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# Navigating the liquid biopsy Minimal Residual Disease (MRD) in non-small cell lung cancer: Making the invisible visible

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ABSTRACT

Liquid biopsy has gained increasing interest in the growing era of precision medicine as minimally invasive technique. Recent findings demonstrated that detecting minimal or molecular residual disease (MRD) in NSCLC is a challenging matter of debate that need multidisciplinary competencies, avoiding the overtreatment risk along with achieving a significant survival improvement. This review aims to provide practical consideration for solving data interpretation questions about MRD in NSCLC thanks to the close cooperation between biologists and oncology clinicians. We discussed with a translational approach the critical point of view from benchside, bedside and bunchside to facilitate the future applicability of liquid biopsy in this setting. Herein, we defined the clinical significance of MRD, focusing on relevant practical consideration about advantages and disadvantages, speculating on future clinical trial design and standardization of MRD technology.

## 1. Introduction

Lung cancer remains the leading cause of cancer deaths worldwide, with non-small cell lung cancer (NSCLC) accounting for 85% of cases (Siegel et al., 2021). Surgical resection with curative intent represents the mainstay for approximately one-quarter of such patients with early-stage NSCLC (Little et al., 2005). Post-operative cisplatin-based chemotherapy became the standard treatment, showing a 5.8% improvement in disease-free survival (DFS) while only yielding a 5-year overall survival (OS) of 5.4% in stage II-III patients with not negligible toxicity (Pignon et al., 2008). In this setting, there are urgently missing biomarkers to select those patients who might benefit the most from adjuvant chemotherapy.

Liquid biopsy has gained increasing interest in the growing era of precision medicine. Blood-based assays for tumor diagnosis and monitoring disease status are therefore attractive, as they are faster to obtain, minimally invasive, and can aid molecular profiling in terms of both prognostic and predictive significance (Russo et al., 2021a).

Minimal or molecular residual disease (MRD) is defined as the blood detection of remaining tumor cells following the administration of initial therapy. The liquid biopsy technology encompasses the evaluation of different blood circulating biomarkers derived from tumor cells and their fragments in such minute quantities that are outside the range of detection of currently used diagnostic medical imaging devices (Pantel and Alix-Panabières, 2019; Powles et al., 2021; Tie et al., 2022) to predict relapse risk (Little et al., 2005; Pignon et al., 2008) and tailor subsequent treatments.

The integration of liquid biopsy platforms in NSCLC has been increasingly studied in recent years in solid cancers (Powles et al., 2021; Tie et al., 2022), with a huge impact on daily clinical practice. Considering its consolidated role in the metastatic setting and the financial burden of relapsed lung cancer disease, in the adjuvant setting MRD could represent an interesting tool for detecting earlier recurrence after curative treatments and for tailoring effective therapies without

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significant toxicities (Pellini and Chaudhuri, 2022a) (Fig. 1).

Recent findings demonstrated that detecting MRD in NSCLC is a challenging matter of debate that need multidisciplinary competencies, avoiding the overtreatment risk along with achieving a significant survival improvement (Russo et al., 2021a).

This review aims to provide practical consideration for solving data interpretation questions about MRD in NSCLC thanks to the close cooperation between biologists and oncology clinicians with a consolidated knowledge in liquid biopsy. We will discuss with a translational approach the critical point of view from benchside, bedside and bunchside to facilitate the future applicability of liquid biopsy in this setting (Fig. 2).

## 2. Methods

A manual search in electronic databases MEDLINE, limiting to English language articles. The search terms used to select the relevant articles included "molecular residual disease", "minimum residual disease" and "Non-Small Cell Lung cancer". The last updated literature search was performed on August 27th, 2022. Record screening was performed to include studies focused on the role of liquid biopsy following the curative treatment in patients with radically resected NSCLC. Review articles, preclinical studies and book chapters were excluded from the review analysis but were used to broaden the literature discussion.

## 3. Results

Data from the relevant articles evaluated are shown in Table 1.

### 3.1. The critical point of view from the bench-side: detection techniques

#### 3.1.1. Integrating different circulating biomarkers for MRD

Liquid biopsies encompass different genomic and/or proteomic analyses of fluid samples (primarily blood) to detect MRD biomarkers (Palmirotta et al., 2018; Rolfo et al., 2018). Each circulating biomarker has different advantages and limitations for MRD detection in NSCLC, as shown in Table 2. For solid malignancies, such as NSCLC, circulating tumor DNA (ctDNA), which is composed of tumor-derived mutations in plasma cell-free DNA (cfDNA), has been predominately used as an MRD biomarker (Pellini and Chaudhuri, 2022a). ctDNA assay allows the identification of known actionable somatic alterations, but also genome-wide copy-number alterations and DNA methylation-based epigenetic signatures.

Circulating tumor cells (CTCs), as different MRD biomarkers, have an intriguing role in the perioperative setting because they could be released during lung surgery. Several reports described an association between CTCs in perioperative blood samples and poorer prognosis (Sawabata et al., 2020), yet few studies have been conducted to longitudinally evaluate CTC after curative surgery.

#### 3.1.2. Standardize MRD detection strategies

Detecting MRD through various biomarkers has shown the potential of more accurately predicting disease prognosis, thus assisting in proficient treatment administration to minimize adverse effects (Coakley et al., 2019a; Russo et al., 2021b).

There is a need for a keen comparison between the quality and quantity of liquid biopsy biomarkers. There are some limitations to currently available liquid biopsies techniques, including the need for appropriate sampling and preservation methods as well as a lack of standardization in analyzing technologies. These limitations produce a significant rate of false positives and negatives, compromising diagnostic accuracy (Lamy et al., 2020).

Table 1 showed several molecular biology techniques used for MRD detection that we summarized as follows.

Over the recent years, different NGS strategies and platforms have been exploited to determine the presence of plasma ctDNA in NSCLC to predict patients' outcomes for different disease settings as early cancer detection and MRD. The benefits of using plasma cell-free NGS-based technologies include having wider access to a larger spectrum of genome

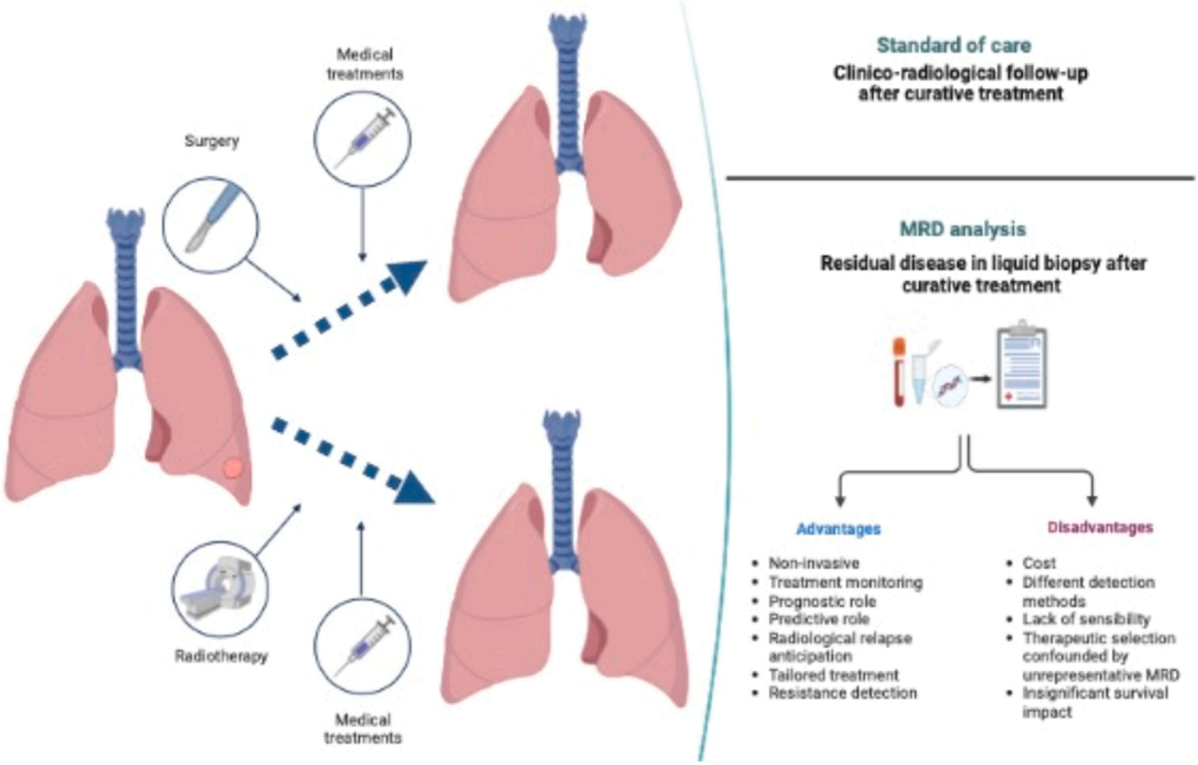


**Fig. 1.** The advantages and disadvantages of utilizing MRD analysis in clinical practice.

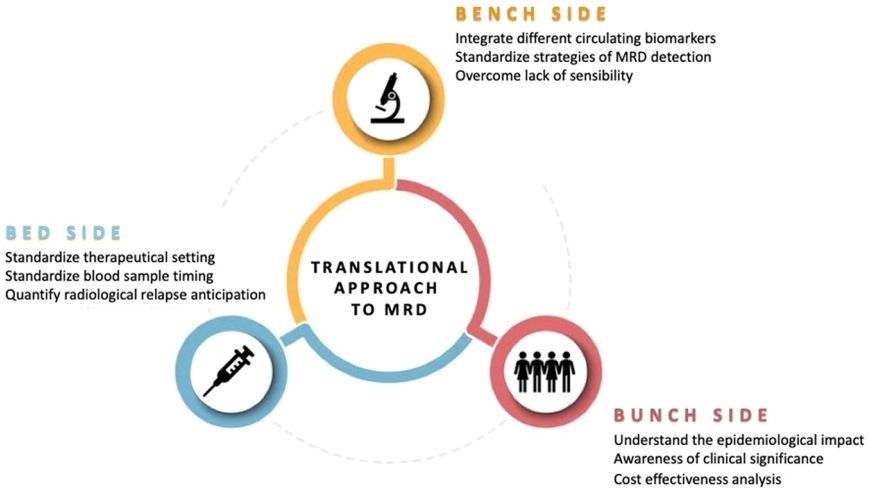


**Fig. 2.** The translational approach to solving data interpretation questions about MRD in our review.

information while combining high-throughput performances with sensitivity depth still representing one of the major challenges in this context (Aravanis et al., 2017; Chin et al., 2019a; Herbreteau et al., 2019). In particular, the two major challenges while working with ctDNA in this setting remain the extremely low levels of circulating nucleic acids and the frequent artifacts deriving from clonal hematopoiesis of indeterminate potential (CHIP) which often compromises the reliability of results. Therefore, several barcoding strategies have been described for filtering out somatic genetic variants that commonly accumulate during aging and clonal expansions of hematopoietic cells not resulting in any neoplastic event (Abbosh et al., 2019). Interestingly, deep sequencing of cell-free DNA and matched white blood cell DNA from cancer and healthy patients demonstrated the presence of several common somatic non-synonymous mutations in genes commonly altered in clonal hematopoiesis as *DNMT3A*, *TET2*, *PPM1D*, and *TP53* (Abbosh et al., 2018a).

As widely described, to better improve the analytical sensitivity and specificity of such technical approaches, prior knowledge of the mutational landscape of the tumor could represent a valuable option. Indeed, the two most applied strategies for NGS-based ctDNA assays could be classified in tumor-naive or tumor-informed approaches (Li and Cui, 2022). A tumor-naïve strategy profits from a pre-specified panel for the discovery and tracking of genetic alterations through a single-step plasma analysis, whereas a tumor-informed strategy benefits from a two-step approach, cutting off non-tumor genetic variants by using wider genomic sequencing techniques (such as Whole-Genome Sequencing [WGS] or Whole-Exome Sequencing [WES], and then using a personalized panel for deeply studying plasma ctDNA. Among tumor-naïve sequencing strategies, Cancer Personalized Profiling by deep Sequencing (CAPP-Seq) represents one of the most spread approaches since 2014 (Abbosh et al., 2018a). CAPP-Seq is a hybrid capture-based NGS assay targeting 128 relevant genes in lung cancer. Over the years several improvements have been made to guarantee the higher performance of CAPP-Seq in the detection of circulating tumor DNA from genomic space optimization to the development of CAPP-Seq strategies to rule out artifacts due to sequencing, as integrated digital error suppression (iDes)- CAPP-Seq with a sensitivity down to 0.004% (Pellini and Chaudhuri, 2022b; Newman et al., 2016). One of the first attempts using a CAPP-Seq strategy and focusing on the reliability of ctDNA detection in the context of an MRD setting is represented by the research work by Chaudhuri et al. The authors demonstrated in a cohort of 37 stage I-III NSCLC and 3 SCLC patients the feasibility of CAPP-Seq to highlight pretreatment ctDNA in 37 of the 40 enrolled patients with a limit of detection of 0.003% considering the mutated fraction with respect to total plasma cfDNA. Interestingly, 17/32 patients with detectable ctDNA at first evaluation (pretreatment) showed poorer outcomes and disease recurrence at surveillance (post-treatment) if compared to patients (N = 15) with undetectable ctDNA at the same time point. Moreover, at several post-treatment timepoints, the authors further demonstrated the positive value of ctDNA in MRD context by reaching 100% sensitivity and specificity in predicting disease relapse. Importantly, in 72% of patients, ctDNA detection during surveillance allowed to anticipate radiological evidence of recurrence with a median of 5.2 months. Overall, these findings suggest that evaluation of ctDNA through high-throughput DNA-sequencing technologies in MRD could be a feasible tool also to identify and stratify patients, among those with a premature detectable ctDNA, that could benefit from additional treatments (Chaudhuri et al., 2017).

The interesting performances of ctDNA in MRD of resectable NSCLC undergoing curative-intent treatment patients have been outlined also in 2020 within the lung TRACERx study, highlighting in the surveillance setting a sensitivity and specificity of 82% and 96% respectively, by using ArcherDx Anchored Multiplexing PCR (PCR) chemistry (Abbosh et al., 2020). As previously mentioned, the main limitations of plasma-based cell-free DNA assays, in particular at early stages of cancer, is represented by the extremely low amount of circulating nucleic acids released by tumors and the unavoidable high risk of background errors during high-throughput sequencing. Commonly, routinely used methods exploited duplex sequencing to reduce background noise through the concordance of mutational events on both DNA complementary strands. Unfortunately, while working with cell-free DNA, the recovery of both original parental strands is quite inefficient due to the independent library preparation and amplification steps they go through. The tumor-informed Phased variant Enrichment and Detection Sequencing (PhasED-Seq) is an interesting attempt to reduce background errors by targeting only "Phased variants" (PVs) defined as a clustered group of genetic variants *in cis* on a single DNA strand. Interestingly, the evidence that PVs occurs also in solid tumors has deeply encouraged the feasibility of this approach also to overcome high-throughput technical issues in the MRD context (Kurtz et al., 2021a). In fact, Kurtz et al., by comparing PhasED-Seq with standard SNV detection sequencing demonstrated the ability of the former to detect ctDNA in 10 of 14 plasma samples in localized NSCLC patients with a limit of detection of 0.000094%. Moreover, the authors compared PhasED-Seq with standard SNV detection sequencing for the detection of ctDNA in MRD of a locally advanced NSCLC. In details, although both methods were able to detect ctDNA levels in pretreatment, only PhasED-Seq highlighted ctDNA MRD during treatment and consolidation therapies (Kurtz et al., 2021a, 2021b; Cohen et al., 2018).

**Table 1**
Data from selected published articles about MRD detection in NSCLC after treatments with curative intent.

| First Author, Year | Patients | Stage (%) | Treatment (%) | Timepoints | Biomarker | Detection method | MRD relevant findings |
|---|---|---|---|---|---|---|---|
| Abbosh C, 2020 (Abbosh et al., 2020) | 78 | I-III | Surgery (100) | NOS | ctDNA | PCR | MRD positivity was present in 37/45 relapse pts and in 1/23 relapse-free pts. Median time from MRD detection to relapse of 151 days. |
| Chaudhuri A, 2017 ( Chaudhuri et al., 2017) | 40 | I (Ohara et al., 2020)<br>II (Ohara et al., 2020)<br>III (Listì et al., 2019) | Surgery (Tie et al., 2022)<br>RT [88]<br>ChT [70] | 56 days (median) | ctDNA | NGS, (CAPP-Seq) | All pts with detectable posttreatment MRD progressed, whereas all pts with undetectable ctDNA remained disease-free. MRD preceded radiographic progression in 72%, by a median of 5,2 months |
| Chen K, 2019 ( Chen et al., 2019a) | 36 | I (Abbosh et al., 2020)<br>II (Yue et al., 2022)<br>> III [66] | Surgery (100) | Various time points | ctDNA | cSMART | ctDNA positive on the third day after R0 resection suffered a poorer RFS |
| Gale D, 2022 ( Gale et al., 2022) | 88 | I (Abbosh et al., 2017b)<br>II (Rolfo et al., 2018)<br>III (Wu et al., 2020) | Surgery [78]<br>CRT (Waldeck et al., 2022)<br>AT (Abbosh et al., 2020) | Various time points | ctDNA | NGS (RaDaR™) | MRD positivity had specificity > 98.5% and preceded recurrence by a median of 212.5 days. MRD (2 weeks to 4 months after treatment end) was associated with shorter RFS and OS. |
| Kuang P, 2020 ( Kuang et al., 2020) | 38 | I (Ohara et al., 2020)<br>II (Cohen et al., 2018)<br>III (Newman et al., 2016) | Surgery (100) | Various time points | ctDNA | NGS | ctDNA in preoperative plasma was in good accordance with tissue analysis. Post-operative and post-chemotherapy ctDNA was detectable in 22% of sample and were both associated with inferior RFS |
| Li H, 2021 (Li et al., 2021) | 65 | I (Ohara et al., 2020)<br>II (Coakley et al., 2019a)<br>III (Chen et al., 2019b) | Surgery (100) | Various time points | ctDNA | NGS (MRD Score) | Longitudinal profiling of mutation and DNA methylation may have the potential for detecting MRD and predicting recurrence. Among relapsed pts, elevated MRD score was observed between 0.5 and 7 months prior to radiologic recurrence |
| Li N, 2022 (Li et al., 2022) | 119 | I (Passiglia et al., 2021)<br>II (Qiu et al., 2021)<br>III (Li et al., 2022) | Surgery (100)<br>AT (Coakley et al., 2019a) | 1 month | ctDNA | NGS | MRD positive after surgery was associated with shorter RFS. Longitudinal MRD-positive pts had significantly shorter RFS and shorter OS. Serial ctDNA detection preceded radiologic disease recurrence by a median lead time of 8.71 months. |
| Markou A, 2022 (Markou et al., 2022) | 42 | I-III | Surgery (100) | NOS | ctDNA<br>CTC | Real-time methylation specific PCR assays | The incidence of relapses was higher in pts with promoter methylation of APC and SLFN11 in plasma-ctDNA and at least one detected methylated gene promoter in CTC or plasma-ctDNA. A combination of DNA methylation analysis in CTC and plasma-ctDNA was associated with worse RFS |
| Moding E, 2020 (Moding et al., 2020) | 65 | IIB-IIIB | CRT (100)<br>Surgery (0) | Various time points | ctDNA | NGS, (CAPP-Seq) | Pts with undetectable MRD had excellent outcomes. Pts with MRD after CRT who received consolidation immune checkpoint inhibition (CICI) had significantly better outcomes than pts who did not receive CICI. |
| Ohara S, 2020 ( Ohara et al., 2020) | 20 | IB–IIB Powles et al., (2021)<br>III (Pignon et al., 2008) | Surgery (100)<br>AT (Pignon et al., 2008; Pantel and Alix-Panabières, 2019) | 6.3 days (median) | ctDNA | NGS, (CAPP-Seq) | Postoperative MRD positivity predicted shorter RFS |
| Peng M, 2020 ( Peng et al., 2020) | 77 | I (Chen et al., 2019b)<br>II (Wu et al., 2020)<br>III (Waldeck et al., 2022)<br>IV (Pignon et al., 2008) | Surgery (100)<br>AT (Aravanis et al., 2017) | 2 weeks and 3, 6, 12, 18 and 24 months | ctDNA | cSMART | MRD-positive patients were associated with a lower RFS and OS, identifying in advance radiographic findings by a median of 12.6 months |
| Qiu B, 2021 ( Qiu et al., 2021) | 116 | I (Chaudhuri et al., 2017)<br>II (Kurtz et al., 2021a)<br>III (Yan et al., 2021)<br>IV (Little et al., 2005) | Surgery (100)<br>AT [77]<br>CRT (Siegel et al., 2021) | within 30 days | ctDNA | NGS | MRD positivity was significantly associated with worse recurrence-free survival. In stage II-III, the MRD positive pts had benefit from AT, while MRD negative had a low risk of relapse regardless of whether or not AT is administered. MRD positivity precedes radiological recurrence by a median of 88 days |
| | 81 | | Surgery (100) | NOS | CTC | | |

(*continued on next page*)

**Table 1** (*continued*)

| First Author, Year | Patients | Stage (%) | Treatment (%) | Timepoints | Biomarker | Detection method | MRD relevant findings |
|---|---|---|---|---|---|---|---|
| Sawabata N, 2020 ( Sawabata et al., 2020) | | I [79]<br>II (Gale et al., 2022)<br>III (Pantel and Alix-Panabières, 2019)<br>IV (Russo et al., 2021a) | | | | ScreenCell® CTC selection kit | The 2-year OS and RFS rates were 96.5% and 94.6% respectively, for the CTC-negative group, versus 80% and 62.5%, respectively, for the CTC-positive group |
| Waldeck S, 2022 ( Waldeck et al., 2022) | 21 | I (Abbosh et al., 2020)<br>II (Frisone et al., 2021)<br>III (Chin et al., 2019b) | Surgery (100)<br>AT (Frisone et al., 2021)<br>CRT (Abbosh et al., 2020) | 1–2weeks | ctDNA | NGS | Positive ctDNA in early postoperative plasma samples was associated with shorter progression-free survival and overall survival |
| Wu CY, 2020 ( Wu et al., 2020) | 41 | I (Chin et al., 2019b)<br>II (Gale et al., 2022)<br>III (Markou et al., 2022)<br>IV (Little et al., 2005) | Surgery (100) | Day 1 and 3 | CTC | Flow cytometry | An early rebound of CTC counts on postoperative days 1 and 3 was associated with recurrence |
| Xia L, 2021 (Xia et al., 2021) | 330 | I-III | Surgery (100) | 3-day and 1-month | ctDNA | NGS | MRD positivity was a strong predictor for disease relapse. MRD-positive pts who received AT had improved RFS over those not receiving AT, whereas MRD-negative pts receiving AT had lower RFS than their counterparts without AT |
| Yue D, 2022 ( Yue et al., 2022) | 22 | I (Wu et al., 2020)<br>II (Ohara et al., 2020)<br>III (Coakley et al., 2019b) | Surgery (100)<br>NAT (100)<br>AT (NOS) | 3–8 days | ctDNA | NGS | MRD after surgery was an independent risk factor for recurrence. MRD preceded radiographic relapse, with a median time of 6.83 months |
| Zhang J, 2022 ( Zhang et al., 2022) | 261 | I (Reglero and Reglero, 2019)<br>II (Moding et al., 2020)<br>III (Gale et al., 2022) | Surgery (100)<br>NAT (Yue et al., 2022)<br>AT (Sawabata et al., 2020) | 1 month and every 3–6 months | ctDNA | NGS | The positive predictive value of longitudinal detectable MRD was 89.1%, with a median lead time of 3.4 months. However, brain-only recurrence was less commonly detected by MRD |

MRD, Molecular residual disease, NAT, neoadjuvant treatment; AT, adjuvant treatment; ctDNA, circulating tumor DNA; RFS, recurrence-free survival; OS, overall survival; NOS, not otherwise specified; Cht, chemotherapy (without specifying NAT or AT); Pts, patients; CRT, chemoradiation; CTC, circulating tumor cells

#### 3.1.3. *Overcoming lack of sensibility of MRD detection*

Unlike hematologic malignancies, NSCLC as other solid tumors has been known to be a multigenic malignancy (Frisone et al., 2021). Furthermore, genetic abnormalities differ during the clinical course, due to acquired resistance to oncological treatments and adaption to microenvironment changes. In this contest, liquid biopsy represents a valid tool for catching spatial and temporal lung cancer molecular heterogeneity, compared to the invasive tissue re-biopsy strategy (Kim et al., 2021).

Considering the lack of sensibility of liquid biopsy methods for detecting low-volume MRD, tracking multiple mutations may have the potential to improve sensitivity and monitor early recurrence (Abbosh et al., 2017a). Several efforts have been made for developing an ultra-sensitive ctDNA MRD detection system to simultaneously detect up to different driver mutations from low plasma sample volume (Xu et al., 2022).

Compared to advanced disease, liquid biopsy lacks the detection of MRD in intrathoracic disease. Although the amount of cfDNA is significantly higher in a cancer patient's blood (Yan et al., 2021), the amount of ctDNA present is small. Furthermore, the amount of mutant allele frequencies observed in early cancer stages is as low as 1%, dropping even further to 0.1% for samples from patients having undergone treatments (Chin et al., 2019b).

Abbosh et al. (2017) discovered that several factors affected the amount of ctDNA detected in patients' blood, including tumor histology and size, rate of proliferation and necrosis, and the invasion of lymph and blood vessel that can lead to metastasis (Abbosh et al., 2017a, 2017b).

The application of MRD in clinical practice is hampered not only by detection techniques but also by detection time and other factors that influence the sensibility, such as CHIP (Abbosh et al., 2018b).

The latest consensus proposes that ctDNA with abundance $\geq 0.02\%$ can be stably detected in the peripheral blood of perioperative NSCLC patients, which is based on the possibility of ctDNA as an MRD indicator (Shihua et al., 2021). However, applying this detection limit the specificity remains high whereas lacking sensibility. This matter could be overcome by future detection strategies that increase the number of genomic alterations detected including methylation and other multiomics technologies.

### 3.2. *The critical point of view from the bed-side: clinical implications*

#### 3.2.1. *Standardizing the therapeutical setting for MRD testing*

Curative treatment in NSCLC differs from staging at diagnosis, including primary lung surgery and definitive chemoradiotherapy (CRT). Furthermore, the perioperative lung cancer landscape is recently complicated by the recent introduction of immune checkpoint inhibitors (ICI) to standard chemotherapy treatment, both in the neoadjuvant setting and as consolidation treatment after CRT.

Literature search about MRD testing included a heterogenous population, in terms of staging at diagnosis and type of curative treatments. The majority of MRD studies have been performed from postoperative blood samples, with different pathological extension and adjuvant treatment addiction rates (ranging from 3% to 77%). Instead, some authors focused on locally-advanced NSCLC that underwent chemoradiotherapy alone treatment (Chaudhuri et al., 2017; Moding et al.,

**Table 2**
Advantages and limitations of different biomarkers used for MRD detection.

| Biomarkers for MRD | Advantages | Limitations |
|---|---|---|
| ctDNA | Potential for determining prognosis and predicting relapse.<br>Faster and inexpensive sequencing methods available.<br>Can provide a complete picture of the heterogeneous tumor genome. | Unstable with a short half-life (several minutes to a few hours).<br>Amount of cfDNA in body fluids is affected by various factors, thus inconsistencies in results.<br>Different diagnostic platforms within different NGS platforms. |
| CTCs | Available detecting devices show significant sensitivity and cell retention.<br>Potential for determining prognosis, early diagnosis and predicting relapse.<br>Capacity for developing xenografts for developing personalized treatments.<br>Ideal for optimum biochemical characterization and study of cancer cells. | Inconsistencies between commercially available detection devices.<br>Difficult to isolate CTCs in forms accessible for molecular testing.<br>Present in lesser amounts in the blood. |
| TEPs | Comparatively greater in numbers.<br>Easier to purify.<br>Can be used for genomic, transcriptomic and proteomic analysis. | Isolating and analyzing technologies not yet fully developed.<br>Activation must be avoided during analysis. |
| miRNAs | Capacity for use in understanding treatment response.<br>Potential for use in personalized medicine.<br>Present in most bodily fluids.<br>Relative stability in blood. | Inconsistencies in operating and storing techniques.<br>Plasma levels misrepresented due to hemolysis. |
| Extracellular vesicles | Potential for determining prognosis and predicting relapse.<br>To determine the effectiveness of treatments.<br>Present in most bodily fluids. | Isolating and analyzing technologies not yet fully developed. |

2020). Only two studies included patients treated with neoadjuvant chemotherapy (Yue et al., 2022; Zhang et al., 2022).

A major benefit of standardizing MRD detection would be the ability to guide clinical choice in the postoperative setting, especially in more stage III NSCLC patients that are affected by a higher risk of relapse after surgery (76%) compared to stage I (45%) (Frisone et al., 2021) to personalize the treatment of localized disease.

MRD demonstrated a prognostic value both after surgery (Abbosh et al., 2020; Chaudhuri et al., 2017; Qiu et al., 2021; Xia et al., 2021; Zhang et al., 2022; Abbosh et al., 2017a; Chen et al., 2019b) and post-adjuvant therapy (Kuang et al.; Qiu et al., 2021).

Post-surgery ctDNA-MRD monitoring confirmed its prognostic role, demonstrating a risk of recurrence of 11 times higher in MRD-positive patients compared to MRD-negative patients (Xia et al., 2021).

Studies that evaluated the post-adjuvant value of MRD indicated that the detection of MRD could select patients that need more intensive post-operative treatments (Powles et al., 2021).

A uniform population selection about the therapeutic setting is preferable for future MRD studies to determine exactly which patients would substantially benefit from adjuvant chemotherapy, considering that patients with undetected MRD may be potentially cured after surgery (Zhang et al., 2022).

#### 3.2.2. *Standardizing the sampling time after curative treatment*

Defining the appropriate MRD timing is an important need for future application in clinical practice limiting the potential MRD toxicity for NSCLC patients. As shown in Table 1, the literature differs in terms of blood sample time points after curative treatments ranging from a few days to months with various follow-up time points.

A recent meta-analysis investigated the prognostic potential of circulating tumor DNA detection in resectable NSCLC, focusing on MRD at different time points. The authors demonstrated that ctDNA detection in a range period of 3 days-2 weeks after surgery could be a more reliable and feasible timing for identifying patients with a higher risk for relapse (Wang et al., 2022).

The DYNAMIC clinical trial observed changes, at different time points, in ctDNA levels of patients after surgery. Although the low ctDNA detection rate (~19%), due to low sensitivity and lack of standardization, this study discovered that MRD-positive patients showed a statistically significant increase in the half-life of ctDNA, as compared to those of MRD-negative patients (Chen et al., 2019c) In fact, they proposed the use of ctDNA, to detect MRD, three days after treatment as a possible basis for deciding subsequent decisions (Chen et al., 2019c) This could be explained by the fact that surgical trauma could influence the real level of ctDNA in the plasma (Waldeck et al., 2022).

In our review we focused on MRD detection after curative treatments, as previously explained. However, some studies evaluated MRD at different timepoints, including the preoperative setting (Kuang et al.; Ohara et al., 2020; Peng et al., 2020; Xia et al., 2021; Abbosh et al., 2017a) showing an independent risk factor for RFS although post-operative MRD evaluation demonstrated a more powerful in predicting the prognosis (Li and Cui, 2022).

#### 3.2.3. *Quantifying radiological relapse anticipation*

The clinical benefit of introducing MRD in practice includes a survival advantage through an earlier relapse identification.

Making unvisible visible, microscopical evidence of relapse in liquid biopsy could help clinicians in personalized oncological treatment or radiological timing during follow-up workup. For early-stage NSCLC patients, an enhanced adjuvant therapy or additional surgery can contribute to the cure. Moreover, when occult distant metastases have occurred, an anticipated first-line treatment could better control a lower tumor burden.

In our review, eight studies showed a descriptive analysis of preceded recurrence with MRD (Abbosh et al., 2020; Chaudhuri et al., 2017; Gale et al., 2022; Li et al., 2022; Peng et al., 2020; Qiu et al., 2021; Yue et al., 2022; Zhang et al., 2022). Among relapsed patients, elevated MRD score was observed with a median value between 3 and 12 months prior to radiological recurrence.

Longitudinal MRD-positive patients demonstrated a significantly shorter survival (Qiu et al., 2021), but the survival influence of MRD-guided therapeutical decisions needs further evidence through randomized controlled trials.

### 3.3. *The critical point of view from the bunch-side: community perspectives*

#### 3.3.1. *Understanding the epidemiological impact of relapsed NSCLC*

Though decades have passed, cancer remains among the leading causes of global mortality rates. Of these, one of the most concerning is lung cancer, accounting for 11.4% of cancer incidences and 18% (the highest) of deaths in 2020 (Sung et al., 2021). In addition, WHO predicts an estimated 30.2 million incidences of cancer in the year 2040, a sharp 56% increase from the number of new cases in 2020 (International Agency for Research on Cancer, 2020).

The increasing prevalence and financial burden of cancer highlight the need to focus on methods for earlier diagnosis. Earlier diagnosis has been linked with better disease outcomes through timely intervention (Phallen et al., 2017).

Further worsening the situation is the occurrence of cancer relapse or reoccurrence, which can lead to a worse prognosis, thus complicating the search for appropriate therapies with long-lasting effects and significantly contributing to increased mortality rates, particularly in

NSCLC (Reglero and Reglero, 2019).

More efforts should be made for enhancing MRD methods that could guide clinical choices, together with clinical and histopathological prognostic factors, to change the dismal behavior of this malignancy.

### 3.3.2. *Awareness of MRD clinical significance in NSCLC*

ctDNA is an adjuvant biomarker capable of both detecting MRD following surgery and defining the clonality of relapsing disease. These data pave the way for clinical trials predicating the escalation of adjuvant chemotherapy in NSCLC patients who exhibit MRD-positive status following surgery.

MRD levels can also be used to determine subsequent treatment after detection of relapse, to detect treatment-resistant mutation, and to be used as a 'surrogate endpoint' in clinical trials (Coakley et al., 2019b).

The literature search showed interesting results about the predictive role of the MRD test, as shown in Table 1. The TRACERx trials are also aiming to evaluate the effectiveness of using ctDNA as a measure of MRD and its subsequent clinical relevance (Bailey et al., 2021). Moding et al. demonstrated that patients with undetectable ctDNA after CRT had better outcomes whether or not they received consolidation ICI. Among such patients, one died from ICI-related pneumonitis, highlighting the potential utility of only treating patients with detectable MRD (Moding et al., 2020). Of note, Zhang et al. revealed in a subgroup analysis that patients with undetectable MRD might not benefit from adjuvant therapy (Zhang et al., 2022).

Qiu et al. investigated the clinical utility of ctDNA with a benefit from adjuvant chemotherapy only in stage II-III patients, the post-surgical ctDNA positive (Qiu et al., 2021). Moreover, LUNGCA-1 trial authors showed similar results, demonstrating a predictive role of MRD positivity after surgery with a significantly improved relapse-free survival over those not receiving adjuvant treatment (Xia et al., 2021).

Finally, the increasing knowledge about MRD techniques and its clinical utility could be applied to enhancing early detection in screening procedures, representing an intriguing research field of interest.

### 3.3.3. *Cost-effectiveness analysis of MRD testing*

An urgent need is to improve liquid biopsy quality and, at the same time, reduce cost methods for bridging MRD into regular clinical practice. Liquid biopsy represents a more feasible alternative to tissue biopsy, with a favorable financial impact in the future. A recent cost-effectiveness analysis of liquid biopsy in NSCLC patients (Englmeier et al., 2022), demonstrated a positive clinical effect accompanied by a moderate cost-effectiveness in metastatic setting.

The economic impact of MRD application in early-stage NSCLC has not been extensively studied. However, our review showed several relevant clinical advantages of using MRD after curative treatments, in spite of intrinsic costs (Pisapia et al., 2021). Firstly, MRD detects earlier recurrence guiding radiological follow-up currently based on unselected criteria. Secondly, early intervention in MRD positive patients could delay recurrence and consequently improve prognosis in disease with well-known dismal outcomes. Furthermore, MRD test could select cured patients that don't have to receive additional therapies, with both financial and clinical benefit (Gristina et al., 2022).

We need further evidence through cost-effective analysis practice, with the aim of evaluating the potential MRD toxicity for NSCLC patients due to anticipated oncological treatments with insignificant survival impact or therapeutic selection confounded by unrepresentative MRD detection (Listì et al., 2019).

### 3.3.4. *On-going future clinical trials*

Several clinical trials on patients with NSCLC are being conducted, with primary or secondary goals to measure MRDs for disease prognosis and/or predicting relapse. Most of the current NSCLC trials (Table 3; data taken from https://beta.clinicaltrials.gov/) are using ctDNA as a measurement for MRD.

**Table 3**
Current clinical trials evaluating the MRD detection in NSCLC.

| Clinical Trial ID number | Study Title | Study Type | MRD outcomes |
|---|---|---|---|
| NCT04585477 | "Adjuvant Durvalumab for Early-Stage NSCLC Patients With ctDNA Minimal Residual Disease" | Intervention (AVENIO ctDNA Surveillance Kit and Durvalumab) | Assessing change/ presence or absence of MRD through level of ctDNA after treatment with durvalumab |
| NCT04758949 | "FL-101 in Surgically Resectable Non-Small Cell Lung Cancer" | Intervention (FL-101, Nivolumab and Placebo) | Assessing presence of MRD (by detecting ctDNA) for probability of relapse in relation to treatment |
| NCT04976296 | "MRD Monitoring in Lung Cancer After Resection" | Observation (MRD diagnostic test) | Prognostic role of MRD. Separating cohort for therapy after surgery based on MRD measurement and determining time difference between relapse detection by MRD and imaging diagnostics |
| NCT05059444 | "ORACLE: Observation of ResiduAl Cancer with Liquid Biopsy Evaluation" | Observation (Guardant Reveal diagnostic test) | Assessing MRD, through ctDNA detection, for prediction of disease relapse at/before clinical detection; for accurately detecting relapse chances; and time lapse between ctDNA detection and clinical detection of relapse |
| NCT05167604 | "Clinical Value of MRD Monitoring for Adjuvant Therapy in Postoperative NSCLC" | Observation | Assessing MRD by measuring level of ctDNA throughout course of study |
| NCT05165160 | "Residual Disease Evaluation of Resected NSCLC by cirDNA Analysis" | Intervention (MiTest) | Detecting ctDNA, before and after surgery, as a measure of MRD for determining chances of relapse |
| NCT04385368 | MERMAID-1 | Intervention | Phase III study assessing the role of ctDNA MRD to predict recurrence and personalize treatment strategies after surgery in patients randomly assigned to adjuvant durvalumab with platinum-based chemotherapy versus placebo plus platinum-based chemotherapy |
| NCT04642469 | MERMAID-2 | Intervention | Phase III study assessing the role of ctDNA MRD to predict recurrence and personalize treatment strategies after surgery with serial ctDNA analysis for up to 2 |

(*continued on next page*)

**Table 3** (*continued*)

| Clinical Trial ID number | Study Title | Study Type | MRD outcomes |
|---|---|---|---|
| | | | years after completion of curative-intent treatment. When ctDNA becomes positive, they are randomly assigned to receive adjuvant durvalumab or placebo for up to 2 years. |
| NCT02998528 | CheckMate-816 | Intervention | Exploratory analysis in a phase III trial that is comparing neoadjuvant nivolumab plus chemotherapy versus chemotherapy alone. Investigators are using the ArcherDx Personalized Cancer Monitoring platform to assess ctDNA dynamics, which they will correlate with response to neoadjuvant systemic therapy. |

## 4. Conclusion

The integration of liquid biopsy platforms has been increasingly studied in NSCLC, with a promising role in the adjuvant setting MRD for earlier recurrence detection after curative treatments and for tailoring effective therapies without predictable toxicities.

The clinical application of MRD is hampered by lack of standardization and sensibility. To date there is no standardized technology for MRD analysis, due to ctDNA low concentration and false positives ascribed to clonal hematopoiesis of undetermined potential. Another data interpretation question is the huge heterogeneity of the included population, in terms of staging at diagnosis and type of curative treatments. The current perioperative lung cancer therapeutical landscape has been recently enhanced by the introduction of ICI to standard chemotherapy treatment, both in the neoadjuvant setting and as consolidation treatment after CRT. Of note, in contrast with previous reports our literature search was extended to different MRD techniques and several curative treatment approaches, including not only patients that underwent surgery but also locally advanced NSCLC that underwent chemoradiotherapy as definitive treatment or neoadjuvant chemotherapy.

A cost-effective analysis of MRD application in NSCLC has not been extensively studied. However, our review showed several relevant clinical advantages of using MRD after curative treatments, in spite of intrinsic costs. Firstly, MRD detects earlier recurrence guiding radiological follow-up, with minimally invasive impact on patients' quality of life (Passiglia et al., 2021). Secondly, early intervention in MRD positive patients could delay recurrence and consequently improve prognosis in disease with well-known dismal outcomes. Moreover, making unvisible visible, the extended molecular analysis of microscopical residual disease could help clinicians in personalizing oncological treatment with a targeted approach. Furthermore, MRD test could select cured patients that don't have to receive additional therapies, with both financial and clinical benefit.

MRD represents an intriguing research field in NSCLC and its intrinsic limits could be overcome by close cooperation between biologists and oncology clinicians with a consolidated knowledge in liquid biopsy. Incorporating the critical point of view from benchside, bedside and bunchside, could lead to a better data interpretation, a more homogeneous population included in clinical trials and a feasible applicability of MRD in the next future.

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## References

Siegel, R.L., Miller, K.D., Fuchs, H.E., Jemal, A., 2021. Cancer Statistics, 2021. CA Cancer J. Clin. 71 (1), 7–33. Jan 1 [cited 2022 Oct 16]. ⟨https://onlinelibrary.wiley.com/doi/full/10.3322/caac.21654⟩.

Little, A.G., Rusch, V.W., Bonner, J.A., Gaspar, L.E., Green, M.R., Webb, W.R., et al., 2005. Patterns of surgical care of lung cancer patients. Ann. Thorac. Surg. 80 (6), 2051–2056 (Available from). ⟨https://pubmed.ncbi.nlm.nih.gov/16305843/⟩.

Pignon, J.P., Tribodet, H., Scagliotti, G. v, Douillard, J.Y., Shepherd, F.A., Stephens, R.J., et al., 2008. Lung adjuvant cisplatin evaluation: a pooled analysis by the LACE Collaborative Group. J. Clin. Oncol. 26 (21), 3552–3559 (Available from). ⟨https://pubmed.ncbi.nlm.nih.gov/18506026/⟩.

Russo A. , Incorvaia L. , del Re M. , Malapelle U. , Capoluongo E. , Gristina V. , et al. The molecular profiling of solid tumors by liquid biopsy: a position paper of the AIOM-SIAPEC-IAP-SIBioC-SIC-SIF Italian Scientific Societies. ESMO Open [Internet]. 2021a Jun 1 [cited 2022 Oct 16];6(3). Available from: ⟨https://pubmed.ncbi.nlm.nih.gov/34091263/⟩.

Pantel, K., Alix-Panabières, C., 2019. Liquid biopsy and minimal residual disease—latest advances and implications for cure. Nat. Rev. Clin. Oncol. 16 (7), 409–424.

Powles, T., Assaf, Z.J., Davarpanah, N., Banchereau, R., Szabados, B.E., Yuen, K.C., et al., 2021. ctDNA guiding adjuvant immunotherapy in urothelial carcinoma. Nat. 595 (7867), 432–597 (Available from). ⟨https://pubmed.ncbi.nlm.nih.gov/34135506/⟩.

Tie, J., Cohen, J.D., Lahouel, K., Lo, S.N., Wang, Y., Kosmider, S., et al., 2022. Circulating Tumor DNA Analysis Guiding Adjuvant Therapy in Stage II Colon Cancer. N. Engl. J. Med. 386 (24), 2261–2272 (Available from). ⟨https://pubmed.ncbi.nlm.nih.gov/35657320/⟩.

Pellini, B., Chaudhuri, A.A., 2022a. Circulating Tumor DNA Minimal Residual Disease Detection of Non–Small-Cell Lung Cancer Treated with Curative Intent. J. Clin. Oncol. 40 (6), 567–575. Feb 20.

Abbosh, C., Frankell, A., Garnett, A., Harrison, T., Weichert, M., Licon, A., et al., 2020. Abstract CT023: Phylogenetic tracking and minimal residual disease detection using ctDNA in early-stage NSCLC: A lung TRACERx study. Cancer Res. 80 (16_Supplement). CT023–CT023. Available from. ⟨https://aacrjournals.org/cancerres/article/80/16_Supplement/CT023/645029/Abstract-CT023-Phylogenetic-tracking-and-minimal⟩.

Chaudhuri, A.A., Chabon, J.J., Lovejoy, A.F., Newman, A.M., Stehr, H., Azad, T.D., et al., 2017. Early Detection of Molecular Residual Disease in Localized Lung Cancer by Circulating Tumor DNA Profiling. Cancer Disco 7 (12), 1394–1403 (Available from). ⟨https://pubmed.ncbi.nlm.nih.gov/28899864/⟩.

Chen, K., Zhao, H., Shi, Y., Yang, F., Wang, L.T., Kang, G., et al., 2019a. Perioperative Dynamic Changes in Circulating Tumor DNA in Patients with Lung Cancer (DYNAMIC). Clin. Cancer Res. 25 (23), 7058–7067 (Available from). ⟨https://pubmed.ncbi.nlm.nih.gov/31439586/⟩.

Gale, D., Heider, K., Ruiz-Valdepenas, A., Hackinger, S., Perry, M., Marsico, G., et al., 2022. Residual ctDNA after treatment predicts early relapse in patients with early-stage non-small cell lung cancer. Ann. Oncol. 33 (5), 500–510. May 1.

Kuang P.P., Li N., Liu Z., Sun T.Y., Wang S.Q., Hu J., et al. Circulating Tumor DNA Analyses as a Potential Marker of Recurrence and Effectiveness of Adjuvant Chemotherapy for Resected Non-Small-Cell Lung Cancer. Available from: ⟨www.frontiersin.org⟩.

Li, H., Ma, Z.L., Li, B., Pan, Y. jian, Xiang, J.Q., Zhang, Y.W., et al., 2021. Potential utility of longitudinal somatic mutation and methylation profiling for predicting molecular residual disease in postoperative non-small cell lung cancer patients. Cancer Med. 10 (23), 8377–8386. Dec 1.

Li, N., Wang, B.X., Li, J., Shao, Y., Li, M.T., Li, J.J., et al., 2022. Perioperative circulating tumor DNA as a potential prognostic marker for operable stage I to IIIA non–small cell lung cancer. Cancer 128 (4), 708–718. Feb 15.

Markou, Londra D., Tserpeli, V., Kollias, Tsaroucha, E., Vamvakaris, I., et al., 2022. DNA methylation analysis of tumor suppressor genes in liquid biopsy components of early stage NSCLC: a promising tool for early detection. Clin. Epigenet. 14 (1) (Available from). ⟨https://pubmed.ncbi.nlm.nih.gov/35538556/⟩.

Moding, E.J., Liu, Y., Nabet, B.Y., Chabon, J.J., Chaudhuri, A.A., Hui, A.B., et al., 2020. Circulating tumor DNA dynamics predict benefit from consolidation immunotherapy in locally advanced non-small cell lung cancer. Nat. Cancer 1 (2), 176–183. Feb 1.

Ohara, S., Suda, K., Sakai, K., Nishino, M., Chiba, M., Shimoji, M., et al., 2020. Prognostic implications of preoperative versus postoperative circulating tumor DNA in surgically resected lung cancer patients: A pilot study. Transl. Lung Cancer Res 9 (5), 1915–1923. Oct 1.

Peng, M., Huang, Q., Yin, W., Tan, S., Chen, C., Liu, W., et al., 2020. Circulating Tumor DNA as a Prognostic Biomarker in Localized Non-small Cell Lung Cancer. Front

Oncol. [Internet] 10, 561598 (Available from). ⟨http://www.ncbi.nlm.nih.gov/pubmed/33042842⟩.
Qiu, B., Guo, W., Zhang, F., Lv, F., Ji, Y., Peng, Y., et al., 2021. Dynamic recurrence risk and adjuvant chemotherapy benefit prediction by ctDNA in resected NSCLC. Nat. Commun. 12 (1). Dec 1.
Sawabata, N., Nakamura, T., Kawaguchi, T., Watanabe, T., Ouji, N.S., Ito, T., et al., 2020. Circulating tumor cells detected only after surgery for non-small cell lung cancer: is it a predictor of recurrence? J. Thorac. Dis. 12 (9), 4623–4632 (Available from). ⟨https://pubmed.ncbi.nlm.nih.gov/33145035/⟩.
Waldeck, S., Mitschke, J., Wiesemann, S., Rassner, M., Andrieux, G., Deuter, M., et al., 2022. Early assessment of circulating tumor DNA after curative-intent resection predicts tumor recurrence in early-stage and locally advanced non-small-cell lung cancer. Mol. Oncol. 16 (2), 527–537. Jan 1.
Wu, C.Y., Lee, C.L., Wu, C.F., Fu, J.Y., Yang, C.T., Wen, C.T., et al., 2020. Circulating tumor cells as a tool of minimal residual disease can predict lung cancer recurrence: A longitudinal, Prospective Trial. Diagnostics 10 (3). Feb 1.
Xia L., Mei J., Kang R., Deng S., Chen Y., Yang Y., et al. Perioperative ctDNA-Based Molecular Residual Disease Detection for Non–Small Cell Lung Cancer: A Prospective Multicenter Cohort Study (LUNGCA-1). Clinical Cancer Research, 2021 Aug 2;
Yue, D., Liu, W., Chen, C., Zhang, T., Ma, Y., Cui, L., et al., 2022. Circulating tumor DNA predicts neoadjuvant immunotherapy efficacy and recurrence-free survival in surgical non-small cell lung cancer patients. Transl. Lung Cancer Res 11 (2), 263–276. Feb 1.
Zhang, J.T., Liu, S.Y., Gao, W., Liu, S.Y.M., Yan, H.H., Ji, L., et al., 2022. Longitudinal Undetectable Molecular Residual Disease Defines Potentially Cured Population in Localized Non-Small Cell Lung. Cancer Cancer Disco 12 (7), 1690–1701 (Available from). ⟨http://www.ncbi.nlm.nih.gov/pubmed/35543554⟩.
Palmirotta, R., Lovero, D., Cafforio, P., Felici, C., Mannavola, F., Pellè, E., et al., 2018. Liquid biopsy of cancer: a multimodal diagnostic tool in clinical oncology. Ther. Adv. Med Oncol. 10, 1758835918794630.
Rolfo C., Mack P.C., Scagliotti G.V., Baas P., Barlesi F., Bivona T.G., et al. Liquid Biopsy for Advanced Non-Small Cell Lung Cancer (NSCLC): A Statement Paper from the IASLC. Journal of Thoracic Oncology, 2018.
Coakley, M., Garcia-Murillas, I., Turner, N.C., 2019a. Molecular residual disease and adjuvant trial design in solid tumors. Clin. Cancer Res. 25 (20), 6026–6034.
Russo, A., Incorvaia, L., Del Re, M., Malapelle, U., Capoluongo, E., Gristina, V., et al., 2021b. The molecular profiling of solid tumors by liquid biopsy: a position paper of the AIOM-SIAPEC-IAP-SIBioC-SIC-SIF Italian Scientific Societies. ESMO Open 6 (3), 100164 (Jun).
Lamy, P.J., van der Leest, P., Lozano, N., Becht, C., Duboeuf, F., Groen, H.J.M., et al., 2020. Mass spectrometry as a highly sensitive method for specific circulating tumor DNA analysis in NSCLC: a comparison study. Cancers (Basel) 12 (10), 3002.
Aravanis, A.M., Lee, M., Klausner, R.D., 2017. Next-generation sequencing of circulating tumor DNA for early cancer detection. Cell [Internet] 168 (4), 571–574 (Available from). ⟨https://pubmed.ncbi.nlm.nih.gov/28187279/⟩.
Chin, R.I., Chen, K., Usmani, A., Chua, C., Harris, P.K., Binkley, M.S., et al., 2019a. Detection of Solid Tumor Molecular Residual Disease (MRD) Using Circulating Tumor DNA (ctDNA). Mol. Diagn. Ther. 23 (3) (Available from). ⟨https://pubmed.ncbi.nlm.nih.gov/30941670/⟩.
Herbreteau, G., Vallée, A., Charpentier, S., Normanno, N., Hofman, P., Denis, M.G., 2019. Circulating free tumor DNA in non-small cell lung cancer (NSCLC): clinical application and future perspectives. Thorac. Dis. (Suppl 1), S113–S126 (doi: 10.21037/jtd.2018.12.18). Available from. ⟨https://doi.org/10.21037/jtd.2018.12.18⟩.
Abbosh, C., Swanton, C., Birkbak, N.J., 2019. Clonal haematopoiesis: a source of biological noise in cell-free DNA analyses. Ann. Oncol. 30 (3), 358–359 (Available from). ⟨https://pubmed.ncbi.nlm.nih.gov/30649226/⟩.
Abbosh, C., Birkbak, N.J., Swanton, C., 2018a. Early stage NSCLC - challenges to implementing ctDNA-based screening and MRD detection. Nat. Rev. Clin. Oncol. 15 (9), 577–586 (Available from). ⟨https://pubmed.ncbi.nlm.nih.gov/29968853/⟩.
Li, F.Q., Cui, J.W., 2022. Circulating tumor DNA-minimal residual disease: An up-and-coming nova in resectable non-small-cell lung cancer. Crit. Rev. Oncol. Hematol. 179 (Available from). ⟨https://pubmed.ncbi.nlm.nih.gov/36031171/⟩.
Pellini, B., Chaudhuri, A.A., 2022b. Circulating Tumor DNA Minimal Residual Disease Detection of Non-Small-Cell Lung Cancer Treated With Curative Intent. J. Clin. Oncol. 40 (6), 567–575 (Available from). ⟨https://pubmed.ncbi.nlm.nih.gov/34985936/⟩.
Newman, A.M., Lovejoy, A.F., Klass, D.M., Kurtz, D.M., Chabon, J.J., Scherer, F., et al., 2016. Integrated digital error suppression for improved detection of circulating tumor DNA. Nat. Biotechnol. 34 (5), 547–555 (Available from). ⟨https://pubmed.ncbi.nlm.nih.gov/27018799/⟩.
Kurtz, D.M., Soo, J., Co Ting Keh, L., Alig, S., Chabon, J.J., Sworder, B.J., et al., 2021a. Enhanced detection of minimal residual disease by targeted sequencing of phased variants in circulating tumor DNA. Nat. Biotechnol. 39 (12), 1537–1547 (Available from). ⟨https://pubmed.ncbi.nlm.nih.gov/34294911/⟩.
Kurtz D.M., Chabon J.J., Sworder B.J.. Abstract #8518: Leveraging phased variants for personalized minimal residual disease detection in localized non-small cell lung cancer. ASCO [Internet]. 2021b [cited 2022 Nov 27]; Available from: ⟨https://foresight-dx.com/documents/KurtzDM_Lung_Poster_ASCO2021.pdf⟩.
Cohen, J.D., Li, L., Wang, Y., Thoburn, C., Afsari, B., Danilova, L., et al., 2018. Detection and localization of surgically resectable cancers with a multi-analyte blood test. Sci. 359 (6378), 926–930 (Available from). ⟨https://pubmed.ncbi.nlm.nih.gov/29348365/⟩.
Frisone, D., Friedlaender, A., Addeo, A., 2021. The Role and Impact of Minimal Residual Disease in NSCLC. Curr. Oncol. Rep. 23 (12), 1–6.
Kim C., Xi L., Cultraro C.M., Wei F., Jones G., Cheng J., et al. Longitudinal Circulating Tumor DNA Analysis in Blood and Saliva for Prediction of Response to Osimertinib and Disease Progression in EGFR-Mutant Lung Adenocarcinoma. Vol. 13, Cancers, 2021.
Abbosh, C., Birkbak, N.J., Wilson, G.A., Jamal-Hanjani, M., Constantin, T., Salari, R., et al., 2017a. Phylogenetic ctDNA analysis depicts early-stage lung cancer evolution. Nat. 545 (7655), 446–451 (Available from). ⟨https://pubmed.ncbi.nlm.nih.gov/28445469/⟩.
Xu, J., Pu, Y., Lin, R., Xiao, S., Fu, Y., Wang, T., 2022. PEAC: An Ultrasensitive and Cost-Effective MRD Detection System in Non-small Cell Lung Cancer Using Plasma Specimen. Front Med (Lausanne) 9. Mar 3.
Yan, Y. yan, Guo, Q. ru, Wang, F. hua, Adhikari, R., Zhu, Z. yan, Zhang, H. yan, et al., 2021. Cell-Free DNA: Hope and Potential Application in Cancer. Front Cell Dev. Biol. 9, 192.
Chin, R.I., Chen, K., Usmani, A., Chua, C., Harris, P.K., Binkley, M.S., et al., 2019b. Detection of solid tumor molecular residual disease (MRD) using circulating tumor DNA (ctDNA). Mol. Diagn. Ther. 23 (3), 311–331.
Abbosh, C., Birkbak, N.J., Wilson, G.A., Jamal-Hanjani, M., Constantin, T., Salari, R., et al., 2017b. Phylogenetic ctDNA analysis depicts early-stage lung cancer evolution. Nature 545 (7655), 446–451 (Apr).
Abbosh, C., Birkbak, N.J., Swanton, C., 2018b. Early stage NSCLC — challenges to implementing ctDNA-based screening and MRD detection. Nat. Rev. Clin. Oncol. 15 (9), 577–586. Sep 1.
Shihua, D., Hongsheng, X., Lin, Y., 2021. Application Value of ctDNA-based MRD Dedection in Early Stage Non-small Cell Lung Cancer after Radical Surgery. Chin. J. Lung Cancer 24 (12), 838–846.
Chen, K., Kang, G., Zhao, H., Zhang, K., Zhang, J., Yang, F., et al., 2019b. Liquid biopsy in newly diagnosed patients with locoregional (I-IIIA) non-small cell lung cancer. Expert Rev. Mol. Diagn. 19 (5), 419–427. May 4.
Wang, B., Pei, J., Wang, S., Cheng, K., Yu, J., Liu, J., 2022. Prognostic potential of circulating tumor DNA detection at different time periods in resectable non-small cell lung cancer: Evidence from a meta-analysis. Crit. Rev. Oncol. Hematol. 177, 103771 (Available from). ⟨https://linkinghub.elsevier.com/retrieve/pii/S1040842822001950⟩.
Chen, K., Zhao, H., Shi, Y., Yang, F., Wang, L.T., Kang, G., et al., 2019c. Perioperative dynamic changes in circulating tumor DNA in patients with lung cancer (DYNAMIC). Clin. Cancer Res. 25 (23), 7058–7067.
Sung, H., Ferlay, J., Siegel, R.L., Laversanne, M., Soerjomataram, I., Jemal, A., et al., 2021. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J. Clin. 71 (3), 209–249.
International Agency for Research on Cancer, 2020. Cancer Tomorrow. World Health Organization,.
Phallen, J., Sausen, M., Adleff, V., Leal, A., Hruban, C., White, J., et al., 2017. Direct detection of early-stage cancers using circulating tumor DNA. Sci. Transl. Med 9 (403) (Aug).
Reglero, C., Reglero, G., 2019. Precision nutrition and cancer relapse prevention: a systematic literature review. Nutrients 11 (11).
Coakley, M., Garcia-Murillas, I., Turner, N.C., 2019b. Molecular residual disease and adjuvant trial design in solid tumors. Clin. Cancer Res. 25 (20), 6026–6034.
Bailey, C., Black, J.R.M., Reading, J.L., Litchfield, K., Turajlic, S., McGranahan, N., et al., 2021. Tracking Cancer Evolution through the Disease Course. Cancer Disco 11 (4).
Englmeier, F., Bleckmann, A., Brückl, W., Griesinger, F., Fleitz, A., Nagels, K., 2022. Clinical benefit and cost-effectiveness analysis of liquid biopsy application in patients with advanced non-small cell lung cancer (NSCLC): a modelling approach. J. Cancer Res Clin. Oncol. 1–17 (Available from). ⟨https://link.springer.com/article/10.1007/s00432-022-04034-w⟩.
Pisapia, P., Pepe, F., Gristina, V., La Mantia, M., Francomano, V., Russo, G., Iaccarino, A., Galvano, A., 2021. A narrative review on the implementation of liquid biopsy as a diagnostic tool in thoracic tumors during the COVID-19 pandemic. Mediastinum 5, 27. https://doi.org/10.21037/med-21-9.
Gristina, V., Barraco, N., La Mantia, M., Castellana, L., Insalaco, L., Bono, M., Perez, A., Sardo, D., Inguglia, S., Iacono, F., Cutaia, S., Bazan Russo, T.D., Francini, E., Incorvaia, L., Badalamenti, G., Russo, A., Galvano, A., Bazan, V., 2022. Clinical Potential of Circulating Cell-Free DNA (cfDNA) for Longitudinally Monitoring Clinical Outcomes in the First-Line Setting of Non-Small-Cell Lung Cancer (NSCLC): A Real-World Prospective Study. Cancers (Basel) 14 (23), 6013. https://doi.org/10.3390/cancers14236013.
Listì, A., Barraco, N., Bono, M., Insalaco, L., Castellana, L., Cutaia, S., Ricciardi, M.R., Gristina, V., Bronte, E., Pantuso, G., Passiglia, F., 2019. Immuno-targeted combinations in oncogene-addicted non-small cell lung cancer. Transl. Cancer Res. 8 (Suppl 1), S55–S63. https://doi.org/10.21037/tcr.2018.10.04. PMID: 35117064; PMCID: PMC8799193.
Passiglia, F., Galvano, A., Gristina, V., Barraco, N., Castiglia, M., Perez, A., La Mantia, M., Russo, A., Bazan, V., 2021. Is there any place for PD-1/CTLA-4 inhibitors combination in the first-line treatment of advanced NSCLC?-a trial-level meta-analysis in PD-L1 selected subgroups. Transl. Lung Cancer Res. 10 (7), 3106–3119. https://doi.org/10.21037/tlcr-21-52.

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