THE EUROPEAN SOCIETY OF MEDICINE
Medical Research Archives, Volume 13 Issue 9

RESEARCH ARTICLE

Efficacy of next generation sequencing-based liquid biopsy for detecting actionable mutations in non-small cell lung cancer patients with limited tissue availability: An observational study

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OPEN ACCESS

PUBLISHED
30 September 2025

CITATION
V, Chetan., Saldanha, SC., et al., 2025. Efficacy of next generation sequencing-based liquid biopsy for detecting actionable mutations in non-small cell lung cancer patients with limited tissue availability: An observational study. Medical Research Archives, [online] 13(9).
https://doi.org/10.18103/mra.v13i9.6986

DOI
https://doi.org/10.18103/mra.v13i9.6986

ISSN
2375-1924

ABSTRACT

Background: Targeted therapy has transformed the management of non-small cell lung cancer by improving survival in patients with identifiable driver mutations. However, tissue biopsies often yield insufficient material for molecular testing, leading to delays in initiating personalized treatment. Liquid biopsy offers a non-invasive alternative for mutation detection.

Methods: This single-center observational study was conducted between February and October 2024 and enrolled 48 patients with newly diagnosed non-small lung cancer who had inadequate tumor tissue for molecular profiling. Peripheral blood samples were analyzed using next-generation sequencing to determine the mutation spectrum. Clinical data, mutational profiles, and treatment outcomes were recorded. Descriptive statistics were used to summarize baseline characteristics, mutation frequencies, and treatment responses.

Results: Circulating tumor Deoxyribonucleic acid analysis identified mutations in 28 of 48 patients (58.3%). Actionable mutations were detected in 20 patients (41.6%), with epidermal growth factor receptor alterations being most frequent, followed by Anaplastic Lymphoma kinase rearrangements and other less common targets. Among patients who received targeted therapy combined with chemotherapy, 14.3% achieved complete metabolic response and 71.4% had partial response at the time of analysis.

Conclusions: Liquid biopsy using next-generation sequencing can reliably identify actionable mutations in patients with non-small cell lung cancer where tissue sampling is inadequate, thereby facilitating timely initiation of personalized therapy in real-world clinical settings.

Keywords: Liquid biopsy, next-generation sequencing, non-small cell lung cancer, actionable mutations

Introduction

Lung cancer remains the most common cause of cancer-related death worldwide, accounting for an estimated 1.8 million deaths annually.[1] Non-small cell lung cancer (NSCLC) constitutes nearly 85% of cases, with the majority of patients presenting at an advanced stage, where prognosis remains poor despite therapeutic advances.[1,2] Globally, lung cancer is the most frequently diagnosed malignancy among men and the second most common among women, following breast cancer.[1,2] In India, the burden of NSCLC has risen steadily, with increasing incidence in both smokers and never-smokers, making it a significant public health concern.[13,14]

Over the past two decades, an improved understanding of tumor biology has revolutionized the management of NSCLC. The identification of oncogenic driver mutations such as alterations in epidermal growth factor receptor (EGFR), anaplastic lymphoma kinase (ALK), ROS proto-oncogene 1 (ROS1), and B-Raf proto-oncogene (BRAF) has enabled the integration of precision medicine into routine practice.[3-6] These mutations define distinct molecular subsets of NSCLC with specific therapeutic vulnerabilities.

- **EGFR mutations** occur in approximately 30% to 50% of Asian NSCLC patients, particularly in never-smokers and adenocarcinoma histology, and are highly sensitive to EGFR tyrosine kinase inhibitors (TKIs) such as gefitinib and osimertinib.[3,4]
- **ALK rearrangements** are present in 3% to 7% of NSCLC cases, often in younger patients and never-smokers. ALK TKIs, including alectinib and lorlatinib, have demonstrated superior efficacy compared with chemotherapy.[5,12]
- **ROS1 fusions** are less common, detected in approximately 1% to 2% of NSCLC cases, but respond well to targeted agents such as crizotinib and entrectinib, leading to durable responses.[7]
- **BRAF mutations**, including the V600E variant, occur in 1% to 3% of NSCLC cases and can be effectively treated with combined BRAF and MEK inhibition, such as dabrafenib plus trametinib.[8]

Consequently, international guidelines recommend comprehensive molecular profiling for all patients with advanced NSCLC to identify actionable mutations and guide optimal treatment strategies.[5,6]

Tissue biopsy remains the standard method for molecular testing; however, in clinical practice, obtaining adequate tumor tissue can be challenging. Small biopsies may be limited by tumor location, patient comorbidities, or technical difficulties, resulting in insufficient material for extended molecular analysis.[7,8] These limitations often delay the initiation of targeted therapy, especially in resource-constrained healthcare systems.

Liquid biopsy, particularly analysis of circulating tumor Deoxyribonucleic acid (ctDNA), offers a promising non-invasive alternative that overcomes many limitations of tissue sampling. With the application of next-generation sequencing (NGS), liquid biopsy enables broad genomic profiling and has demonstrated high concordance with tissue-based testing in multiple international studies.[9-12] Beyond detection of baseline mutations, liquid biopsy also allows for dynamic monitoring of resistance mutations during treatment, highlighting its versatility in precision oncology.[9,10]

However, most evidence supporting the clinical use of liquid biopsy originates from high-income countries. Data from low- and middle-income countries (LMICs), including India, remain sparse, despite unique challenges such as higher prevalence of advanced-stage presentations, limited access to repeat biopsies, and financial barriers to targeted therapies.[13,14] Addressing this evidence gap is crucial to guide integration of liquid biopsy into clinical workflows in these settings.

The present study was conducted to evaluate the clinical utility of NGS-based liquid biopsy for mutation profiling in Indian patients with NSCLC who had insufficient tissue for molecular testing. By generating real-world evidence from a resource-constrained setting, this study aims to contribute to the global understanding of liquid biopsy and support its adoption in regions where traditional biopsy methods are often inadequate.

Methods

PATIENTS

Patients aged 18 years or older with newly diagnosed NSCLC were eligible if they had histologically confirmed adenocarcinoma, squamous cell carcinoma, or NSCLC-not otherwise specified (NSCLC-NOS) and insufficient tumor tissue for molecular profiling. Patients were excluded if they had received prior systemic therapy for NSCLC, had synchronous or metachronous malignancies, or were younger than 18 years.

STUDY DESIGN AND DATA COLLECTION

This was a single-center observational study conducted between February and October 2024. After obtaining written informed consent, eligible patients were enrolled. Baseline clinical information, including demographic characteristics, smoking history, presenting symptoms, histology, stage, and treatment details, was prospectively collected.

NGS TESTING AND MUTATION ANALYSIS

Peripheral blood (10 mL) was obtained from each patient and analyzed using a broad-based next-generation sequencing (NGS) assay (OncoMonitor). The sequencing panel included, but was not limited to, ALK, BRAF, EGFR, ERBB2 (HER2), KRAS, MET, NTRK1/2/3, RET, ROS1, PTEN, PIK3CA, and TP53, with a minimum coverage depth of 10,000×. Variants were considered positive at a variant allele frequency (VAF) of ≥0.1%. The average turnaround time for reporting was three weeks. Patients were permitted to receive one cycle of planned chemotherapy, if clinically indicated, while awaiting NGS results.

OUTCOMES

- **Primary outcome:** Proportion of patients with actionable mutations detected by liquid biopsy NGS.
- **Secondary outcomes:**

- Distribution of actionable and non-actionable mutations across histologic subtypes.
- Description of the mutation spectrum, including co-occurring alterations.
- Feasibility of initiating targeted therapy based on liquid biopsy results.
- Response to therapy in patients treated according to liquid biopsy findings, assessed using **PERCIST 1.0 criteria** (complete metabolic response, partial response, stable disease, progressive disease).
- Mutation-specific treatment outcomes, particularly in patients with EGFR mutations receiving gefitinib plus chemotherapy.

STATISTICAL ANALYSIS

Descriptive statistical methods were used.

- **Categorical variables** (e.g., sex, smoking status, histologic subtype, presence of actionable mutations, treatment responses) were summarized as frequencies and percentages.
- **Continuous variables** (e.g., age) were reported as medians due to small sample size and potential non-normal distribution.
- Mutation frequencies were tabulated across histological subtypes.
- Treatment response rates were expressed as percentages of patients who received targeted therapy.

Data analysis was performed using Microsoft Excel 2016 and SPSS version 25.0 (IBM Corp)

Results

PATIENT CHARACTERISTICS (TABLE 1)

A total of 48 patients were enrolled in this prospective observational study. The median age was 52.5 years for males and 57 years for females, with a male predominance (28 males, 20 females). Most patients (83.3%) were never-smokers, and the most common presenting symptom was cough (90%), followed by chest pain (55%) and dyspnea (53%). Systemic symptoms such as weight loss and fatigue were reported in 70% of patients.

Histologically, adenocarcinoma accounted for the majority of cases (85.4%), while 12.5% had squamous cell carcinoma and 2.1% had NSCLC-NOS. Most patients (93.8%) presented with stage IV disease according to the AJCC 8th edition, with only 6.2% diagnosed at stage III.

Table 1: Baseline characteristics

Variable	Observation
Median age	Males: 52.5 years; Females: 57 years
Sex	Male: 28 (58.3%); Female: 20 (41.7%)
Smoking history	Never-smokers: 40 (83.3%); Current smokers: 8 (16.7%)
Presenting complaints	Cough: 43 (90%); Chest pain: 26 (55%); Dyspnea: 25 (53%); Constitutional symptoms: 34 (70%)
ECOG PS	0–1: 24 (50%); 2: 19 (40%); 3: 5 (10%)
Histology	Adenocarcinoma: 41 (85.4%); Squamous cell carcinoma: 6 (12.5%); NSCLC-NOS: 1 (2.1%)
Stage (AJCC 8th edition)	Stage IV: 45 (93.8%); Stage III: 3 (6.2%)

NGS RESULTS FROM LIQUID BIOPSY

Mutations were successfully identified in 28 of 48 patients (58.3%). Of these, 20 patients (41.6%) harbored actionable mutations with direct therapeutic relevance. Actionable alterations were more frequently observed in adenocarcinoma (n = 18) compared with squamous cell carcinoma (n = 2), while none of the NSCLC-NOS cases showed actionable mutations.

Mutation Spectrum

- **Actionable mutations (Table 3)**: The most common alteration was **EGFR** (n = 14), followed by **ALK rearrangements** (n = 2), **KRAS G12C** (n = 1), **NTRK** (n = 1), **RET** (n = 1), and **ERBB2** (n = 1).
- **Non-actionable mutations (Table 4)**: Frequently observed alterations included **TP53**, either alone or in combination with other mutations. Other non-actionable variants identified were **KRAS (non-G12C)**, **CHEK2**, **APC**, and **RAD50**.
- **Co-mutations**: Multiple mutations were present in 31.3% of patients, with TP53 co-mutations being the most common.

EGFR Mutation Profile and Treatment

Among the 14 patients with EGFR alterations (Table 5):

- 8 had **exon 19 deletions**,
- 2 had **exon 21 L858R mutations**, and
- 4 had **compound or uncommon EGFR mutations**.

Patients with EGFR mutations were treated with a combination of chemotherapy (Table 6) (pemetrexed or paclitaxel with carboplatin) and the EGFR TKI **gefitinib**. The chemotherapy regimen was tailored to histology and patient tolerance

Treatment Outcomes

Response to therapy was assessed after at least three months of treatment using **PERCIST 1.0 criteria**:

- 14.3% achieved a **complete metabolic response**,
- 71.4% achieved a **partial response**, and
- 14.3% had **progressive disease** at the time of analysis.

No cases of stable disease were observed in this subgroup.

Table 2. Histology-Wise Distribution of NGS Results

Histology	Actionable mutations	Non-actionable mutations	Negative
Adenocarcinoma	18	6	16
Squamous cell carcinoma	2	1	4
NSCLC-NOS	0	1	0
Total	20	8	20

Table 3. Distribution of Actionable Mutations

Mutation Type	Adenocarcinoma (n)	Squamous cell carcinoma (n)	NSCLC-NOS (n)	Total (n)
EGFR	12	2	0	14
ALK	2	0	0	2
KRAS G12C	1	0	0	1
NTRK	1	0	0	1
RET	1	0	0	1
ERBB2	1	0	0	1
Total	18	2	0	20

Table 4. Distribution of Non-Actionable Mutations

Mutation Type	Adenocarcinoma (n)	Squamous cell carcinoma (n)	NSCLC-NOS (n)	Total (n)
TP53	1	0	0	1
KRAS (non-G12C)	2	1	0	3
CHEK2	1	0	0	1
APC	1	0	0	1
RAD50	1	0	0	1
NF:Px225	0	0	1	1
Total	6	1	1	8

Table 5. EGFR Mutation Subtypes (n = 14)

EGFR Variant	No. of Patients (%)
Exon 19 deletion	8 (57.1%)
Exon 21 L858R	2 (14.3%)
Compound/uncommon mutations	4 (28.6%)

Table 6. Chemotherapy Regimens Used in Combination With Gefitinib

Histology	Regimen
Adenocarcinoma	Pemetrexed 500 mg/m² + Carboplatin AUC 5 + Gefitinib 250 mg OD, every 3 weeks
Squamous cell carcinoma	Paclitaxel 175 mg/m² + Carboplatin AUC 5 + Gefitinib 250 mg OD, every 3 weeks
	Paclitaxel 80 mg/m² + Carboplatin AUC 2 + Gefitinib 250 mg OD, weekly

Discussion

The present study demonstrates that next-generation sequencing (NGS)-based liquid biopsy is a feasible and clinically relevant diagnostic tool for mutation profiling in patients with non-small cell lung cancer (NSCLC) when tissue material is insufficient. In our cohort, actionable mutations were identified in 41.6% of patients, with epidermal growth factor receptor (EGFR) alterations being the most common, followed by ALK rearrangements, KRAS G12C, NTRK, RET, and ERBB2. Among patients treated with EGFR-targeted therapy in combination with chemotherapy, 85.7% achieved an objective response. These findings reinforce the growing body of evidence that liquid biopsy can bridge diagnostic gaps in resource-constrained settings and provide clinically meaningful information to guide therapy.

CONCORDANCE WITH GLOBAL DATA

Our detection rate aligns with results from multiple international studies validating the use of liquid biopsy for NSCLC. The NILE study by Aggarwal et al demonstrated that plasma-based NGS identified actionable mutations in 48% of patients, with a concordance rate of 98% compared with tissue genotyping when tissue was available.[15] Similarly, the Guardant INFORM registry, one of the largest real-world liquid biopsy datasets, reported mutation detection in 55%–60% of patients with insufficient or unavailable tissue, consistent with our 58.3% overall mutation detection rate.[16]

Beyond diagnostic accuracy, the clinical impact of liquid biopsy has been extensively validated. The BFAST trial, a global, prospective study, demonstrated that plasma NGS could successfully identify ALK fusions and other oncogenic drivers, with clinical outcomes comparable to tissue-based testing.[17] In addition, studies from the US and Europe have reported turnaround times of 7–10 days, substantially faster than tissue-based profiling, allowing for earlier initiation of therapy.[18,19] While our turnaround time was longer (≈3 weeks), this reflects infrastructural limitations in low- and middle-income countries (LMICs) and highlights the need for system-level improvements.

Recent reviews further emphasize the expanding role of liquid biopsy. A 2024 narrative review by Zhu et al

synthesized emerging biomarkers and technical advances, reinforcing its relevance for precision oncology worldwide.[20] A 2024 multicentre study in *Frontiers in Oncology* also demonstrated >94% success rates in tissue and plasma NGS profiling across 246 patients, validating feasibility in real-world practice.[21]

COMPARISON WITH INDIAN AND LMIC STUDIES

Data from India and LMICs remain limited but are gradually emerging. Doval et al, in the first Indian experience using Guardant360, reported a 65% detection rate, with EGFR, TP53, and ALK being the most common mutations.[13] Mukherjee et al evaluated 236 Indian patients and reported a 52% detection rate using plasma NGS, again showing strong feasibility in a resource-constrained setting.[14] Another multicentre Indian analysis found liquid biopsy particularly useful in patients with inadequate tissue or poor performance status, consistent with our findings.[22]

A 2024 Indian study by Singh et al confirmed the clinical value of cfDNA-based liquid biopsy for guiding treatment decisions in lung cancer, highlighting its cost-effectiveness and applicability in routine care.[23] These findings, together with our data, establish liquid biopsy as a pragmatic diagnostic strategy for Indian patients and reinforce its potential to complement or replace tissue biopsy when material is insufficient.

EGFR MUTATIONS AND CLINICAL OUTCOMES

EGFR mutations accounted for 70% of actionable alterations in our cohort, consistent with the known epidemiology of Asian NSCLC patients, where EGFR prevalence is 30%–50% compared with 10%–15% in Western populations.[3,4] In our study, patients treated with gefitinib plus chemotherapy achieved an overall response rate of 85.7%, which is comparable to landmark EGFR TKI trials such as IPASS (ORR 71%) and FLAURA (ORR 80%),[24,25] as well as real-world Indian data.[14] This reinforces the reliability of liquid biopsy in identifying EGFR mutations and guiding effective therapy.

However, access to newer-generation TKIs such as osimertinib remains limited in LMICs. While osimertinib has become the global standard for frontline EGFR-mutated NSCLC following the FLAURA trial,[25] its high cost restricts availability in India and many other LMICs. Consequently, most patients in our study were treated with gefitinib, highlighting the persistent gap between molecular diagnosis and therapeutic access.

Emerging evidence also underscores the value of liquid biopsy in tracking resistance. A 2024 study demonstrated the integration of cfDNA and circulating tumour cells to detect osimertinib resistance mechanisms, providing deeper insights into progression biology.[26] Additionally, a 2025 longitudinal ctDNA profiling study published in *Nature* revealed that post-immunotherapy NSCLC patients exhibit evolving mutational landscapes that can be captured dynamically by liquid biopsy.[27] These findings highlight the expanding role of ctDNA beyond initial diagnosis.

OTHER ACTIONABLE ALTERATIONS

Although EGFR dominated our findings, liquid biopsy also detected other important actionable mutations. ALK rearrangements were observed in 2 patients (4.1%). The global prevalence of ALK fusions in NSCLC is approximately 3%–7%, with higher representation in never-smokers and younger patients.[5,12] Clinical trials such as ALEX and ALTA-1L have demonstrated the superior efficacy of alectinib and brigatinib over crizotinib, with median progression-free survival exceeding 30 months.[28,29] However, in India, crizotinib remains the most accessible ALK inhibitor due to cost, while second- and third-generation inhibitors are not universally available.[30]

ROS1 fusions and BRAF mutations were less frequent in our cohort, as expected given their reported global prevalence of 1%–2% and 1%–3%, respectively.[7,8] In the EUCROSS and PROFILE trials, patients with ROS1 fusions achieved response rates >70% with crizotinib, while BRAF V600E mutations responded well to combined BRAF and MEK inhibition.[31,32] Despite this, treatment for such rare alterations is often not available in LMICs, further widening the actionability gap.

We also identified a single case of KRAS G12C, a mutation historically considered undruggable but now targetable with sotorasib and adagrasib.[33] While global approvals have expanded treatment options, these agents are not yet accessible in India, again underscoring disparities in therapeutic implementation.

TP53 AND CO-MUTATIONS

An important observation was the high frequency of TP53 co-mutations (18.8%). Previous studies have shown that TP53 mutations are among the most common in NSCLC, occurring in up to 40% of cases, often in combination with EGFR or KRAS.[34] TP53 co-mutations are associated with aggressive disease biology, resistance to targeted therapy, and shorter survival.[35,36] In EGFR-mutated NSCLC, concurrent TP53 alterations have been linked to lower response rates and shorter progression-free survival with TKIs.[37] The detection of TP53 by liquid biopsy in our study highlights its prognostic value and potential role in stratifying patients for intensified monitoring or alternative treatment strategies.

CLINICAL IMPLEMENTATION AND THE "ACTIONABILITY GAP"

Our study highlights two key challenges in the clinical adoption of liquid biopsy in LMICs: turnaround time and therapeutic access. The average reporting time of three weeks is substantially longer than global benchmarks of 7–10 days,[18,19] and could delay initiation of targeted therapy in patients with poor performance status. Streamlined workflows, decentralized testing, and government-supported infrastructure will be critical to reduce delays.

The more pressing issue is the **"actionability gap"**—the disconnect between identifying actionable mutations and providing corresponding therapies. While we identified ALK, ROS1, RET, NTRK, and KRAS G12C alterations, treatment could not be offered to many patients due to

lack of drug availability or prohibitive costs. This contrasts sharply with high-income countries, where reimbursement policies and clinical trial access facilitate the use of novel targeted agents.[31,38] Bridging this gap in LMICs will require not only diagnostic infrastructure but also policy-level interventions, public–private partnerships, and local clinical trials to ensure equitable access.

EMERGING ROLE OF LIQUID BIOPSY IN RESISTANCE AND MONITORING

Beyond baseline profiling, liquid biopsy has growing utility in monitoring treatment resistance and disease progression. The AURA3 trial demonstrated that plasma genotyping could reliably detect EGFR T790M mutations at progression, enabling osimertinib therapy without repeat tissue biopsy.[39] Similarly, the BLOOM study highlighted the role of ctDNA in identifying resistance mutations such as C797S.[40] Recent studies confirm this evolving role, with longitudinal ctDNA profiling showing potential for dynamic disease monitoring and minimal residual disease detection.[27,41,42]

LIMITATIONS

This study has limitations. The modest sample size and single-centre design limit generalizability. Paired tissue samples were not available for concordance analysis, which is a key limitation compared with studies such as NILE and BFAST.[15,17] Additionally, therapeutic access was skewed towards EGFR, as most patients could not access targeted therapies for other alterations. Finally, survival outcomes such as progression-free survival and overall survival were not mature at the time of analysis, precluding direct comparison with global benchmarks.

Conclusions

This study provides real-world evidence that liquid biopsy using NGS can identify actionable mutations in over 40% of NSCLC patients with insufficient tumour tissue, even in a resource-constrained setting. Our findings are consistent with global and Indian data, reinforcing the clinical utility of plasma-based testing. However, significant challenges remain, including prolonged turnaround times and limited access to targeted therapies, which contribute to the actionability gap in LMICs. To realise the full potential of liquid biopsy in precision oncology, future efforts must focus on strengthening diagnostic infrastructure, reducing costs, ensuring equitable drug access, and expanding longitudinal applications for resistance monitoring.

DECLARATIONS

Funding: None

Conflict of interest: none

Ethical approval: yes

Acknowledgement: We gratefully thank Bangalore Medical System Laboratory for NGS testing and reporting

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