Trends in Cancer

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Review

Implementing circulating tumor DNA as a prognostic biomarker in resectable non-small cell lung cancer

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Systemic treatment of resectable non-small cell lung cancer (NSCLC) is evolving with emerging neoadjuvant, perioperative, and adjuvant immunotherapy approaches. Circulating tumor DNA (ctDNA) detection at clinical diagnosis, during neoadjuvant therapy, or after resection may discern high-risk patients who might benefit from therapy escalation or switch. This Review summarizes translational implications of data supporting ctDNA-based risk determination in NSCLC and outstanding questions regarding ctDNA validity/utility as a prognostic biomarker. We discuss emerging ctDNA capabilities to refine clinical tumor–node–metastasis (TNM) staging in lung adenocarcinoma, ctDNA dynamics during neoadjuvant therapy for identifying patients deriving suboptimal benefit, and postoperative molecular residual disease (MRD) detection to escalate systemic therapy. Considering differential relapse characteristics in landmark MRD-negative/MRD-positive patients, we propose how ctDNA might integrate with pathological response data for optimal postoperative risk stratification.

Highlights

Evaluation of circulating tumor DNA (ctDNA) may meet the need for biomarker-driven optimization of therapeutic allocation in patients with resectable non-small cell lung cancer (NSCLC).

Potential uses for ctDNA evaluation in this setting include at clinical diagnosis to refine tumor–node–metastasis staging, during neoadjuvant therapy as an early endpoint or to detect upfront resistance requiring treatment changes, and for guiding adjuvant treatment decisions.

Increased sensitivity with second-generation versus first-generation tumor-informed ctDNA assays is resulting in ctDNA detection in a higher proportion of patients with lung adenocarcinoma and greater prognostic capability. Conclusive data regarding impact of increased sensitivity on molecular residual disease (MRD) detection is outstanding.

Patterns of relapse differ between landmark MRD-positive and MRD-negative patients with NSCLC, with the former group enriched for poor prognosis patients with non-intracranial distant micrometastatic disease who are at risk of early disease relapse.

ctDNA in NSCLC

Multiple studies have demonstrated that neoadjuvant, perioperative, or adjuvant immune **checkpoint inhibitor (CPI)** (see Glossary) therapy, alone or with chemotherapy, can improve outcomes for patients with resectable NSCLC [1]. Encouraging outcomes with neoadjuvant immune CPI therapy in patients with resectable NSCLC might reflect improved therapeutic activity when primary tumor and draining lymph nodes remain *in situ* [2–4]; exposure to adjuvant immunotherapy might consolidate antitumor immunity, suppress micrometastatic disease, and prevent surgery-related inflammatory signals capable of driving tumor progression [1,5,6]. However, some immunotherapy-eligible patients are cured by surgical resection alone, whereas some high-risk patients with micrometastatic disease exhibit resistance to perioperative anti-**programmed cell death ligand 1 (PD-L1)** CPIs, reinforcing the need for biomarker-driven optimization of therapeutic allocation in this setting in which cure is achievable.

Implementation of novel biomarkers for detecting or predicting the presence of residual tumor will be required to refine this therapeutic approach. Recent advances in liquid biopsies are providing a noninvasive approach to tumor sampling. Tumor cells shed nucleic acids, freely or associated with other structures such as vesicles into body fluids, including blood. This **circulating tumor DNA (ctDNA)** refers to the fraction of cell-free DNA in a patient's blood that originates from a tumor and can be characterized by genomic analysis methods [7–9]. Recent findings suggest that ctDNA could be used as a prognostic biomarker in resectable NSCLC, including for patients who experience adverse clinical outcomes despite receiving standard-of-care therapies. Use of ctDNA may improve risk stratification beyond clinical TNM staging (Asamura, H et al., 2023,

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IASLC, conference), which, despite its validated prognostic value [10,11], carries the risk of under- and over-treatment when used for guiding systemic treatment (Box 1).

This Review discusses uses for ctDNA as a tool to optimize risk stratification in resectable NSCLC (Figure 1, Key figure). These uses include diagnostic ctDNA detection as a tool to enrich for high-risk patients [12–15] (Figure 1A), ctDNA persistence during neoadjuvant therapy to detect upfront therapeutic resistance and potentially identify patients requiring switch in treatment strategy [16,17] (Figure 1B), and MRD detection to define patients with micrometastatic disease who have not been cured requiring treatment escalation [18] (Figure 1C).

Uses of ctDNA

Diagnostic ctDNA detection and relapse risk

ctDNA may have a role in NSCLC risk stratification at diagnosis, when clinical TNM staging is performed and resectability assessed. In 2020, ctDNA detection with tumor-informed cancer personalized profiling by deep sequencing (CAPP-seq) in 48 pre-intervention plasma samples differentiated poor from good outcome stage I patients based on ctDNA level [low vs high, hazard ratio (HR) 9.34] [13]. It was subsequently demonstrated that diagnostic ctDNA detection in lung adenocarcinoma significantly associated with poorer **relapse-free survival (RFS) (**280 patients,

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Box 1. Potential for over-/undertreatment based on TNM staging alone

The 8th TNM classification system [10,11], soon to undergo revisions to N and M stage as part of the 9th iteration for lung cancer (Asamura, H et al., 2023, IASLC, conference), is the central prognostic parameter currently used in patients with solid tumors to guide additional therapy following curative-intent treatment of early-stage tumors. However, despite the extensively validated prognostic implications of TNM stage on patient outcomes [10,11], using TNM stage to guide systemic treatment carries the risk of both patient undertreatment and patient overtreatment.

Overtreatment

In resectable NSCLC, historical adjuvant studies showed that more than 40% of patients with stage IB–III disease are cured by surgery alone. In the Adjuvant Navelbine International Trialist Association (ANITA) trial, of 433 patients randomized to receive no systemic therapy postresection, 42% had no disease progression reported during follow-up (median 77 months) [40]. Therefore, the 'all-comers' approach to adjuvant therapy administration based on TNM stage that is currently implemented will result in over-treatment of some patients and the consequent risk of unnecessary toxicity. This is increasingly important considering that emerging therapeutic modalities with the potential to transition into an early-stage setting might result in high-grade toxicities beyond those observed with chemotherapy and anti-PD-(L)1 CPI therapy, necessitating judicious patient selection to prevent unwarranted treatment burden.

Undertreatment

Patients with stage I NSCLC typically do not receive neoadjuvant or adjuvant systemic therapy, yet 5–30% (depending on stage IA1–IB status) suffer a postresection RFS event within 5 years of resection [10,61]. These patients may experience clinical understaging, whereby the final pathological stage is higher than the clinical stage due to the limitations of noninvasive clinical staging. A meta-analysis of 698 patients from several randomized controlled trials showed that clinical TNM staging misclassified nodal status in 38% [62]. Conceivably, some patients with clinical stage I NSCLC who have occult nodal disease are potentially under-treated because they do not qualify for emerging neoadjuvant regimens. Therefore, there is a requirement to identify high-risk clinical stage I patients and design interventional studies to ascertain whether systemic intervention can improve curative outcomes in this population.

Despite optimal management using emerging systemic therapy approaches, a sizeable proportion of patients with stage II–III NSCLC experience disease relapse and so might benefit from systemic therapy escalation. In KEYNOTE-671 patients with resectable stage II–IIIB NSCLC received perioperative pembrolizumab or placebo plus neoadjuvant chemotherapy; in the interventional arm, an estimated 37.6% of patients had experienced an EFS event by 2 years postenrollment [30], with similar landmark EFS outcomes observed in pivotal trials investigating perioperative durvalumab [63] or nivolumab [16,33]. Most events in these studies are disease progression or relapse, which might be prevented using targeted therapeutic escalation beyond the standard of care and emerging anti-PD-L1 CPI treatment options. There exists a need for biomarker-driven identification of patients with disease with primary or acquired resistance to CPI therapy, which would enable novel therapeutic modalities to be trialed in these high-risk populations.

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Key figure

Circulating tumor DNA (ctDNA) intended uses in early-stage resectable non-small cell lung cancer

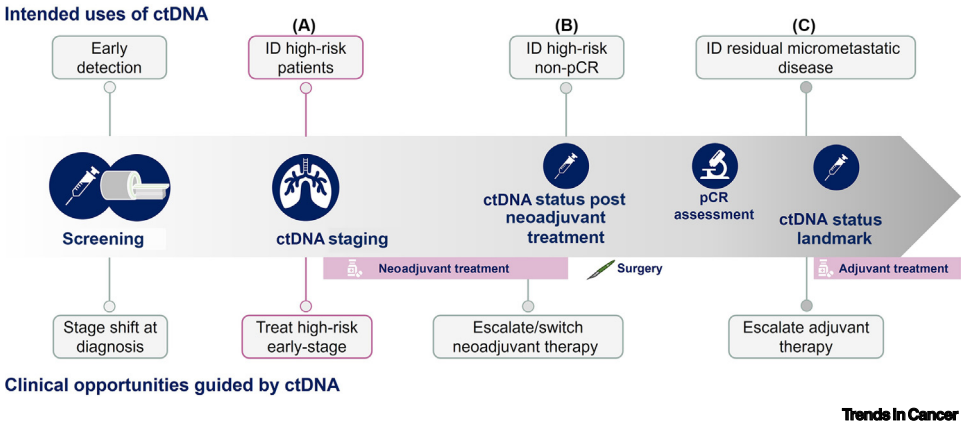

Figure 1. (A) Diagnostic ctDNA in lung adenocarcinoma enriches for high-risk patients whose tumors exhibit a propensity for local and distant clinically occult micrometastases; ctDNA evaluation might therefore refine clinical tumor–node–metastasis staging. (B) Absence of ctDNA clearance during neoadjuvant therapy could reflect upfront therapeutic resistance and might identify patients requiring a treatment switch. (C) Molecular residual disease detection (trace quantities of ctDNA in the blood) is attractive for guiding adjuvant treatment decisions following curative-intent procedures. Abbreviations: ctDNA, circulating tumor DNA; pCR, pathological complete response.

HR 6.0); however, this effect was not as apparent in lung squamous cell carcinoma (43 patients, HR 2.4) [15]. Additionally, data from the Phase 2 NADIM study support baseline ctDNA levels (prior to neoadjuvant chemoimmunotherapy) as prognostic in patients with stage IIIA NSCLC [19], suggesting preoperative ctDNA detection refines clinical TNM staging, particularly in lung adenocarcinoma. The prognostic implications of pre-intervention ctDNA detection might become increasingly important with wider uptake of blood-based diagnostic and early-detection efforts, catalyzed by projects such as the NHS-Galleri study (evaluation of blood-based multicancer early detection) [20].

This use of ctDNA was evaluated within the TRACERx study in 197 patients with preoperative ctDNA data using a tumor-informed, personalized approach based on anchored-multiplex PCR technology [12]. Of 88 patients with stage I–III lung adenocarcinoma, the 59% with no ctDNA detected at diagnosis prior to surgery experienced excellent overall survival (OS; 2-year rate, 90%) versus patients with high (above cohort median; 2-year OS, 24%) or low (below cohort median; 2-year OS 63%) blood ctDNA levels. Findings were similar for RFS. Of 81 patients with non-adenocarcinoma, OS in seven ctDNA-negative patients was indistinguishable from OS in those with low or high blood ctDNA levels, suggesting that a subgroup of ctDNA-negative patients with good OS did not exist in these NSCLC histological subtypes. Additionally, diagnostic ctDNA-positive patients were more likely to have clinically occult mediastinal lymph node disease, supporting ctDNA evaluation as a means of inferring risk of clinical under-staging.

The biology associated with ctDNA detection in lung adenocarcinoma was explored by leveraging genomic and transcriptomic multiregion sequencing data of primary tumors. In diagnostic ctDNA-positive lung adenocarcinomas, proliferation-associated genes (e.g., *AURKA* and *AURKB* [21]) and

Glossary

Checkpoint inhibitor (CPI): immunotherapy drugs that prevent checkpoint proteins on cancer cells from binding to checkpoints on T cells and thereby switching off the immune response to cancer cells.
Circulating tumor DNA (ctDNA): the component of cell-free DNA in the bloodstream that originates from shedding by tumor tissue.
Clinical utility: the ability of a test to provide information to healthcare providers enabling them to act upon test results to improve patient management.
Clinical validity: the ability of a test to correctly classify a patient with respect to a diagnostic, prognostic, or predictive category.
Disease-free survival (DFS): length of time during which a patient is free from recurrence of their lung cancer.
Event-free survival (EFS): length of time during which a patient remains alive and without progression of their lung cancer (and, in some definitions, without other specified events occurring).
Major pathological response (MPR): presence of ≤10% residual viable tumor cells in primary tumor.
Pathological complete response (pCR): absence of any residual viable tumor cells in resection specimens, including primary tumor and all sampled lymph nodes.
Programmed cell death 1 (PD-1): immune checkpoint protein on the surface of T cells that switches off the immune response upon binding to a ligand, e.g., PD-L1.
Programmed cell death ligand-1 (PD-L1): one of the PD-1 checkpoint protein ligands expressed by tumor cells to bind to PD-1 and switch off the immune response to the tumor cell.
Relapse-free survival (RFS): length of time during which a patient remains alive and without relapse/progression of their lung cancer.
Residual viable tumor (RVT): the percentage of viable tumor cells in a tumor tissue or lymph node sample on pathologic examination.
Tumor-informed test: personalized assay based on mutations present in an individual patient's tumor.
Tumor-naïve test: assay utilizing a preselected panel of mutations for analyzing all patients.

proliferation-associated transcriptomic-associated pathways were upregulated. ctDNA positivity associated with increased chromosomal instability versus diagnostic ctDNA-negative patients [22,23]. Since high tumor proliferation rate (by *Ki67*) [24,25] and chromosomal instability [26] are thought to negatively associate with prognosis in resectable NSCLC, these data provide a biological rationale for the association between diagnostic ctDNA detection and poor clinical outcome. Similar associations between proliferation-related gene-set upregulation and pretreatment ctDNA detection have been made in early-stage breast cancer, supporting the existence of ctDNA shedding in other glandular tumors [27].

Important questions remain regarding diagnostic ctDNA as a prognostic biomarker in lung adenocarcinoma (see Outstanding questions). First, can a level of performance similar to that of tumor-informed assays be achieved? This level of performance is desirable for a ctDNA test aimed at refining clinical TNM staging, since tissue availability for **tumor-informed testing** will be limited in the early-diagnostic versus postresection setting, potentially reducing true **clinical utility** of this biomarker approach. The prognostic implications of ctDNA status derived from a tumor-informed personalized ctDNA detection approach (PROPHET) versus a **tumor-naïve approach** were evaluated in 151 patients with stage I–III NSCLC [28]. The ctDNA-positive rate was higher with PROPHET versus tumor-naïve technology (42% vs 17%). ctDNA positivity with both technologies had prognostic implications, which were greater with PROPHET (HR 14.11) than the tumor-naïve approach (HR 4.37), attributable to a lower event rate in the PROPHET-defined ctDNA-negative population versus that defined by the tumor-naïve technology, suggesting that improved sensitivity for low ctDNA levels (with tumor-informed technology) may decrease clinical false-negative rates (ctDNA-negative patients who experience lung relapse) when leveraging diagnostic ctDNA as a prognostic biomarker. Therefore, although diagnostic ctDNA as a preoperative risk biomarker may be valid with less sensitive tumor-naïve technologies (which have clear feasibility and operational advantages as clinical tools), improvements in assay sensitivity may be required for optimal risk stratification.

Second, would additional technical sensitivity beyond first-generation tumor-informed assays increase the validity of associations between preoperative ctDNA detection and relapse in lung adenocarcinoma (see Outstanding questions)? Data from the post-half-way point of TRACERx using a second-generation tumor-informed assay [14], analytical validation of which demonstrates a 95% limit of detection (LOD) of 3.45 ppm [29] (lower than ~80 ppm with the assay in the 2023 TRACERx publication [12]), showed that the fraction of 171 patients with lung adenocarcinomas detected preoperatively was greater than in the 2023 publication [12], with ctDNA detected in 81% versus 42% [12]. This increased sensitivity was particularly apparent in patients with stage I lung adenocarcinoma (52% vs 14% ctDNA-positive). Critically, in adenocarcinomas in which ctDNA was pre-operatively detected at <80 ppm (95% LOD achieved at higher levels of cfDNA input with anchored-multiplex PCR approach [12]) or not detected at all, OS and RFS were significantly worse in the former, suggesting that ctDNA detection at LODs below those of first-generation tumor-informed assays is clinically meaningful in this setting. The 5-year OS was 100% among patients with ctDNA-negative adenocarcinoma, supporting the idea that greater assay technical sensitivity might improve discrimination of clinical low-risk adenocarcinoma patients.

ctDNA dynamics during neoadjuvant therapy and outcome

With neoadjuvant nivolumab approved based on **event-free survival (EFS)** outcomes from the CheckMate 816 trial [16], perioperative pembrolizumab approved by the US Food and Drug Administration based on outcomes from the KEYNOTE-671 trial [30], and positive EFS outcomes reported from other Phase 3 trials (AEGEAN [31], NeoTORCH [32], and CheckMate 77T [33]) of perioperative anti-PD-L1 CPI therapy, it will be important to characterize ctDNA dynamics in neoadjuvant treatment settings as a biomarker of neoadjuvant systemic therapy efficacy.

Assessments of ctDNA dynamics during neoadjuvant therapy have been performed in NSCLC. In CheckMate 816 [16], a tumor-informed assay was used to track ctDNA dynamics in stage IB–IIIA NSCLC patients (American Joint Committee on Cancer [AJCC] Staging Manual, 7th edition) receiving neoadjuvant chemotherapy or chemoimmunotherapy for three cycles. Clearance of ctDNA after two cycles of neoadjuvant chemoimmunotherapy was associated with achievement of a **pathological complete response (pCR)** (11/24 patients with ctDNA clearance vs 0/19 without ctDNA clearance). The ability of ctDNA clearance to predict longer-term clinical outcomes such as EFS was less clear, although both chemoimmunotherapy (HR 0.60) and chemotherapy (HR 0.63) arms showed a numerical improvement in EFS based on HR point estimates, albeit with wide confidence intervals due to small patient numbers [16]. ctDNA clearance was associated with **residual viable tumor (RVT)** in resection specimens, with the highest clearance rates (80%) observed in the 0–10% RVT primary tumor group [34]. In the AEGEAN study [17], 186 stage IIA–IIIB (N2) patients (AJCC Staging Manual, 8th edition) treated with neoadjuvant chemotherapy and perioperative durvalumab or placebo were analyzed, using a tumor-informed assay to track ctDNA dynamics during neoadjuvant treatment. Tumor-informed panels were created from pre-neoadjuvant tumor biopsies to minimize bias, and blood samples from baseline, day 1 of cycles 2–4, and prior to surgery were analyzed. Neoadjuvant durvalumab plus chemotherapy resulted in greater ctDNA clearance prior to surgery versus chemotherapy alone [17]. Among baseline ctDNA-positive patients, all who achieved pCR and >90% of those who achieved a **major pathological response (MPR)** had ctDNA clearance by cycle 4 of neoadjuvant treatment, whereas patients without ctDNA clearance were not likely to achieve pCR (negative predictive value >84%, cycle 2, day 1), suggesting potential for guiding treatment escalation/de-escalation decisions.

Outstanding issues remain regarding ctDNA persistence during neoadjuvant therapy as a high-risk biomarker in patients with resectable NSCLC (see Outstanding questions). First, generating tumor-informed ctDNA fingerprints could be challenging pre-operatively (prior to neoadjuvant therapy), as the reliance on tumor biopsy material rather than resection specimens to source tumor tissue may limit the proportion of patients evaluable for biomarker testing. To ensure accessibility, it will be necessary either to devise technical and clinical strategies to enable this class of assay to be used routinely in the neoadjuvant setting or to validate tumor-naïve ctDNA detection approaches as neoadjuvant response biomarkers. Second, further data are required to definitively validate associations between neoadjuvant ctDNA clearance and endpoints including RVT, EFS, **disease-free survival (DFS)**, and OS. As discussed previously, ctDNA clearance in the neoadjuvant setting was associated with EFS in CheckMate 816 [16], although interpretation was limited by the small numbers of evaluable patients. In the single-arm NADIM study of neoadjuvant nivolumab and chemotherapy plus adjuvant nivolumab, progression-free survival (HR 0.26) and OS (HR 0.04) were improved in patients with ctDNA clearance following neoadjuvant therapy [19]. These substantially differentiated outcomes by ctDNA clearance may reflect the benefit of continuing adjuvant CPI therapy, including in patients with ctDNA clearance after neoadjuvant therapy; however, assay differences and small numbers of evaluable patients limit interpretation. Larger cohorts will be required to determine whether ctDNA clearance can be used prognostically and potentially to guide subsequent treatment. If persistent ctDNA prior to surgery enriches for high-risk disease (in terms of propensity for relapse), this information could guide postoperative adjuvant therapy selection, particularly if persistent ctDNA indicates upfront therapy resistance, thus providing a rationale for switching treatment. Additionally, ctDNA dynamics during neoadjuvant therapy might be a novel early endpoint for efficacy to support regulatory decision-making, as discussed previously [35].

Landmark MRD detection in NSCLC

Multiple studies have demonstrated the validity of postoperative ctDNA detection as a marker of residual cancer [15,18,36–39]. The landmark timepoint relates to ctDNA testing immediately after

surgery but before adjuvant therapy, representing a valid timepoint at which ctDNA could be integrated into adjuvant therapy decision-making. In the 2023 TRACERx study [12], landmark MRD-positive status was defined as ctDNA detection within 120 days post-resection, prior to adjuvant therapy. Of 108/131 patients with an eligible landmark blood sample, 27 (25%) were landmark MRD-positive (Figure 2); these patients exhibited significantly poorer DFS (HR 6.8) and OS (HR 5.3) compared to landmark MRD-negative patients, with 93% of landmark MRD-positive patients experiencing disease recurrence. The clinical sensitivity of the MRD landmark timepoint for relapse was 49%; that is, 51% of patients who relapsed were landmark MRD-negative.

The pattern of disease relapse differed between landmark MRD-positive and landmark MRD-negative patients (Figure 3A). Additionally, relapse events in landmark MRD-positive patients tended to occur earlier than in landmark MRD-negative patients, and patients who relapsed who were landmark MRD-positive versus landmark MRD-negative exhibited significantly shorter OS after surgery (Figure 3B). These data suggest that landmark MRD positivity detected by a first-generation tumor-informed ctDNA assay (95% LOD ~80 ppm) enriches for poor prognosis patients with nonintracranial distant micrometastatic disease who are at risk of early disease relapse. Conversely, patients with localized residual disease and more indolent relapse kinetics were less likely to be landmark MRD-positive.

Given these associations between landmark MRD status and pattern of relapse, what are potential implications for the clinical utility of MRD to guide treatment escalation or de-escalation in NSCLC (see Outstanding questions)? Historical trial data have demonstrated that adjuvant chemotherapy prevents local and non-intracranial distant relapse [40,41], which could have implications for MRD as a tool to guide post-operative cytotoxic therapy. In TRACERx, landmark MRD-positive patients exhibited a nonintracranial distant metastatic relapse pattern. This observation, coupled with the efficacy of adjuvant chemotherapy in reducing nonintracranial metastasis, supports landmark ctDNA evaluation as a tool for determining further escalation of cytotoxic therapy (e.g., with emerging antibody–drug conjugate modalities) to improve outcomes. However, de-escalation of cytotoxic therapy based on landmark MRD status might lead to undertreatment, given that MRD-negative patients who relapse are enriched for a local (intrathoracic) pattern of relapse. As adjuvant chemotherapy also prevented local relapses in historical trials, cytotoxic de-escalation

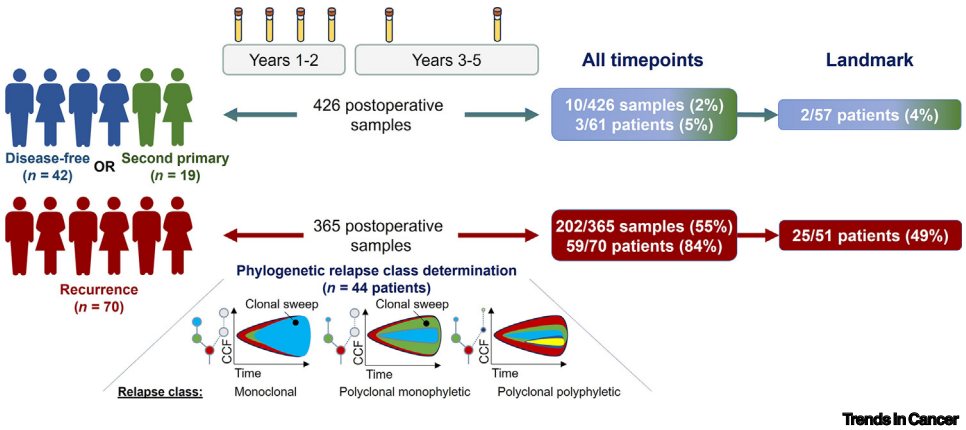

Figure 2. Overview of the TRACERx 2023 ctDNA cohort [12]. Multiple postoperative blood samples were obtained from 131 patients in TRACERx and analyzed using a first-generation tumor-informed ctDNA assay (95% LOD ~80 ppm). Landmark molecular-residual-disease-positive status was defined as ctDNA detection within 120 days postresection, prior to adjuvant therapy if administered. Abbreviations: CCF, cancer cell fraction; ctDNA, circulating tumor DNA; LOD, limit of detection.

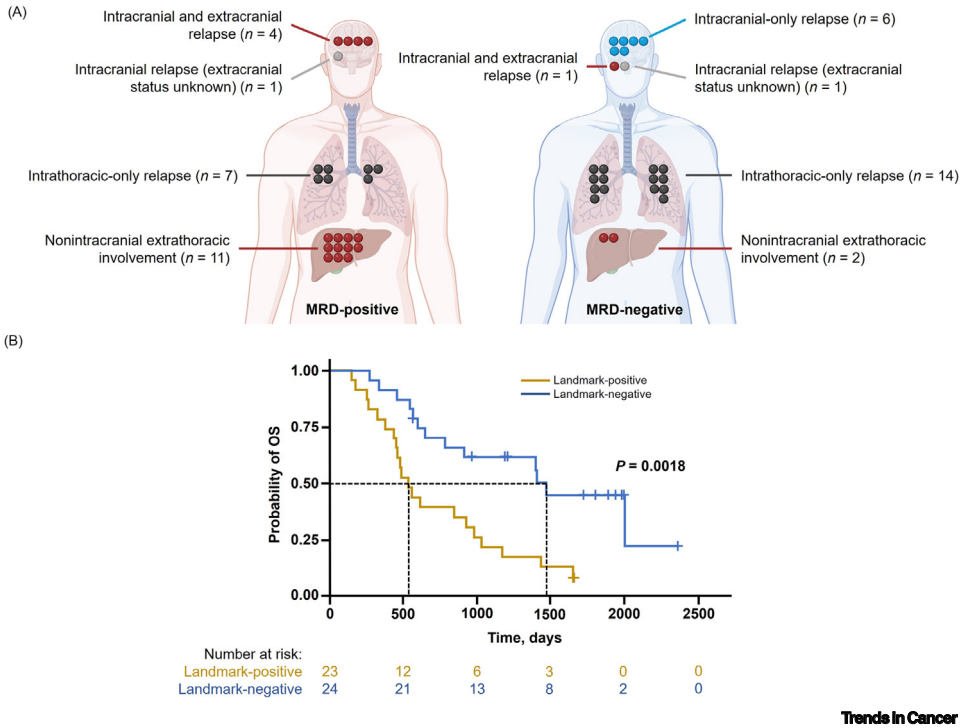

Figure 3. Sites of relapse and Kaplan–Meier analysis of overall survival (OS) by landmark molecular residual disease (MRD) status in patients with non-small cell lung cancer in TRACERx. (A) Site of relapse in MRD-positive and MRD-negative patients in TRACERx. In landmark MRD-positive patients, relapses were more likely to include extrathoracic disseminated disease, whereas in landmark MRD-negative patients, intracranial-only or intrathoracic-only relapses were more likely. Created with BioRender.com. (B) Kaplan–Meier analysis of OS in TRACERx patients who relapsed and were MRD-positive or MRD-negative at the landmark of 120 days postresection (for methodology, see TRACERx 2023 publication [12]) Relapse events in MRD-positive patients tended to occur earlier than in MRD-negative patients [15/21 (71%) relapse events within 1 year of surgery vs 8/26 (31%) events occurring >1 year after surgery were in MRD-positive patients], and patients who relapsed who were landmark MRD-positive versus MRD-negative exhibited significantly shorter OS from surgery.

approaches based on the MRD LOD implemented in TRACERx might undertreat patients destined to experience local NSCLC relapse. The enrichment of brain-only metastasis observed in landmark MRD-negative patients who relapse (Figure 3B) becomes increasingly important when considering adjuvant targeted therapy in NSCLC. Efficacy of a third-generation epidermal growth factor receptor (EGFR) inhibitor may be in part related to a reduction of intracranial metastases following surgical resection [42]; early data from the ALINA study (adjuvant alectinib vs chemotherapy in resectable ALK+ NSCLC) demonstrated a HR of 0.22 for central nervous system DFS [43]. Therefore, use of MRD to de-escalate targeted therapy in EGFR+ or ALK+ disease could prevent patients benefiting from adjuvant therapy-associated inhibition of intracranial progression due to the inability of current MRD assays to define patients with occult intracranial disease as high-risk.

Ultimately, assessment of MRD for de-escalating therapy will require prospective, randomized trials exploring survival endpoints within MRD-positive and MRD-negative populations, such as a study in patients with stage II colon cancer demonstrating noninferior RFS with ctDNA-guided management (which resulted in a reduction in adjuvant chemotherapy administration relative to standard management) [44]. The closest available data in NSCLC come from a retrospective biomarker analysis of the Phase 3 IMpower010 study of adjuvant atezolizumab post-standard-of-care adjuvant chemotherapy versus best supportive care [45]. Analyses of blood samples obtained

prior to adjuvant chemotherapy showed that 20% versus 22% in the atezolizumab versus comparator arms were MRD-positive. MRD status was strongly prognostic in both the atezolizumab (median DFS not reached vs 19.1 months, MRD-negative vs MRD-positive patients) and comparator (median not reached vs 7.9 months) arms. Importantly, DFS benefit with atezolizumab was observed in both MRD-positive and MRD-negative patients, particularly those with PD-L1 tumor cell expression ≥1%.

These data, generated using a first-generation tumor-informed ctDNA assay, support the prognostic value of MRD in NSCLC and highlight the potential utility of MRD to guide adjuvant therapy escalation beyond CPI monotherapy. However, de-escalation based on MRD is not currently warranted due to demonstrated treatment benefit in MRD-negative patients.

An outstanding question relates to the impact of increased LOD beyond first-generation tumor-informed assays on MRD clinical sensitivity. It will be important to establish whether moving from a 95% LOD of 80 ppm [12] to ~1 ppm with emerging second-generation tumor-informed MRD approaches might increase ctDNA assay capability for identifying landmark residual disease beyond the 49% observed previously [12]. Given that tumor volume associates with ctDNA level in blood [12,46,47], and that higher ctDNA levels in blood associate with improved assay sensitivity, improvements in assay sensitivity might facilitate detection of lower-burden micrometastatic disease at early postoperative timepoints (Figure 4). Alternatively, proliferative activity within micrometastatic disease, rate of tumor cell death at a metastatic site, and the capability of ctDNA to enter the peripheral circulation (relevant for intracranial-only metastases) may prevent large improvements in landmark MRD sensitivity, even if higher-sensitivity assays are leveraged in patients with metastatic NSCLC. Preliminary data and modeling suggest improvements in technical sensitivity might translate into improved MRD clinical sensitivity [48,49]; for example, whereas a first-generation assay detected only 28% of patients as MRD-positive, a second-generation assay (phasED-seq [50]) identified 56% of the same patients as MRD-positive. Consequently, MRD status by second-generation assay had much greater prognostic value for DFS [48]. In contrast, other preliminary data suggest clinical sensitivity for relapse of an MRD landmark might be similar between first- and second-generation MRD assays (49% [12] vs

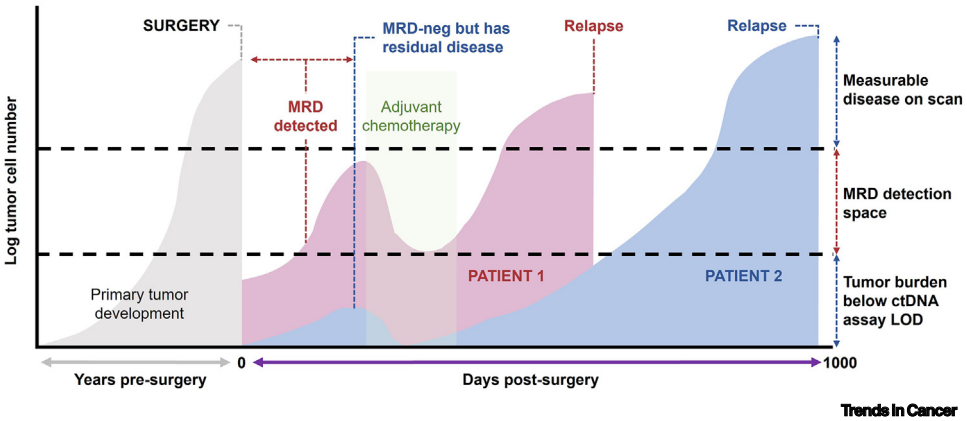

Figure 4. Possible effect of micrometastatic tumor volume on landmark molecular residual disease (MRD) status in resectable non-small cell lung cancer. Patient 1 is landmark MRD-positive because micrometastatic disease burden at the point of MRD testing is sufficient to result in a circulating tumor DNA (ctDNA)-positive status (possibly due to multiple sites of metastatic disease). In contrast, Patient 2 has residual postoperative metastatic disease, yet the disease volume is too low to result in ctDNA levels conducive to an MRD-positive result. This patient is MRD-negative but subsequently relapses.

54% [14] sensitivity, analyses performed in separate TRACERx cohorts with variation in tumor stage, histology, and NSCLC recurrence patterns that might also impact landmark sensitivity). Therefore, the relationship between clinical and technical sensitivity for MRD detection in NSCLC remains unclear.

Beyond prognosis

Other possible uses of ctDNA in patients with resectable NSCLC include as a relapse surveillance tool and for noninvasive determination of actionable mutation status to guide targeted therapy. Early evidence supports ctDNA detection as capable of adjudicating equivocal imaging findings on post-definitive treatment imaging and as a tool to escalate to nonstandard (e.g., positron emission tomography) imaging to detect relapse earlier [12,37,51]. For noninvasive genotyping, ctDNA evaluation represents a complementary route to tumor genotyping in metastatic NSCLC (with relatively high levels of tumor–plasma concordance [52]), but in early-stage NSCLC, ctDNA-based genotyping is not routine [53] due to higher levels of discordance driven by lower plasma ctDNA levels and higher ctDNA assay false-negative rates [54].

Challenges in implementing ctDNA as a prognostic biomarker

Despite interest in leveraging ctDNA as a prognostic biomarker in resectable NSCLC, there are challenges regarding its broad implementation. From a clinical perspective, data support the **clinical validity** of ctDNA as a prognostic biomarker (across use-cases outlined in this review), but broader uptake of ctDNA testing will require demonstration of clinical utility. Clinical utility describes demonstration that a biomarker can improve patient management (versus management without the test). To demonstrate utility, test results must be acted upon in prospective studies, and patient-centric endpoints related to test impact on clinical decision-making and outcomes collected [55]. One route to demonstrating clinical utility includes interventional clinical trials. For example, IMvigor011 (NCT04660344) is randomizing patients with muscle-invasive bladder cancer who are ctDNA-positive after cystectomy to adjuvant atezolizumab versus placebo [56]. This interventional study was implemented based on a *post hoc* exploratory analysis of IMvigor010 showing improved patient outcomes only in ctDNA-positive patients receiving adjuvant atezolizumab and not in all patients irrespective of ctDNA status [57]. However, interventional trials are costly and take many years. Additionally, it will become increasingly important to assess ctDNA assays through economic evaluation analyses to support local reimbursement decisions and ultimately expand access to ctDNA testing [58].

Concluding remarks and future perspectives

There is broad acceptance regarding the putative clinical validity of MRD as a biomarker in solid tumors. Additionally, there is a shift towards using neoadjuvant therapy in NSCLC, accompanied by emerging data to support persistent ctDNA detection during neoadjuvant therapy as a biomarker of high-risk RVT. MRD or neoadjuvant ctDNA response could be integrated with pathological response data (pCR or MPR) in the post-neoadjuvant resection specimen to optimize risk stratification for patients with resectable NSCLC, including defining high-risk metastatic patients (Figure 5). Preliminary outcomes data by MPR and postoperative MRD status from the LCMC3 study in 32 patients treated with neoadjuvant atezolizumab support this concept. Within the non-MPR group, MRD-positive status further differentiated patients who had poor DFS from patients who had no detectable MRD in the postoperative setting and had better outcomes [59]. Therefore, either pre- or postoperative ctDNA evaluation coupled with pathological response assessment could enable novel switch clinical development strategies. Additionally, integration of pathological outcomes and MRD measurement might refine individualized imaging surveillance approaches for patients who have undergone resection (e.g., frequency and/or modality, and anatomical focus of cross-sectional imaging) (Figure 5) given that data from TRACERx indicate a

Outstanding questions

With regards to the clinical utility of diagnostic ctDNA as a prognostic biomarker in lung adenocarcinoma, can a similar performance be achieved with tumor-naïve (blood-only) ctDNA tests as with tumor-informed assays?

Will additional technical sensitivity beyond first-generation tumor-informed assays increase the clinical validity of associations between preoperative ctDNA detection and relapse in lung adenocarcinoma, and increase ctDNA assay capability for identifying postoperative landmark residual disease in NSCLC?

How challenging will it be to generate tumor-informed ctDNA fingerprints prior to neoadjuvant therapy, given that tumor tissue will need to derive from tumor biopsy material rather than resection specimens, and will this limit the proportion of patients evaluable for biomarker testing?

What is the association between neoadjuvant ctDNA clearance and clinical endpoints including RVT, EFS, DFS, and OS?

Given the associations between landmark MRD status and pattern of relapse, what are the implications for the clinical utility of using MRD to guide treatment de-escalation in NSCLC?

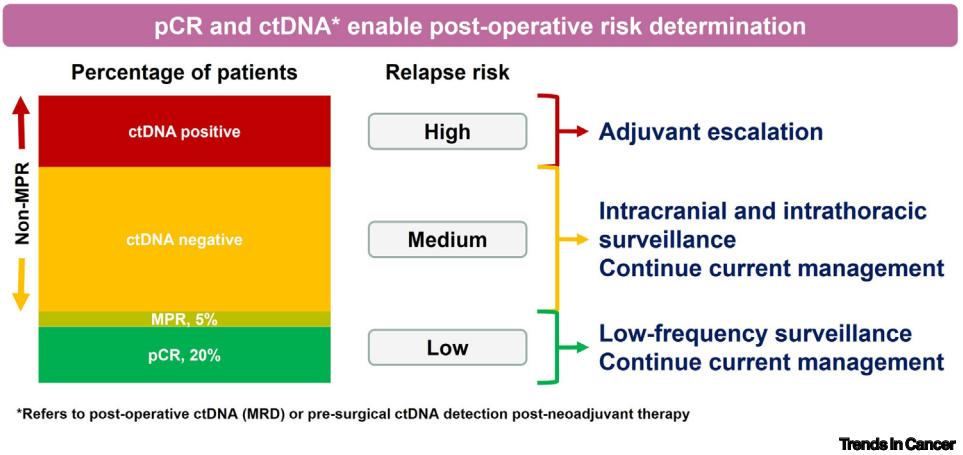

Figure 5. A future postoperative non-small cell lung cancer population might be risk-stratified based on a combination of circulating tumor DNA (ctDNA) status and pathological complete response (pCR) status. For example, patients who achieve major pathological response (MPR) with neoadjuvant treatment exhibit improved long-term outcomes. However, event-free survival events nevertheless occur in this population following current neoadjuvant-only treatment options; postoperative molecular residual disease (MRD) testing might identify these high-risk patients. For patients classified as non-pCR/MPR, persistent ctDNA detection during neoadjuvant therapy might identify patients exhibiting suboptimal response to standard of care anti-programmed death 1 therapy; this could inform selection of appropriate adjuvant therapy for perioperative regimens. Postoperative MRD detection in the same population would indicate occult metastatic disease, defining high-risk metastatic patients.

distinct pattern of relapse in MRD-negative patients, supporting intracranial and intrathoracic surveillance in this latter population (Figure 3).

Diagnostic or pre-intervention ctDNA testing for risk stratification beyond clinical TNM staging also has potential to guide perioperative systemic therapy for clinically under-staged patients. This strategy might have the greatest clinical utility in patients with stage I lung adenocarcinoma, a population expected to increase with broader uptake of low-dose computed tomography screening [60].

In conclusion, the past 7 years have seen a significant increase in evidence supporting ctDNA as a high-risk biomarker that might refine treatment decision-making for patients with early-stage NSCLC. However, optimal integration of ctDNA into clinical management strategies for patients with resectable NSCLC will require considerations including: accessible, economically viable testing platforms that do not disrupt a patient's diagnostic or treatment pathway; data to support utility of ctDNA-based management; improved understanding of the relationship between assay technical performance and clinical utility across use-cases outlined here; and a view on how ctDNA can build upon existing risk biomarker strategies.

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Author contributions

C.A., D.H, and C.S. conceptualised the study. C.A. visualized the study. C.A., D.H, G.J.D., and C.S. investigated the study. All authors wrote, reviewed, and edited the paper.

Declaration of interests

C.A. was formerly an employee of, and held stocks/shares in, AstraZeneca, and is currently an employee of SAGA Diagnostics. D.H., G.J.D., D.G., L.H., and J.S.R.-F. are employees of, and hold stocks/shares in, AstraZeneca. J.R.M.B. has no

conflicts of interest to disclose. D.G. is the cofounder of Inivata, acquired by NeoGenomics in 2021, and holds shares in AstraZeneca and GSK. C.S. is the co-founder of, a Scientific Advisory Board (SAB) member for, and has stock options in Achilles Therapeutics; has stock options in Epic Bioscience, Relay Therapeutics, and Bicycle Therapeutics; held stock options in Apogen Biotechnologies and GRAIL until June 2021; was a member of the American Association for Cancer Research (AACR) Board of Directors until April 2022; is chief investigator of AstraZeneca's MeRmaiD I and II clinical trials and co-chief investigator of the NHS Galleri Trial funded by GRAIL; has received honoraria from Amgen, AstraZeneca, Boehringer Ingelheim, Bristol-Myers Squibb, GlaxoSmithKline, Illumina, MSD, Novartis, Pfizer, and Roche; has received consulting fees from Genentech, Sarah Canon Research Institute, GRAIL (SAB), Medicxi, Bicycle Therapeutics (SAB), Metabomed – until June 2021, China Innovation Centre of Roche (CICor) – (formerly Roche Innovation Centre-Shanghai), Relay Therapeutics (SAB), and Saga Diagnostics (SAB); has received grants or funds from AstraZeneca, Boehringer Ingelheim, Bristol-Myers Squibb, Invitae (formerly Archer Dx), Ono Pharmaceuticals, Personalis, and Pfizer; and holds the following patents – US20190106751A1 – Methods for lung cancer detection; PCT/EP2016/071471 – 'immune checkpoint intervention' in cancer; PCT/EP2016/059401 – Method for treating cancer; PCT/US2017/028013 – Methods for lung cancer detection; PCT/GB2018/051912 – Method for identifying responders to cancer treatment; PCT/GB2018/052004 – Analysis of HLA alleles in tumors and the uses thereof; PCT/GB2020/050221 – Method of predicting survival rates of patients with cancer; PCT/GB2017/053289 – Method of detecting tumor recurrence; PCT/EP2021/059989 – Modulation of T cell cytotoxicity and related therapy; and PCT/EP2022/077987 – Methods and systems for tumor monitoring; PCT/EP2023/059039 – Analysis of HLA alleles transcriptional deregulation.

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