Early real-world experience monitoring circulating tumor DNA in resected early-stage non–small cell lung cancer

Travis K. Martin, DO,[a,b] Aaron Dinerman, MD,[c] Sumedha Sudhaman, PhD,[d] Griffin Budde, PharmD,[d] Charuta C. Palsuledesai, PhD,[d] Michael Krainock, MD, PhD,[d] Minetta C. Liu, MD,[d] Emy Smith, ACNP,[e] Leonidas Tapias, MD,[f] Eitan Podgaetz, MD,[f] and Gary Schwartz, MD[f]

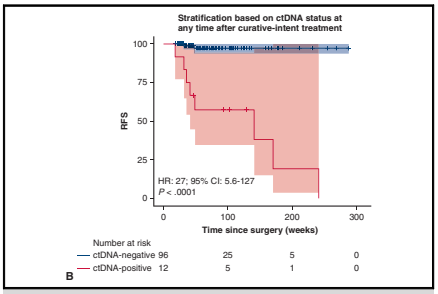
ABSTRACT

Objective: The study objective was to evaluate the impact of monitoring circulating tumor DNA on the detection and management of recurrence in patients with resected early-stage non–small cell lung cancer.

Methods: Between October 2021 and March 2023, postoperative circulating tumor DNA was monitored in patients with non–small cell lung cancer (N = 108). Longitudinal blood samples (n = 378 samples) were collected for prospective circulating tumor DNA analysis at 3-month intervals after curative-intent resection. A tumor-informed assay was used for the detection and quantification of circulating tumor DNA. The primary outcome measure was a circulating tumor DNA–positive result. The secondary outcome measure was changes in practice after a circulating tumor DNA–positive result.

Results: The mean age of the patients in this cohort was 68.1 years. Of the 108 patients, 12 (11.1%) were circulating tumor DNA positive at least at 1 timepoint post-surgery, of whom 8 (66.7%) had a clinically evident recurrence and the remaining 4 had limited clinical follow-up. Of the 10 patients with recurrent disease, 8 demonstrated circulating tumor DNA positivity and the remaining 2 patients had brain-only metastases. Postoperative clinical care was altered in 100% (12/12) of circulating tumor DNA–positive patients, with 58.3% (7/12) receiving an early computed tomography scan and 100% (12/12) receiving an early positron emission tomography computed tomography scan as part of their surveillance strategy. Among the patients who received an early positron emission tomography scan, 66.6% (8/12) were positive for malignant features.

Conclusions: Routine monitoring of tumor-informed circulating tumor DNA after curative intent therapy improved patient risk stratification and prognostication. (J Thorac Cardiovasc Surg 2024;■:1-11)



Postcurative treatment ctDNA positivity was significantly associated with poor RFS.

CENTRAL MESSAGE

Longitudinal, tumor-informed ctDNA monitoring after curative-intent treatment for early-stage NSCLC led to improved patient risk stratification and prognostication.

PERSPECTIVE

Longitudinal ctDNA monitoring using a personalized, tumor-informed assay is a valuable prognostic tool in patients with early-stage NSCLC for early detection of recurrence and can inform clinical decision-making during postresection surveillance. Further studies are warranted to assess the impact of ctDNA-guided adjuvant treatment on patient outcomes.

See Commentary on page XXX.
See Discussion page XXX.

THOR

Lung cancer is the second most common type of cancer and the leading cause of cancer-related mortality worldwide.[1] In the United States alone, the American Cancer Society estimates a total of 238,340 new cases and 127,070 deaths due to lung cancer in 2023.[2] Non–small cell lung cancer (NSCLC) constitutes approximately 80 to 85% of all lung

From the [a]Dignity Health East Valley General Surgery Residency, Chandler Regional Medical Center, Chandler, Ariz; [b]Texas College of Osteopathic Medicine, University of North Texas Health Science Center, Ft Worth, Tex; Departments of [c]General Surgery, [e]Thoracic Surgery, Baylor University Medical Center, Dallas, Tex; [d]Natera, Inc, Austin, Tex; and [f]Department of Thoracic Surgery, University Hospitals Cleveland Medical Center, Cleveland, Ohio.

Informed consent was obtained for all patients as part of inclusion in the study.

Data Availability Statement: Baylor University Medical Center holds the sole responsibility for the accuracy of the clinical data and can provide data on reasonable request.

Read at the 103rd Annual Meeting of The American Association for Thoracic Surgery, Los Angeles, California, May 6-9, 2023.

Received for publication May 7, 2023; revisions received Dec 20, 2023; accepted for publication Jan 7, 2024.

Address for reprints: Travis K. Martin, DO, Texas College of Osteopathic Medicine, University of North Texas Health Science Center, 3500 Camp Bowie Blvd, Ft Worth, TX 76107 (E-mail: traviskohlmartin@gmail.com).

0022-5223/$36.00

https://doi.org/10.1016/j.jtcvs.2024.01.017

Abbreviations and Acronyms

CHIP = clonal hematopoiesis of indeterminate potential
CT = computed tomography
ctDNA = circulating tumor DNA
HR = hazard ratio
MRD = minimal residual disease
MTM = mean tumor molecule
NSCLC = non–small cell lung cancer
PET = positron emission tomography
RFS = recurrence-free survival

Scanning this QR code will take you to the table of contents to access supplementary information. To view the AATS Annual Meeting Webcast, see the URL next to the webcast thumbnail.

cancer diagnoses in the United States. Approximately 25 to 30% of patients with NSCLC are diagnosed with early-stage disease (stage I-II).[3,4] Patients with early-stage NSCLC typically undergo complete surgical resection for curative intent. However, 30 to 55% of patients develop disease recurrence within the first 5 years of surgery, with the chance of NSCLC recurrence most significant during the first 2 years after the curative-intent treatment.[5,6] Five-year survival after lobectomy for stage I NSCLC ranges from 45 to 65% for all histological subtypes collectively, with many of these patients ultimately dying of the disease because of post-resection recurrence.[7] Because of the known risk of recurrence in patients with early-stage NSCLC, surveillance after definitive treatment is recommended. The current surveillance protocols include a chest computed tomography (CT) with or without contrast every 6 months for the first 2 to 3 years, followed subsequently by annual low-dose noncontrast-enhanced chest CT for patients with stage I or II NSCLC treated with surgery with or without chemotherapy.[8,9] Clinical follow-ups for surveillance typically mirror this time course. However, radiographic surveillance is complicated by nonspecific findings. Recurrent NSCLC disease has been shown to be detected by CT scan with a 94% sensitivity and 87% specificity, and a negative predictive value of 99%. However, positive predictive value has been reported as only 53%.[10]

The ability to identify patients at increased risk for recurrence after complete resection would allow for personalized clinical care, including early/frequent surveillance and intervention strategies potentially improving patient outcomes. Circulating tumor DNA (ctDNA) has emerged as a minimally invasive blood-based biomarker to assess recurrence risk in various malignancies.[11-16] Specifically in patients with unresected and resected NSCLC, ctDNA-based minimal residual disease (MRD) detection has been reported to be predictive of recurrence.[17-21] However, the optimum time frame for ctDNA monitoring and whether treatment personalization after ctDNA-based MRD detection can lead to improved outcomes remain to be explored. In this study, we sought to determine the prognostic value of longitudinal, tumor-informed ctDNA testing and its impact on postresection surveillance and management in patients with early-stage NSCLC.

MATERIAL AND METHODS

Study Design and Patient Characteristics

We performed a retrospective analysis of real-world data in patients with early-stage (I-II) NSCLC treated at a tertiary referral center between October 2021 and March 2023. Patients were defined as early-stage based on pathologic stage. Blood samples were collected longitudinally at 3-month intervals after initial collection. ctDNA-based MRD was assessed within 6 months from the time of surgery and before the initiation of adjuvant therapy. In addition, clinicopathologic information was collected retrospectively for all available patients (Figure 1). Patients were followed up and received interventions as determined by standard clinical practice guidelines and at the discretion of the principal investigators. The complete clinical course for patients included in this study is depicted in Figure 2. The inclusion criteria included patients with early-stage, resected NSCLC disease, availability of pathology and at least 1 CT scan report postoperatively, and availability of at least 1 ctDNA measurement postoperatively. The exclusion criteria included patients with stage III and IV NSCLC, history of a second primary tumor, or history of NSCLC recurrence before the initiation of ctDNA monitoring. ctDNA analysis was performed retrospectively in collaboration with Natera, Inc.

Informed consent was obtained for all patients as part of inclusion in the study. This study was approved under the Baylor University Medical Center Umbrella Institutional Review Board (Approval Number: 019-516; date: January 20, 2020) and conducted in accordance with the Declaration of Helsinki.

Personalized Circulating Tumor DNA Assessment

ctDNA testing was conducted at the discretion of the provider and carried out using Natera's standard commercial ordering pathway. Briefly, a personalized, tumor-informed, multiplex polymerase chain reaction next-generation sequencing assay (Signatera, Natera, Inc) was used for the detection of ctDNA (Figure 3), as previously published.[11] Briefly, whole-exome sequencing performed on formalin-fixed, paraffin-embedded tumor blocks, and matched normal blood samples were used to select up to 16 patient-specific, somatic single-nucleotide variants. Multiplex polymerase chain reaction primers targeting the selected single-nucleotide variants were designed, synthesized, and used to track ctDNA in the corresponding patient's plasma samples. Plasma samples with at least 2 tumor-specific variants detected out of 16 were defined as ctDNA positive, and ctDNA concentration was reported in mean tumor molecules (MTMs) per milliliter of plasma.

Study Objectives and Outcomes

The primary objective of the study was to evaluate whether a positive ctDNA result was associated with recurrence-free survival (RFS). For MRD analysis, patients who had ctDNA results within 6 months after

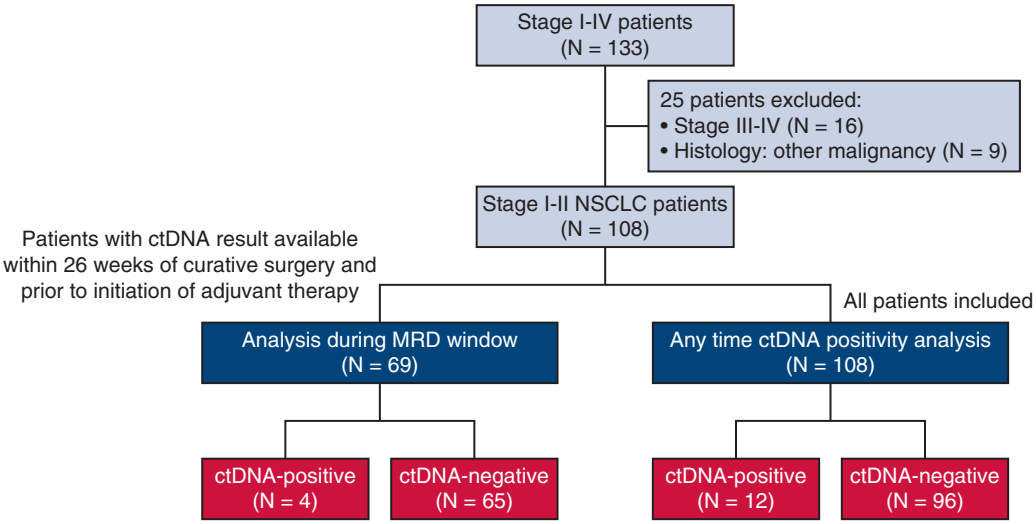


FIGURE 1. Flow diagram depicting an overview of number of patients included in the survival analysis. *NSCLC*, Non–small cell lung cancer; *ctDNA*, circulating tumor DNA; *MRD*, molecular residual disease.

curative-intent resection and before the start of adjuvant treatment were included. For any time ctDNA-positivity analysis, patients with ctDNA positivity at any time point after curative-intent treatment were included. The secondary objective was evaluating changes in practice after a positive ctDNA test that resulted in (1) new referral for consideration of adjuvant therapy, (2) more frequent radiographic surveillance, or (3) consideration for early biopsy. Recurrent disease was defined as at least 1 positive imaging with high clinical suspicion for recurrent disease confirmed via biopsy when the clinical situation was amenable to the procedure. The lead-time for ctDNA-based detection of recurrence was calculated as the time between the date of first positive ctDNA test and the date of first radiologic recurrence.

Statistical Analysis

Based on retrospective clinicopathologic data, RFS was determined from the date of surgery to the first documented sign of radiologic recurrence. The Kaplan–Meier method was used to estimate RFS probabilities, and a log-rank test was used to estimate *P* values. Hazard ratios (HRs) were obtained from Cox regression analysis. Survival analyses were conducted using Rv4.1.2 (http://www.r-project.org; survival, survminer, coxphf packages).

RESULTS

Patient Characteristics

A total of 378 longitudinal plasma samples (n) were collected from 108 patients (N). The average patient age was 68.1 years. The histologic subtypes included adenocarcinoma (N = 87, n = 299), squamous cell carcinoma (N = 16, n = 57), and neuroendocrine carcinoma (N = 5, n = 22). The adenocarcinoma cohort was subdivided into acinar, lepidic, papillary, solid, micropapillary, and mucinous histologic subtypes. Video-assisted thoracoscopic surgery (VATS) was performed in 92.6% (100/108) of patients, with lobectomy accounting for 85.2% (92/108). A subset of patients (16.7%; 18/108) received adjuvant chemotherapy for high-risk features with stage I or stage II disease. Complete demographic, surgical, histologic, and staging characteristics of the patient cohort are detailed in Table 1.

Circulating Tumor DNA Detection During Minimal Residual Disease Window

Of the 69 patients who had ctDNA results available within the MRD window, ctDNA-based MRD was detected in 5.8% (4/69). Of these, 75% (3/4) had confirmed disease recurrence and showed a median lead time (ctDNA detection to radiographic recurrence) of 28 weeks (range, 15.4-30.1 weeks). Compared with MRD-negative patients, those with MRD positivity had a significantly inferior RFS and were 53 times more likely to recur (HR, 53, 95% CI, 5.5-513, $P < .0001$; Figure 4, *A*). A subanalysis in only stage I patients revealed similar findings (Figure E1).

Circulating Tumor DNA Detection During Follow-up

During the follow-up period, 9.3% of patients (10/108) developed disease recurrence, with a median time from resection to recurrence of 44.3 weeks (range, 18.1-240.9 weeks). Of these 10 patients with recurrence, 8 were ctDNA positive during longitudinal testing and 7 of these tested ctDNA positive before radiographic recurrence with a median lead time of 23.9 weeks (range, 10.1-30.8 weeks). Of these 8, 6 recurrences were confirmed via biopsy. The remaining 2 patients had brain metastasis that was detected via magnetic resonance imaging and the recurrence for 1 patient was confirmed via biopsy. In the longitudinal setting, ctDNA positivity at any time point after curative-intent treatment displayed markedly reduced RFS (HR, 27, 95% CI, 5.6-127, $P < .0001$; Figure 4, *B*). On analyzing data for patients with stage I and stage II disease separately, we observed a

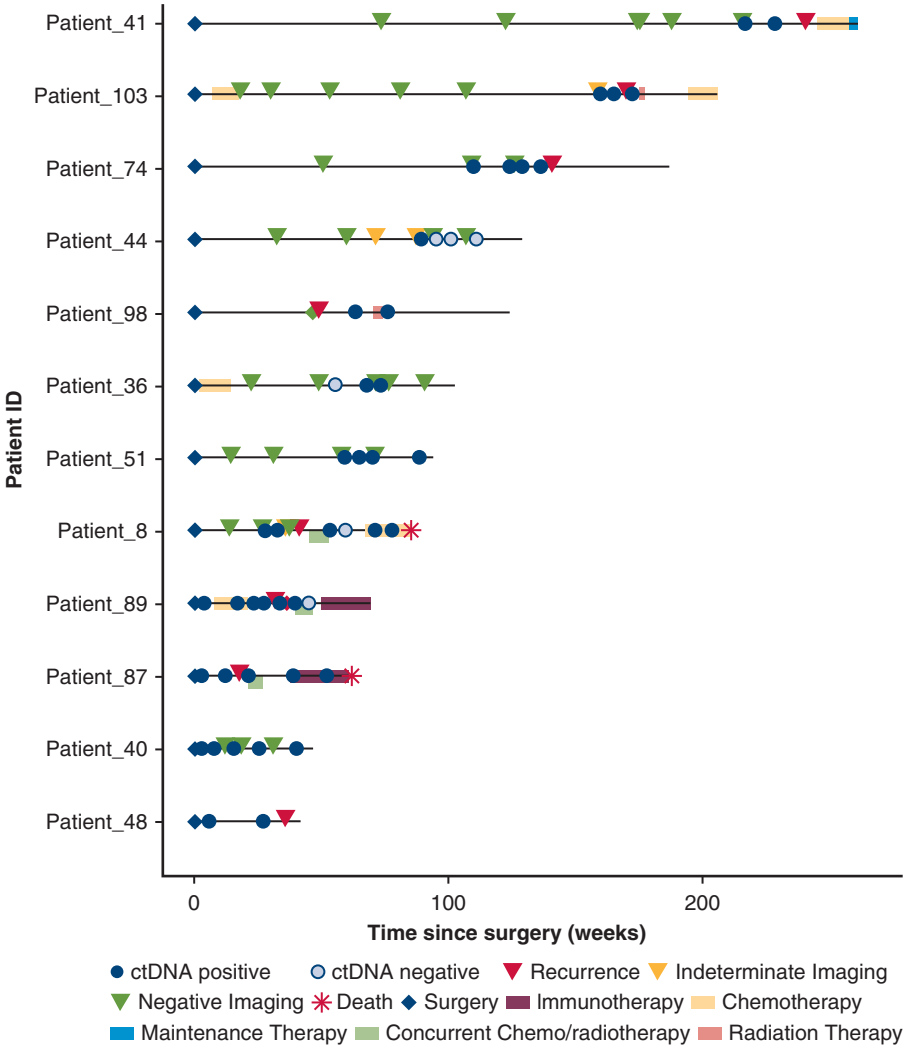


FIGURE 2. Patient overview plot depicting treatment regimens, longitudinal ctDNA analysis, and imaging findings for all patients in the cohort. *Top*: all patients with ctDNA positivity at least at 1 time point. *Bottom*: patients who were persistently ctDNA negative. *ctDNA*, Circulating tumor DNA.

statistically significant association of any time ctDNA-positivity with poor RFS in patients with both stage I (Figure E2) and stage II (Figure E3) disease. Among patients who had a recurrence or a follow-up for a minimum of 52 weeks (1 year), serial monitoring using this tumor-informed ctDNA assay identified recurrence with a sensitivity of 80% (8/10) and specificity of 95.6% (65/68). Likewise, sensitivity and specificity of 80% (8/10) and 95.8% (23/24), respectively, were observed for detecting a recurrence among the patients who had recurrence or a follow-up for more than 104 weeks (2 years).

Adjuvant chemotherapy was administered in 16.7% of patients (18/108) with stage II or stage I disease (high-risk feature). Among these patients, 16.7% (3/18) had ctDNA positivity after completion of adjuvant therapy. Of these 3 patients, 2 ultimately developed biopsy-proven recurrent disease.

Impact of Circulating Tumor DNA Detection on Surveillance Strategy

Postoperative radiographic surveillance was altered in 100% (12/12) of ctDNA-positive patients, with 58.3% (7/12) receiving an earlier surveillance CT scan and 100% (12/12) receiving an early positron emission tomography (PET) scan. Among the patients who received an early PET scan, 66.6% (8/12) were positive for malignant features. Ultimately, early biopsy was obtained in 87.5% (7/8) of PET-positive cases, with 85.7% (6/7) of those early biopsies leading to a confirmed recurrence. These patients were subsequently directed into therapy. The patient with confirmed recurrence on PET who did not undergo biopsy was additionally referred for therapy. Of the ctDNA-positive patients, 33% (4/12) have not demonstrated radiographic recurrence (median follow-up, 6.4 weeks; range, 5.1-29.1 weeks) but were still directed

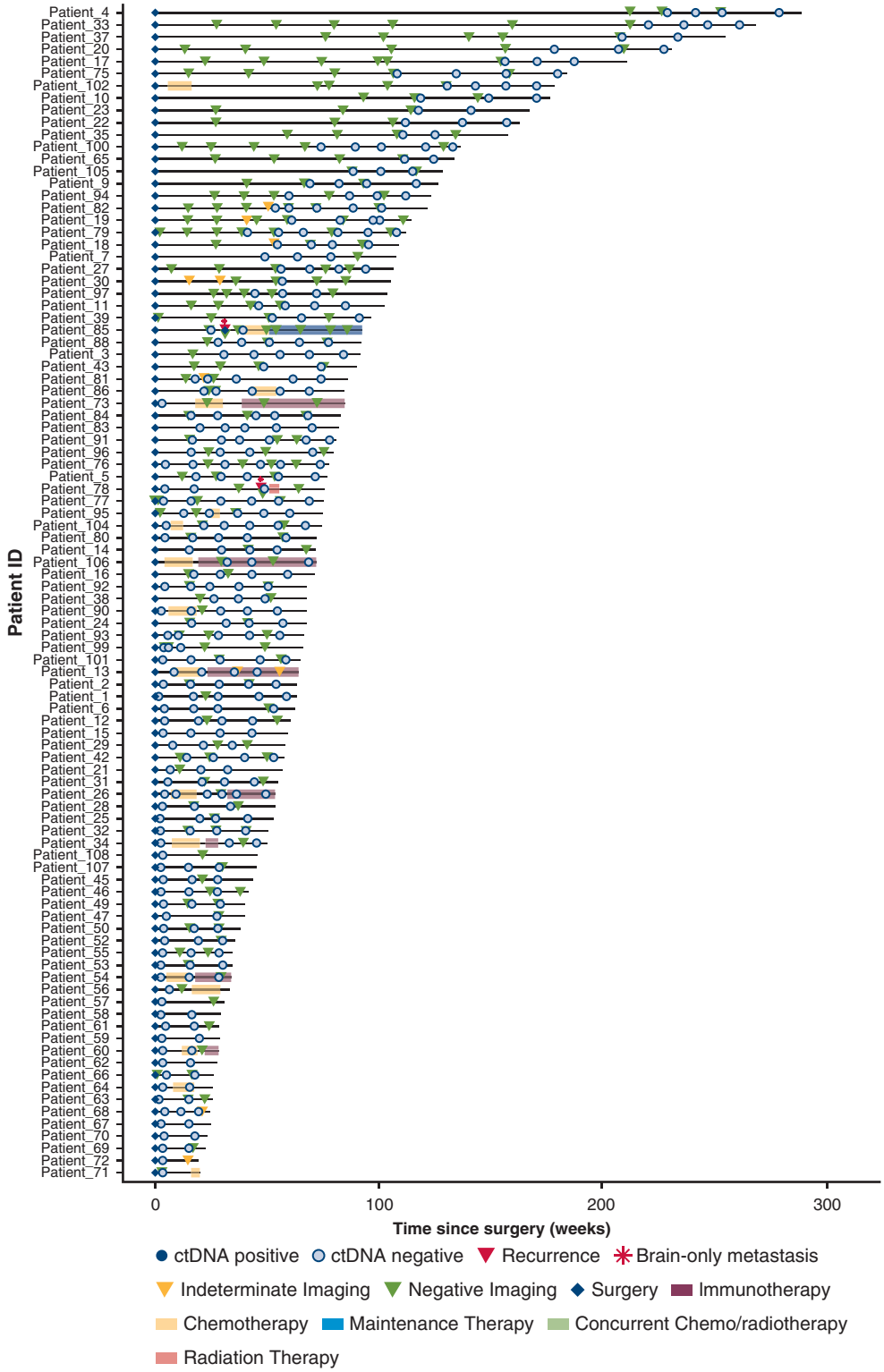


FIGURE 2. (*continued*).

into earlier interval surveillance. Because of the short clinical follow-up and low ctDNA levels observed for these patients (median, 0.31 MTM/mL; range, 0.04-0.86 MTM/mL), an extended follow-up is warranted with continued ctDNA testing to assess any rise in disease burden.

TABLE 1. Complete demographic, surgical, histologic, and staging characteristics of the patient cohort

Characteristics	Categories		ctDNA (+)	ctDNA (−)	Total
Cases			12	96	108
Recurrences			8 (66.7%)	2 (2.1%)	10 (9.3%)
Median follow-up time, wk (range)			98.1 (42.1-261.1)	67.6 (19.2-287.6)	71.4 (19.2-287.6)
Age (mean, y)			66.2	68.4	68.1
Gender	Male		4 (25.0%)	36 (37.5%)	40 (37.0%)
	Female		8 (75.0%)	60 (62.5%)	68 (63.0%)
Diagnosis method	Biopsy		1 (8.3%)	43 (44.8%)	44 (40.2%)
	Surgery		11 (91.7%)	53 (55.2%)	64 (59.3%)
Surgical approach	VATS		9 (75.0%)	91 (94.8%)	100 (92.6%)
	Open		0 (0.0%)	3 (3.1%)	3 (2.8%)
	VATS → Open		3 (25.0%)	2 (2.1%)	5 (4.6%)
Resection	Wedge		2 (16.7%)	12 (12.5%)	14 (13.0%)
	Lobectomy		9 (75.0%)	83 (86.5%)	92 (85.2%)
	Bilobectomy		1 (8.3%)	1 (1.0%)	2 (1.9%)
Laterality	Right		8 (66.7%)	54 (56.3%)	62 (57.4%)
	Left		4 (33.3%)	42 (43.8%)	46 (42.6%)
Surgical margins	Negative		12 (100%)	96 (100%)	108 (100%)
	Positive		0 (0%)	0 (0%)	0 (0%)
Lymph nodes	Median No. of nodes collected		11	11	11
Lymph node stations	Median No. of stations sampled		4.5	5	5
Histology	Adenocarcinoma	Acinar	10 (83.3%)	7 (70.0%)	77 (80.2%)	52 (67.5%)	87 (80.6%)	59 (67.8%)
		Lepidic	0 (0.0%)		5 (6.5%)		5 (5.8%)
		Papillary	2 (20.0%)		7 (9.1%)		9 (10.3%)
		Solid	0 (0.0%)		9 (11.7%)		9 (10.3%)
		Micropapillary	0 (0.0%)		0 (0.0%)		0 (0.0%)
		Mucinous	1 (10.0%)		4 (5.2%)		5 (5.8%)
	Squamous cell		0 (0.0%)		16 (16.7%)		16 (14.8%)
	Neuroendocrine		2 (16.7%)		3 (3.1%)		5 (4.6%)
Staging	IA1		0 (0.0%)		6 (6.3%)		6 (5.6%)
	IA2		7 (58.3%)		40 (41.7%)		47 (43.5%)
	IA3		1 (8.3%)		18 (18.8%)		19 (17.6%)
	IB		1 (8.3%)		15 (15.6%)		16 (14.8%)
	IIA		0 (0.0%)		3 (3.1%)		3 (2.8%)
	IIB		3 (25.0%)		14 (14.6%)		17 (15.7%)
LVI status	Positive		7 (58.3%)		19 (19.8%)		26 (24.1%)
	Negative		5 (41.7%)		77 (80.2%)		82 (75.9%)
VPI status	Positive		0 (0.0%)		14 (14.6%)		14 (13.0%)
	Negative		12 (100%)		82 (85.4%)		94 (87%)
STAS status	Positive		3 (25.0%)		15 (15.6%)		18 (16.7%)
	Negative		9 (75%)		81 (84.4%)		90 (83.3%)

ctDNA, Circulating tumor DNA; *VATS*, video-assisted thoracoscopic surgery; *VATS → Open*, a surgery that began with a VATS approach and then later converted to open thoracotony approach (during the same operation); *LVI*, lymphovascular invasion; *VPI*, visceral pleural invasion; *STAS*, spread through air spaces.

DISCUSSION

This study investigated a commercially available, personalized, tumor-informed ctDNA assay in a real-world cohort of patients with early-stage NSCLC after curative-intent resection. Because of the tumor-informed approach, the assay capitalizes on the knowledge of the mutational landscape of the primary tumor tissue, allowing for increased detection of ctDNA levels after curative-intent resection. The assay detects MRD with high sensitivity and specificity as previously published.[20] ctDNA analysis can be complicated by clonal hematopoiesis of indeterminate potential (CHIP). CHIP is a phenomenon that leads to increased accumulation of somatic mutations in hematopoietic stem cells. CHIP can be present in low levels and may account for nontumor-related mutations detected in plasma samples. This can cause confounding results in the ctDNA assays. However, the method used in this study significantly reduces the false-positive rates by filtering CHIP mutations, thereby providing greater confidence that the ctDNA detected in plasma is tumor-related rather than background artifact caused by CHIP.[20]

In this study, we investigated the utility of a tumor-informed ctDNA assay in a real-world cohort of patients with NSCLC for post–curative-intent resection surveillance. This study demonstrates that ctDNA-based MRD detection and ctDNA detection at any time during surveillance are highly prognostic of poor outcomes and associated with significantly worse RFS. In addition, serial testing with this assay identified recurrence with high sensitivity (80%) and specificity (96%) in patients with early-stage disease who had recurrence or at least 1 year of follow-up. These observed sensitivity and specificity are comparable to those reported in the literature for ctDNA-based detection of recurrence in patients with stage I and III NSCLC.[20,22] The data in this study build on prior published studies supporting the feasibility of ctDNA-based MRD detection and surveillance in patients with NSCLC and demonstrate a median follow-up of 71.4 weeks (range, 19.3-287.6 weeks) as well as a medium-sized population. Because prognosis and clinical management differ by disease stages, we stratified our cohort by stage as appropriate (Figures E1-E3). Across stages, we observed an overall consistent ability of ctDNA positive status to predict an increased risk of recurrence.

In the current schema of cancer recurrence and metastasis, it has been hypothesized that micro-metastatic cells, unaccounted by conventional staging and undetectable by radiographic imaging such as CT or PET, may be present in the systemic circulation at the time of primary resection for early-stage NSCLC. Studies have shown an association between the presence of disseminated tumor cells or circulating tumor cells during resection of NSCLC and patient outcomes.[23-25] However, it remains unclear whether these cells are capable of proliferative activity or simply remain dormant.[26] Detection of MRD and assessment of ctDNA dynamics through serial monitoring may enable early detection of disease recurrence.

Our study addressed its primary objectives: measuring ctDNA in the postresection surveillance period and observing the impact of ctDNA testing on clinical decision-making after curative-intent resection for NSCLC. With exclusion of a single case where no postoperative ctDNA testing was done before clinical relapse, the median lead time was 23.9 weeks (10.1-30.8 weeks) when compared with confirmation of recurrence by radiographic imaging. Prior retrospective studies in patients with NSCLC using tumor-informed ctDNA assays have reported a median lead time of approximately 70 to 165 days (10-23.5 weeks).[18,20,27]

The clinical decision to begin adjuvant therapy after curative resection is currently determined by a combination of TNM staging supplemented with pathologic high-risk features. However, the benefits of adjuvant therapy should be weighed against the potential risk of toxicity and the patient's ability to physiologically tolerate adjuvant treatment. Previous studies have shown that the benefit of adjuvant chemotherapy for patients with early-stage NSCLC translates into an absolute 5-year survival advantage of 4 to 5.4%.[28] However, to date, no biomarkers are yet approved for identifying high-risk patients who are likely to benefit from adjuvant therapy. Our data indicate that ctDNA-based MRD detection and longitudinal ctDNA monitoring may help identify a subpopulation of patients who may be most suitable to benefit from adjuvant therapy.

In this study, among the 10 patients with recurrence, 2 were persistently ctDNA negative and eventually had recurrence. Of note, both these patients developed singular distant brain metastasis. Prior studies have reported similar findings, demonstrating that the brain was the site of recurrence in approximately 20% of the patients with locally advanced NSCLC.[29,30] Other studies have reported lack of molecular evidence of recurrence in cases with brain oligometastatic disease, especially with tracking ctDNA in plasma samples.[31] This has historically been attributed to the blood-brain barrier, which prevents ctDNA of brain metastasis origin from crossing into blood vessels. However, recent studies indicate that tracking ctDNA in cerebrospinal fluid might improve the ability to detect brain metastases.[32,33]

During our study, we observed that the patients who received negative ctDNA results during surveillance were relatively more comforted compared with patients with negative radiographic results, whereas patients with positive ctDNA results experienced anxiety. Some of the ongoing, observational clinical trials are collecting data on the impact of ctDNA testing results on patients'

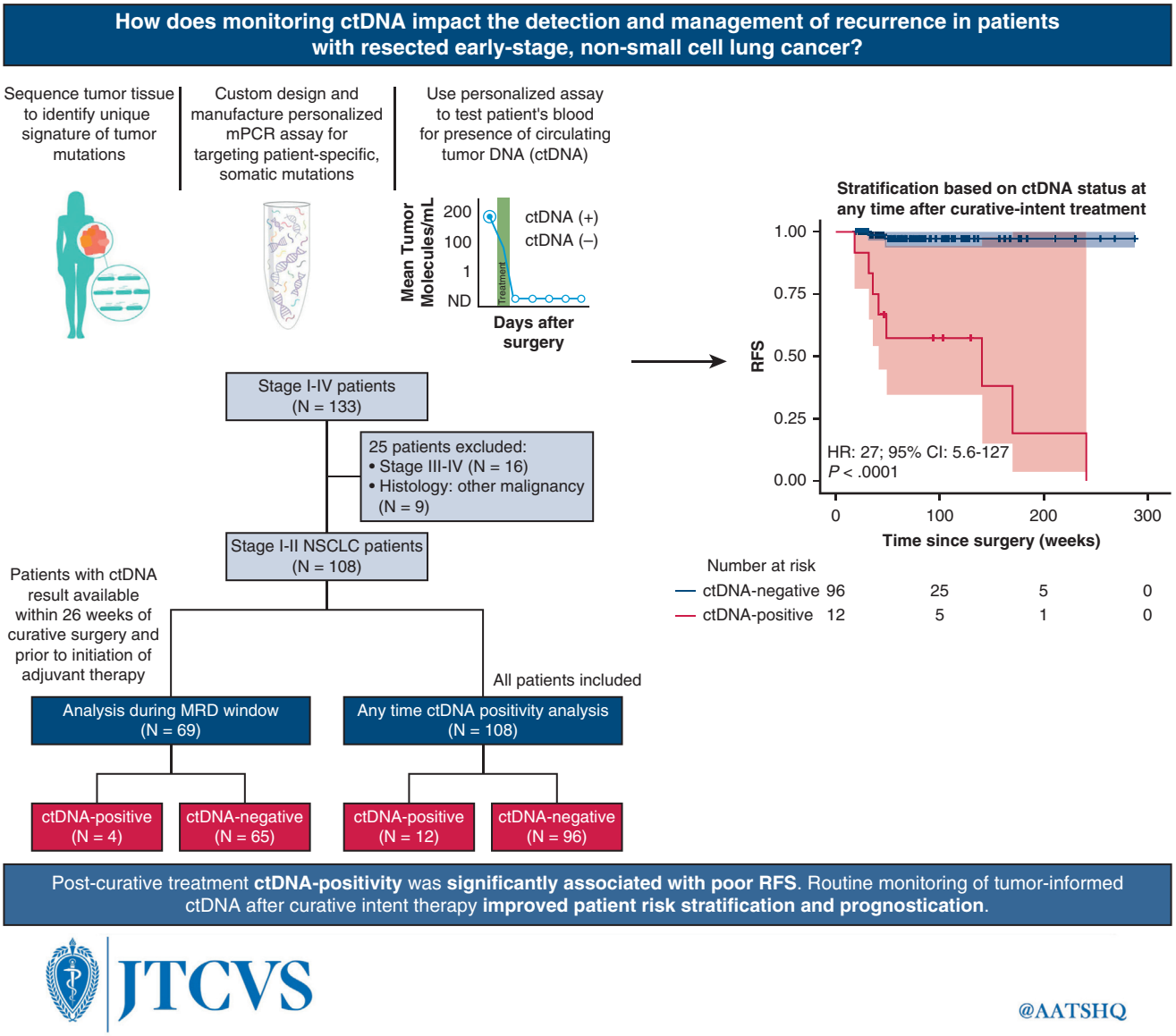


FIGURE 3. Graphical abstract: Summary of study design and key results. *mPCR*, Multiplex polymerase chain reaction; *NSCLC*, non–small cell lung cancer; *MRD*, molecular residual disease; *RFS*, recurrence-free survival; *HR*, hazard ratio.

anxiety about cancer recurrence.[34,35] We believe the potential psychosocial effects of ctDNA testing could be ameliorated if/when therapeutic options become available for patients with ctDNA positivity suggestive of molecular recurrence.

Study Limitations

Limitations of this study include patient noncompliance with regularly scheduled surveillance intervals, leading to lapses or delays between time points in the patient's monitoring after curative-intent treatment. Because the secondary objective of the study was to assess the impact of ctDNA on clinical care, the providers were not blinded to the ctDNA results of patients to make an informed clinical decision on early surveillance/adjuvant treatment. This lack of blinding could contribute an inherent bias. However, radiographic data interpretation of surveillance scans was provided by radiologists who were blinded to patient ctDNA status.

CONCLUSIONS

We highlight both the prognostic and practice-changing role of serial monitoring using a tumor-informed ctDNA assay after the resection of early-stage NSCLC. This study

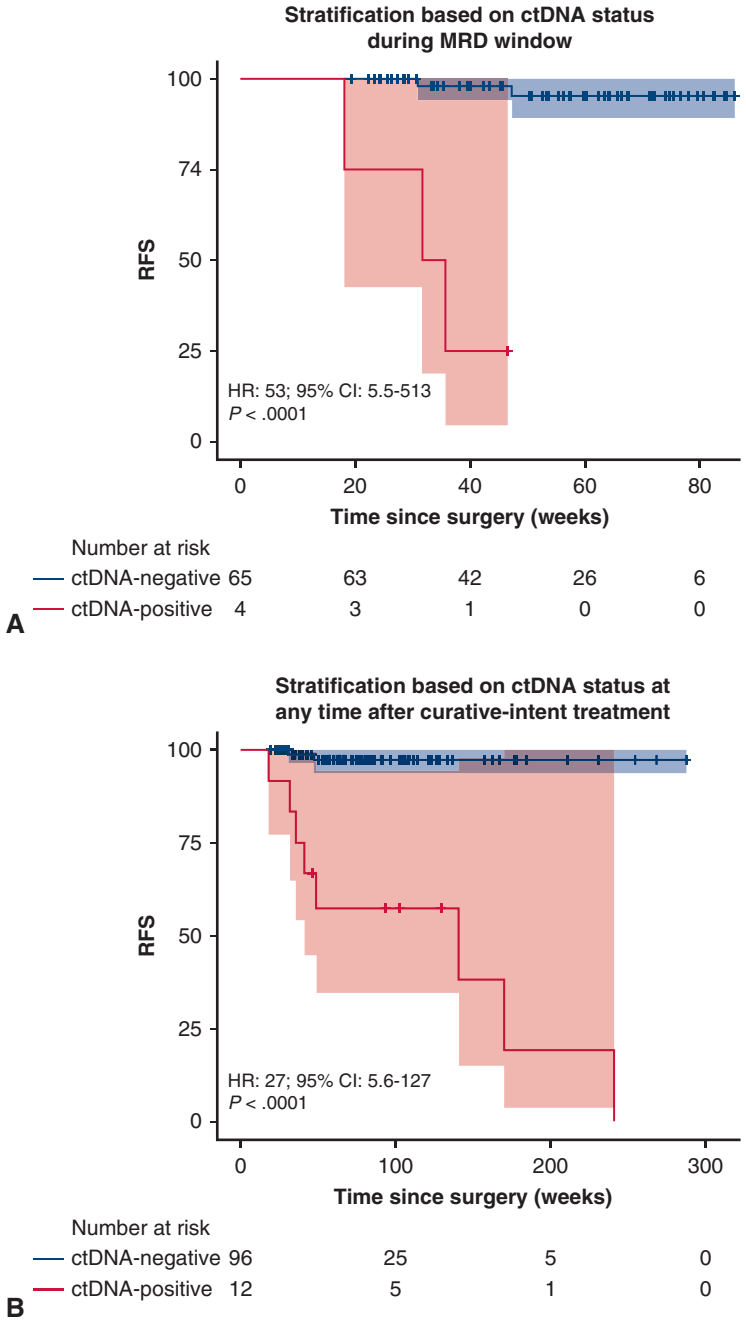
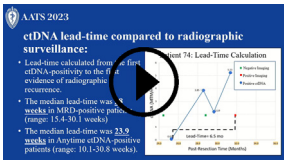


FIGURE 4. Kaplan–Meier analysis of RFS in patients with stage I and II non–small cell lung cance stratified by (A) MRD status (N = 69) and (B) ctDNA status any time after curative-intent surgery (N = 108). *ctDNA*, Circulating tumor DNA; *MRD*, molecular residual disease; *RFS*, recurrence-free survival; *HR*, hazard ratio.

serves to benchmark any time ctDNA detection frequency as it relates to overall RFS survival in this population. Additionally, our study exemplifies the real-life use of ctDNA and its impact on the surveillance and management of early-stage, resected NSCLC. This could aid in the refinement and development of future prospective studies to validate our findings. Given ctDNA's role in other solid tumors and current evidence in NSCLC, we envision ctDNA will improve outcomes and aid in prognostication for early-stage NSCLC. However, further multicenter studies with larger cohorts and longer median follow-ups are required to define clinical best practices more accurately, including potential initiation of systemic therapy based on biomarker detection or elevation.

Webcast

You can watch a Webcast of this AATS meeting presentation by going to: https://www.aats.org/resources/early-real-world-experience-monitoring-circulating-tumor-dna-in-resected-early-stage-non-small-cell-lung-cancer.



Conflict of Interest Statement

S.S., G.B., C.C.P, M.K., and M.C.L. are employees at Natera, Inc, with stock or options to own stock. M.C.L. received grants (funding to Mayo Clinic) from Eisai, Exact Sciences, Genentech, Genomic Health, GRAIL, Menarini Silicon Biosystems, Merck, Novartis, Seattle Genetics, and Tesaro, and travel support from AstraZeneca, Genomic Health, and Ionis. All other authors reported no conflicts of interest.

The *Journal* policy requires editors and reviewers to disclose conflicts of interest and to decline handling or reviewing manuscripts for which they may have a conflict of interest. The editors and reviewers of this article have no conflicts of interest.

References

1. Sung H, Ferlay J, Siegel RL, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*. 2021;71(3):209-249. https://doi.org/10.3322/caac.21660
2. Siegel RL, Miller KD, Wagle NS, Jemal A. Cancer statistics, 2023. *CA Cancer J Clin*. 2023;73(1):17-48. https://doi.org/10.3322/caac.21763
3. Cancer of the lung and bronchus (invasive). In: Howlader N, Noone AM, Krapcho M, et al., eds. *SEER Cancer Statistics Review, 1975-2014*. National Cancer Institute; 2017.
4. Kocher F, Hilbe W, Seeber A, et al. Longitudinal analysis of 2293 NSCLC patients: a comprehensive study from the TYROL registry. *Lung Cancer*. 2015;87(2):193-200. https://doi.org/10.1016/j.lungcan.2014.12.006
5. Uramoto H, Tanaka F. Recurrence after surgery in patients with NSCLC. *Transl Lung Cancer Res*. 2014;3(4):242-249. https://doi.org/10.3978/j.issn.2218-6751.2013.12.05
6. Lou F, Sima CS, Rusch VW, Jones DR, Huang J. Differences in patterns of recurrence in early-stage versus locally advanced non-small cell lung cancer. *Ann Thorac Surg*. 2014;98(5):1755-1761. https://doi.org/10.1016/j.athoracsur.2014.05.070
7. Al-Kattan K, Sepsas E, Fountain SW, Townsend ER. Disease recurrence after resection for stage I lung cancer. *Eur J Cardiothorac Surg*. 1997;12(3):380-384.
8. Ettinger DS, Wood DE, Aisner DL, et al. Non-small cell lung cancer, version 3.2022. *J Natl Compr Canc Netw*. 2022;20(5):497-530. https://doi.org/10.6004/jnccn.2022.0025
9. Jaklitsch MT, Jacobson FL, Austin JHM, et al. The American Association for Thoracic Surgery guidelines for lung cancer screening using low-dose computed tomography scans for lung cancer survivors and other high-risk groups. *J Thorac Cardiovasc Surg*. 2012;144(1):33-38. https://doi.org/10.1016/j.jtcvs.2012.05.060
10. Van Meerbeeck JP, Sirimsi H. Cons: long-term CT-scan follow-up is not the standard of care in patients curatively treated for an early stage non-small cell lung cancer. *Transl Lung Cancer Res*. 2015;4(4):479-483. https://doi.org/10.3978/j.issn.2218-6751.2015.07.06
11. Reinert T, Henriksen TV, Christensen E, et al. Analysis of plasma cell-free DNA by ultradeep sequencing in patients with stages I to III colorectal cancer. *JAMA Oncol*. 2019;5(8):1124-1131. https://doi.org/10.1001/jamaoncol.2019.0528
12. Yang J, Gong Y, Lam VK, et al. Deep sequencing of circulating tumor DNA detects molecular residual disease and predicts recurrence in gastric cancer. *Cell Death Dis*. 2020;11(5):346. https://doi.org/10.1038/s41419-020-2531-z
13. Liang H, Huang J, Wang B, Liu Z, He J, Liang W. The role of liquid biopsy in predicting post-operative recurrence of non-small cell lung cancer. *J Thorac Dis*. 2018;10:S838-S845. https://doi.org/10.21037/jtd.2018.04.08
14. Christensen E, Birkenkamp-Demtröder K, Demtröder D, et al. Early detection of metastatic relapse and monitoring of therapeutic efficacy by ultra-deep sequencing of plasma cell-free DNA in patients with urothelial bladder carcinoma. *J Clin Oncol*. 2019;37:1547-1557. https://doi.org/10.1200/JCO.18
15. Magbanua MJM, Swigart LB, Wu HT, et al. Circulating tumor DNA in neoadjuvant-treated breast cancer reflects response and survival. *Ann Oncol*. 2021;32(2):229-239. https://doi.org/10.1016/j.annonc.2020.11.007
16. Coombes RC, Armstrong AC, Ahmed S, et al. Early detection of residual breast cancer through a robust, scalable and personalized analysis of circulating tumour DNA (ctDNA) antedates overt metastatic recurrence. *Cancer Res*. 2019;79(4_Supplement):P4-01-02. https://doi.org/10.1158/1538-7445.SABCS18-P4-01-02
17. Pellini B, Chaudhuri AA. Circulating tumor DNA minimal residual disease detection of non-small-cell lung cancer treated with curative intent. *J Clin Oncol*. 2022;40:567-575. https://doi.org/10.1200/JCO.21
18. Chen K, Zhao H, Shi Y, et al. Perioperative dynamic changes in circulating tumor DNA in patients with lung cancer (dynamic). *Clin Cancer Res*. 2019;25(23):7058-7067. https://doi.org/10.1158/1078-0432.CCR-19-1213
19. Moding EJ, Liu Y, Nabet BY, et al. Circulating tumor DNA dynamics predict benefit from consolidation immunotherapy in locally advanced non-small cell lung cancer. *Nat Cancer*. 2020;1(2):176-183. https://doi.org/10.1038/s43018-019-0011-0
20. Abbosh C, Birkbak NJ, Wilson GA, et al. Phylogenetic ctDNA analysis depicts early-stage lung cancer evolution. *Nature*. 2017;545(7655):446-451. https://doi.org/10.1038/nature22364
21. Chaudhuri AA, Chabon JJ, Lovejoy AF, et al. Early detection of molecular residual disease in localized lung cancer by circulating tumor DNA profiling. *Cancer Discov*. 2017;7(12):1394-1403. https://doi.org/10.1158/2159-8290.CD-17-0716
22. Li RY, Liang ZY. Circulating tumor DNA in lung cancer: real-time monitoring of disease evolution and treatment response. *Chin Med J (Engl)*. 2020;133(20):2476-2485. https://doi.org/10.1097/CM9.0000000000001097
23. Pantel K, Brakenhoff RH, Brandt B. Detection, clinical relevance and specific biological properties of disseminating tumour cells. *Nat Rev Cancer*. 2008;8(5):329-340. https://doi.org/10.1038/nrc2375
24. Sienel W, Seen-Hibler R, Mutschler W, Pantel K, Passlick B. Tumour cells in the tumour draining vein of patients with non-small cell lung cancer: detection rate and clinical significance. *Eur J Cardiothorac Surg*. 2003;23:451-456. https://doi.org/10.1016/S1010-7940(02)00865-5
25. Tanaka F, Yoneda K, Kondo N, et al. Circulating tumor cell as a diagnostic marker in primary lung cancer. *Clin Cancer Res*. 2009;15(22):6980-6986. https://doi.org/10.1158/1078-0432.CCR-09-1095
26. Bessa X, Elizalde JI, Boix L, et al. Lack of prognostic influence of circulating tumor cells in peripheral blood of patients with colorectal cancer. *Gastroenterology*. 2001;120(5):1084-1092. https://doi.org/10.1053/gast.2001.23245
27. Qiu B, Guo W, Zhang F, et al. Dynamic recurrence risk and adjuvant chemotherapy benefit prediction by ctDNA in resected NSCLC. *Nat Commun*. 2021;12(1):6770. https://doi.org/10.1038/s41467-021-27022-z
28. Pignon JP, Tribodet H, Scagliotti GV, et al. Lung adjuvant cisplatin evaluation: a pooled analysis by the LACE collaborative group. *J Clin Oncol*. 2008;26(21):3552-3559. https://doi.org/10.1200/JCO.2007.13.9030
29. Besse B, Massard C, Haddad V, et al. ERCC1 influence on the incidence of brain metastases in patients with non-squamous NSCLC treated with adjuvant cisplatin-based chemotherapy. *Ann Oncol*. 2011;22(3):575-581. https://doi.org/10.1093/annonc/mdq407
30. Ceresoli GL, Reni M, Chiesa G, et al. Brain metastases in locally advanced non-small cell lung carcinoma after multimodality treatment: risk factors analysis. *Cancer*. 2002;95(3):605-612. https://doi.org/10.1002/cncr.10687
31. Aldea M, Hendriks L, Mezquita L, et al. Circulating tumor DNA analysis for patients with oncogene-addicted NSCLC with isolated central nervous system progression. *J Thorac Oncol*. 2020;15:383-391. https://doi.org/10.1016/j.jtho.2019.11.024

32. Bettegowda C, Sausen M, Leary RJ, et al. Detection of circulating tumor DNA in early- and late-stage human malignancies. *Sci Transl Med*. 2014;6(224):224ra24. https://doi.org/10.1126/scitranslmed.3007094
33. Ma C, Yang X, Xing W, Yu H, Si T, Guo Z. Detection of circulating tumor DNA from non-small cell lung cancer brain metastasis in cerebrospinal fluid samples. *Thorac Cancer*. 2020;11(3):588-593. https://doi.org/10.1111/1759-7714.13300
34. Kasi PM, Sawyer S, Guilford J, et al. BESPOKE study protocol: a multicentre, prospective observational study to evaluate the impact of circulating tumour DNA guided therapy on patients with colorectal cancer. *BMJ Open*. 2021; 11:e047831. https://doi.org/10.1136/bmjopen-2020-047831
35. Kasi PM, Chakrabarti S, Sawyer S, et al. BESPOKE IO protocol: a multicentre, prospective observational study evaluating the utility of ctDNA in guiding immunotherapy in patients with advanced solid tumours. *BMJ Open*. 2022; 12:e060342. https://doi.org/10.1136/bmjopen-2021-060342

Key Words: biomarker, ctDNA, early stage, molecular/minimal residual disease, non–small cell lung cancer, recurrence, surveillance

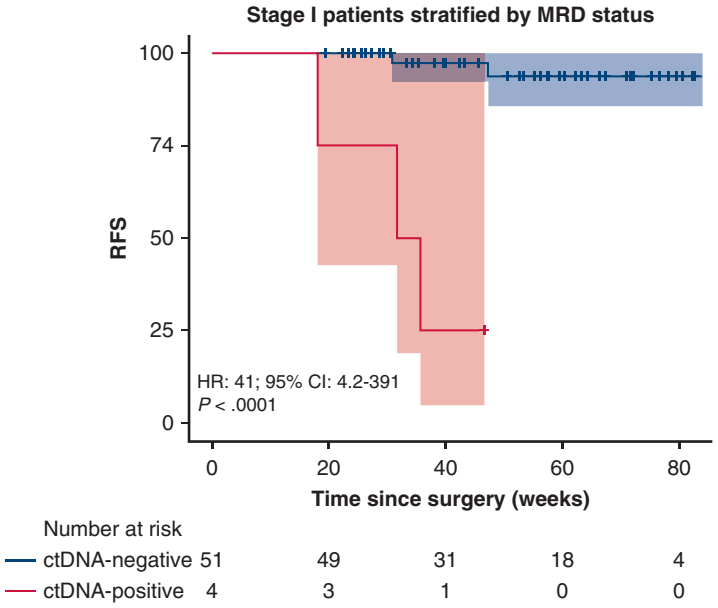


FIGURE E1. Kaplan–Meier analysis of RFS in only patients with stage I NSCLC cancer stratified by MRD status (N = 55). *MRD*, Molecular residual disease; *RFS*, recurrence-free survival; *HR*, hazard ratio; *ctDNA*, circulating tumor DNA.

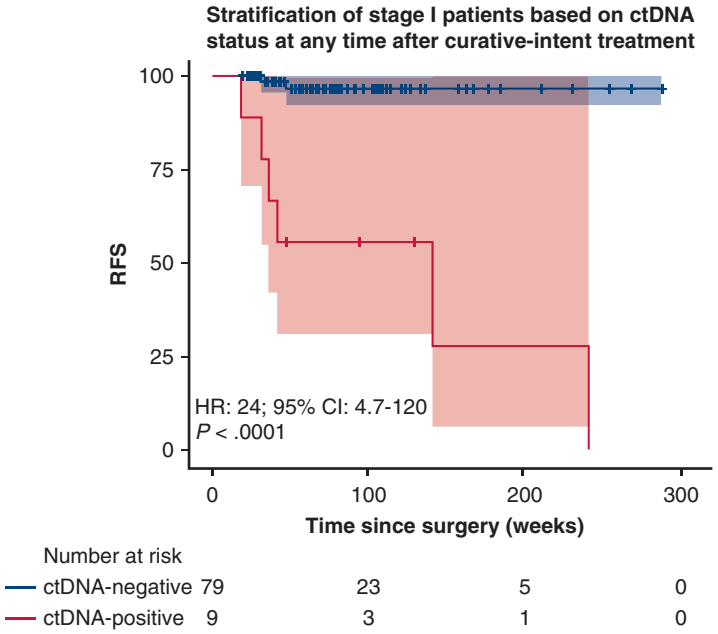


FIGURE E2. Kaplan–Meier analysis of RFS in only patients with stage I lung cancer stratified by status any time after curative-intent surgery (N = 88). *ctDNA*, Circulating tumor DNA; *RFS*, recurrence-free survival; *HR*, hazard ratio.

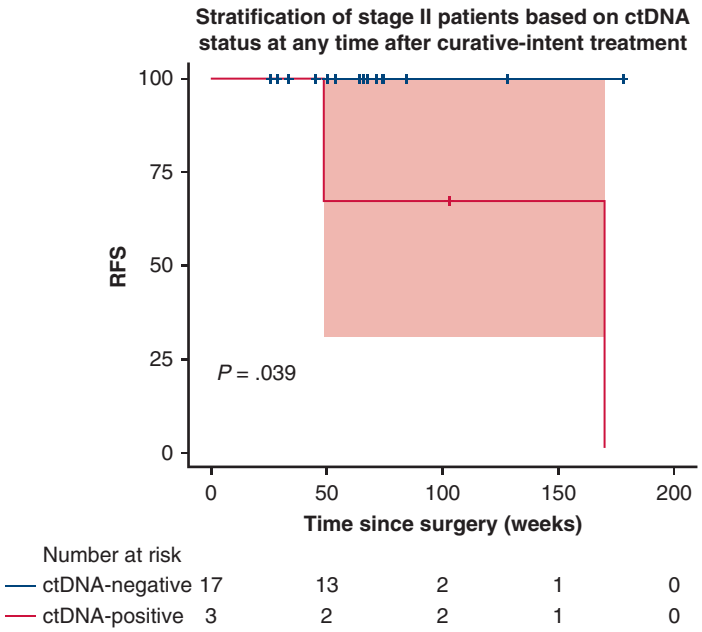


FIGURE E3. Kaplan–Meier analysis of RFS in only patients with stage II lung cancer stratified by status any time after curative-intent surgery (N = 20). *ctDNA*, Circulating tumor DNA; *RFS*, recurrence-free survival.