review articles

Circulating Tumor DNA Minimal Residual Disease Detection of Non–Small-Cell Lung Cancer Treated With Curative Intent

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abstract

Circulating tumor DNA (ctDNA) minimal residual disease (MRD) is a powerful biomarker with the potential to improve survival outcomes for non–small-cell lung cancer (NSCLC). Multiple groups have shown the ability to detect MRD following curative-intent NSCLC treatment using next-generation sequencing–based assays of plasma cell-free DNA. These studies have been modest in size, largely retrospective, and without thorough prospective clinical validation. Still, when restricting measurement to the first post-treatment timepoint to assess the clinical performance of ctDNA MRD detection, they have demonstrated sensitivity for predicting disease relapse ranging between 36% and 100%, and specificity ranging between 71% and 100%. When considering all post-treatment follow-up timepoints (surveillance), including those beyond the initial post-treatment measurement, these assays' performances improve with sensitivity and specificity for identifying relapse ranging from 82% to 100% and 70% to 100%, respectively. In this manuscript, we review the evidence available to date regarding ctDNA MRD detection in patients with NSCLC undergoing curative-intent treatment and the ongoing prospective studies involving ctDNA MRD detection in this patient population.

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INTRODUCTION

Liquid biopsy refers to the analysis of bodily fluid for cancer detection.[1,2] For solid malignancies such as non–small-cell lung cancer (NSCLC), these assays usually measure circulating tumor DNA (ctDNA), which is composed of tumor-derived mutations in plasma cell-free DNA (cfDNA).[3-5] Assays in development also measure blood-based tumor mutational burden, genome-wide copy-number alterations, and methylation signatures.[6-12] ctDNA testing already plays an important role in clinical practice, allowing the identification of actionable tumor-derived somatic alterations from plasma that guide treatment decisions in patients with metastatic NSCLC.[13] Nonetheless, these assays are not yet ready to be used as predictive biomarkers to guide treatment in patients with localized NSCLC outside of a research context.

In 2020, the US Food and Drug Administration approved the use of two ctDNA assays to identify genomic alterations in patients with advanced-stage solid malignancies[14,15]: Guardant 360 and FoundationOne Liquid CDx are hybrid capture–based next-generation sequencing (NGS) assays that enable the detection of mutations present in 73 and 311 genes, respectively.[16,17] These assays can be applied to plasma samples from patients with advanced NSCLC; however, they lack sensitivity for the detection of ctDNA minimal residual disease (MRD) following curative-intent treatment. Although commercially available assays have not yet achieved US Food and Drug Administration approval to be used in clinical practice to detect MRD in NSCLC, several research groups and companies are studying and developing new and promising technologies for ctDNA MRD detection in patients with solid tumors.[2,3,9,10,12,18-35] Here, we review the current state of the field regarding ctDNA MRD detection in NSCLC and discuss possible applications and barriers for ctDNA MRD utility in patients with NSCLC undergoing curative-intent treatment.

ctDNA MRD FOR UNRESECTABLE NSCLC

In 2017, Chaudhuri et al[10] published one of the first studies demonstrating that ctDNA MRD could be detected in the plasma of patients with NSCLC following curative-intent treatment and that its detection preceded disease relapse. In this study, 37 patients diagnosed with stage I-III NSCLC and three patients with limited-stage small-cell lung cancer were enrolled. Among these, 33 patients had stage II or III NSCLC, most of whom underwent concurrent chemoradiation (CRT) treatment. Using a hybrid capture–based NGS assay known as Cancer Personalized Profiling by deep Sequencing (CAPP-Seq), which targets 128 genes recurrently mutated in lung cancer, they detected pretreatment ctDNA in 37 of 40 patients.[10]

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CONTEXT

Key Objective

Can circulating tumor DNA (ctDNA) be used for minimal residual disease (MRD) detection in patients with non–small-cell lung cancer (NSCLC) treated with a curative-intent and predict disease relapse?

Knowledge Generated

We comprehensively reviewed studies conducted in patients with NSCLC who underwent curative-intent treatment in which different ctDNA technologies were applied to detect MRD. The ctDNA assays' sensitivity to predict disease relapse ranged from 36%-100% when restricting ctDNA MRD measurement to the initial post-treatment timepoint, but improved to 82%-100% when considering all available post-treatment follow-up timepoints. ctDNA MRD (+) patients had significantly higher rates of disease relapse and worse survival outcomes compared with their ctDNA MRD (–) counterparts.

Relevance

Single-institution studies suggest that ctDNA MRD detection after curative-intent NSCLC treatment predicts disease relapse and is associated with worse outcomes. Larger prospective studies are ongoing and will need to confirm these findings.

Among 32 evaluable patients, 17 individuals with detectable ctDNA at the first timepoint within 4 months of treatment completion (MRD+) had shorter freedom from progression (FFP) and disease-specific survival when compared with the ones with negative ctDNA MRD at this timepoint (n = 15).[10] All 17 patients with detectable MRD relapsed or died of NSCLC, whereas 1 of 15 patients with undetectable ctDNA MRD in the same follow-up period developed disease relapse (Table 1). Reflective of the ultrasensitive nature of CAPP-Seq, ctDNA MRD levels as low as 0.003% (as a percentage of total plasma cfDNA) were detectable.[10]

The authors also performed post-treatment monitoring of ctDNA at multiple timepoints in 37 evaluable patients (surveillance).[10] They observed 100% sensitivity and 100% specificity for predicting recurrence using this ever-positive versus never-positive ctDNA surveillance framework (Table 2). Importantly, detection of post-treatment surveillance ctDNA preceded radiographic relapse in 72% of patients by a median of 5.2 months.[10] The authors also developed an equation to predict blood-based tumor mutational burden using CAPP-Seq data and further identified actionable mutations within their ctDNA data. In an exploratory analysis, they used these data to identify multiple patients who developed disease progression who might have benefited from additional treatment at the MRD timepoint (ie, immune checkpoint blockade or tyrosine kinase inhibitors).[10] These findings demonstrate that ctDNA can be detected after curative-intent treatment, suggest that ctDNA MRD and ctDNA surveillance can identify recurrence earlier than imaging, and that the oncogenomic features of ctDNA MRD could facilitate actionability to prevent relapse. Conversely, they also suggested that patients with undetectable ctDNA MRD after definitive-intent treatment are likely cured and may not need consolidation therapy.

Five months after the work of Chaudhuri et al[10] was published, durvalumab was approved as consolidation immunotherapy following CRT in patients with unresectable NSCLC on the basis of the findings of the PACIFIC trial.[36] Although durvalumab significantly improved disease-free survival and overall survival (OS) following CRT, a third of patients on the placebo arm were still alive after receiving CRT alone at 5 years.[37] Given the costs and potentially severe adverse effects associated with a year of immune checkpoint inhibitor (ICI) therapy,[38] it is important to develop biomarkers to facilitate greater treatment personalization.

In this context, Moding et al[22] conducted a retrospective study assessing whether ctDNA MRD could be used as a biomarker to stratify which patients treated with CRT benefit from consolidation ICI. In this study, 65 patients with unresectable stage IIB-IIIB NSCLC who were treated with CRT ± consolidation ICI were analyzed. Among these, 28 patients received consolidation ICI following CRT. Plasma samples were collected pre-CRT, post-CRT, and early-on consolidation ICI (median of 11 weeks into ICI treatment) for those who received it.[22] Using CAPP-Seq, the authors showed that 78% and 75% of patients in the no consolidation and consolidation cohorts harbored detectable ctDNA pretreatment, respectively.[22]

Among patients treated with CRT in the no consolidation group who had detectable pretreatment ctDNA (58% of whom were previously included in Chaudhuri et al[10]), 17 had detectable ctDNA MRD after CRT and all recurred within 12 months of follow-up, whereas among the patients with undetectable ctDNA MRD after CRT (n = 12), only one developed disease relapse.[22] In the immunotherapy consolidation group, 22 patients with detectable pretreatment ctDNA had evaluable samples for ctDNA MRD analysis after completion of CRT.[22] Among them, 13 had undetectable ctDNA MRD after CRT, of whom two relapsed within 12 months. Among the remaining nine patients with detectable ctDNA MRD after CRT, those with ctDNA levels that then decreased from pre-ICI to early-on ICI achieved longer FFP (median of

TABLE 1. ctDNA Performance to Detect MRD in Patients With NSCLC

Author (Year)	No.[a]	Clinical Stage	Treatment	ctDNA Assay	Sensitivity, %[b]	Specificity, %[b]
Chaudhuri et al (2017)[c]	32	IB-IIIB	CRT or RT and/or surgery ± chemo	CAPP-Seq	94	100
Abbosh et al (2017)[d]	24	IA-IIIB	Surgery ± chemo ± PORT	Signatera	36	90
Chen et al (2019)[e]	25	IIB-IIIB	Surgery ± chemo	cSMART	44	88
Zviran et al (2020)[f]	22	IA-III	Surgery ± chemo and RT	MRDetect	100	71

Abbreviations: CAPP-Seq, Cancer Personalized Profiling by deep Sequencing; chemo, chemotherapy; CRT, chemoradiation; cSMART, circulating single-molecule amplification and resequencing technology; ctDNA, circulating tumor DNA; MRD, minimal residual disease; NSCLC, non–small-cell lung cancer; PORT, postoperative radiotherapy; RT, radiotherapy.

[a]No. depicts the number of patients eligible for this analysis.

[b]Sensitivity and specificity are for lung cancer relapse using ctDNA detection status acquired only from the MRD timepoint. The MRD timepoint was after lung cancer surgery for Abbosh et al, Chen et al, and Zviran et al, and typically after CRT for Chaudhuri et al.

[c]Chaudhuri et al[10]: Source of data in the original manuscript: Figure 3b, Figure S1, Table S1.

[d]Abbosh et al[20]: Source of data in the original manuscript: Figure 4, Extended Data Figure 5, Extended Data Figure 6.

[e]Chen et al[23]: Source of data in the original manuscript: Figure S5.

[f]Zviran et al[9]: Source of data in the original manuscript: Figure 5e.

approximately 22 months) compared with those with increasing ctDNA levels in the same period (median FFP of approximately 5 months).[22] Also, there were two patients in whom ctDNA MRD went from detectable to undetectable after approximately 2 months of consolidation ICI, both of whom achieved long intervals of disease-free survival.[22]

A major challenge for detecting ctDNA MRD after curative-intent NSCLC treatment remains the low levels of ctDNA in plasma, often at levels below 0.1% (as a percentage of total cfDNA).[3,10,20,32] Additionally, clonal hematopoiesis of indeterminate potential (CHIP) is present at low levels in plasma and can be a confounder of ctDNA detection specificity.[2,3,39,40] Therefore, ctDNA MRD assays need to have superb sensitivity and specificity to detect tumoral genomic alterations that are often present at ultralow levels in plasma, while also not falsely calling CHIP mutations. Background error rates can further compromise the performance of NGS-based ctDNA assays.[21,24]

To decrease the error profile inherent to most ctDNA assays that involve deep sequencing, a novel method focused on tracking multiple mutations on a single cfDNA molecule (phased variants or PVs) called Phased Variant Enrichment and Detection Sequencing (PhasED-Seq) was recently developed.[21,24] PhasED-Seq was applied to 14 plasma samples from five patients with localized NSCLC and compared with a standard single-nucleotide variant (SNV)-based ctDNA method. Using the standard SNV-based method, ctDNA was detectable in five of 14 plasma cfDNA samples, whereas PhasED-Seq detected ctDNA in 10 of the 14 samples to levels as low as 0.000094%.[24] The authors then compared both methods for ctDNA MRD detection in a patient with locally advanced lung adenocarcinoma. SNV-based ctDNA analysis and PhasED-Seq detected similar ctDNA levels pretreatment in this patient, but ctDNA MRD during CRT, in between CRT and ICI consolidation, and during ICI consolidation treatment was not detected using the standard SNV-based approach, whereas it was detectable using PhasED-Seq.[21,24] These findings suggest that PhasED-Seq may have superior sensitivity to detect ctDNA in the MRD setting in patients with NSCLC compared with traditional SNV-based ctDNA analysis.

ctDNA MRD FOR RESECTABLE NSCLC

As part of the prospective tumor evolution NSCLC TRACERx study (NCT01888601), Abbosh et al[20] applied multiplex polymerase chain reaction (PCR) to plasma cfDNA samples

TABLE 2. ctDNA Performance to Detect Disease in the Surveillance Setting in Patients With NSCLC

Author (Year)	No.[a]	Clinical Stage	Treatment	ctDNA Assay	Sensitivity, %[b]	Specificity, %[b]
Chaudhuri et al (2017)[c]	37	IB-IIIB	CRT or RT and/or surgery ± chemo	CAPP-Seq	100	100
Abbosh et al (2017)[d]	24	IA-IIIB	Surgery ± chemo	Signatera	93	70
Abbosh et al (2020)[e]	78	I-III	Surgery ± chemo	ArcherDx	82	96

Abbreviations: CAPP-Seq, Cancer Personalized Profiling by deep Sequencing; chemo, chemotherapy; CRT, chemoradiation; ctDNA, circulating tumor DNA; NSCLC, non–small-cell lung cancer; RT, radiotherapy.

[a]No. depicts the number of patients eligible for this analysis.

[b]Sensitivity and specificity are for lung cancer relapse using ctDNA detection status acquired at multiple surveillance timepoints after surgery or CRT (ever positive *v* never positive). Only studies with multiple post-treatment surveillance timepoints are included here.

[c]Chaudhuri et al[10]: Source of data in the original manuscript: Figure S1, Table S1.

[d]Abbosh et al[20]: Source of data in the original manuscript: Figure 4, Extended Data Figure 5, Extended Data Figure 6.

[e]Abbosh et al[35]: Source of data: Abstract CT023 (doi: 10.1158/1538-7445.AM2020-CT023).

following surgical resection to assess the ability to identify ctDNA and predict relapse in patients with resectable NSCLC undergoing curative-intent treatment. Using genomic data from whole-exome sequencing of the primary tumor, personalized PCR panels were created for each individual to target a median of 18 SNVs per patient using Signatera technology (Natera, San Carlos, CA). The analytical sensitivity (derived from in vitro experimentation) of this assay to detect ctDNA was shown to be > 99% for SNVs at frequencies above 0.1%.[20]

Twenty-four patients were included in this study. All patients had resectable NSCLC stage IA-IIIB, half of whom also received adjuvant chemotherapy following surgical resection and some received postoperative radiation treatment.[20] Presurgical and postsurgical plasma samples were analyzed for ctDNA detection. If at least two tumor-informed SNVs were detected in plasma, the ctDNA assay was considered positive. During a median follow-up of 775 days, 14 patients developed disease relapse, and 13 of 14 (93%) patients had detectable ctDNA before or at the time of clinical relapse (surveillance+; Table 2).[20] However, only 36% of patients who developed disease relapse had detectable ctDNA MRD at the first timepoint after surgery; by contrast, 90% of patients with undetectable ctDNA MRD at the first timepoint after surgery did not develop disease relapse (Table 1).[20]

Interestingly, three patients with detectable ctDNA within 30 days after surgery who then received adjuvant chemotherapy had increasing ctDNA levels following chemotherapy and all developed disease relapse within 1 year.[20] Among the 10 patients without relapse, ctDNA was detected in three individuals in at least one timepoint (surveillance+).[20] These combined findings demonstrate that ctDNA detection during follow-up has high sensitivity to identify disease relapse, while maintaining modest specificity (Table 2). Furthermore, these findings suggest that ctDNA dynamics may inform us regarding chemotherapy resistance as illustrated by the three patients with rising ctDNA levels despite adjuvant chemotherapy.

In 2020, updated findings from the TRACERx study were presented at the American Association for Cancer Research Annual Meeting.[35] Seventy-eight patients with NSCLC stage I-III were included in this analysis. Patient-specific anchored multiplex PCR panels were generated using ArcherDx technology.[35] These panels targeted a median of 196 clonal and subclonal variants detected in primary tumor tissue by multiregion whole-exome sequencing. Forty-five patients developed disease recurrence, and ctDNA was detected at or before clinical relapse in 37 of them (82%; surveillance+; Table 2).[35] Interestingly, 10 of 78 patients were diagnosed with other primary malignancies during clinical follow-up, and ctDNA was not detected in their plasma, reflective of the specificity of the ArcherDx tumor-informed assay.[35] In the 23 patients who remained relapse-free during a median follow-up of 1,184 days, ctDNA was detected in only one of the 199 timepoints analyzed (Table 2).[35] These combined findings suggest promising applications of personalized tumor-informed ctDNA surveillance following NSCLC resection.

In November of 2016, the DYNAMIC study (NCT02965391), a prospective observational study, began enrolling patients with suspected lung malignancy before surgical resection to assess the half-life of ctDNA after surgery.[23] Plasma samples were collected at multiple timepoints: (1) immediately before surgery, (2) 5 minutes, 30 minutes, and 2 hours after surgery, and (3) 1, 3, and 30 days after surgery. The authors applied circulating single-molecule amplification and resequencing technology (cSMART), a PCR-based NGS platform, to plasma cfDNA samples to detect eight commonly mutated genes in NSCLC (*EGFR*, *KRAS*, *ERBB2*, *PIK3CA*, *MET*, *TP53*, *ALK*, and *RET*).[23]

Among the 205 enrolled patients, 36 (18%) had ctDNA detected in their preoperative plasma samples. From these, 26 patients were evaluable for postsurgical ctDNA analysis.[23] Plasma samples collected preoperatively through 2 hours after surgery were used for ctDNA half-life calculations, whereas plasma collected between 1 and 30 days after surgery was used for MRD analysis.[23] Plasma ctDNA concentrations exhibited a rapid decrease trend after surgical resection with mutant allele fractions dropping from an average of 2.72% to 0.17% from immediately before surgery to 2 hours after surgery. The median ctDNA half-life was 35 minutes, and the half-life was longer in patients with detectable ctDNA between 1 and 30 days after surgery than in patients with undetectable ctDNA within this timeframe (103.2 minutes *v* 29.7 minutes, respectively; $P = .001$).[23]

Among 25 evaluable patients, during a median follow-up of 532 days, 9 of 25 developed disease recurrence, of which 4 of 9 (44%) had detectable ctDNA MRD 3 days postoperatively, whereas 14 of 16 (88%) patients without relapse had negative ctDNA MRD at this same timepoint (Table 1).[23] While there was no difference in recurrence-free survival (RFS) between patients with detectable versus undetectable ctDNA 1 day after surgery, patients with detectable ctDNA MRD 3 days after surgery had shorter RFS compared with their undetectable counterparts (median RFS = 278 *v* 637 days, respectively; $P = .002$).[23] Also, ctDNA results did not change from 3 to 30 days after surgery as illustrated by 7 of 25 patients who had samples interrogated at both the 3- and 30-day timepoints.[23] Finally, patients with detectable ctDNA MRD either 3 or 30 days after surgery had shorter OS than those with negative ctDNA at the same postoperative timepoints (median OS = 434 *v* 720 days, respectively; $P = .018$).[23] These findings suggest that detectable postoperative ctDNA as early as 3 days after lung cancer surgery can accurately stratify patients on the basis of relapse risk and be prognostic for survival.

Although the above studies used a limited panel for targeted deep sequencing to detect ctDNA MRD, Zviran et al aimed to investigate whether WGS of cfDNA would enable MRD detection in patients with early-stage NSCLC

undergoing surgical resection.[9] The authors performed WGS on tumor tissue to identify SNVs to calculate a cumulative genome-wide tumor signal from plasma.[9] They demonstrated that by integrating more than 10,000 SNVs, the probability of detecting ctDNA tumor fractions in plasma to levels as low as 10^{-5} was $>85\%$ with $20\times$ depth of coverage and $>99\%$ with $50\times$ depth of coverage.[9] They also performed genome-wide analysis to calculate a tumor signal from copy-number alterations, which was combined with the detected SNVs to generate a single statistical detection score. They named this assay MRDetect and tested its application in a cohort of 22 patients with lung adenocarcinoma undergoing surgery.[9]

Plasma samples were collected before and approximately 2.5 weeks after surgery in these 22 patients with stage I-III NSCLC. With a median follow-up of 18 months, 12 of 22 patients had negative ctDNA MRD following surgery, and none developed relapse.[9] Among the 10 patients with detectable ctDNA MRD, 50% developed disease recurrence (Table 1). Finally, patients with detectable ctDNA MRD following surgery had significantly worse RFS when compared with their ctDNA MRD-negative counterparts ($P = .009$).[9] These findings suggest that this tumor-informed plasma WGS approach could be used as a prognostic ctDNA MRD biomarker for early-stage NSCLC.

ONGOING STUDIES

With the proof-of-concept evidence available to date suggesting ctDNA MRD is a prognostic and possibly a predictive biomarker, different groups have begun prospective studies assessing the role of ctDNA MRD to predict recurrence and personalize treatment strategies. AstraZeneca opened two studies in collaboration with ArcherDx: MERMAID-1 and MERMAID-2. MERMAID-1 (NCT04385368) is a phase III trial where patients with resectable stage II-III NSCLC, who are ctDNA MRD-positive after surgery, are randomly assigned to receive adjuvant durvalumab in combination with platinum-based chemotherapy versus placebo plus platinum-based chemotherapy. MERMAID-2 (NCT04642469) is a phase III study where patients with stage II-III NSCLC who undergo standard-of-care surgical resection $\pm$ neoadjuvant and/or adjuvant therapy are followed with serial ctDNA analysis for up to 2 years after completion of curative-intent treatment and, if and when ctDNA becomes positive, they are randomly assigned to receive adjuvant durvalumab or placebo for up to 2 years.

In collaboration with Stanford, AstraZeneca also opened a phase II trial assessing ctDNA MRD in patients with stage I-III NSCLC treated with curative intent (NCT04585477). In this trial, patients with positive ctDNA MRD, detected using the AVENIO ctDNA surveillance kit (CAPP-Seq technology) following curative-intent surgery or stereotactic body radiotherapy, receive up to 1 year of adjuvant durvalumab, whereas those with negative ctDNA MRD receive no further treatment beyond the standard of care. This type of study has the potential to improve our ability to precisely recommend adjuvant systemic treatment for patients with early-stage NSCLC and improve upon the tumor size cut-point of 4 cm currently used clinically in early-stage node-negative patients.[41] In another study using the AVENIO ctDNA detection platform (NCT04585490), patients with unresectable stage III NSCLC with positive ctDNA MRD following CRT receive durvalumab plus four cycles of platinum-doublet chemotherapy, whereas patients with no detectable ctDNA MRD after CRT receive standard-of-care consolidation treatment with durvalumab.

Other studies are prospectively collecting blood samples for ctDNA analysis as part of phase II and phase III trials to correlate ctDNA MRD with outcomes and treatment responses. At Washington University, a phase II study is investigating local and regional control with hypofractionated magnetic resonance imaging–guided adaptive radiotherapy with concurrent chemotherapy followed by consolidation durvalumab (NCT03916419). In this trial, as part of an exploratory analysis, investigators are collecting plasma samples at six timepoints (pre-, on- and serially post-treatment) for ctDNA analysis using CAPP-Seq. They plan on correlating ctDNA levels with radiographic progression, progression-free survival, and OS.

Also, as part of an exploratory analysis in CheckMate-816 (NCT02998528), a phase III trial that is comparing neoadjuvant nivolumab plus chemotherapy versus chemotherapy alone in terms of safety and efficacy in patients with resectable stage IB-IIIA NSCLC, blood is being collected for ctDNA analysis before each cycle of systemic therapy in the neoadjuvant setting. Investigators are using the ArcherDx Personalized Cancer Monitoring platform[42] to assess ctDNA dynamics, which they will correlate with response to neoadjuvant systemic therapy.[43] Preliminary results on 90 patients were presented at the 2021 American Association for Cancer Research Annual Meeting and demonstrated that 87 patients had detectable ctDNA before neoadjuvant treatment and 56% cleared ctDNA levels from cycle 1 to cycle 3 in the nivolumab plus chemotherapy group versus 34% in the chemotherapy group.[43] Also, there was an association between ctDNA clearance and pathologic complete response.[43]

Provided that the aforementioned prospective studies confirm that ctDNA is a prognostic and predictive biomarker for recurrence and response to therapy, respectively, in the future, the treatment paradigm for patients with NSCLC treated with curative-intent may change toward ctDNA-driven personalization (Fig 1). One possible outcome would be the incorporation of ctDNA MRD testing into standard clinical practice to potentially increase cure rates while mitigating toxicity risk through personalized adjuvant and consolidation therapy escalation versus de-escalation.

DISCUSSION AND FUTURE DIRECTIONS

Despite aggressive multimodality treatment approaches, patients with stage I-III NSCLC still have a high rate of

disease relapse after curative-intent treatment.[37,44] It is thus of paramount importance to identify which patients are at increased risk for disease relapse and investigate strategies that can mitigate this and potentially improve cure rates. Although pretreatment ctDNA levels are not always detectable, some studies suggest that detectable ctDNA pretreatment is associated with worse FFP and survival.[21,45] This suggests that detectable ctDNA before curative-intent strategies may be a marker for micrometastatic disease and could be a useful biomarker to select patients for neoadjuvant treatment (Fig 1).

Although ctDNA analysis has the potential to identify patients for treatment personalization to maximize chance of cure while decreasing the risk of overtreatment, its application into clinical practice for patients with early-stage NSCLC has some major barriers. One primary issue is the low sensitivity of detecting ctDNA in stage I NSCLC.[32] The Circulating Cell-free Genome Atlas (NCT02889978) study, which used methylation sequencing of cfDNA followed by machine learning, demonstrated a pretreatment sensitivity of approximately 24% for detecting stage I lung cancer.[11] Another machine learning–based ctDNA detection approach called Lung cancer likelihood in plasma (Lung-CLiP)[47] demonstrated stage IA pretreatment detection sensitivity of 18%.[47] Highlighting the challenge of detecting early-stage disease, Abbosh et al[32] estimated that a ctDNA assay lower limit of detection as low as 20 parts per million may be required to detect lung cancer nodules measuring 1.2 cm in size.

Another challenge of detecting ctDNA reliably is CHIP. CHIP leads to mutations detectable in cfDNA that originate from hematopoietic cells and can result in false-positive plasma genotyping as cancer-related genes such as *TP53*, *KRAS*, *JAK2*, *GNAS*, and *IDH2* may also be involved in CHIP.[40,46] CHIP mutational frequency has been shown to be 92% in approximately 60-year-old patients with CHIP-associated variants enriched at low allele fractions.[39] Thus, as ctDNA assays achieve greater sensitivity and ultralow limits of detection, there is a risk of also detecting more CHIP mutations. The primary solution to filtering CHIP variants from ctDNA results is through parallel sequencing of peripheral blood mononuclear cells to similar depths as plasma cfDNA. Indeed, currently available commercial

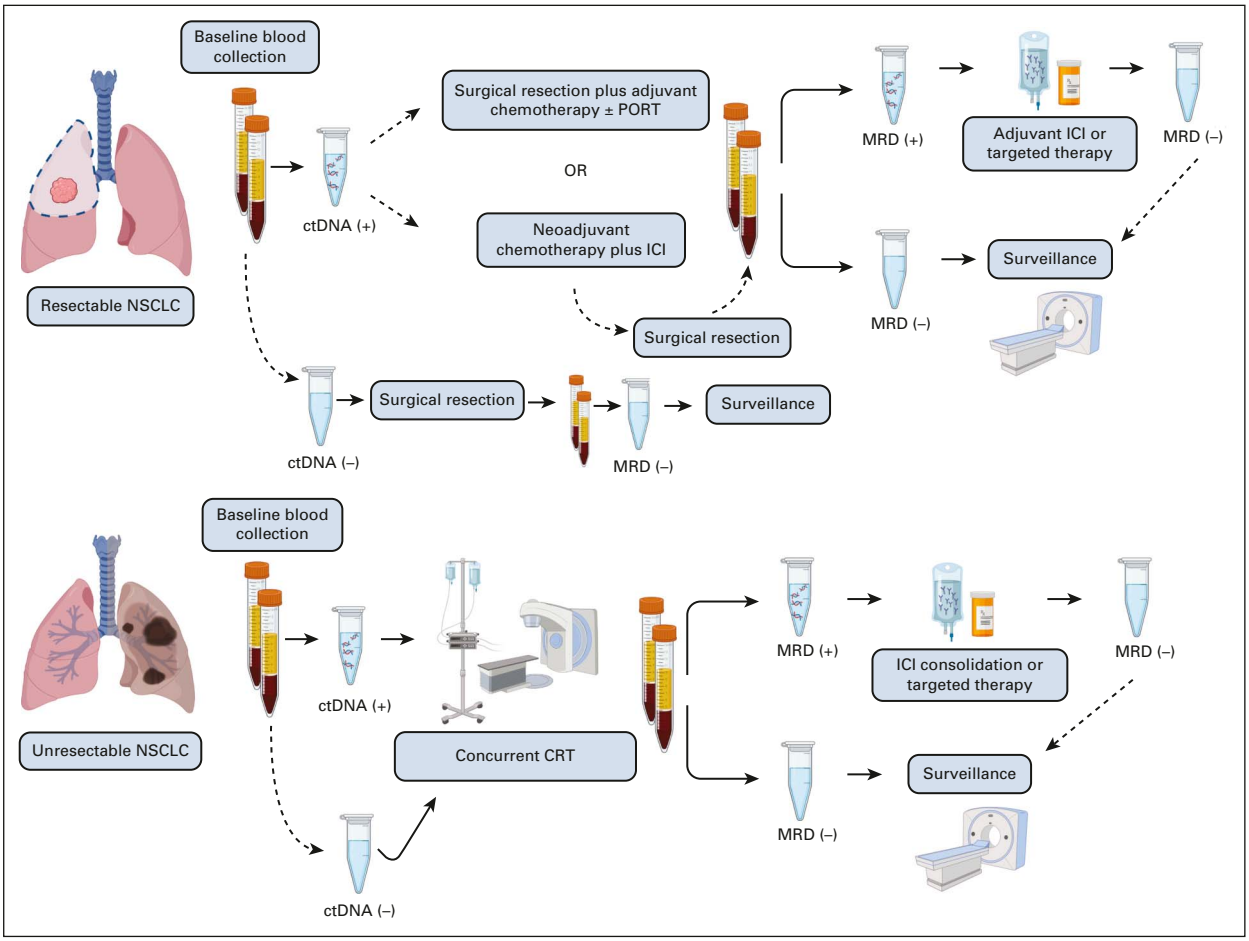

FIG 1. Proposed clinical trial designs for early-stage and locally advanced NSCLC using ctDNA as a biomarker for treatment personalization. CRT, chemoradiation; ctDNA, circulating tumor DNA; ICI, immune checkpoint inhibitor; MRD, minimal residual disease; NSCLC, non–small-cell lung cancer; PORT, postoperative radiotherapy.

ctDNA assays may not be suitable for MRD testing in the clinical setting because of inadequate filtering of CHIP variants that affect assay specificity, as well as limits of detection that are not low enough to ensure high assay sensitivity.

The technologies applied for ctDNA MRD detection can also affect assay performance. ctDNA assays can be broadly categorized as either tumor-informed or tumor-naive, where tumor-informed approaches require initial sequencing of tumor tissue to create personalized assays to interrogate plasma samples for the presence of mutations known a priori.[48] In contrast, tumor-naive approaches rely on mutation calling from plasma and do not require pretreatment tumor sequencing for assay development.[49]

Limitations of the tumor-informed compared with tumor-naive approach include (1) inability to detect clonal variants that emerge during follow-up, including mutations that confer treatment resistance; (2) potential for limited application because of insufficient tumor material for sequencing; (3) potential for geographic tumor heterogeneity to bias assay design and performance; (4) increased turnaround time because of assay personalization; and (5) practical limitations related to tumor tissue access in patients diagnosed in one clinical practice setting but treated in another setting.[12,48] The advantage of a tumor-informed assay is greater confidence that the mutations detected in plasma are tumor-specific rather than artifactual. The ability to track multiple tumor-specific mutations simultaneously using personalized tumor-informed assays can also optimize both assay sensitivity and specificity and increase the chance of success compared with off-the-shelf tumor-naive assay designs.[48-50] Future studies of lung cancer will have to weigh these practical and technical considerations when deciding upon which ctDNA MRD technology to utilize.

Another major issue in the field is the lack of standardization regarding what is the optimal timepoint for ctDNA MRD interrogation. Assay sensitivity and specificity vary substantially depending on whether ctDNA is analyzed at a single post-treatment timepoint or integrated across multiple timepoints (Tables 1 and 2).[10,20,22,23,35] Additionally, the ctDNA MRD data presented from Chen et al,[23] Abbosh et al,[20] and Zviran et al[9] are for resectable disease following surgery, whereas the respective data from Chaudhuri et al[10] and Moding et al[22] are primarily from unresectable disease after completion of chemotherapy and radiotherapy. Although the ctDNA technologies, treatment modalities and analysis timepoints across these studies varied (Table 1), ctDNA MRD detection status remained highly prognostic.[9,10,20,22,23,35] Still, it remains to be proven whether treatment personalization according to ctDNA MRD detection status will translate to improved patient outcomes.

Given the heterogeneity and modest size of published cohorts, as well as the paucity of predictive data, it remains unclear what the optimal post-treatment timepoint is for ctDNA MRD assessment for an oncologist to make a determination about whether to offer additional treatment. Addressing this question will require collaboration across scientific and commercial entities, similar to the ongoing ctMoniTR harmonization effort of post-ICI ctDNA data in advanced-stage NSCLC.[51] It is also critical to continue prospective clinical trial work specifically testing whether ctDNA MRD is a robust predictive biomarker (Fig 1), similar in structure to MERMAID-1, which is testing whether ctDNA MRD detection after lung cancer surgery can be used to personalize the administration of adjuvant immunotherapy. Thus, more studies with larger patient populations and multiple early post-treatment timepoints are needed to better understand ctDNA MRD and its predictive association with NSCLC recurrence and survival.

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Circulating Tumor DNA Minimal Residual Disease Detection of Non–Small-Cell Lung Cancer Treated With Curative Intent

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