

Review

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Integrating ctDNA testing for *EGFR* analysis in advanced non-small cell lung cancer: strategies for clinical laboratories

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Abstract: Epidermal growth factor receptor gene (*EGFR*) molecular testing is essential for guiding targeted therapies in patients with advanced non-small cell lung cancer (NSCLC). Between 15 and 40 % of patients with NSCLC carry mutations in *EGFR* that are sensitive to tyrosine kinase inhibitors (TKIs). Due to the significant clinical benefits, identifying patients eligible for TKI therapy is crucial for optimizing treatment. While tumor tissue has been considered the gold standard for this testing, adequate material for *EGFR* molecular study cannot be obtained in up to 30 % of patients. In this context, circulating tumor DNA (ctDNA) analysis offers a guideline-recommended non-invasive method to detect *EGFR* mutations. Despite its promise, the widespread adoption of ctDNA analysis faces challenges for integration into clinical practice. This review provides a comprehensive synthesis of current knowledge on the clinical utility of *EGFR* molecular analysis in ctDNA, alongside its relationship with other circulating biomarkers widely implemented in clinical laboratories, such as serum tumor markers (STMs). It delves into the technical considerations, interpretation of results, and other challenges associated with ctDNA analysis, offering valuable insights into its integration into laboratory workflows.

Introduction

Precision oncology has profoundly influenced the management of lung cancer (LC), which was the most frequently diagnosed cancer in 2022, responsible for almost 2.5 million new cases worldwide and the leading cause of cancer-related mortality [1]. Identification of molecular mechanisms involved in LC has led to the development and clinical implementation of targeted therapies, especially for non-small cell lung cancer (NSCLC).

The epidermal growth factor receptor (*EGFR*) gene represents the most prevalent and first targetable oncogenic gene in NSCLC. *EGFR* mutations occur in about 15–40 % of cases. *EGFR* tyrosine kinase inhibitors (*EGFR*-TKIs) have become the first-line systemic therapy for patients with *EGFR*-activating mutations as they lead to improvements in survival and quality of life [2]. Therefore, molecular testing is mandatory for advanced NSCLC to identify patients eligible for *EGFR*-TKI therapy [3].

However, the use of this testing in the clinical routine is often limited by the difficulty in obtaining adequate tumor tissue, which is considered the gold standard for molecular studies. In up to 30 % of LC patients, sufficient material for *EGFR* molecular analysis is unavailable [4]. Additionally, the invasive nature of tissue biopsies makes continuous monitoring impractical. In this context, “liquid biopsy” (LB) strategies have emerged as non-invasive or minimally invasive methods for analyzing tumor characteristics from biological fluids like blood [5]. These methods detect tumor-derived analytes, including circulating tumor DNA (ctDNA), circulating tumor cells, circulating free RNA, and extracellular vesicles.

This review will primarily focus on ctDNA analysis due to its extensive validation and broad acceptance in clinical settings. Analyzing *EGFR* mutations in ctDNA is an established method for identifying patients who would benefit

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from EGFR-TKIs. ctDNA testing is particularly valuable when tissue samples are unavailable or when fast turn-around time (TAT) is needed for urgent treatment decisions. However, the routine implementation of ctDNA analysis in clinical laboratories still faces significant challenges. Laboratory professionals play a crucial role in ensuring the reliability and optimal interpretation of these results, making them essential for successful integration into clinical practice.

This review aims to provide a comprehensive synthesis of current knowledge regarding the clinical utility of *EGFR* molecular analysis in ctDNA. From a clinical laboratory perspective, the review will explore technical aspects, clinical applications, benefits, and ongoing challenges associated with ctDNA analysis, while offering practical guidelines to effectively integrate ctDNA testing into routine practice.

EGFR: structure, signaling and clinical implications

The EGFR is a 170-kDa transmembrane glycoprotein belonging to a family of four cell-surface receptors: (EGFR or HER1), HER2, HER3 and HER4. Early studies on the EGFR pathway were initiated with the discovery of epidermal growth factor (EGF) by Cohen and Levi-Montalcini in 1962 [6, 7]. Since then, biochemical and genetic studies have elucidated its role in regulating cellular growth, detailing the intricate cascade of intracellular signals following ligand-receptor interaction.

The oncoprotein EGFR is composed of three domains: an extracellular domain (rich in carbohydrates that facilitate ligand binding), a transmembrane domain (hydrophobic), and a cytoplasmic domain (with tyrosine kinase activity and phosphorylation sites). Ligands such as EGF and transforming growth factor α (TGF α) can bind to EGFR. Upon ligand binding, EGFR dimerizes, forming homodimers, and heterodimers with HER2 or other family members. This dimerization facilitates autophosphorylation, activating the downstream signaling [8]. The activation of this cascade induces cell proliferation and plays a significant role in cell survival and differentiation.

Therefore, EGFR overexpression or inappropriate activation can contribute to cancer development and progression. Increased EGFR signaling can result from *EGFR* gene amplification, protein overexpression, or specific activating mutations. EGFR is frequently overexpressed in various solid tumors, including lung, head and neck, breast, kidney,

colon, ovarian, prostate, brain, and bladder cancers [9]. This overexpression has been associated with increased tumor aggressiveness and a reduced response to conventional therapies.

In NSCLC, EGFR deregulation is prevalent, characterized by protein overexpression in 85 % of cases, gene mutations in 10–40 %, and gene amplification in approximately 10 %. Protein overexpression (assessed by immunohistochemistry) or gene copy number (assessed by fluorescence *in situ* hybridization) lack consistent prognostic or predictive value. In contrast, the detection of *EGFR* mutations has proven clinical utility as biomarkers for predicting response to EGFR-TKIs [10]. These somatic mutations, which result in constitutive activation of EGFR kinase activity, are more frequent in women, patients with adenocarcinoma, Asian ethnicity, and those who have never smoked. The most common activating *EGFR* mutations in NSCLC are exon 19 deletions (>50 %) followed by the p.L858R point mutation in exon 21 (~40 %), both of which are associated with a positive response to EGFR-TKI therapy. Mutations in exons 18 and 20 account for the remaining 10 % of *EGFR* mutations in NSCLC [11] (Figure 1).

The p.T790M point mutation represents the most common mechanism of acquired resistance, occurring in 50–60 % of patients treated with first- and second-generation EGFR-TKIs. In cases of atypical *EGFR* mutations, treatment response may vary compared to classical mutations. For instance, exon 20 insertions are typically associated with resistance to standard EGFR-TKIs, although the response can be heterogeneous [12, 13].

Methodologies for molecular characterization of *EGFR* gene in circulating tumor DNA

Methodologies for detecting *EGFR* mutations in ctDNA can be categorized into two groups: targeted and non-targeted approaches (as detailed in Table 1). Targeted methods include real-time polymerase chain reaction-based techniques (RT-PCR), the amplification refractory mutation system (ARMS), and digital PCR (dPCR) methods such as droplet digital PCR (ddPCR) and BEAMing (beads, emulsions, amplification, and magnetics). These methods provide excellent specificity but are limited to detecting deletions and point mutations pre-defined in their design.

Regarding analytical sensitivity, ARMS and RT-PCR are known for their fast and precise detection capabilities, with

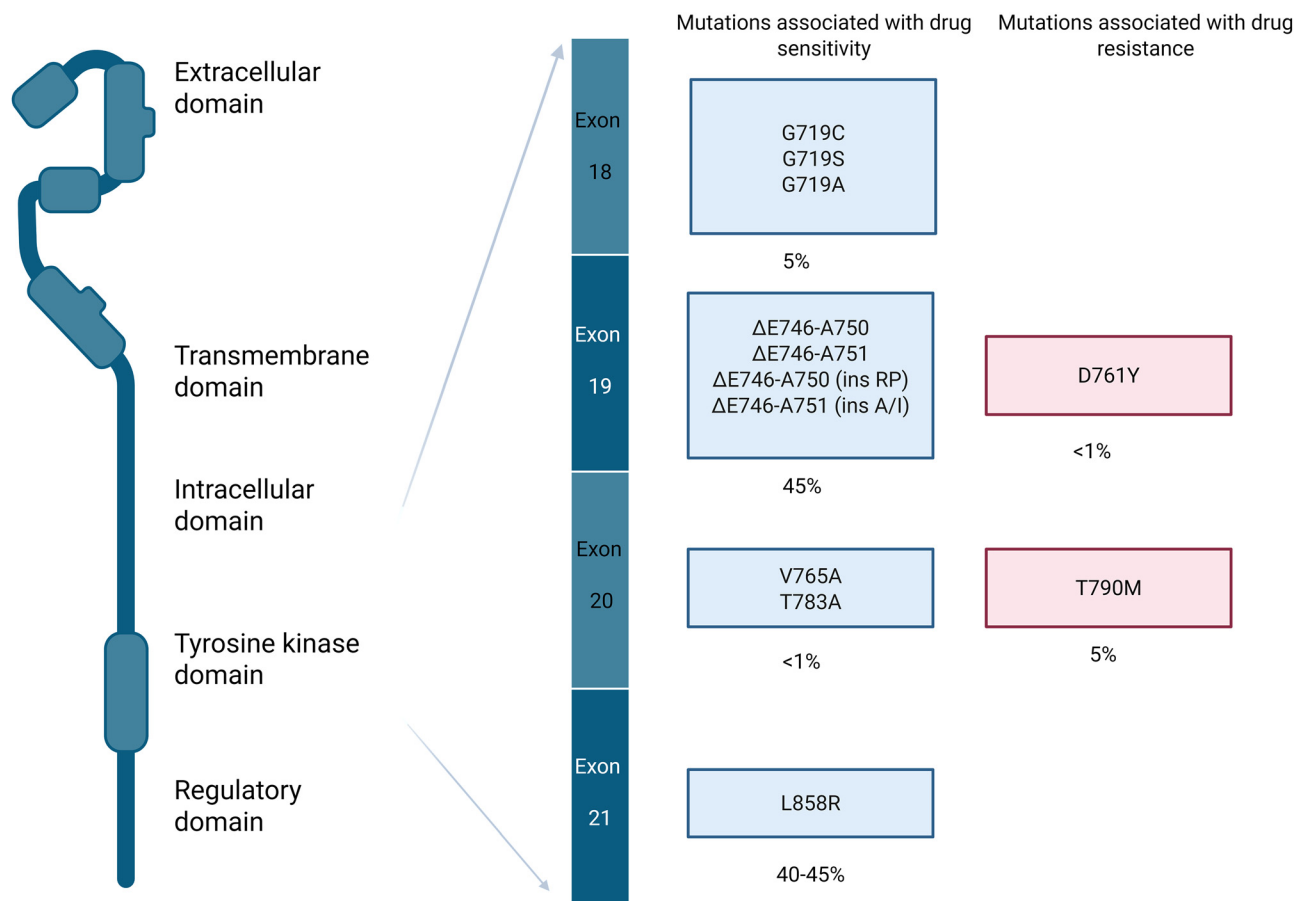


Figure 1: Clinical relevance of key *EGFR* mutations in non-small cell lung cancer. The Figure illustrates the clinical relevance of key *EGFR* mutations in non-small cell lung cancer (NSCLC). Mutations are grouped by their location within specific exons (18–21) and their association with drug sensitivity or resistance. Mutations in blue boxes are linked to increased drug sensitivity to *EGFR* tyrosine kinase inhibitors (TKIs), with their corresponding prevalence percentages noted. Mutations in red boxes are associated with drug resistance. Exon 19 deletions and the L858R mutation in exon 21 are the most prevalent drug-sensitive mutations, while T790M in exon 20 is the most common resistance mutation. The domains of the *EGFR* protein (extracellular, transmembrane, intracellular, tyrosine kinase, and regulatory) are also shown for structural context. Adapted from: Sharma SV, et al.: Epidermal growth factor receptor mutations in lung cancer, *Nat Rev Cancer* 7: 169–181, 2007 and Bai Y, et al.: Molecular genetics of solid tumors, in *Henry's Clinical Diagnosis and Management by Laboratory Methods*, 24th ed, pp. 1579–1587, Elsevier, 2022. Figure created with Biorender.

a limit of detection (LOD) ranging from 0.1 to 5 % in most commercial assays. These methods generate qualitative or semi-quantitative results. In contrast, dPCR offers enhanced sensitivity, achieving a LOD of 0.1–1 % by partitioning the sample into numerous discrete reactions. This approach minimizes background interference and improves the detection of low-abundance mutations. Unlike the previous methods, dPCR provides a direct quantitative result, enabling precise determination of variant allele frequencies (VAF).

Next-generation sequencing (NGS) offers a non-targeted approach that enables comprehensive genomic profiling, allowing for the detection of a wide range of mutations in

EGFR and other relevant genes while providing quantitative results. NGS also allows the identification of novel mutations not detected by targeted assays. Despite historically facing challenges with lower sensitivity and specificity due to the inherent error rates in DNA polymerase and sequencing processes, advancements like deep sequencing, molecular barcoding, and error-correction algorithms have significantly improved their performance. These developments have led to unprecedented levels of sensitivity (LOD from 0.1 to 0.5 %).

In terms of turnaround time (TAT), the broader scope of NGS results in a longer TAT and requires complex computational analysis compared to targeted approaches such as

Table 1: List of methodologies used for *EGFR* molecular analysis in plasma (ctDNA).

Method	Mutations studied	LoD	CE-IVD	FDA	Ref.
Targeted strategies					
RT-PCR/ARMS					
EGFR mutation test v2 (Roche)	47 mutations: Exons 18, 19, 20, 21 + T790M	25–100 copies/mL	Yes	Yes	[14]
Idylla™ ctEGFR mutation assay (Biocartis)	49 mutations: Exons 18, 19, 20, 21 + T790M	<5 %	Yes	No	[15]
Therascreen EGFR plasma RGQ PCR kit (Qiagen)	29 mutations: Deletions Exon 19, L858R, T790M	0.81–17.5 %	Yes	No	[16]
ctDNA EGFR mutation detection kit (entroGen)	58 mutations: Exons 18, 19, 20, 21 + T790M + C797S	0.1–0.5 %	Yes	No	[17]
Easy EGFR (Diatech Pharmacogenetics)	T790M and C797S	<0.5 %	Yes	No	[18]
AmoyDX® EGFR mutation detection test (Amoy diagnostics)	29 mutations: Exons 18, 19, 20, 21 + T790M	1 %	Yes	No	[19]
The SuperARMS EGFR mutation detection kit (Amoy diagnostics)	31 mutations: Exons 18, 19, 20, 21 + T790M	0.2–0.8 %	Yes	No	[20]
PANAMutyper-R-EGFR (Panagene)	29 mutations: Exons 18, 19, 20, 21 + T790M	0.1–0.5 %	Yes	No	[21]
dPCR and beaming					
ddPCR Custom assays (Bio-Rad)	L858R, T790M, C797S, deletions in exon 19	0.1–0.4 %	RUO	No	[22]
ddPCR EGFR Exon 19 deletions screening kit (Bio-Rad)	15 exon 19 deletions	<0.5 %	RUO	No	[23]
Oncobeam EGFR kit v2 (Sysmex)	36 mutations, including T790M and C797S	0.1 %	RUO	No	[24]
Non-targeted Strategies^a					
Oncomine precision assay (OPA) (Thermo Fisher Scientific)	DNA hotspots, CNV and intra-genic fusions	0.1–0.2 %	RUO	No	[25]
Oncomine™ lung ctDNA assay (Thermo Fisher Scientific)	T790M, C797S, L858R, exon 19 del	0.1 %	RUO	No	[26]
AVENIO ctDNA Targeted/Expanded/Surveillance kit v(Roche)	SNV, Indel, CNV	0.5–1 %	RUO	No	[27]
TruSight Oncology 500 ctDNA v2 (Illumina)	Small variants, CNV, DNA fusions	0.2–0.5 %	RUO	No	[28]
Guardant360 CDx (Guardant Health, Inc)	SNV, InDels, CNV	0.2–1.8 %	Yes	Yes	[29]
FoundationOne liquid CDx (Foundation medicine, Roche)	Complete exonic coverage (substitutions, indels) and introns (7, 15, 24–27)	0.4–0.8 %	Yes	Yes	[30]

ARMS, amplification refractory mutation system; CE-IVD, *In Vitro* Diagnostic Medical Device (approved for use in the European Union); CNV, copy number variants; ddPCR, droplet digital PCR; dPCR, digital polymerase chain reaction; FDA, U.S. Food and Drug Administration (approved for clinical use in the United States); Indel, insertion/deletion mutation; LoD, limit of detection; RT-PCR, reverse transcription polymerase chain reaction; RUO, research use only; SNV, single nucleotide variant. Information derived from the manufacturer's specifications. ^aIn broad coverage panels, information regarding mutation types is focused on the *EGFR* gene. The LoD ranges rely on ctDNA input and the type of variant analyzed.

RT-PCR or dPCR. While NGS methods have a TAT of 1–2 weeks, Idylla and other PCR methods have a TAT of approximately seven days [31–33]. In summary, while targeted approaches are highly effective for detecting specific mutations with low detection limits and fast TAT, NGS provides a more comprehensive overview of the mutational landscape.

According to the 2022 report from the European Molecular Quality Network (EMQN), targeted approaches are used by the majority of laboratories, with RT-PCR being the predominant method (51 %). Among these, the cobas® *EGFR* Mutation Test v2 (Roche) was the most commonly employed assay, used by 27 % of all laboratories. This test was the first LB assay approved by the U.S. Food and Drug Administration (FDA) in 2016. Meanwhile, NGS strategies are also commonly adopted, with the Oncomine™ Lung cfDNA Assay (Thermo Fisher Scientific) being the most frequently used panel [34].

The rapid advancement of NGS technologies in recent years has significantly reduced sequencing costs while enhancing accuracy. Moreover, as the list of actionable gene alterations with FDA-approved therapies continues to grow (*EGFR*, *ALK*, *ROS1*, *BRAF*, *RET*, *MET*, *NTRK*, *KRAS*, *HER2*), there is a corresponding increase in the recommendation for broader NGS panels. These panels are not only more cost-effective than sequential single-gene testing in tissue samples but are also gaining prominence in plasma-based testing. The International Association for the Study of Lung Cancer (IASLC) and the European Society for Medical Oncology (ESMO) recommend adopting broad-based plasma ctDNA analysis by NGS, in advanced NSCLC [34–36]. In regions with a high prevalence of *EGFR* mutations, an initial targeted analysis remains pertinent as the first step in molecular testing. However, if a negative result is obtained by single-gene testing, it is advisable to proceed with serial testing for additional actionable biomarkers.

Comparison of plasma ctDNA and tissue samples for *EGFR* molecular analysis

Tumor tissue has historically been considered the gold standard for *EGFR* molecular analysis. However, it has inherent limitations, including its invasive nature and challenges associated with obtaining biopsies from hard-to-reach locations or patients with complex clinical conditions. Additionally, tumor tissue samples may yield insufficient or degraded DNA, complicating the analysis. *EGFR* analysis in ctDNA obtained from plasma has shown high concordance with tissue samples, addressing some of these limitations.

Plasma-based ctDNA analysis has several advantages: it is minimally invasive, demonstrates high concordance with tissue results, offers a rapid TAT and allows for repeated testing over time, providing a more comprehensive representation of tumor heterogeneity and clonal evolution. However, potential drawbacks include the risk of false negatives and higher costs when used alongside tissue testing [35].

When comparing plasma-based ctDNA testing with tumor tissue analysis, studies have shown a high level of concordance for *EGFR* mutations, as well as other guideline-recommended biomarkers [37, 38]. González de Aledo-Castillo et al. reported a concordance of 95.2 % for plasma cfDNA testing using the Cobas® *EGFR* test in NSCLC patients at stages III and IV, compared with tissue analysis. In their prospective study, 39.9 % of patients lacked accessible tumor tissue for molecular characterization. In this scenario, plasma-based *EGFR* testing facilitated therapy initiation in 34.8 % of these patients, emphasizing the utility of ctDNA when tissue biopsy is not feasible [14].

Regarding the analytical performance, plasma-based ctDNA analysis generally demonstrates high specificity across platforms (>96 %). However, sensitivity varies considerably (58–100 %), depending on the platform and the mutations studied [35]. For instance, T790M mutations are typically detected at lower frequencies in plasma ctDNA compared to the primary sensitizing mutations (L858R or exon 19 deletions), leading to reduced sensitivity (41–90.5 %) [35]. A recent meta-analysis reported a pooled sensitivity of 68 % (95 % CI=60–75 %) and specificity of 98 % (95 % CI=95–99 %) [39].

Another significant advantage of plasma-based ctDNA analysis is the shorter TAT compared with tissue genotyping, which has a median TAT of 12 days (range 1–54 days) [40, 41]. Overall, employing a plasma-based strategy, significantly improves the availability of test results before the initial patient visit (85 vs. 9 %, $p<0.0001$), thus reducing time-to-treatment for patients with advanced NSCLC [42].

Plasma-based ctDNA analysis also offers the possibility of monitoring treatment response and disease progression longitudinally in patients receiving *EGFR*-TKIs [43]. Tissue re-biopsies can be risky due to the patient's health status or tumor location; plasma analysis is a safer alternative to assess *EGFR* mutation status during treatment [44]. Additionally, ctDNA analysis can provide valuable insights into the mechanisms of resistance, such as *MET* amplification, revealing tumor heterogeneity and clonal evolution in osimertinib-resistant cases with *EGFR* mutations (AURA3 trial, NCT02151981) [45, 46]. Nevertheless, it is important to note that ctDNA analysis may not identify all resistance mechanisms, such as histologic transitions, which require morphological examination of tissue.

False negatives remain the weakness of ctDNA analysis; tissue biopsy remains necessary when LB tests yield negative results. Sensitivity may fluctuate based on both analytical and biological factors. Like other circulating markers, such as serum tumor markers (STMs), the biological characteristics of the tumor – such as stage, tumor burden, and vascularization – can influence the amount of ctDNA released into circulation, which impacts sensitivity. Tumors with low ctDNA shedding are observed in 15–32 % of NSCLC patients [47], and sensitivity is further reduced in early-stage NSCLC, dropping below 30 % [48]. False negative results can also arise from technique's limitations, underscoring the increasing focus on high-sensitive methods like NGS. Some studies have explored alternative matrices, such as bronchial washing fluid, which has shown significantly higher sensitivity for detecting *EGFR* mutations than plasma [49, 50]. False positives are uncommon but can occur, particularly when using broad-spectrum methods like NGS, which analyze multiple genes. These false positives can arise from germline variants or non-tumor sources such as clonal hematopoiesis of indeterminate potential (CHIP). While CHIP can influence mutations in genes like *TP53* and *KRAS*, it does not notably impact the analysis of *EGFR* mutations [51].

In conclusion, *EGFR* analysis in plasmatic ctDNA offers significant advantages in terms of non-invasiveness, accessibility, and monitoring capability, but it also has limitations. Plasma ctDNA analysis does not replace tissue genotyping and should be considered a complementary tool in the therapeutic decision-making for advanced NSCLC.

Preanalytical and postanalytical factors

The pre-analytical phase plays a critical role in influencing cfDNA results. Adhering to established guidelines for sample collection, handling, transport, and cfDNA extraction is essential to achieve optimal standardization and reliability [52]. The main challenges affecting the quality of cfDNA analysis include: (1) the heterogeneous content of blood, which complicates cfDNA isolation, (2) enzymatic degradation and clotting, (3) the instability of naked DNA in biological environments, (4) contamination of cfDNA by genomic DNA (gDNA), and (5) the difficulty in detecting the small, specific fraction of ctDNA within the cfDNA pool.

Regarding cfDNA isolation, plasma is the preferred specimen over serum to avoid contamination with gDNA from disrupted leukocytes during clotting [53]. Blood should be collected in tubes with cell stabilizer (e.g. Streck®) or

EDTA, which is the recommended anticoagulant. When EDTA is used, plasma isolation should be performed within 4 h [53]. When samples need to be transported to different centers, it is preferable to use commercial cell stabilizer tubes, as they prevent blood cell lysis and allow processing times to be extended for several days at room temperature. A two-step plasma centrifugation process is recommended: first, low-speed centrifugation to concentrate blood cells, followed by high-speed centrifugation to remove cell debris. For the extraction method, ready-to-use automated kits are preferred for routine applications [52]. It is also advised to perform quality control on the cfDNA extract to assess both cfDNA quantity and fragmentation levels [54].

In the post-analytical phase, the interpretation and reporting of *EGFR* variants in ctDNA should be consistent with standard criteria for somatic variant interpretation [55, 56]. It is also crucial to consider the unique characteristics of ctDNA and adhere to specific guidelines for ctDNA analysis [37, 57], ensuring accurate detection and reporting of *EGFR* mutations relevant to NSCLC.

Molecular reports for cfDNA should be clear and concise, emphasizing clinically significant information. Genetic alterations should be described using standardized nomenclature as per Human Genome Variation Society (HGVS) guidelines (<http://varnomen.hgvs.org>). Additionally, reports should include simplified, colloquial terms to enhance clarity and understanding. For instance, an *EGFR* gene alteration should be listed as “c.2369C>T (p.Thr790Met)” and can optionally be described as the “T790M mutation”. Reports should provide essential details to guide clinical decision-making, emphasizing the clinical significance of identified variants concerning FDA/EMA-approved therapies. Key methodological information should be included at the end of the report: the alterations tested, limitations, allelic frequencies, coverage, and other pertinent technical data. The language used in reporting should highlight the possibility of discrepancies with tumor testing, particularly in situations where a variant is not identified in plasma ctDNA [37]. Considering the potential risk of false negatives, *EGFR* results in ctDNA should not be reported merely as negative. Instead, they should be described as “non-informative” or “not detected”. This terminology reflects the possibility that an *EGFR* mutation may be present in the tissue but not detectable in the plasma due to biological factors or technical limitations.

Finally, Molecular Tumor Boards are crucial for interpreting complex cases, requiring the collaboration of multidisciplinary experts. These boards ensure a thorough evaluation of molecular findings, with laboratory professionals playing a crucial role in ensuring the correct interpretation of molecular alterations, especially when addressing variants of uncertain significance or conflicting results.

Clinical utility and guideline recommendations across diverse clinical scenarios

National and international guidelines recommend ctDNA genotyping as a surrogate for tissue genotyping in the molecular analysis of *EGFR* and other relevant biomarkers in advanced NSCLC [58, 59]. This approach is advised when tissue samples are unavailable, both for initial diagnosis and for monitoring resistance. Decision algorithms, provided by organizations such as IASLC, help guide the selection of plasma- or tissue-based testing strategies. IASLC recommends a “plasma-first” strategy when a tissue sample is unavailable, a “complementary approach” when tissue is available but is scant or of uncertain adequacy for genotyping, and a “sequential approach” when tissue genotyping is incomplete [35].

By contrast, tissue-based testing remains the preferred method for early stages NSCLC (stages I–III), which accounts for 25–30 % of patients. Osimertinib, a third-generation *EGFR*-TKI, was approved by the FDA in 2020 to reduce the recurrence rate post-surgery in patients with stage IB to IIIA NSCLC with *EGFR* activating mutations (*EGFR*+), based on the ADAURA trial, which demonstrated significantly improved disease-free survival (DFS) [60]. In the neo-adjuvant setting, anti-*EGFR* therapies may reduce tumor size and improve resection rates, though, ongoing trials like NeoADAURA are critical to further refining treatment strategies in this context.

In early-stage NSCLC, ctDNA is not routinely recommended, and its clinical utility remains under investigation. Its potential lies in its value as a prognostic biomarker for assessing the risk of recurrence. Many patients experience recurrence following surgery, and post-surgical cfDNA monitoring, offers an opportunity for earlier detection, enabling prompt intervention or closer follow-up before radiological signs of recurrence appear. In NSCLC-*EGFR*+ patients, the presence or absence of ctDNA before and after surgery is a strong prognostic indicator, with ctDNA negativity or clearance being associated with better DFS. Notably, most patients with undetectable minimal residual disease through longitudinal ctDNA monitoring did not experience recurrence [61].

In the screening context, there is a critical need for non-invasive screening methods to improve early detection of LC in high-risk and general populations. Research into circulating biomarkers, including proteins, autoantibodies, gene expression profiles, and microRNAs, shows promise for early detection and distinguishing cancer from non-

cancerous nodules [62]. Currently, none of these approaches are approved for clinical use. Blood tests utilizing LB techniques can identify various surgically resectable cancers by detecting cfDNA mutations in key oncogenes, such as *EGFR*, and analyzing circulating proteins. However, their sensitivity for detecting these alterations in early-stage lung cancer (LC) remains relatively low [63].

Combining different biomarkers and techniques with clinical features offers the most promising strategy to increase detection sensitivity and specificity. A recent study demonstrated that integrating cfDNA fragmentation analysis with clinical risk factors and STM concentration (CEA) significantly enhanced LC detection across various stages and subtypes, including 91 % of stage I/II and 96 % of stage III/IV, at 80 % specificity [64].

Correlation between *EGFR* status and serum tumor markers

Tumoral circulome represents the analysis of genetic biomarkers (cfDNA) in addition to other tumor-derived biomarkers, such as proteins and other molecules released into circulation. This novel multi-omic approach is gaining interest in the precision oncology field [65]. STMs are glycoproteins secreted into the bloodstream from the tumor cells or tumor microenvironment. STMs are routinely measured in clinical laboratories for the management of epithelial neoplasms; however, their clinical utility in LC remains controversial. Prospective studies have shown that combining six STMs (CEA, CYFRA 21.1, CA 15.3, SCC, NSE, and ProGRP) with clinical parameters represents the most accurate approach for detecting LC in symptomatic patients [66]. Additionally, recent findings indicate that integrating STM with ctDNA analysis is a promising strategy for early diagnosis of LC [63, 67]. The combination of STMs and ctDNA has predictive value, with elevated levels being linked to poorer prognosis and shorter progression-free survival following *EGFR*-TKI treatment [68]. However, there is currently insufficient evidence to include none of these blood biomarkers in clinical guidelines for LC diagnosis.

Regarding molecular features, several STMs have been linked to *EGFR* mutation status or other molecular alterations, as summarized in Table 2. Most studies have found an association between elevated levels of CEA and CA 15-3, and/or decreased levels of CYFRA 21-1 and SCC, with *EGFR* mutations [69–75]. This aligns with evidence suggesting that specific markers predict certain histology: CEA and CA 15-3 for adenocarcinoma, where *EGFR* mutations are more prevalent, and CYFRA 21-1 and SCC for squamous cell

Table 2: Summary of studies evaluating the association between serum tumor markers and *EGFR* molecular status.

Title	Cohort	STMs	Main conclusions	Year	Ref
Distinction of <i>ALK</i> fusion gene and <i>EGFR</i> mutation-positive lung cancer with tumor markers	306 LC Stage III/IV 43.8 % <i>ALK</i> + 56.2 % <i>EGFR</i> +	CEA CYFRA 21-1	Higher proportion of <i>ALK</i> + patients were CYFRA21-1 positive Higher CYFRA 21-1: CEA ratios were observed in <i>ALK</i> + patients compared to <i>EGFR</i> + patients.	2024	[69]
The predictive value of serum tumor markers for <i>EGFR</i> mutation in non-small cell lung cancer patients with non-stage IA	6711 NSCLC Stage IA and non-stage IA 57.9 % <i>EGFR</i> +	CEA CYFRA 21-1 SCC	Significant associations with <i>EGFR</i> mutations in non-stage IA. No STM predictors in stage IA. Combination of STMs with clinical factors effectively predicts <i>EGFR</i> mutations.	2024	[70]
Dynamic monitoring serum tumor markers to predict molecular features of <i>EGFR</i> -mutated LC during targeted therapy	303 LC Stages III–IV 43 % <i>EGFR</i> +	CEA CYFRA 21-1 NSE CA125 CA153 SCC ctDNA	<i>EGFR</i> mutations: Significantly associated with female gender, abnormal CEA and CA153 levels, and normal SCC level. T790M mutation: More common in patients with abnormal CEA levels. Baseline STM levels and variations: May suggest secondary T790M mutation	2022	[71]
Establishment and evaluation of <i>EGFR</i> mutation prediction model based on Tumor markers and CT features in NSCLC	148 NSCLC Stages I–IV 51 % <i>EGFR</i> +	CEA CYFRA 21-1 NSE CA 125 CA 19.9	Predictive factors for <i>EGFR</i> mutation: Non-smoking status, high CEA and low CYFRA21-1 CYFRA21-1 levels: Higher in the wild-type group, CA 19.9 levels: Higher in the <i>EGFR</i> mutation group.	2022	[72]
Value of serum tumor markers for predicting <i>EGFR</i> mutations and positive <i>ALK</i> expression in 1089 Chinese non-small-cell lung cancer patients: A retrospective analysis	1089 NSCLC <i>EGFR</i> status 50.1 % <i>EGFR</i> +(1088 evaluated)	CEA CA 125 SCC CYFRA 21-1 FERR	<i>EGFR</i> mutations associated with ADC, never-smoker status, and negative CA 125, SCC, FERR, CYFRA 21-1. Multivariate analysis demonstrated that ADC, never-smoker status, and negative CA 125 and SCC results were predictors of <i>EGFR</i> mutations	2020	[73]
Value of serum tumor markers for predicting <i>EGFR</i> mutations in non-small cell lung cancer patients	143 NSCLC 44.06 % <i>EGFR</i> +	CEA CYFRA 21-1 NSE SCC ProGRP	STMs are associated with mutant <i>EGFR</i> status and could be integrated with other clinical factors to facilitate the classification of <i>EGFR</i> mutation status among NSCLC patients. Univariate logistic regression analysis showed that gender, smoking status, histological type, SCC, and proGRP levels were significantly correlated with <i>EGFR</i> mutations.	2020	[80]
Combining PET/CT with serum tumor markers to improve the evaluation of histological type of suspicious lung cancers	201 LC Stages I–IV 50 % <i>EGFR</i> +(16 evaluated)	CEA CYFRA 21-1 NSE SCC	No significant difference between different <i>EGFR</i> mutation statuses in SUVmax, CEA, CYFRA 21-1, SCC-Ag or NSE	2017	[76]
Predictive and prognostic value of CYFRA 21-1 for advanced non-small cell lung cancer treated with <i>EGFR</i> -TKIs	95 NSCLC Stages IIB–IV 57 % <i>EGFR</i> +	CEA CYFRA 21-1	Serum CYFRA 21-1 level may be a predictive factor for patients with NSCLC treated with <i>EGFR</i> -TKIs, regardless of <i>EGFR</i> mutation status. Elevated serum CYFRA 21-1 was associated with shorter PFS and OS of patients with NSCLC treated with <i>EGFR</i> -TKI.	2017	[79]
Correlation between <i>EGFR</i> gene mutation, cytologic tumor markers, 18F-FDG uptake in non-small cell lung cancer	61 NSCLC Stages I–IV 49.1 % <i>EGFR</i> +	Serum and cytologic CEA CYFRA 21-1 SCC	No significant difference in STM levels between wild-type and mutant <i>EGFR</i> . c-CYFRA levels: Significantly higher in patients with <i>EGFR</i> mutations compared to those with wild-type <i>EGFR</i> .	2016	[77]
Predictive and prognostic value of preoperative serum tumor markers is <i>EGFR</i> mutation-specific in resectable non-small-cell lung cancer	1016 NSCLC I–IIIA 25 % <i>EGFR</i> +(979 evaluated)	CEA CYFRA 21-1 SCC NSE	There is no difference in CEA or CYFRA21-1 levels between <i>EGFR</i> -positive and wild-type adenocarcinoma patients. CYFRA21-1 serves as a predictive and prognostic marker in resectable adenocarcinoma patients with <i>EGFR</i> mutations, particularly in the <i>EGFR</i> del19 or L858R groups. CEA is an independent predictive and prognostic factor only for <i>EGFR</i> wild-type adenocarcinoma and <i>EGFR</i> L858R adenocarcinoma patients.	2015	[78]

Table 2: (continued)

Title	Cohort	STMs	Main conclusions	Year	Ref
Monitoring of carcinoembryonic antigen levels is predictive of <i>EGFR</i> mutations and efficacy of <i>EGFR</i> -TKI in patients with lung adenocarcinoma	70 ADC Stage IIA–IV 62.9 % <i>EGFR</i> +	CEA	High-level CEA is independently associated with <i>EGFR</i> gene mutation The variation types of CEA level could help us to predict the efficacy of <i>EGFR</i> -TKI in patients harboring <i>EGFR</i> mutation within only one month of TKI therapy	2014	[74]
Correlation between <i>EGFR</i> mutations and serum tumor markers in lung adenocarcinoma patients	70 ADC Stages I–IV 38.6 % <i>EGFR</i> +	CEA CA 242	Serum CEA and CA242 levels are associated with the presence of <i>EGFR</i> mutations.	2013	[75]

For studies where the stage is not provided, this information is not specified in the cohort section. ADC, adenocarcinoma; ALK, anaplastic lymphoma kinase; CEA, carcinoembryonic antigen; CA125, carbohydrate antigen 125; CA153, carbohydrate antigen 153; CA 242, carbohydrate antigen 242; CYFRA 21-1, cytokeratin-19 fragments; c-CYFRA 21-1, cytologic CYFRA 21.1; ctDNA, circulating tumor DNA; *EGFR*, epidermal growth factor receptor; FERR, ferritin; LC, lung cancer; NSE, neuron-specific enolase; OS, overall survival; PFS, progression free survival; SCC, squamous cell carcinoma antigen; STM, serum tumor markers; TKI, tyrosine kinase inhibitor.

carcinoma [66]. By contrast, other studies found no significant differences between *EGFR* status and STM [76–78]; particularly in early-stage patients where expression in circulation and, therefore, the sensitivity is low [70]. During follow-up of *EGFR*-TKI therapy, dynamic STMs may predict the effectiveness of treatment [79], including the presence of secondary T790M mutation.

All this evidence suggests that STMs could be integrated with other clinical factors to predict *EGFR* status and combined ctDNA + STMs monitoring can offer insights into therapy response, enhancing personalized treatment decisions. However, there is considerable variability among studies highlighting the need for ongoing research into their clinical utility.

A promising strategy can be driven by recent advances in artificial intelligence and machine learning, enabling the efficient combination of markers into multi-marker models, integrating clinical and imaging data. This strategy facilitates the analysis of large volumes of clinical and imaging data to identify patterns that can predict the presence and progression of cancer [81, 82]. These advancements pave the way for the development of clinical decision support tools and open up new opportunities for more effective, personalized cancer treatments.

Conclusions

This review provides practical guidelines for integrating ctDNA analysis into real-world clinical settings. *EGFR* mutations are critical therapeutic targets in advanced NSCLC, and the limited availability of tissue samples highlights the need for non-invasive approaches, such as ctDNA analysis in plasma. Current guidelines recommend ctDNA genotyping as a surrogate for tissue genotyping when tissue samples are

unavailable, with decision algorithms guiding the choice between plasma- or tissue-based testing strategies.

Clinical laboratories play a crucial role in ensuring reliable molecular analysis of *EGFR* in ctDNA, addressing both pre-analytical and post-analytical phases. While targeted methods remain the most common approach in clinical practice due to their robustness and rapid TAT, NGS based methods are becoming increasingly important for more comprehensive mutational profiling. This shift is driven by the evolving landscape of targeted therapies and the growing need for comprehensive testing to identify resistance mechanisms.

Beyond its established role in advanced NSCLC, ctDNA analysis is being explored for earlier stages of the disease. The potential use of *EGFR*-TKI therapies in both neoadjuvant and adjuvant settings highlights the need for non-invasive *EGFR* testing in early-stage NSCLC to guide targeted treatment. While ctDNA analysis has shown prognostic utility post-surgery, its application in early stages and LC screening is currently limited by sensitivity issues. Tumoral circulome analysis, which integrates molecular alterations, cfDNA fragmentation, proteins and other biomolecules released by tumor cells into the circulation, is emerging as a promising non-invasive method. This approach could significantly enhance early detection sensitivity and precision, as well as improve personalized treatment strategies.

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References

- Bray F, Laversanne M, Sung H, Ferlay J, Siegel RL, Soerjomataram I, et al. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 2024;74:229–63.
- Wu Y-L, Zhou C, Liam C-K, Wu G, Liu X, Zhong Z, et al. First-line erlotinib versus gemcitabine/cisplatin in patients with advanced EGFR mutation-positive non-small-cell lung cancer: analyses from the phase III, randomized, open-label, ENSURE study. *Ann Oncol* 2015;26:1883–9.
- Planchard D, Popat S, Kerr K, Novello S, Smit E, Faivre-Finn C, et al. ESMO clinical practice guidelines for mNSCLC. *Ann Oncol* 2019;29:iv192–37.
- Goldman JW, Noor ZS, Remon J, Besse B, Rosenfeld N. Are liquid biopsies a surrogate for tissue EGFR testing? *Ann Oncol* 2018;29:i38–46.
- Arechederra M, Ávila MA, Berasain C. Liquid biopsy for cancer management: a revolutionary but still limited new tool for precision medicine. *Adv Lab Med/Av en Med Lab* 2020;1:20200009.
- Cohen S, Alfred P, Sloan Jr. The epidermal growth factor (EGF). *Award. Cancer* 1983;51:1787–91.
- Cohen S. Isolation of a mouse submaxillary gland protein accelerating incisor eruption and eyelid opening in the new-born animal. *J Biol Chem* 1962;237:1555–62.
- Lewis TS, Shapiro PS, Ahn NG. Signal transduction through MAP kinase cascades. *Adv Cancer Res* 1998;74:49–139.
- Rajaram P, Chandra P, Ticku S, Pallavi B, Rudresh K, Mansabdar P. Epidermal growth factor receptor: role in human cancer. *Indian J Dent Res* 2017;28:687.
- Fukuoka M, Wu Y-L, Thongprasert S, Sunpaweravong P, Leong S-S, Sriuranpong V, et al. Biomarker analyses and final overall survival results from a phase III, randomized, open-label, first-line study of gefitinib versus carboplatin/paclitaxel in clinically selected patients with advanced non-small-cell lung cancer in Asia (IPASS). *J Clin Oncol* 2011;29:2866–74.
- Sharma SV, Bell DW, Settleman J, Haber DA. Epidermal growth factor receptor mutations in lung cancer. *Nat Rev Cancer* 2007;7:169–81.
- Wu JY, Wu SG, Yang CH, Gow CH, Chang YL, Yu CJ, et al. Lung cancer with epidermal growth factor receptor exon 20 mutations is associated with poor gefitinib treatment response. *Clin Cancer Res* 2008;14:4877.
- Robichaux JP, Le X, Vijayan RSK, Hicks JK, Heeke S, Elamin YY, et al. Structure-based classification predicts drug response in EGFR-mutant NSCLC. *Nature* 2021;597:732–7.
- González de Aledo-Castillo JM, Arcocha A, Victoria I, Martínez-Puchol AI, Sánchez C, Jares P, et al. Molecular characterization of advanced non-small cell lung cancer patients by cfDNA analysis: experience from routine laboratory practice. *J Thorac Dis* 2021;13:1658–70.
- Gilson P, Saurel C, Salleron J, Husson M, Demange J, Merlin J-L, et al. Evaluation of the Idylla ctEGFR mutation assay to detect EGFR mutations in plasma from patients with non-small cell lung cancers. *Sci Rep* 2021;11:10470.
- Szpechcinski A, Bryl M, Wojcik P, Czyzewicz G, Wojda E, Rudzinski P, et al. Detection of EGFR mutations in liquid biopsy samples using allele-specific quantitative PCR: a comparative real-world evaluation of two popular diagnostic systems. *Adv Med Sci* 2021;66:336–42.
- Jensen SG, Epistolio S, Madsen CL, Kyneb MH, Riva A, Paganotti A, et al. A new sensitive and fast assay for the detection of EGFR mutations in liquid biopsies. *Chalmers J., editor PLoS One* 2021;16:e0253687.
- Dono M, De Luca G, Lastraioli S, Anselmi G, Dal Bello MG, Coco S, et al. Tag-based next generation sequencing: a feasible and reliable assay for EGFR T790M mutation detection in circulating tumor DNA of non small cell lung cancer patients. *Mol Med* 2019;25:15.
- Amoy Diagnostics. AmoyDx EGFR 29 mutations detection kit ADAE 29 mutations detection kit. AD. Available from: <https://www.amoydiagnostics.com/products/amoydx-egfr-29-mutations-detection-kit> [Accessed 23 Mar 2024].
- Cui S, Ye L, Wang H, Chu T, Zhao Y, Gu A, et al. Use of SuperARMS EGFR mutation detection kit to detect EGFR in plasma cell-free DNA of patients with lung adenocarcinoma. *Clin Lung Cancer* 2018;19:e313–22.
- Han AL, Kim HR, Choi KH, Hwang KE, Zhu M, Huang Y, et al. Comparison of cobas EGFR mutation test v2 and PANAMutyper-R-EGFR for detection and semi-quantification of epidermal growth factor receptor mutations in plasma and pleural effusion supernatant. *Ann Lab Med* 2019;39:478–87.
- Zhu G, Ye X, Dong Z, Lu YC, Sun Y, Liu Y, et al. Highly sensitive droplet digital PCR method for detection of EGFR-activating mutations in plasma cell-free DNA from patients with advanced non-small cell lung cancer. *J Mol Diagn* 2015;17:265–72.
- Bio-Rad Laboratories. ddPCR EGFR exon 19 deletions screening kit. Available from: <https://www.bio-rad.com/es-es/sku/12002392-ddpcr-egfr-exon-19-deletions-screening-kit?ID=12002392>.
- Romero A, Jantus-Lewintre E, García-Peláez B, Royuela A, Insa A, Cruz P, et al. Comprehensive cross-platform comparison of methods for non-invasive EGFR mutation testing: results of the RING observational trial. *Mol Oncol* 2021;15:43–56.
- Low S-K, Ariyasu R, Uchibori K, Hayashi R, Chan HT, Chin YM, et al. Rapid genomic profiling of circulating tumor DNA in non-small cell lung cancer using OncoPrint Precision Assay with Genexus™ integrated sequencer. *Transl Lung Cancer Res* 2022;11:711–21.
- De Luca G, Lastraioli S, Conte R, Mora M, Genova C, Rossi G, et al. Performance of the OncoPrint™ lung cfDNA assay for liquid biopsy by NGS of NSCLC patients in routine laboratory practice. *Appl Sci* 2020;10:2895.
- Schouten RD, Vessies DCL, Bosch LJW, Barlo NP, van Lindert ASR, Cillessen SAGM, et al. Clinical utility of plasma-based comprehensive molecular profiling in advanced non-small-cell lung cancer. *JCO Precis Oncol* 2021;5:1112–21.
- Harter J, Buth E, Johaenning J, Battke F, Kopp M, Zelba H, et al. Analytical performance evaluation of a 523-gene circulating tumor DNA assay for next-generation sequencing-based comprehensive tumor profiling in liquid biopsy samples. *J Mol Diagn* 2024;26:61–72.
- Baumli JM, Li BT, Velcheti V, Govindan R, Curioni-Fontecedro A, Dooms C, et al. Clinical validation of Guardant360 CDx as a blood-based companion diagnostic for sotorasib. *Lung Cancer* 2022;166:270–8.
- Woodhouse R, Li M, Hughes J, Delfosse D, Skoletsky J, Ma P, et al. Clinical and analytical validation of FoundationOne Liquid CDx, a novel 324-Gene cfDNA-based comprehensive genomic profiling assay for cancers of solid tumor origin. *PLoS One* 2020;15:e0237802.
- Sharma S, Satapathy A, Aggarwal A, Dewan A, Jain E, Katara R, et al. Comparison of epidermal growth factor receptor mutation detection turnaround times and concordance among real-time polymerase chain reaction, high-throughput next-generation sequencing and the

- Biocartis Idylla™ platforms in non-small cell lung carcinomas. *Pathol Res Pract* 2021;220:153394.
32. Behnke A, Cayre A, De Maglio G, Giannini G, Habran L, Tarsitano M, et al. FACILITATE: a real-world, multicenter, prospective study investigating the utility of a rapid, fully automated real-time PCR assay versus local reference methods for detecting epidermal growth factor receptor variants in NSCLC. *Pathol Oncol Res* 2023;29:1610707.
 33. Dagogo-Jack I, Azzolli CG, Fintelmann F, Mino-Kenudson M, Farago AF, Gainor JF, et al. Clinical utility of rapid EGFR genotyping in advanced lung cancer. *JCO Precis Oncol* 2018;2:1–13.
 34. Fairley JA, Cheetham MH, Patton SJ, Rouleau E, Denis M, Dequeker EMC, et al. Results of a worldwide external quality assessment of cfDNA testing in lung Cancer. *BMC Cancer* 2022;22:759.
 35. Rolfo C, Mack PC, Scagliotti GV, Baas P, Barlesi F, Bivona TG, et al. Liquid biopsy for advanced non-small cell lung cancer (NSCLC): a statement paper from the IASLC. *J Thorac Oncol* 2018;13:1248–68.
 36. Mosele F, Remon J, Mateo J, Westphalen CB, Barlesi F, Lolkema MP, et al. Recommendations for the use of next-generation sequencing (NGS) for patients with metastatic cancers: a report from the ESMO Precision Medicine Working Group. *Ann Oncol* 2020;31:1491–505.
 37. Pascual J, Attard G, Bidard F-C, Curigliano G, De Mattos-Arruda L, Diehn M, et al. ESMO recommendations on the use of circulating tumour DNA assays for patients with cancer: a report from the ESMO Precision Medicine Working Group. *Ann Oncol* 2022;33:750–68.
 38. Raez LE, Brice K, Dumais K, Lopez-Cohen A, Wietecha D, Izquierdo PA, et al. Liquid biopsy versus tissue biopsy to determine front line therapy in metastatic non-small cell lung cancer (NSCLC). *Clin Lung Cancer* 2023;24:120–9.
 39. Wang N, Zhang X, Wang F, Zhang M, Sun B, Yin W, et al. The diagnostic accuracy of liquid biopsy in EGFR-mutated NSCLC: a systematic review and meta-analysis of 40 studies. *SLAS Technol* 2021;26:42–54.
 40. Sacher AG, Paweletz C, Dahlberg SE, Alden RS, O’Connell A, Feeney N, et al. Prospective validation of rapid plasma genotyping for the detection of EGFR and KRAS mutations in advanced lung cancer. *JAMA Oncol* 2016;2:1014.
 41. Lee Y, Clark EW, Milan MSD, Champagne C, Michael KS, Awad MM, et al. Turnaround time of plasma next-generation sequencing in thoracic oncology patients: a quality improvement analysis. *JCO Precis Oncol* 2020:1098–108. <https://doi.org/10.1200/po.20.00121>.
 42. Thompson JC, Aggarwal C, Wong J, Nimgaonkar V, Hwang W-T, Andronov M, et al. Plasma genotyping at the time of diagnostic tissue biopsy decreases time-to-treatment in patients with advanced NSCLC – results from a prospective pilot study. *JTO Clin Res Rep* 2022;3:100301.
 43. Ho H-L, Jiang Y, Chiang C-L, Karwowska S, Yerram R, Sharma K, et al. Efficacy of liquid biopsy for disease monitoring and early prediction of tumor progression in EGFR mutation-positive non-small cell lung cancer. *Lin C-C., editor PLoS One* 2022;17:e0267362.
 44. Hou T, Zeng J, Xu H, Su S, Ye J, Li Y. Performance of different methods for detecting T790M mutation in the plasma of patients with advanced NSCLC after developing resistance to first-generation EGFR-TKIs in a real-world clinical setting. *Mol Clin Oncol* 2022;16:88.
 45. Zheng X, Zhang G, Li P, Zhang M, Yan X, Zhang X, et al. Mutation tracking of a patient with EGFR-mutant lung cancer harboring de novo MET amplification: successful treatment with gefitinib and crizotinib. *Lung Cancer* 2019;129:72–4.
 46. Deng L, Kiedrowski LA, Ravera E, Cheng H, Halmos B. Response to dual crizotinib and osimertinib treatment in a lung cancer patient with MET amplification detected by liquid biopsy who acquired secondary resistance to EGFR tyrosine kinase inhibition. *J Thorac Oncol* 2018;13:e169–72.
 47. Gray JE, Okamoto I, Sriuranpong V, Vansteenkiste J, Imamura F, Lee JS, et al. Tissue and plasma EGFR mutation analysis in the FLAURA trial: osimertinib versus comparator EGFR tyrosine kinase inhibitor as first-line treatment in patients with EGFR-mutated advanced non-small cell lung cancer. *Clin Cancer Res* 2019;25:6644–52.
 48. Shong LY-W, Deng J-Y, Kwok H-H, Lee NC-M, Tseng SC-Z, Ng L-Y, et al. Detection of EGFR mutations in patients with suspected lung cancer using paired tissue-plasma testing: a prospective comparative study with plasma ddPCR assay. *Sci Rep* 2024;14:25701.
 49. Lee SH, Kim EY, Kim T, Chang YS. Compared to plasma, bronchial washing fluid shows higher diagnostic yields for detecting EGFR-TKI sensitizing mutations by ddPCR in lung cancer. *Respir Res* 2020;21:142.
 50. Nair VS, Hui AB-Y, Chabon JJ, Esfahani MS, Stehr H, Nabat BY, et al. Genomic profiling of bronchoalveolar lavage fluid in lung cancer. *Cancer Res* 2022;82:2838–47.
 51. Genovese G, Kähler AK, Handsaker RE, Lindberg J, Rose SA, Bakhoum SF, et al. Clonal hematopoiesis and blood-cancer risk inferred from blood DNA sequence. *N Engl J Med* 2014;371:2477–87.
 52. Meddeb R, Pisareva E, Thierry AR. Guidelines for the preanalytical conditions for analyzing circulating cell-free DNA. *Clin Chem* 2019;65:623–33.
 53. Lee T, Montalvo L, Chrebtow V, Busch MP. Quantitation of genomic DNA in plasma and serum samples: higher concentrations of genomic DNA found in serum than in plasma. *Transfusion* 2001;41:276–82.
 54. Devonshire AS, Whale AS, Gutteridge A, Jones G, Cowen S, Foy CA, et al. Towards standardisation of cell-free DNA measurement in plasma: controls for extraction efficiency, fragment size bias and quantification. *Anal Bioanal Chem* 2014;406:6499–512.
 55. Mehta N, He R, Viswanatha DS. Correspondence on “standards for the classification of pathogenicity of somatic variants in cancer (oncogenicity): joint recommendations of clinical Genome resource (ClinGen), cancer genomics consortium (CGC), and variant interpretation for cancer consortium (VICC)” by Horak et al. *Genet Med* 2022;24:1986–8.
 56. Li MM, Datto M, Duncavage EJ, Kulkarni S, Lindeman NI, Roy S, et al. Standards and guidelines for the interpretation and reporting of sequence variants in cancer: a joint consensus recommendation of the Association for Molecular Pathology, American Society of Clinical Oncology, and College of American Pathologists. *J Mol Diagn* 2017;19:4–23.
 57. Heitzer E, van den Broek D, Denis MG, Hofman P, Hubank M, Mouliere F, et al. Recommendations for a practical implementation of circulating tumor DNA mutation testing in metastatic non-small-cell lung cancer. *ESMO Open* 2022;7:100399.
 58. Majem M, Juan O, Insa A, Reguart N, Trigo JM, Carcereny E, et al. SEOM clinical guidelines for the treatment of non-small cell lung cancer (2018). *Clin Transl Oncol* 2019;21:3–17.
 59. Passaro A, Leighl N, Blackhall F, Popat S, Kerr K, Ahn MJ, et al. ESMO expert consensus statements on the management of EGFR mutant non-small-cell lung cancer. *Ann Oncol* 2022;33:466–87.
 60. Koch AL, Vellanki PJ, Drezner N, Li X, Mishra-Kalyani PS, Shen YL, et al. FDA approval summary: osimertinib for adjuvant treatment of surgically resected non-small cell lung cancer, a collaborative project orbis review. *Clin Cancer Res* 2021;27:6638–43.
 61. Jung H-A, Ku BM, Kim YJ, Park S, Sun J-M, Lee S-H, et al. Longitudinal monitoring of circulating tumor DNA from plasma in patients with curative resected stages I to IIIA EGFR-mutant non-small cell lung cancer. *J Thorac Oncol* 2023;18:1199–208.

62. Seijo LM, Peled N, Ajona D, Boeri M, Field JK, Sozzi G, et al. Biomarkers in lung cancer screening: achievements, promises, and challenges. *J Thorac Oncol* 2019;14:343–57.
63. Cohen JD, Li L, Wang Y, Thoburn C, Afsari B, Danilova L, et al. Detection and localization of surgically resectable cancers with a multi-analyte blood test. *Science* 2018;359:926–30.
64. Mathios D, Johansen JS, Cristiano S, Medina JE, Phallen J, Larsen KR, et al. Detection and characterization of lung cancer using cell-free DNA fragmentomes. *Nat Commun* 2021;12:5060.
65. Wu J, Hu S, Zhang L, Xin J, Sun C, Wang L, et al. Tumor circulome in the liquid biopsies for cancer diagnosis and prognosis. *Theranostics* 2020;10:4544–56.
66. Molina R, Marrades RM, Augé JM, Escudero JM, Viñolas N, Reguart N, et al. Assessment of a combined panel of six serum tumor markers for lung cancer. *Am J Respir Crit Care Med* 2016;193:427–37.
67. Ren S, Zeng G, Yi Y, Liu L, Tu H, Chai T, et al. Combinations of plasma cfDNA concentration, integrity and tumor markers are promising biomarkers for early diagnosis of non-small cell lung cancer. *Heliyon* 2023;9:e20851.
68. Zheng J, Wang Y, Hu C, Zhu M, Ii J, Lin C, et al. Predictive value of early kinetics of ctDNA combined with cfDNA and serum CEA for EGFR-TKI treatment in advanced non-small cell lung cancer. *Thorac Cancer* 2022;13:3162–73.
69. Akita T, Ariyasu R, Kakuto S, Miyadera K, Kiritani A, Tsugitomi R, et al. Distinction of ALK fusion gene- and EGFR mutation-positive lung cancer with tumor markers. *Thorac Cancer* 2024;15:788–96.
70. Du W, Qiu T, Liu H, Liu A, Wu Z, Sun X, et al. The predictive value of serum tumor markers for EGFR mutation in non-small cell lung cancer patients with non-stage IA. *Heliyon* 2024;10:e29605.
71. Chen Z, Liu L, Zhu F, Cai X, Zhao Y, Liang P, et al. Dynamic monitoring serum tumor markers to predict molecular features of EGFR-mutated lung cancer during targeted therapy. *Cancer Med* 2022;11:3115–25.
72. Zhang H, He M, Wan R, Zhu L, Chu X. Establishment and evaluation of EGFR mutation prediction model based on tumor markers and CT features in NSCLC. Bhagyaveni MA., editor *J Healthc Eng* 2022;2022:1–6.
73. Wang S, Ma P, Ma G, Lv Z, Wu F, Guo M, et al. Value of serum tumor markers for predicting EGFR mutations and positive ALK expression in 1089 Chinese non-small-cell lung cancer patients: a retrospective analysis. *Eur J Cancer* 2020;124:1–14.
74. Zhang Y, Jin B, Shao M, Dong Y, Lou Y, Huang A, et al. Monitoring of carcinoembryonic antigen levels is predictive of EGFR mutations and efficacy of EGFR-TKI in patients with lung adenocarcinoma. *Tumor Biol* 2014;35:4921–8.
75. Pan J-B, Hou Y-H, Zhang G-J. Correlation between EGFR mutations and serum tumor markers in lung adenocarcinoma patients. *Asian Pac J Cancer Prev* 2013;14:695–700.
76. Jiang R, Dong X, Zhu W, Duan Q, Xue Y, Shen Y, et al. Combining PET/CT with serum tumor markers to improve the evaluation of histological type of suspicious lung cancers. Chen C-T., editor *PLoS One* 2017;12:e0184338.
77. Cho A, Hur J, Moon YW, Hong SR, Suh YJ, Kim YJ, et al. Correlation between EGFR gene mutation, cytologic tumor markers, 18F-FDG uptake in non-small cell lung cancer. *BMC Cancer* 2016;16:224.
78. Zhi Q, Wang Y, Wang X, Yue D, Li K, Jiang R. Predictive and prognostic value of preoperative serum tumor markers in resectable adenosquamous lung carcinoma. *Oncotarget* 2016;7:64798–809.
79. Takeuchi A, Oguri T, Sone K, Ito K, Kitamura Y, Inoue Y, et al. Predictive and prognostic value of CYFRA 21-1 for advanced non-small cell lung cancer treated with EGFR-TKIs. *Anticancer Res* 2017;37:5771–6.
80. Wen L, Wang S, Xu W, Xu X, Li M, Zhang Y, et al. Value of serum tumor markers for predicting EGFR mutations in non-small cell lung cancer patients. *Ann Diagn Pathol* 2020;49:151633.
81. Nené NR, Ney A, Nazarenko T, Blyuss O, Johnston HE, Whitwell HJ, et al. Serum biomarker-based early detection of pancreatic ductal adenocarcinomas with ensemble learning. *Commun Med* 2023;3:10.
82. Capobianco E. High-dimensional role of AI and machine learning in cancer research. *Br J Cancer* 2022;126:523–32.

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