutations), *MET* amplification, or emergent fusions may contribute to resistance. Similarly, in *ALK*- and *ROS1*-driven lung cancers, specific resistance mutations, such as *ALK* G1202R or *ROS1* G2302R, may be detected in plasma and direct the use of more specific inhibitors of resistance mutations (e.g. lorlatinib, repotrectinib). Caveats to this

approach include the lower sensitivity of plasma testing than tumour (e.g. *MET* amplification), and the need for tissue to diagnose histologic transformation. However, repeat tumour biopsy and successful tissue NGS after progression on osimertinib are not possible in many patients, supporting a complementary approach.[16]

Liquid Biopsy to Resolve Diagnostic Uncertainty

Interpreting diagnostic imaging in the setting of potential recurrence or progression can be challenging. For example, ground glass changes, parenchymal thickening, and growing atelectatic lesions may be related to cancer progression or treatment complications, such as pneumonitis and post-surgical or post-radiation change. While obtaining pathologic confirmation via biopsy or other invasive methods is the gold standard, liquid biopsy may help resolve diagnostic uncertainty. For example, for patients with tumours with oncogene addiction, a liquid biopsy of ctDNA may detect the return of the original mutation or the appearance of a resistance mutation. The TRACERx study in patients with early-stage lung cancer demonstrated that ctDNA using a tumour-informed assay predicted relapse in 79% of equivocal cases with lymph node enlargement on imaging.[17] Further validation of this approach will help facilitate its routine clinical use.

Emerging Uses - Liquid Biopsy for Treatment Monitoring and Minimal Residual Disease (MRD) Detection

Monitoring Treatment Response in Advanced Disease

Liquid biopsy using plasma is an ideally suited disease monitoring approach during treatment. The presence of plasma ctDNA is a strong prognostic marker across all stages of the disease, with higher levels corresponding with greater tumour burden, greater metastatic potential, and worse prognosis.[18] Clearance or reduction of ctDNA levels is also prognostic, as it is associated with therapy response and better outcomes in advanced and early stages of lung cancer treated with all types of therapy available.[19-21]

The APPLE trial explored the utility of serial monitoring of T790M using plasma ctDNA in patients with advanced EGFR-mutant NSCLC. Molecular progression was identified in 17% of patients in the plasma monitoring arm before radiologic progression, and this early detection of T790M enabled a timely switch from gefitinib to osimertinib.[22] However, median progression-free and overall survival were not significantly different between the arms and the impact of early switching on patient quality of life or symptoms has not been reported.

The optimal cut-off for changes in ctDNA levels to initiate treatment modifications remains under exploration.[23] Studies using adaptive designs to escalate treatment based on ctDNA response after initial therapy are underway. Yu *et al*. are leading a study in which patients that do not clear ctDNA after initial osimertinib are randomized to continue osimertinib alone or add chemotherapy (NCT04410796). Anagnastou *et al*. are studying patients receiving initial pembrolizumab, randomizing those without molecular response to continue immunotherapy alone or add chemotherapy (NCT04093167).

Use of Minimal Residual Disease (MRD) in Early-Stage NSCLC

The detection of ctDNA in plasma, either pre- or post-curative therapy, is a strong prognostic marker in early-stage NSCLC. Chauduri *et al*. demonstrated that MRD detection by ctDNA precedes radiographic detection by a median of 5.2 months in 72% of patients, which was confirmed by other studies.[19]

However, the clinical utility of using MRD to guide treatment decisions remains uncertain. In the adjuvant setting, ctDNA detection using sensitive tumour-informed assays post-surgery is prognostic but cannot identify a population that does not require adjuvant therapy.[24] Clearance of ctDNA after preoperative chemo-immunotherapy has been shown to strongly associate with pathologic complete response (pCR) in several studies, although it may not be specific enough as a single predictive variable.[25,26] Withholding further adjuvant treatment from those that achieve ctDNA clearance has not yet been shown to be safe. Ongoing studies examine the utility of escalating adjuvant therapy in patients with resected Stage NSCLC (NCT04966663) and de-escalating therapy in those with Stage II NSCLC.

Limitations of Liquid Biopsy

Despite the many uses of liquid biopsy in lung cancer, some limitations have yet to be overcome. The lower sensitivity of ctDNA NGS

compared to tumour tissue NGS is the main challenge for the current clinical use of liquid biopsy in lung cancer.[5] In cases with negative ctDNA NGS results, performing additional tissue NGS is recommended. Plasma testing is also less sensitive for the assessment of certain genomic alterations, such as fusions and copy number gain (e.g., *MET* amplification), and the use of RNA-based assays and switching to tissue testing in the case of negative results are recommended.

False-negative results with liquid biopsy are most commonly associated with low tumour DNA shedding to a level below an assay's technical limit of detection.[1] This is important for patients with low tumour burden (especially in those with <1 cm^3 of solid tumour) and those with minimal tumour shedding (e.g. isolated central nervous system [CNS] metastasis).[27] There are additional considerations in the collection and processing of specimens to ensure that DNA or RNA is not significantly degraded before analysis.[1] False positive results can occur in the context of genomic heterogeneity of the tumour. Somatic mutations from the proliferation of clonal blood cell populations may lead to false positive results, known as clonal hematopoiesis of indeterminate potential (CHiP). These variants can be mistakenly identified as cancer-associated mutations, and should be corrected for by using leukocyte sequencing or bioinformatic methods. Fortunately, CHiP alterations do not overlap with current actionable alterations in lung cancer, although they are relevant in treatment response monitoring and MRD detection.

As the use of liquid biopsy moves to early-stage disease, more sensitive assays will be required, although with improved sensitivity may come with a higher risk of false positive results. This may be overcome through use of tumour-informed assays.[28] However, generating tumour-informed assays requires tissue, time, and greater cost, which may limit uptake in routine clinical use. Novel tumour-informed and uninformed ("off the shelf") assays are under development, including uninformed assays for lung cancer screening.[29]

Cost remains an important barrier to reimbursement and widespread implementation in many countries. Single gene assays performed with droplet digital polymerase chain reaction (ddPCR) are less expensive and faster to perform than broader NGS assays. They can be highly sensitive but have limited application.[30] The increased cost of testing with using NGS may be offset by subsequent treatment costs.[7] In addition, the expertise required for these technologies may further restrict routine clinical uptake, with the need to standardize pre-analytical, analytical, and post-analytical methods to ensure consistency.

Summary

Liquid biopsy is an important tool for clinicians treating patients with lung cancer to ensure access to precision medicine and optimal treatment outcomes. Liquid biopsy using plasma ctDNA testing is now recommended by international guidelines for routine use in advanced treatment-naïve NSCLC and as a triage test in tumours resistant to targeted therapies (**Table 1**). Liquid biopsy has been consistently shown to improve the rate of complete genotyping, lead to faster genomic results, and accelerate time to treatment. These factors, in turn, lead to better patient outcomes, less need for repeat biopsies, and fewer missed opportunities for precision medicine. Guidelines do not yet recommend the use of ctDNA for treatment monitoring, including for MRD in early-stage disease, nor for use in adapting treatment. There is active ongoing research to demonstrate and guide clinical utility in these areas.

Despite the advantages of liquid biopsy, there are limitations, including its lower sensitivity, leading to false-negative results and increased testing costs compared to tissue NGS. As the field of liquid biopsy in lung and other cancers continues to evolve, ongoing research will lead to expanded indications for the utilization of liquid biopsy in routine clinical practice.

Indication for ctDNA use	ESMO	NCCN	IASLC	ASCO
Validated, sensitive ctDNA assays can be used to genotype advanced NSCLC and other advanced cancers	✓	✓	✓	✓
Initial genotyping with ctDNA assays should be considered when tissue is unavailable and/or rapid results are needed	✓	✓	✓	✓
Validated ctDNA assays can be used in TKI resistance (NSCLC)	✓	✓	✓	✓
Caveats – ctDNA assays limited by false-negative results, lower sensitivity for fusions, copy number variants → if negative results, reflex to tissue testing	✓	✓	✓	✓
Use of ctDNA/CTC assays to detect minimal residual disease; treatment monitoring not yet recommended	X	X	X	X

Table 1. Current guideline recommendations for liquid biopsy in lung cancer [1-4]; *courtesy of Nadia Ghazali, BMed and Natasha B. Leighl, MD, MMSc, FRCPC, FASCO*

Abbreviations: ASCO: Americal Society of Clinical Oncology; **CTC:** circulating tumour cell; **ctDNA:** circulating tumour DNA; **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; **TKI:** tyrosine kinase inhibitor.

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