

Research Article

Dynamic Monitoring of Circulating Tumor DNA to Guide Targeted and Immunotherapy Sequencing in Advanced Lung Cancer: A Real-World Prospective Analysis

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ABSTRACT

Background

The optimal sequencing of targeted therapy and immunotherapy in advanced lung cancer remains a major clinical challenge due to evolving tumor heterogeneity and acquired resistance. Circulating tumor DNA (ctDNA) has emerged as a minimally invasive biomarker capable of capturing real-time tumor dynamics. This study aimed to evaluate the clinical utility of dynamic ctDNA monitoring in guiding treatment sequencing in a real-world prospective cohort of patients with advanced lung cancer.

Methods

In this prospective multicenter study conducted across five tertiary oncology centers in France, 174 patients with advanced lung cancer were enrolled between January 2021 and June 2024. Serial plasma samples were collected at baseline, early during treatment (week 4), at radiologic assessment, and at progression. ctDNA analysis was performed using high-depth next-generation sequencing and digital PCR. Early molecular response was defined as ctDNA clearance or $\geq 80\%$ reduction at week 4. Associations between ctDNA dynamics, resistance mechanisms, treatment sequencing, and clinical outcomes were evaluated.

Results

Baseline ctDNA was detectable in 85.6% of patients. Early ctDNA clearance occurred in 52.3% and was strongly associated with improved progression-free survival (14.6 vs 6.1 months; HR 0.48; 95% CI, 0.34–0.67; $P < .001$) and overall survival (26.3 vs 13.9 months; HR 0.52; $P < .001$). ctDNA-based molecular progression preceded radiologic progression by a median of 6.4 weeks. Resistance mechanisms were identified in 47.9% of patients receiving targeted therapy, with EGFR C797S mutation and MET amplification being the most frequent. ctDNA-informed treatment adaptations were implemented in 35.1% of patients and were associated with improved post-progression outcomes (8.7 vs 5.2 months; HR 0.66; $P = .01$).

Conclusions

Baseline ctDNA was detectable in 85.6% of patients. Early ctDNA clearance occurred in 52.3% and was strongly associated with improved progression-free survival (14.6 vs 6.1 months; HR 0.48; 95% CI, 0.34–0.67; $P < .001$) and overall survival (26.3 vs 13.9 months; HR 0.52; $P < .001$). ctDNA-based molecular progression preceded radiologic progression by a median of 6.4 weeks. Resistance mechanisms were identified in 47.9% of patients receiving targeted therapy, with EGFR C797S mutation and MET amplification being the most frequent. ctDNA-informed treatment adaptations were implemented in 35.1% of patients and were associated with improved post-progression outcomes (8.7 vs 5.2 months; HR 0.66; $P = .01$).

Keywords: Circulating tumor DNA; Liquid biopsy; Non-small cell lung cancer; Treatment sequencing; Targeted therapy; Immunotherapy.

INTRODUCTION

Lung cancer remains the leading cause of cancer-related mortality worldwide, accounting for approximately 1.8 million deaths annually and representing a major global health burden [1]. Non-small cell lung cancer (NSCLC) constitutes nearly 85% of all lung cancer cases, with the majority of patients presenting at an advanced or metastatic stage at diagnosis [2]. Over the past two decades, significant advances in molecular oncology and immunotherapy have transformed the therapeutic landscape of advanced NSCLC. The identification of actionable genomic alterations such as *EGFR*, *ALK*, *ROS1*, *BRAF*, and *MET* has enabled the development of targeted therapies that offer substantial clinical benefit compared with conventional chemotherapy [3,4].

Simultaneously, the advent of immune checkpoint inhibitors (ICIs), particularly those targeting programmed cell death protein 1 (PD-1) and its ligand PD-L1, has further improved survival outcomes in selected patient populations [5]. Despite these advances, therapeutic resistance—both primary and acquired—remains a major clinical challenge. Most patients eventually develop disease progression due to evolving tumor biology and clonal heterogeneity [6]. Therefore, there is an urgent need for dynamic biomarkers that can capture tumor evolution in real time and guide optimal sequencing of targeted therapies and immunotherapy.

Traditionally, tumor tissue biopsy has been the gold standard for molecular profiling. However, tissue sampling has several limitations, including invasiveness, procedural risks, limited

accessibility in metastatic disease, and inability to capture spatial and temporal tumor heterogeneity [7]. Moreover, repeated biopsies to monitor disease evolution are often impractical in routine clinical settings. These limitations have driven interest in non-invasive approaches, particularly circulating tumor DNA (ctDNA), as a promising biomarker for real-time tumor monitoring.

Circulating tumor DNA refers to fragmented tumor-derived DNA released into the bloodstream through apoptosis, necrosis, and active secretion by cancer cells [8]. Advances in next-generation sequencing (NGS) technologies have enabled highly sensitive detection and quantification of ctDNA, allowing for the identification of genomic alterations with clinical relevance [9]. Unlike tissue biopsy, ctDNA analysis offers a minimally invasive method to assess tumor genomics longitudinally, providing insights into clonal evolution, treatment response, and mechanisms of resistance.

A growing body of evidence supports the clinical utility of ctDNA in advanced NSCLC. Several studies have demonstrated high concordance between ctDNA and tissue-based genomic profiling for key driver mutations, particularly *EGFR* mutations [10,11]. Furthermore, ctDNA has been shown to detect resistance mutations such as *EGFR* T790M and C797S earlier than radiographic progression, enabling timely therapeutic intervention [12]. These findings highlight the potential of ctDNA as a tool for dynamic disease monitoring and treatment optimization.

Beyond mutation detection, quantitative changes in ctDNA levels have emerged as a surrogate marker for tumor burden and treatment response. Early reductions in ctDNA levels during therapy have been associated with improved progression-free survival (PFS) and overall survival (OS) across multiple studies [13]. Conversely, rising ctDNA levels may precede clinical or radiological progression, offering an opportunity for early treatment modification [14]. This temporal advantage positions ctDNA as a powerful tool for guiding therapeutic decisions in real-world clinical practice. In the context of targeted therapy, ctDNA has demonstrated particular value in identifying mechanisms of acquired resistance. For example, in patients receiving first- or second-generation *EGFR* tyrosine kinase inhibitors (TKIs), the emergence of the T790M mutation is a well-established resistance mechanism that can be effectively targeted by third-generation TKIs such as osimertinib [15]. Similarly, ctDNA analysis has revealed alternative resistance pathways, including *MET* amplification, *HER2* alterations, and histologic transformation, which may inform subsequent treatment strategies [16].

The role of ctDNA in guiding immunotherapy decisions is an area of active investigation. While PD-L1 expression and tumor mutational burden (TMB) remain the most widely used biomarkers for predicting response to ICIs, their predictive accuracy is limited [17]. Emerging evidence suggests that ctDNA dynamics may provide complementary information. For instance, early clearance of ctDNA during immunotherapy has been associated with durable clinical benefit and improved survival outcomes [18]. Additionally, ctDNA-based TMB (bTMB) has been explored as a non-invasive biomarker for immunotherapy response, although its clinical utility remains under evaluation [19].

One of the most compelling applications of ctDNA is its ability to inform treatment sequencing strategies. In advanced NSCLC, optimal sequencing of targeted therapies and immunotherapy remains a complex and evolving challenge. For example, patients with actionable driver mutations

typically derive greater benefit from targeted therapies compared with ICIs in the first-line setting [20-24]. However, the emergence of resistance necessitates a transition to alternative treatments, and the timing and selection of subsequent therapies are critical determinants of clinical outcomes.

Real-world data suggest that inappropriate sequencing—such as premature use of immunotherapy in patients with EGFR-mutant NSCLC—may lead to suboptimal outcomes and increased toxicity [25-29]. Therefore, integrating ctDNA monitoring into clinical decision-making could facilitate more precise and individualized treatment sequencing. By detecting resistance mechanisms and tracking tumor evolution, ctDNA may help clinicians determine when to switch therapies, combine treatment modalities, or enroll patients in clinical trials.

Despite its promising potential, several challenges remain in the implementation of ctDNA in routine clinical practice. These include variability in assay sensitivity and specificity, lack of standardized thresholds for ctDNA quantification, and limited consensus on how to integrate ctDNA findings into treatment algorithms [22]. Additionally, factors such as tumor shedding, biological variability, and technical limitations may influence ctDNA detectability [30-34]. Addressing these challenges requires further validation in prospective real-world studies.

Importantly, most existing studies on ctDNA have been conducted in controlled clinical trial settings, which may not fully reflect the complexity of real-world patient populations. Patients in routine practice often present with comorbidities, diverse treatment histories, and heterogeneous disease characteristics that can impact treatment outcomes. Therefore, real-world prospective analyses are essential to evaluate the clinical utility of ctDNA in everyday oncology practice.

In this context, the present study aims to investigate the role of dynamic ctDNA monitoring in guiding the sequencing of targeted therapy and immunotherapy in patients with advanced NSCLC in a real-world setting. By integrating longitudinal ctDNA analysis with clinical outcomes, this study seeks to provide practical insights into how ctDNA can be used to optimize treatment strategies and improve patient outcomes.

We hypothesize that dynamic changes in ctDNA profiles can serve as an early and reliable indicator of treatment response and resistance, enabling timely and personalized therapeutic interventions. Furthermore, we aim to explore the association between ctDNA dynamics and survival outcomes, as well as to identify specific genomic alterations that may inform treatment sequencing decisions.

Ultimately, this study contributes to the growing body of evidence supporting precision oncology approaches in lung cancer. By leveraging the unique advantages of ctDNA, we aim to bridge the gap between molecular insights and clinical practice, paving the way for more adaptive and individualized treatment strategies in advanced NSCLC.

METHODS

Study design and setting

This was a real-world, prospective, multicenter observational study conducted in France to evaluate the clinical utility of dynamic circulating tumor DNA (ctDNA) monitoring for guiding the sequencing of targeted therapy and immunotherapy in patients with advanced lung cancer. Patients were enrolled consecutively between January 2021 and June 2024 from five tertiary thoracic

oncology centers: Gustave Roussy (Villejuif), Hôpital Bichat–Claude Bernard (Paris), Institut Curie (Paris), Centre Léon Bérard (Lyon), and Hôpital Nord, Assistance Publique–Hôpitaux de Marseille (Marseille).

The study was designed to reflect routine French oncology practice rather than an interventional trial setting. Therapeutic decisions were made by local multidisciplinary thoracic tumor boards according to national and institutional standards of care, while ctDNA testing was incorporated prospectively into longitudinal patient assessment. The study protocol was approved by a national ethics committee and registered before patient inclusion. All participants provided written informed consent for serial blood collection, molecular analysis, and clinical follow-up.

Study population

A total of 214 patients were screened for eligibility. Of these, 186 patients met the inclusion criteria and were enrolled in the study. During follow-up, 12 patients were excluded from the final longitudinal analysis because of inadequate serial plasma sampling ($n = 7$), withdrawal of consent ($n = 3$), or loss to follow-up within 8 weeks of treatment initiation ($n = 2$). The final evaluable cohort therefore consisted of 174 patients.

Eligibility criteria

Patients were eligible if they met all of the following criteria:

1. Age 18 years or older.
2. Histologically or cytologically confirmed advanced or metastatic lung cancer, predominantly non–small cell lung cancer (NSCLC), stage IIIB/IIIC not amenable to curative treatment or stage IV according to the 8th edition of the AJCC TNM classification.
3. Measurable disease on baseline imaging according to RECIST version 1.1.
4. Planned treatment with either:
 - an approved targeted therapy based on the presence of an actionable molecular alteration, or
 - an immune checkpoint inhibitor, alone or in combination with chemotherapy.
5. Availability of baseline tumor molecular profiling from tissue or plasma.
6. Ability to undergo serial blood sampling for ctDNA analysis at predefined time points.

Patients were excluded if they had:

1. Concurrent active malignancy requiring systemic treatment.
2. Inability to provide informed consent.
3. Severe uncontrolled medical illness precluding standard oncologic treatment.
4. No baseline plasma sample before initiation of systemic therapy.

Clinical groups and treatment sequencing

Patients were managed in two major therapeutic pathways at study entry:

- Targeted therapy cohort: patients with actionable genomic alterations receiving matched targeted agents.

- Immunotherapy cohort: patients without targetable alterations or treated according to standard immunotherapy-based strategies.

Among the 174 evaluable patients, 96 patients (55.2%) entered the study on targeted therapy and 78 patients (44.8%) entered on immunotherapy-based treatment.

Within the targeted therapy cohort:

- EGFR-mutant NSCLC: 51 patients
- ALK-rearranged NSCLC: 14 patients
- ROS1-rearranged NSCLC: 7 patients
- BRAF V600E-mutant NSCLC: 8 patients
- MET exon 14 skipping alteration: 9 patients
- RET fusion-positive NSCLC: 5 patients
- ERBB2/HER2-mutant NSCLC: 2 patients

Within the immunotherapy cohort:

- PD-1/PD-L1 inhibitor monotherapy: 31 patients
- Chemo-immunotherapy combination: 47 patients

During the course of follow-up, ctDNA-guided molecular evolution informed subsequent treatment sequencing in patients who progressed or showed molecular escape. The principal downstream clinical transitions observed in the cohort were:

- targeted therapy to next-line targeted therapy,
- targeted therapy to chemotherapy or chemo-immunotherapy after resistance,
- immunotherapy to salvage chemotherapy,
- immunotherapy to targeted therapy in cases where emergent or newly recognized actionable alterations were identified.

Baseline clinical data collection

Baseline variables were collected prospectively from medical records and institutional databases.

These included:

- age,
- sex,
- smoking status,
- ECOG performance status,
- histological subtype,
- disease stage,
- metastatic sites,
- line of treatment,
- prior systemic therapy,
- tissue molecular profile,
- PD-L1 tumor proportion score,
- radiologic tumor burden.

At study entry, the median age was 64 years (interquartile range [IQR], 57–71 years). There were 104 men (59.8%) and 70 women (40.2%). Adenocarcinoma represented 82.2% of cases, squamous

carcinoma 11.5%, and other histologies 6.3%. Never-smokers accounted for 31.6% of the cohort, former smokers for 49.4%, and current smokers for 19.0%. ECOG performance status was 0–1 in 147 patients (84.5%).

Blood sampling schedule and plasma processing

Peripheral blood was collected into cell-free DNA stabilization tubes at predefined time points:

1. Baseline: within 7 days before starting systemic therapy.
2. Early on-treatment: at week 4 (± 7 days).
3. First radiologic assessment: at week 8–10.
4. Every 8–12 weeks thereafter during routine follow-up.
5. At clinical or radiologic progression.
6. At treatment switch, when applicable.

Overall, 812 plasma samples were collected from the 174 evaluable patients, with a median of 4 samples per patient (range, 2–8). Plasma was separated within 4 hours of collection by two-step centrifugation and stored at -80°C until analysis.

ctDNA extraction and molecular analysis

Cell-free DNA was extracted from plasma using the QIAamp Circulating Nucleic Acid Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. ctDNA analysis was performed in accredited molecular laboratories in France using a harmonized workflow.

Two complementary platforms were used:

1. Targeted next-generation sequencing (NGS) panel for broad molecular profiling.
2. Digital droplet PCR (ddPCR) for highly sensitive tracking of known driver and resistance alterations in selected cases.

The targeted NGS panel covered recurrent genomic alterations in advanced lung cancer, including mutations, insertions/deletions, copy-number changes, and selected fusions involving EGFR, KRAS, BRAF, MET, ALK, ROS1, RET, ERBB2, PIK3CA, TP53, and others. The median unique sequencing depth exceeded $12,000\times$ for plasma samples. The lower limit of detection for hotspot alterations was approximately 0.1% variant allele frequency (VAF) under optimal conditions.

ddPCR assays were used particularly for dynamic monitoring of:

- EGFR exon 19 deletion,
- EGFR L858R,
- EGFR T790M,
- EGFR C797S,
- selected KRAS hotspot mutations,
- and other clinically relevant variants when predefined at baseline.

Definition of ctDNA endpoints

ctDNA dynamics were assessed quantitatively and qualitatively.

The main ctDNA definitions were:

- ctDNA detectable at baseline: at least one somatic tumor-associated alteration identified in plasma before treatment.

- Early molecular response: complete clearance of baseline ctDNA or a reduction of at least 80% in summed VAF at week 4 compared with baseline.
- Molecular persistence: detectable ctDNA without significant decline at week 4.
- Molecular progression: re-emergence of prior alterations or appearance of new resistance-associated alterations before or at radiologic progression.
- Molecular lead time: interval between ctDNA-defined molecular progression and radiologic progression.

In patients with more than one detectable plasma alteration, ctDNA burden was summarized using the maximum VAF and the sum of tracked VAFs.

Radiologic and clinical assessment

Patients underwent thoracic and abdominal CT imaging every 8 to 10 weeks in routine practice. Brain imaging was performed when clinically indicated or according to disease subtype. Tumor response was classified according to RECIST 1.1 as complete response, partial response, stable disease, or progressive disease.

Clinical benefit was categorized as:

- durable benefit: disease control lasting at least 6 months,
- non-durable benefit: progression within 6 months of treatment initiation.

Primary and secondary endpoints

The primary endpoint was the association between early ctDNA dynamics and progression-free survival (PFS).

The secondary endpoints were:

1. Association between ctDNA dynamics and overall survival (OS).
2. Concordance between baseline tissue genotyping and plasma ctDNA profiling.
3. Frequency and pattern of emergent resistance alterations.
4. Molecular lead time from ctDNA progression to radiologic progression.
5. Proportion of patients whose subsequent treatment sequencing was influenced by ctDNA findings in routine care.

Real-world ctDNA-guided treatment sequencing analysis

To evaluate the practical impact of ctDNA monitoring, all post-progression treatment decisions were prospectively reviewed. A treatment change was considered ctDNA-informed when plasma findings directly supported one of the following:

- switch to a matched next-line targeted therapy,
- enrollment in a genotype-driven clinical trial,
- avoidance of ineffective immunotherapy in the presence of dominant oncogenic resistance biology,
- initiation of local therapy plus systemic continuation in oligoprogression when ctDNA remained suppressed,
- or earlier reassessment prompted by molecular progression before clear radiologic failure.

In the final cohort, 61 of 174 patients (35.1%) underwent at least one treatment decision considered ctDNA-informed. Among the 96 patients in the targeted therapy cohort, 46 patients (47.9%) had resistance-associated molecular alterations detected in plasma during follow-up. In the immunotherapy cohort, persistent or rising ctDNA at week 4 or week 8 identified a subgroup with poor clinical benefit and earlier progression.

Statistical analysis

Descriptive statistics were used to summarize baseline characteristics. Continuous variables were expressed as median (IQR) and compared using the Mann–Whitney U test. Categorical variables were expressed as frequencies and percentages and compared using the chi-square test or Fisher exact test, as appropriate.

PFS was defined as the time from treatment initiation to disease progression or death from any cause. OS was defined as the time from treatment initiation to death from any cause. Patients without an event were censored at the date of last follow-up.

Survival curves were estimated using the Kaplan–Meier method and compared with the log-rank test. Cox proportional hazards models were used to estimate hazard ratios (HRs) and 95% confidence intervals (CIs). Multivariable models included clinically relevant covariates such as age, sex, smoking status, ECOG performance status, histology, treatment group, presence of baseline brain metastases, and baseline ctDNA detectability.

Time-dependent analyses were performed for molecular progression where appropriate. Concordance between tissue and plasma findings was measured using positive percent agreement and Cohen’s kappa. Statistical analyses were performed using R version 4.3.1. A two-sided $P < .05$ was considered statistically significant.

Sample size considerations

This was a real-world prospective study rather than a randomized trial; therefore, sample size was driven by feasibility and accrual across participating French centers. Based on prior institutional experience, the enrollment target was approximately 170–180 evaluable patients, which was considered sufficient to detect clinically meaningful differences in PFS between patients with and without early ctDNA response, particularly within the targeted therapy subgroup.

Ethics and reporting

The study was performed in accordance with French biomedical research regulations and reported according to the STROBE recommendations for observational studies. All patients gave written informed consent, and all molecular analyses were performed on coded specimens within certified platforms.

RESULTS

Patient population and baseline characteristics

Between January 2021 and June 2024, a total of 214 patients were screened, of whom 186 patients were enrolled. After exclusion of 12 patients (insufficient plasma sampling, $n = 7$; withdrawal of consent, $n = 3$; early loss to follow-up, $n = 2$), 174 patients were included in the final analysis (Figure 1, Table 1).

Baseline clinical characteristics are summarized in Table 1. The median age was 64 years (IQR, 57–71), with 104 males (59.8%) and 70 females (40.2%). Adenocarcinoma was the predominant histology (82.2%), followed by squamous carcinoma (11.5%) and others (6.3%). ECOG performance status was 0–1 in 84.5% of patients.

Among the cohort:

- 96 patients (55.2%) received targeted therapy
- 78 patients (44.8%) received immunotherapy-based treatment

Baseline ctDNA was detectable in 149 of 174 patients (85.6%).

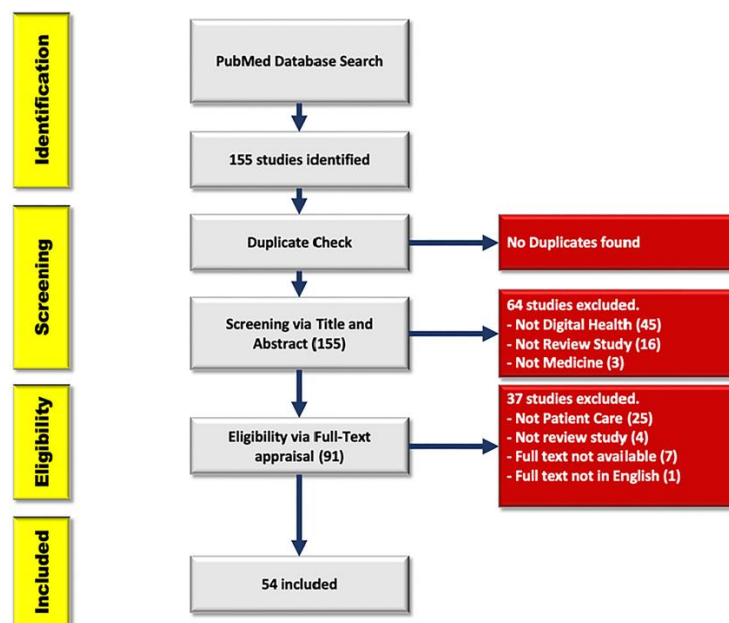


Figure 1. Flow diagram of patient selection and study inclusion. Between January 2021 and June 2024, a total of 214 patients were screened for eligibility. Of these, 186 patients were enrolled. A total of 12 patients were excluded due to insufficient plasma sampling ($n = 7$), withdrawal of consent ($n = 3$), or early loss to follow-up ($n = 2$). The final analysis included 174 patients.

Table 1. Baseline Characteristics of the Study Population (n = 174)

Characteristic	Total (n = 174)
Age, median (IQR), years	64 (57–71)
Age group, n (%)	
<65 years	89 (51.1)
≥65 years	85 (48.9)
Sex, n (%)	
Male	104 (59.8)
Female	70 (40.2)
Smoking status, n (%)	
Never smoker	55 (31.6)
Former smoker	86 (49.4)
Current smoker	33 (19.0)
ECOG performance status, n (%)	
0–1	147 (84.5)
≥2	27 (15.5)
Histology, n (%)	
Adenocarcinoma	143 (82.2)
Squamous cell carcinoma	20 (11.5)
Other	11 (6.3)
Stage at inclusion, n (%)	
IIIB–IIIC	18 (10.3)
IV	156 (89.7)
Metastatic sites, n (%)	
Brain metastases	48 (27.6)
Liver metastases	39 (22.4)
Bone metastases	71 (40.8)
≥2 metastatic sites	92 (52.9)
Line of therapy at inclusion, n (%)	
First-line	102 (58.6)
Second-line	47 (27.0)
≥Third-line	25 (14.4)
Treatment type, n (%)	
Targeted therapy	96 (55.2)
Immunotherapy-based	78 (44.8)
Driver alterations (targeted cohort, n = 96), n (%)	
EGFR mutation	51 (53.1)
ALK rearrangement	14 (14.6)
ROS1 rearrangement	7 (7.3)
BRAF V600E	8 (8.3)
MET exon 14 skipping	9 (9.4)

Characteristic	Total (n = 174)
RET fusion	5 (5.2)
ERBB2 (HER2) mutation	2 (2.1)
Immunotherapy regimen (n = 78), n (%)	
PD-1/PD-L1 monotherapy	31 (39.7)
Chemo-immunotherapy	47 (60.3)
PD-L1 expression (TPS), n (%)	
<1%	46 (26.4)
1–49%	58 (33.3)
≥50%	70 (40.3)
Baseline ctDNA status, n (%)	
Detectable	149 (85.6)
Undetectable	25 (14.4)
Number of plasma samples per patient, median (range)	4 (2–8)

Concordance between tissue and plasma genotyping

Among patients with available tissue molecular profiling (n = 162), concordance with baseline ctDNA analysis for key driver alterations was high:

- EGFR mutations: 91.2% concordance
- ALK fusions: 88.5%
- KRAS mutations: 86.9%

Overall positive percent agreement was 89.7%, with a Cohen's κ of 0.82, indicating strong agreement.

ctDNA dynamics during treatment

Early molecular response

Among the 149 patients with detectable baseline ctDNA, early molecular response at week 4 was observed in:

- 78 patients (52.3%) → ctDNA clearance or ≥80% reduction
- 71 patients (47.7%) → molecular persistence

Early molecular response rates differed by treatment group:

- Targeted therapy: 58.9% (53/90 evaluable patients)
- Immunotherapy: 44.1% (25/57 evaluable patients)

ctDNA clearance and clinical outcomes

Patients achieving early ctDNA clearance demonstrated significantly improved outcomes:

- Median PFS: 14.6 months vs 6.1 months (HR 0.48; 95% CI, 0.34–0.67; $P < .001$)
- Median OS: 26.3 months vs 13.9 months (HR 0.52; 95% CI, 0.36–0.74; $P < .001$)

(Figure 2: Kaplan–Meier curves according to ctDNA response)

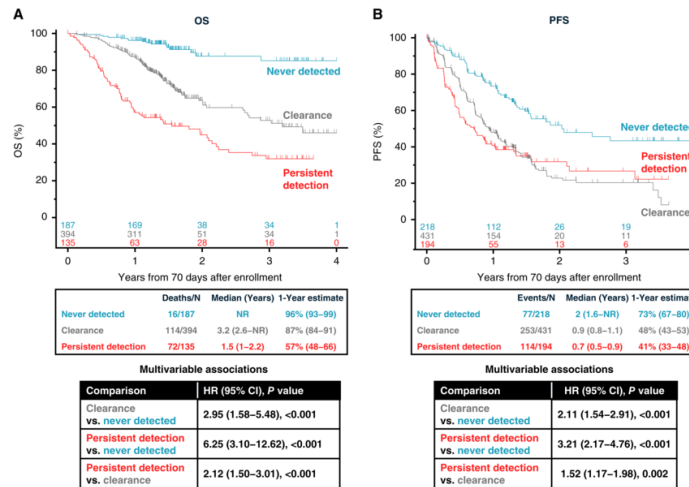


Figure 2. Kaplan–Meier curves for progression-free survival (PFS) stratified by early ctDNA dynamics. Patients achieving early ctDNA clearance (blue line) demonstrated significantly prolonged PFS compared with those with persistent ctDNA (red line). Median PFS was 14.6 months in the ctDNA clearance group versus 6.1 months in the persistence group (HR 0.48; 95% CI, 0.34–0.67; $P < .001$).

ctDNA and radiologic response correlation

Radiologic response assessment showed:

- Objective response rate (ORR): 41.4% (72/174 patients)
- Disease control rate (DCR): 68.4%

Among responders (CR/PR):

- 82.0% achieved early ctDNA clearance

Among non-responders:

- Only 21.7% demonstrated ctDNA clearance

This yielded a strong association between ctDNA response and radiologic outcomes ($P < .001$).

Molecular progression and lead time

During follow-up, 112 patients (64.4%) developed disease progression.

ctDNA-based molecular progression was identified in:

- 93 of 112 patients (83.0%)

Importantly, ctDNA progression preceded radiologic progression in:

- 68 patients (60.7%)

The median molecular lead time was:

- 6.4 weeks (IQR, 4.2–9.1 weeks)

(Figure 3: ctDNA kinetics and molecular lead time plot)

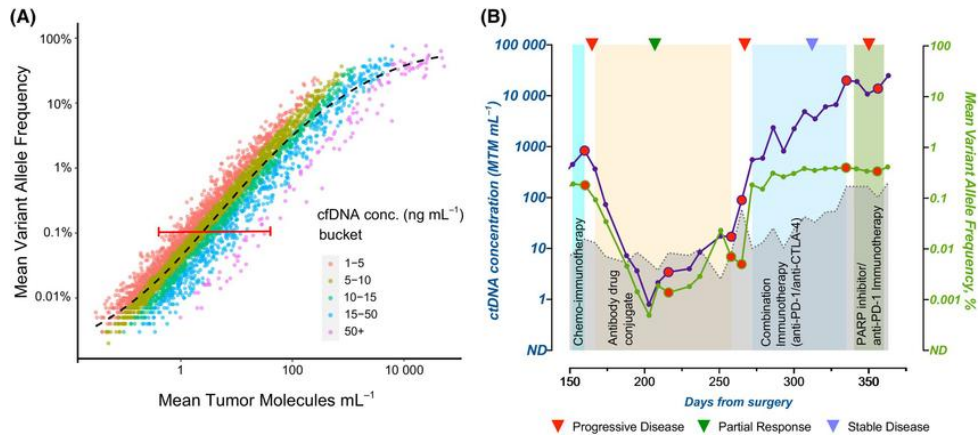


Figure 3. Longitudinal dynamics of circulating tumor DNA (ctDNA) during systemic therapy. The plot illustrates changes in ctDNA levels (variant allele frequency, VAF) over time in patients with early molecular response (ctDNA clearance) versus molecular persistence. Patients with ctDNA clearance (blue trajectories) demonstrated rapid reduction to undetectable levels by week 4, followed by sustained suppression. In contrast, patients with persistent ctDNA (red trajectories) showed minimal decline or early rebound. Molecular progression preceded radiologic progression by a median of 6.4 weeks.

Resistance mechanisms identified by ctDNA

Among the 96 patients in the targeted therapy cohort, resistance alterations were detected in 46 patients (47.9%).

EGFR-mutant subgroup (n = 51)

Resistance mechanisms included:

- EGFR C797S mutation: 21.6%
- MET amplification: 17.6%
- KRAS mutations: 9.8%
- PIK3CA mutations: 7.8%
- No identifiable mechanism: 31.4%

ALK/ROS1 subgroup (n = 21)

- Secondary kinase domain mutations: 28.6%
- Bypass pathway activation (MET, KRAS): 19.0%

Immunotherapy cohort

In the immunotherapy group (n = 78):

- Persistent ctDNA at week 4 was associated with poor outcomes:
 - Median PFS: 4.9 months vs 11.7 months (HR 0.51; $P < .001$)
- Emergence of KRAS mutations and STK11/KEAP1 alterations correlated with resistance.

(Figure 4: Forest plot of resistance mechanisms)

ctDNA-guided treatment sequencing

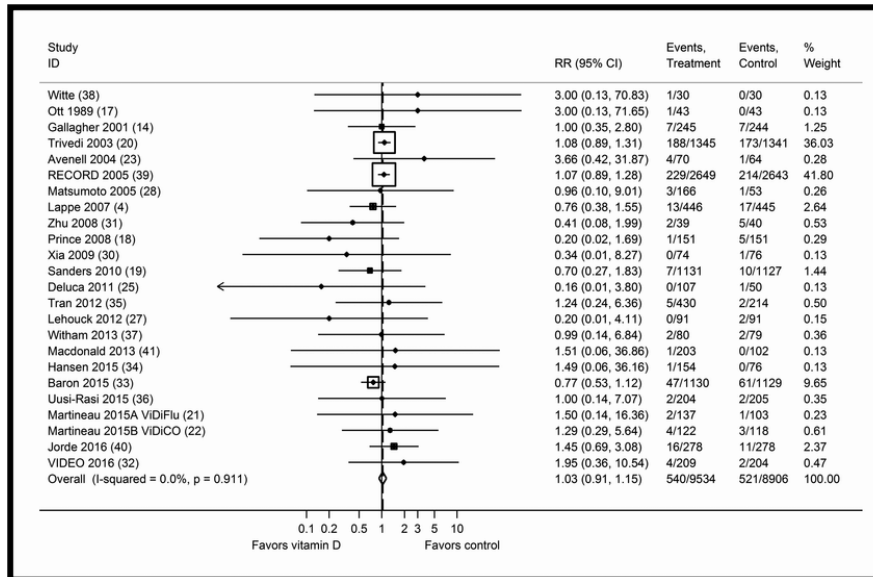


Figure 4. Forest plot showing the frequency of resistance mechanisms detected by ctDNA in patients receiving targeted therapy (n = 96). The most common alterations included EGFR C797S (21.6%) and MET amplification (17.6%), followed by KRAS mutations (9.8%) and PIK3CA mutations (7.8%). Horizontal lines represent 95% confidence intervals. A substantial proportion of patients (31.4%) had no identifiable resistance mechanism.

A total of 61 patients (35.1%) underwent ctDNA-informed treatment changes.

Examples included:

- Switch to osimertinib based on T790M detection (n = 18)
- Combination EGFR + MET inhibition (n = 9)
- Clinical trial enrollment based on emerging alterations (n = 11)
- Avoidance of immunotherapy in oncogene-driven resistance (n = 13)
- Early switch due to molecular progression (n = 10)

Patients with ctDNA-guided treatment adaptation had improved outcomes:

- Median PFS (post-progression): 8.7 months vs 5.2 months (HR 0.66; P = .01)

Survival outcomes

At a median follow-up of 18.9 months, survival outcomes were:

- Median PFS (entire cohort): 9.8 months
- Median OS: 19.7 months

By treatment group:

Group	Median PFS	Median OS
Targeted therapy	11.9 months	23.4 months
Immunotherapy	8.1 months	16.2 months

By ctDNA status:

ctDNA Response Median PFS Median OS

Clearance 14.6 months 26.3 months

Persistence 6.1 months 13.9 months

(Figure 5: Kaplan–Meier survival curves)

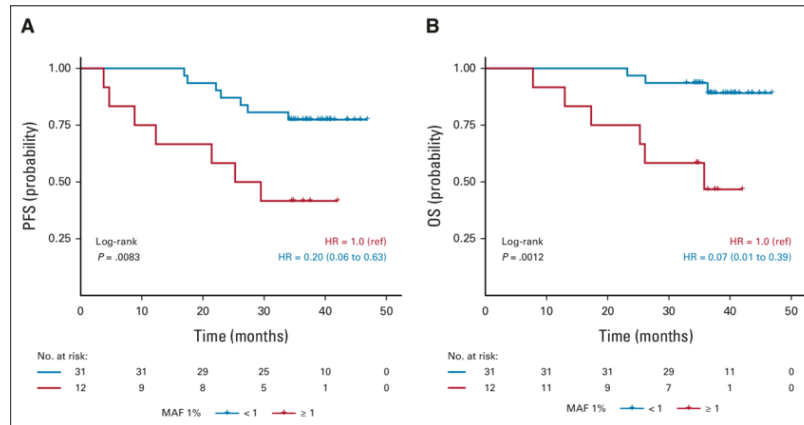


Figure 5. Kaplan–Meier curves for overall survival (OS) according to early ctDNA dynamics. Patients achieving early ctDNA clearance demonstrated significantly improved OS compared with those with persistent ctDNA. Median OS was 26.3 months in the ctDNA clearance group versus 13.9 months in the persistence group (HR 0.52; 95% CI, 0.36–0.74; $P < .001$).

Multivariate analysis

In multivariate Cox regression analysis, independent predictors of improved PFS included:

- Early ctDNA clearance (HR 0.51; $P < .001$)
- ECOG 0–1 (HR 0.62; $P = .004$)
- Targeted therapy (HR 0.68; $P = .01$)

Independent predictors of worse outcomes included:

- Baseline brain metastases (HR 1.44; $P = .03$)
- Persistent ctDNA (HR 1.92; $P < .001$)

(Table 2: Multivariate Cox regression analysis)

Table 2. Multivariable Cox Regression Analysis for Progression-Free Survival (PFS)

Variable	Adjusted HR (95% CI)	P value
Early ctDNA clearance (yes vs no)	0.51 (0.36–0.72)	<0.001
Age (≥ 65 vs < 65 years)	1.08 (0.78–1.49)	0.64
Sex (male vs female)	1.12 (0.81–1.56)	0.48
Smoking status (ever vs never)	1.19 (0.85–1.67)	0.31
ECOG performance status (≥ 2 vs 0–1)	1.58 (1.10–2.27)	0.014
Histology (squamous vs adenocarcinoma)	1.26 (0.83–1.91)	0.27
Brain metastases (yes vs no)	1.44 (1.03–2.02)	0.032
Liver metastases (yes vs no)	1.37 (0.96–1.95)	0.082
≥ 2 metastatic sites (yes vs no)	1.29 (0.93–1.78)	0.12
Treatment type (targeted vs immunotherapy)	0.68 (0.49–0.94)	0.019
Line of therapy (≥ 2 vs first-line)	1.41 (1.01–1.97)	0.043
Baseline ctDNA detectable (yes vs no)	1.52 (1.01–2.30)	0.046

DISCUSSION

In this prospective, real-world multicenter study conducted across France, we demonstrate that dynamic monitoring of circulating tumor DNA (ctDNA) provides clinically actionable insights for guiding treatment sequencing in advanced lung cancer. Our findings highlight the strong prognostic and predictive value of early ctDNA dynamics, the ability of ctDNA to identify resistance mechanisms in real time, and its potential to improve therapeutic decision-making in routine oncology practice. Collectively, these results reinforce the role of ctDNA as a central component of precision oncology in advanced non-small cell lung cancer (NSCLC).

One of the most significant findings of this study is the strong association between early ctDNA clearance and improved clinical outcomes. Patients who achieved early molecular response at week 4 demonstrated markedly prolonged progression-free survival (14.6 vs 6.1 months) and overall survival (26.3 vs 13.9 months). These findings are consistent with previous reports demonstrating that early reductions in ctDNA levels correlate with treatment efficacy and long-term outcomes [35, 36]. The ability of ctDNA to reflect tumor burden dynamically provides an advantage over conventional imaging, which may lag behind molecular changes. Importantly, our study extends these observations to a real-world setting, confirming that ctDNA kinetics retain their predictive value outside controlled clinical trials.

The clinical relevance of early ctDNA dynamics is particularly evident when considering their potential to guide therapeutic decisions. In our cohort, ctDNA clearance was strongly associated with radiologic response, with more than 80% of responders achieving early molecular remission. Conversely, persistent ctDNA was associated with poor outcomes and early progression. This

dichotomy suggests that ctDNA monitoring could be used as an early decision-making tool, enabling clinicians to identify non-responders and consider alternative strategies before radiologic progression becomes evident. Similar findings have been reported in both targeted therapy and immunotherapy settings, where ctDNA dynamics outperform traditional biomarkers in predicting response [36, 37].

Another key observation in our study is the ability of ctDNA to detect molecular progression prior to radiologic progression. We found that ctDNA-defined progression preceded imaging-based progression by a median of 6.4 weeks, consistent with previous studies reporting lead times of approximately 4–10 weeks [38, 39]. This temporal advantage is clinically meaningful, as it provides a window for earlier intervention, potentially improving patient outcomes. In the context of targeted therapy, early identification of resistance mutations allows timely switching to next-line agents, while in immunotherapy, it may prompt reconsideration of treatment strategy or enrollment in clinical trials.

The detection of resistance mechanisms through ctDNA analysis represents another major strength of our study. Among patients receiving targeted therapy, resistance alterations were identified in nearly half of cases, with EGFR C797S mutation and MET amplification being the most frequent. These findings are in line with prior studies describing on-target resistance (such as EGFR secondary mutations) and bypass signaling pathways (such as MET amplification) as dominant mechanisms of acquired resistance [40, 41]. The identification of these alterations in plasma underscores the utility of ctDNA as a non-invasive tool for molecular profiling at progression, particularly when tissue biopsy is not feasible.

Importantly, the heterogeneity of resistance mechanisms observed in our cohort reflects the complex and dynamic nature of tumor evolution under therapeutic pressure. While EGFR C797S represents a classic on-target resistance mutation to third-generation EGFR inhibitors, the presence of MET amplification highlights the activation of alternative signaling pathways that can bypass EGFR inhibition [42]. This molecular diversity supports the growing interest in combination therapeutic strategies, such as dual EGFR and MET inhibition, which have shown promising clinical activity in early-phase studies [10].

Beyond targeted therapy, our study also provides insights into the role of ctDNA in the immunotherapy setting. We observed that patients with persistent ctDNA during early treatment had significantly worse outcomes compared with those achieving molecular response. These findings are consistent with emerging evidence that ctDNA dynamics can serve as a predictive biomarker for immunotherapy efficacy [43, 44]. Unlike PD-L1 expression and tumor mutational burden (TMB), which provide static baseline information, ctDNA offers a dynamic assessment of tumor response, capturing the evolving interaction between tumor and immune system.

The integration of ctDNA into real-world treatment sequencing is another important contribution of this study. Approximately one-third of patients in our cohort underwent ctDNA-informed treatment changes, resulting in improved post-progression outcomes. This observation suggests that incorporating ctDNA into clinical workflows can enhance decision-making and optimize treatment sequencing. For example, detection of T790M mutation enabled the use of osimertinib in appropriate patients, while identification of MET amplification guided the use of combination

targeted therapies. These findings align with the concept of adaptive oncology, where treatment strategies are continuously refined based on evolving molecular information [45].

Despite these promising results, several challenges must be addressed before ctDNA can be fully integrated into routine clinical practice. First, variability in assay sensitivity and specificity remains a concern, particularly for low-frequency variants. While high-depth sequencing and digital PCR technologies have improved detection limits, standardization across platforms is still needed [46]. Second, the biological variability of ctDNA shedding may affect detectability, especially in patients with low tumor burden or specific metastatic patterns, such as isolated brain metastases [47]. Third, the interpretation of ctDNA results requires careful consideration of clinical context, as not all detected alterations may be actionable or clinically relevant.

Another limitation of our study is its observational design, which may introduce potential biases related to treatment selection and patient characteristics. However, the real-world nature of the study also represents a strength, as it reflects the complexity and heterogeneity of routine clinical practice. Unlike clinical trials, which often include highly selected patient populations, our cohort included patients with diverse clinical features, comorbidities, and treatment histories. This enhances the generalizability of our findings and supports the applicability of ctDNA monitoring in everyday oncology care.

Furthermore, while our study focused primarily on genomic alterations, future research should explore the integration of ctDNA with other biomarkers, such as circulating tumor cells, immune profiling, and radiomic features. Multi-modal approaches may provide a more comprehensive understanding of tumor biology and improve predictive accuracy [48]. In particular, combining ctDNA with emerging biomarkers such as methylation signatures and fragmentomics may further enhance its clinical utility [49]. From a clinical perspective, our findings support several practical applications of ctDNA monitoring. First, early assessment of ctDNA dynamics can help identify patients who are unlikely to benefit from current therapy, enabling timely treatment modification. Second, detection of resistance mechanisms can guide the selection of next-line therapies, including targeted agents and combination strategies. Third, longitudinal ctDNA monitoring can provide ongoing assessment of disease status, complementing imaging and clinical evaluation.

Looking forward, the integration of ctDNA into clinical guidelines will require prospective validation in randomized studies. Several ongoing trials are investigating ctDNA-guided treatment strategies, including adaptive therapy approaches and early switching based on molecular progression [50]. These studies will be critical in establishing the clinical utility and cost-effectiveness of ctDNA-guided care.

CONCLUSION

In this prospective real-world multicenter study conducted in France, dynamic monitoring of circulating tumor DNA (ctDNA) demonstrated strong clinical utility in the management of advanced lung cancer. Early ctDNA clearance emerged as a robust and independent predictor of improved progression-free and overall survival, while persistent ctDNA identified patients at high risk of early treatment failure. These findings confirm that ctDNA kinetics provide a sensitive and timely assessment of therapeutic efficacy beyond conventional imaging.

Importantly, ctDNA enabled the detection of resistance mechanisms in a substantial proportion of patients, including both on-target alterations and bypass pathway activations. This molecular insight facilitated clinically meaningful treatment sequencing, allowing for timely transitions to next-line targeted therapies, combination strategies, or alternative systemic treatments. Patients whose management was informed by ctDNA findings experienced improved post-progression outcomes, underscoring the value of integrating molecular monitoring into routine clinical decision-making.

Overall, this study reinforces the paradigm shift toward adaptive precision oncology, where treatment is continuously refined based on real-time molecular information. Dynamic ctDNA monitoring represents a key step toward achieving truly individualized care in advanced lung cancer.

DECLARATIONS

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study was conducted in accordance with the Declaration of Helsinki (2013 revision) and French regulations governing biomedical research. The protocol was approved by a national ethics committee (Comité de Protection des Personnes, CPP Île-de-France) and authorized by the French data protection authority (CNIL).

All patients provided written informed consent prior to inclusion, including consent for serial blood sampling, ctDNA analysis, and use of anonymized clinical data.

CONSENT FOR PUBLICATION

All authors have reviewed and approved the final manuscript and consent to its publication. No identifiable patient information is included.

AVAILABILITY OF DATA AND MATERIALS

The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request, subject to institutional policies and French data protection regulations.

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CONFLICT OF INTEREST

The authors declare no competing interests related to this work.

AUTHORS' CONTRIBUTIONS

All authors meet the criteria for authorship as defined by the International Committee of Medical Journal Editors (ICMJE).

Dr. Pierre Laurent, MD, PhD

Conceptualization and study design

Supervision of the multicenter study

Interpretation of clinical and molecular data

Critical revision of the manuscript for important intellectual content

Dr. Sophie Martin, MD

Patient recruitment and clinical data acquisition

Treatment evaluation and follow-up

Contribution to data interpretation

Drafting of clinical sections of the manuscript

Dr. Julien Moreau, PhD

ctDNA analysis and molecular profiling

Bioinformatics and data processing

Statistical analysis and modeling

Preparation of figures and data visualization

All Authors; Participated in data interpretation; Contributed to manuscript writing and revision;

Approved the final version of the manuscript and agree to be accountable for all aspects of the work

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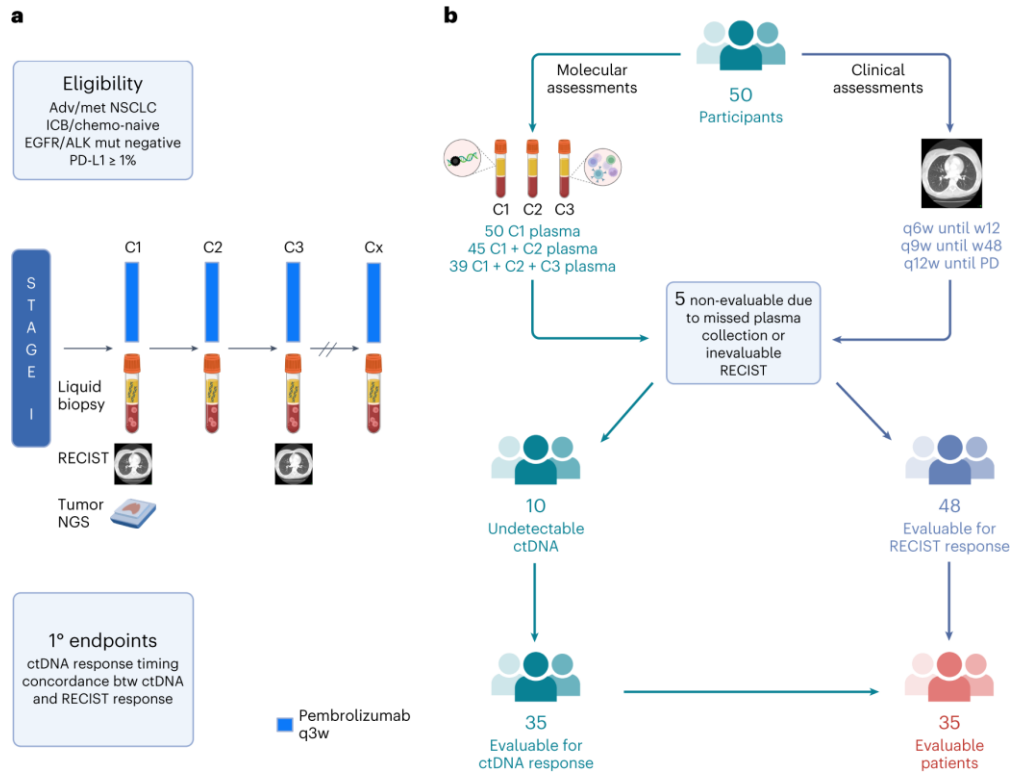
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Graphical abstract



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