ORIGINAL ARTICLE

Clinical Significance of Perioperative Minimal Residual Disease Detected by Circulating Tumor DNA in Patients With Lung Cancer With a Long Follow-up Data: An Exploratory Study

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ABSTRACT

Introduction: Molecular residual disease detected by circulating tumor DNA (ctDNA) has been reported to be predictive of patients' outcomes in various types of cancers after curative intent treatment. Nevertheless, additional detailed information regarding the association of longitudinal ctDNA detection with long-term follow-up in lung cancer is needed. Here, we report on a cohort of patients with NSCLC who underwent definitive surgery and ctDNA analysis in the pre-operative, adjuvant, and surveillance settings.

Method: Plasma samples were collected from 46 patients with clinical stage II-III NSCLC before surgery (n = 46), after surgery (n = 45), and every six months until two years thereafter (n = 78). A clinically validated, personalized, tumor-informed 16-plex polymerase chain reaction–next-generation sequencing assay was used for the detection and quantification of ctDNA in retrospectively analyzed plasma samples.

Results: Circulating tumor DNA was detected in the first postoperative (within 51 days after surgery) plasma samples in 13% (6/45) of patients (landmark analysis). All of them had disease recurrence within a median of 9.1 months. These patients had shorter recurrence-free and overall survivals than those without detectable ctDNA at a landmark time point ($p < 0.01$) and in multivariate analyses ($p < 0.03$). Longitudinally (considering all post-operative follow-up time points), ctDNA was detected in 13 patients, all of whom experienced disease recurrence (positive predictive value = 100%). Three patients who had central nervous system–only metastases did not have detectable ctDNA.

Conclusions: The presence of ctDNA post-surgery or during surveillance identifies patients with NSCLC at high risk of recurrence. Serial testing is important to detect disease recurrence earlier (lead-time: 3.2 months).

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Introduction

Although the recent application of targeted therapy and immunotherapy in the perioperative management of patients with NSCLC greatly improved the survival outcome,[1–3] some patients may require more intensive treatment. Meanwhile, some patients are cured by surgery alone, such that postoperative therapies may be safely de-escalated or even omitted. Currently, there are no reliable biomarkers available to accurately risk-stratify patients with NSCLC after curative-intent surgery and facilitate decision-making regarding treatment escalation and de-escalation.

Circulating tumor DNA (ctDNA)-based detection of molecular residual disease (MRD) after surgery[4,5] has recently emerged as a promising prognostic tool in various solid tumors, including NSCLC. There are several techniques to detect ctDNA, including polymerase chain reaction (PCR)–based[6–8] and next-generation sequencing (NGS)–based[9,10] approaches including the bespoke NGS.[11,12] There are several advantages and disadvantages to these methods, including the sensitivity and costs; nevertheless, it is currently unknown how different techniques will influence the outcomes. In a previous pilot study of 20 patients with lung cancer at Kindai University, using NGS-based Cancer Personalized Profiling by deep Sequencing analysis, most patients with ctDNA positivity post-surgery (within 3–12 days) experienced early disease recurrence within six months.[13] Nevertheless, even in patients without detectable ctDNA at the landmark time point after pulmonary resection, longitudinal (surveillance) analysis of ctDNA may be a useful tool to detect disease recurrence earlier than clinical or radiological detection. This is particularly true when considering assays with higher sensitivity of ctDNA detection. We therefore evaluated the prognostic value of a personalized, tumor-informed ctDNA assay. We correlated the presence of ctDNA through longitudinal testing with various clinicopathologic features and patient outcomes with a long follow-up period (median: 47.5 months, range: 4.8-68 months).

Patients and Methods

Study Cohort

Fifty patients with clinical (c) stage IIA–IIIA NSCLC (Tumor, Node, and Metastasis eighth edition) who underwent pulmonary resection between January 2018 and June 2020 at our institution were assessed for inclusion in the study. Patients who received neoadjuvant chemotherapy or who had advanced malignancies other than lung cancer within the previous five years were excluded. Out of 50 patients, four (8%) were excluded from the study; one patient withdrew the consent, and plasma samples from the other three patients did not pass the quality check. As a result, the data from 46 patients were analyzed in this study. The preoperative nodal staging was performed based on both fluorodeoxyglucose–positron emission tomography/computed tomography (CT) and enhanced CT findings. Written informed consent was obtained from each patient. This study was approved by the Institutional Review Board of Kindai University Faculty of Medicine (30-009).

Sample Collection and Extraction of Cell-free DNA

Biospecimens were prospectively collected and stored as part of the clinical protocol. Preoperative blood samples were collected within 48 hours before surgery. The first postoperative blood samples were collected at a median of seven days (range: 2–51 days) after surgical resection/before the initiation of adjuvant treatment and this time point was referred to as the landmark. Thereafter, postoperative blood samples were collected longitudinally every six months (surveillance). For each time point, 8.5 mL of blood was collected in the Cell-Free DNA Collection Tube (Roche Diagnostics, Mannheim, Germany). Plasma was separated by centrifugation within one week and stored at -80°C until use.

ctDNA Detection Using the Signatera Assay

A clinically validated, personalized, tumor-informed, 16-plex PCR-NGS assay (Signatera, Natera Inc.) was used for the detection and quantification of ctDNA in plasma samples as previously described.[14] Briefly, tumor tissue (frozen tumor tissue [n = 28] or formalin-fixed, paraffin-embedded tissue [n = 22]) and matched normal blood samples were subjected to whole exome sequencing. A set of up to 16 patient-specific, somatic single-nucleotide variants from whole exome sequencing were selected for multiplex PCR testing to track ctDNA in the corresponding patients' banked plasma samples (median plasma volume of 4.3 mL, range 2.4 mL–6.4 mL). Plasma samples with at least 2 out of 16 detectable variants were defined as ctDNA-positive and the concentration was measured in mean tumor molecules /mL of plasma.

Statistical Methods

The chi-square test was used to compare differences in categorical variables. Recurrence-free survival (RFS) was defined as the interval between surgery and recurrence or death from any cause. Overall survival (OS) was

defined as the interval between the date of surgery and the date of death from any cause. Recurrence-free survival and OS were estimated by the Kaplan–Meier method and compared using the log-rank test. The multivariate Cox proportional hazards model was used to assess the individual contribution of other independent clinical risk factors contributing to the survival outcome. A p value lower than 0.05 was considered statistically significant. All data were analyzed using JMP version 15.0 software (SAS Institute, Cary, NC).

Results

Patient Characteristics

The cohort consisted of 36 male individuals (78.2%) and 10 female individuals (27.8%) with a median age of 72.5 (range: 50–89) years, therefore we set the cut-off point at 73 years in this study. The subtypes included 15 squamous cell carcinomas (41.7%), 26 adenocarcinomas (56.5%), two adenosquamous cell carcinomas (4.3%), and three others (6.5%) (combined small cell carcinoma and squamous cell carcinoma, large cell neuroendocrine carcinoma, and adenoid cystic carcinoma). The details of patient clinical characteristics are summarized in Table 1. In this cohort, 17 patients (37%) received adjuvant chemotherapy whereas 29 patients (63%) did not.

Pre-operative ctDNA Analysis

Of 46 patients, 27 (59%) had detectable ctDNA preoperatively (Table 1). Factors that significantly affected ctDNA positivity were histology (80% in squamous cell carcinoma, 48% in non-squamous cell carcinoma, $p = 0.04$) and histologic grade (49% in grade 1–2, 100% in grade 3–4, $p < 0.01$). Patients with preoperative ctDNA-positivity tended to have a shorter RFS (hazard ratio [HR] = 1.55, $p = 0.31$) (Fig. 1*A*) and OS (HR = 1.57, $p = 0.19$) (Fig. 1*D*); nevertheless, these differences did not reach statistical significance.

Landmark ctDNA Analysis

Of 45 patients with a ctDNA test available at the landmark time point, six were ctDNA positive. ctDNA-positivity at the landmark time point was significantly associated with histological grade (8% in grades 1–2,

Table 1. Correlation Between Patient Characteristics and Positive Rate for Preoperative and Landmark ctDNA

		Preoperative Time Point			Landmark		
Variables	Category	Number of Patients	ctDNA Detection, n (%)	p Value	Number of Patients[a]	ctDNA Detection (%)	p Value
Age	≥73 y	23	12 (52)	0.37	23	3 (13)	0.95
	<73 y	23	15 (65)		22	3 (14)	
Sex	Male	36	22 (61)	0.53	35	6 (17)	0.16
	Female	10	5 (50)		10	0 (0)	
Smoking history	Smoker	36	22 (61)	0.53	35	6 (17)	0.16
	Nonsmoker	10	5 (50)		10	0 (0)	
CT size	≥4.0 cm	38	21 (55)	0.3	37	4 (11)	0.28
	<4.0 cm	8	6 (75)		8	2 (25)	
Clinical stage	IIA-IIB	29	16 (55)	0.53	28	3 (11)	0.51
	IIIA	17	11 (65)		17	3 (18)	
Pathological invasion size	≥4.0 cm	20	11 (55)	0.66	20	1 (5)	0.14
	<4.0 cm	26	16 (62)		25	5 (20)	
Pathological nodal status	N0	27	14 (52)	0.48	26	2 (8)	0.28
	N1	8	5 (63)		8	1 (13)	
	N2	11	8 (73)		11	3 (27)	
Pathological stage	IA3-IIB	30	15 (50)	0.1	29	2 (7)	0.09
	IIIA-IIIB	16	12 (75)		16	4 (25)	
Histology	Squamous cell carcinoma	15	12 (80)	0.04	15	3 (20)	0.87
	Nonsquamous cell carcinoma	31	15 (48)		30	3 (10)	
Grade	1, 2	37	18 (49)	<0.01	36	3 (8)	0.05
	3, 4	9	9 (100)		9	3 (33)	

[a]One patient was excluded because the single nucleotide polymorphism concordance quality control did not pass.
CT, computed tomography; ctDNA, circulating tumor DNA.

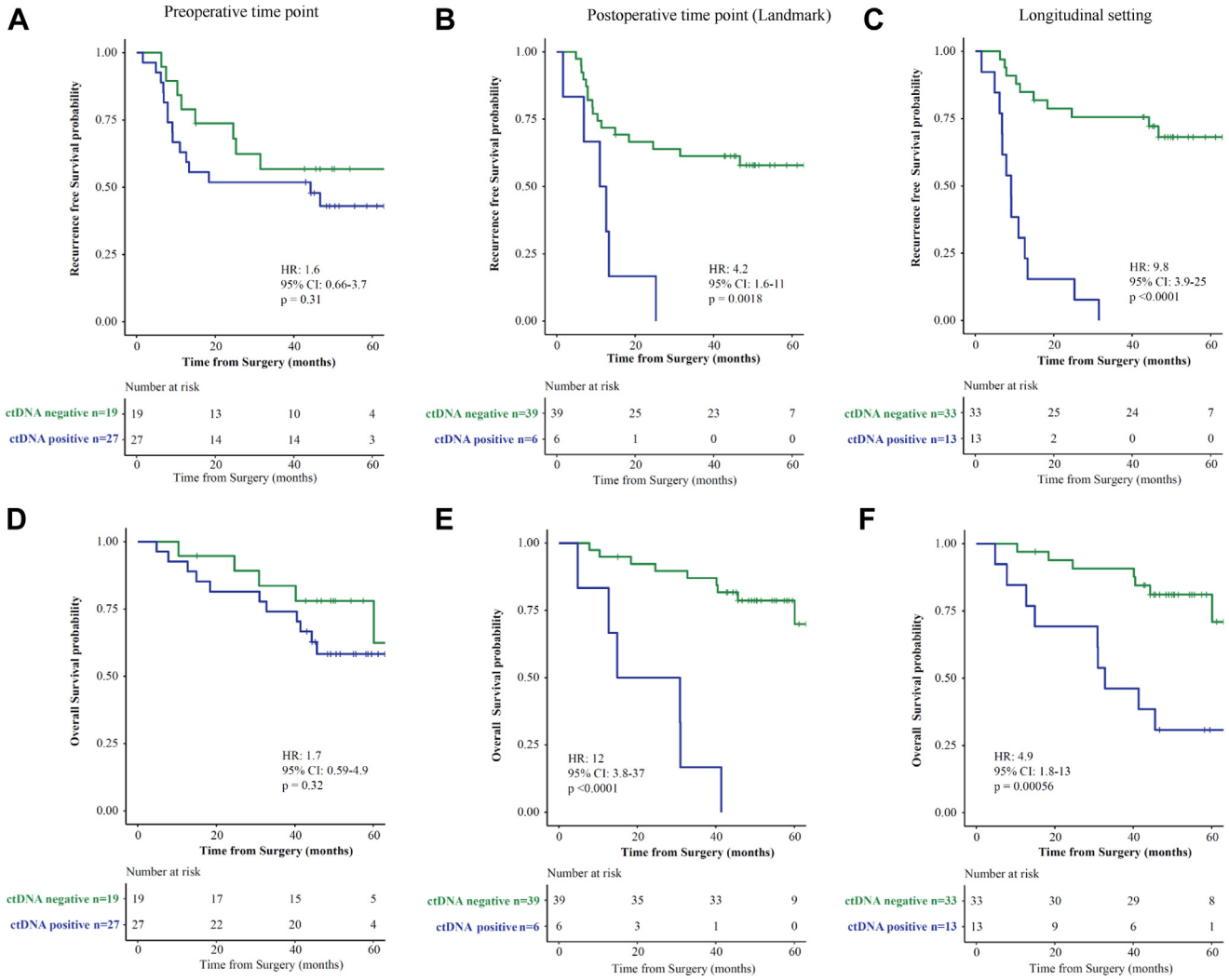

Figure 1. Association of ctDNA status with RFS and OS in our cohort. (*A*, *D*) Comparison of patients with and without preoperative ctDNA positivity on RFS and OS; (*B*, *E*) Comparison of patients with and without postoperative ctDNA positivity on RFS and OS; (*C*, *F*) Comparison of patients with and without longitudinal ctDNA positivity on RFS and OS. ctDNA, circulating tumor DNA; OS, overall survival; RFS, recurrence-free survival.

33% in grades 3–4, $p \leq 0.05$). In addition, patients with higher pathologic stage disease tended to have a higher incidence of landmark ctDNA positivity (7% in p-stage I–II, 25% in stage III, $p = 0.09$) (Table 1).

In this study with a median follow-up of 47.5 months (range: 4.8-68 months, Fig. 2), 19 patients had disease recurrence (locoregional, n = 9; distant extra-cranial ± central nervous system [CNS], n = 7; CNS only, n = 3 [Fig. 3]). All six patients (100%) who were ctDNA-positive at the landmark time point experienced disease recurrence within two years after surgery (median 9.1 months), resulting in a positive predictive value of 100%. On the other hand, 13 of the 39 patients (33.3%) with ctDNA negativity at the landmark developed disease recurrence, of which only three patients had a distant extracranial recurrence, the remaining 10 patients had locoregional (n = 7) and CNS-only (n = 3) recurrence. All the patients who did not experience disease recurrence were ctDNA negative at landmark (specificity = 100%); nevertheless, it should be noted that ctDNA negativity does not guarantee any recurrence, as 13 out of 19 patients who had disease recurrence were negative for ctDNA.

In survival analyses, patients with positive ctDNA at the landmark time point had significantly poorer RFS (HR = 4.18, $p < 0.01$) and OS (HR = 14.4, $p < 0.01$) compared with those who were ctDNA-negative at landmark (Figs. 1*B* and *E*). In the multivariate analysis, ctDNA-positivity at the landmark time point was an independent predictor of poor RFS and OS (Table 2).

Next, we performed a competing risk analysis to account for non-cancer deaths observed in the dataset

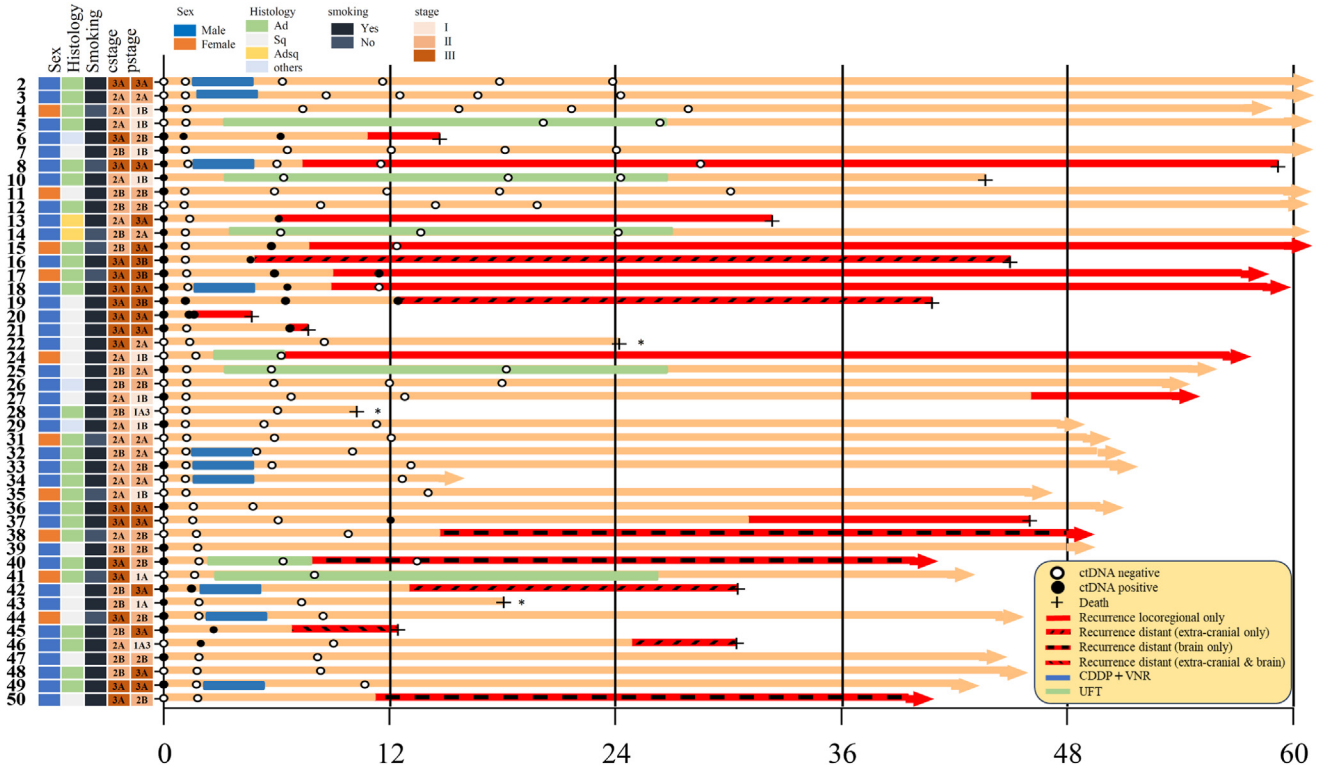

Figure 2. Swimmer plot for each patient depicting pre- and postoperative ctDNA status and disease status by imaging. The survival periods of each patient are summarized according to recurrence and death and adjuvant therapy data. The asterisk indicates non-cancer-related deaths. ctDNA, circulating tumor DNA.

(Supplementary Fig. 1). The competing risk analysis established a significantly increased hazard ($p = 0.0009$) for disease recurrence associated with postsurgical ctDNA-positivity (landmark time point); whereas, the significance of pre-surgery ctDNA positivity was not identified ($p = 0.25$).

Longitudinal ctDNA Analysis

Of the 16 patients with extracranial distant recurrences, 13 patients (81.3%) were ctDNA positive with longitudinal testing starting any time after surgery. Three patients with CNS-only metastases remained serially ctDNA negative (Fig. 3, Supplementary Fig. 2). Notably, patients with ctDNA positivity at any time point post-surgery were 16 times more likely to have extracranial recurrence (HR = 16, $p < 0.0001$), compared with those who were serially ctDNA negative post-surgery (Supplementary Fig. 2). The median lead-time for ctDNA detection before radiological recurrence was 3.2 months (ranges: 0–24.3 months). All patients who were recurrence-free until the end of follow-up were serially ctDNA negative (specificity = 100%).

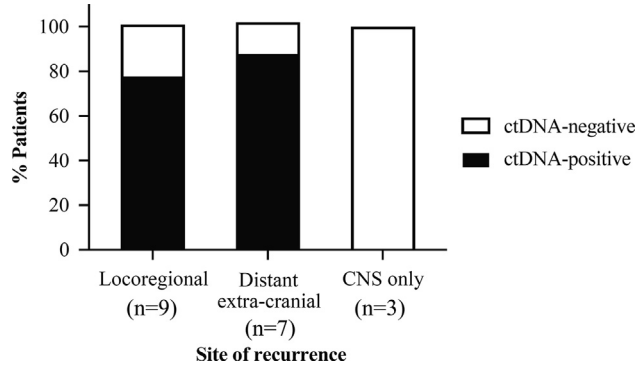

Figure 3. ctDNA detection rates varied among the patients who recurred based on the site of recurrence. ctDNA, circulating tumor DNA.

In the univariate and multivariate analyses, patients with ctDNA-positivity at any time point after surgery (longitudinal ctDNA positive) had significantly inferior RFS (HR = 9.76, $p < 0.01$) and OS (HR = 7.60, $p < 0.01$) compared with those who were serially ctDNA-negative (Figs. 1*C* and *F*; Table 2).

There were six patients without detectable ctDNA at time points preceding radiologic relapses "Pt 8, 24, 27, 38, 40, and 50" (Fig. 2). Of these six patients, three had squamous and non-squamous histology, each; and two, three, and one patient had p-stages I, II, and III disease, respectively. When we looked at the recurrence site, three of the six patients had brain-only metastases (Fig. 3).

Table 2. Univariate and Multivariate Cox Regression Analysis of Recurrence-Free Survival and Overall Survivals Including ctDNA Status at the Landmark Time Point and in the Longitudinal Setting

		Analysis at the Landmark Time Point												Analysis in the Longitudinal Setting											
		Recurrence-Free Survival						Overall Survival						Recurrence-Free Survival						Overall Survival					
		Univariate			Multivariate[a]			Univariate			Multivariate[a]			Univariate			Multivariate[a]			Univariate			Multivariate[a]		
Variables	Category	HR	95% CI	*p* Value	HR	95% CI	*p* Value	HR	95% CI	*p* Value	HR	95% CI	*p* Value	HR	95% CI	*p* Value	HR	95% CI	*p* Value	HR	95% CI	*p* Value	HR	95% CI	*p* Value
Age	<73 y	1.6	0.27-1.43	0.27				1.33	0.47-3.76	0.58				1.6	0.27-1.43	0.27				1.33	0.47-3.76	0.58			
	≥73 y	1						1						1						1					
Sex	Male	1.35	0.46-3.96	0.59						[b]				1.35	0.46-3.96	0.59						[b]			
	Female	1												1											
Smoking history	Smoker	1.37	0.47-4.03	0.57				5.18	0.68-39.5	0.11				1.37	0.47-4.03	0.57				5.18	0.68-39.5	0.11			
	Nonsmoker	1						1						1						1					
Pathologic stage	IIIA-IIIB	3.71	1.62-8.50	<0.01	3.23	1.34-7.80	<0.01	3.11	0.11-8.75	0.03	2.26	0.70-7.27	0.17	3.71	1.62-8.50	<0.01	1.37	0.50-3.80	0.54	3.11	0.11-8.75	0.03	0.52	0.11-2.48	0.41
	IA3-IIB	1						1						1						1					
Histology	Squamous cell carcinoma	1.4	0.58-3.20	0.43				1.69	0.60-4.77	0.32				1.4	0.58-3.20	0.43				1.69	0.60-4.77	0.32			
	Nonsquamous cell carcinoma	1						1						1						1					
Grade	3, 4	2.09	0.82-5.35	0.12				1.24	0.35-4.39	0.74				2.09	0.82-5.35	0.12				1.24	0.35-4.39	0.74			
	1, 2	1						1						1						1					
Landmark ctDNA	Positive	4.18	1.58-11.1	<0.01	3.02	1.10-8.35	0.03	14.4	4.23-49.3	<0.01	10.6	2.94-38.0	<0.01	9.76	3.89-24.5	<0.01	8.18	2.76-24.3	<0.01	7.6	2.57-22.5	<0.01	12.6	2.45-64.3	<0.01
	Negative	1						1						1						1					

[a]Only factors with *p* value less than 0.05 in the backward stepwise procedures were included.
[b]Sex was removed from multivariate analysis because there was no event in female group.
CI, confidence interval; CT, computed tomography; ctDNA, circulating tumor DNA; HR, hazard ratio.

Case Report: ctDNA to Predict Recurrence and Monitor Response to Therapy

A 69-year-old male patient (Patient 18; Fig. 4) underwent left upper lobectomy for lung adenocarcinoma (p-stage IIIA). He had detectable preoperative ctDNA; nevertheless, ctDNA was not detected in the blood drawn on postoperative day (POD) 9. Nevertheless, ctDNA turned positive on POD 200 with no evidence of recurrence by CT. Subsequent CT examination performed on POD 272 revealed metastasis to the mediastinal lymph nodes (lead-time: 72 days). He received concurrent chemoradiotherapy which was initiated on POD 290 followed by durvalumab for one year. Circulating tumor DNA cleared during the course of durvalumab as evidenced by a negative result on POD 348. The patient remained recurrence-free until the last follow-up (POD 1748). This case emphasizes the clinical utility of longitudinal ctDNA analysis as a tool to detect recurrence ahead of imaging and monitor the response to treatment.

Discussion

We evaluated a tumor-informed ctDNA assay as a tool for MRD detection after pulmonary resection (landmark analysis) and a tool for early detection of disease recurrence (any time after resection). A recent study by Chen et al.[15] reported that the ctDNA detected in plasma samples one day after surgery was not predictive of tumor recurrence probably due to incomplete degradation of ctDNA present before surgery, but that ctDNA detected in plasma three days after surgery was predictive. Our results are in accordance with this observation; all six patients who were ctDNA positive at the landmark time point taken within 51 days after surgery had disease recurrence. The positive predictive value and specificity during both landmark and longitudinal analyses were 100%. Even at stage IA3, one patient who was ctDNA positive at the landmark time point relapsed, suggesting that adjuvant chemotherapy may be applicable at stage I. Longitudinally, 6/7 extracranial distant relapses were detectable with ctDNA, in contrast to the three cases with brain-only relapses and two with locoregional recurrences that remained undetected. In other words, these show that the landmark ctDNA analysis with Signatera assay found 31.6% sensitivity. This number is approximately compatible with reported data in surgically resected patients with NSCLC; sensitivity for predicting recurrence ranges between 33% and 100%.[16,17] Nevertheless, the observed sensitivity was lower than we expected. In any case, the sensitivity of landmark ctDNA detection by currently available platforms even with the use of a tumor-informed approach is not high enough to exclude patients who will be cured by surgery alone from candidates for adjuvant therapy. In an exploratory study of Impower 010 in which adjuvant atezolizumab prolonged disease-free survival (DFS) of the patients with resected stages II to III NSCLC, ctDNA positivity detected by Signatera assay was a poor prognostic factor for DFS; nevertheless, atezolizumab reported DFS benefit irrespective of ctDNA status if PD-L1 was expressed in greater than or equal to 1%.[18] There are several factors that potentially influenced the sensitivity of the present study. The timing and frequency of plasma evaluation varied in this cohort, as did the time from the last ctDNA test to the end of follow-up/clinical detection of relapse. The longer follow-up period of this study compared with previous studies (median 54 months versus 16–36 months),[19,20] allowed us to capture late recurrences. Finally, the shedding of ctDNA from the tumor to the bloodstream is impacted not only by tumor burden but also by tumor biology (genomic subtype, proliferation, and cell turnover rate) and anatomic location of relapse.[21,22] Lower ctDNA shedding and detection rates have been reported in patients with NSCLC with adenocarcinoma histology compared with squamous cell histology,[23,24] in cases of isolated locoregional recurrences,[25] and in CNS-only recurrences impacted by the blood-brain-barrier.[25,26]

Our results indicate that the presence of ctDNA is strongly indicative of disease recurrence and reports the value of longitudinal testing; nevertheless, before ctDNA-guided decisions regarding the personalization of adjuvant systemic therapy can be made, further studies with well-defined sample collection schedules and metrics are needed. In addition, as one of the limitations, it should be noted that our study has been conducted before the era of perioperative immune checkpoint inhibitors (ICIs) or tyrosine kinase inhibitors (TKIs). Therefore, further data is essential in this area involving patients treated with perioperative ICIs or TKIs to meet the current standard of therapy for the early stage. Nevertheless, our data will still have the impact of historical control against studies that analyze MRD status in patients with NSCLC who receive perioperative ICIs or TKIs. Although we observed a sensitivity of 93% (13/14) for distant recurrence outside of the CNS with serial testing, the lower ctDNA shedding rate observed in this cancer type, reflective of its unique biology, along with frequent locoregional and CNS-only recurrences, further complicate adjuvant decision making based on a single blood draw. As technology continues to evolve new approaches will help further refine patient risk stratification and guide adjuvant therapy decision-making, including a tumor-informed approach using a greater number of gene mutations identified by whole genome sequencing,[27,28] in addition to involving other liquid data such as

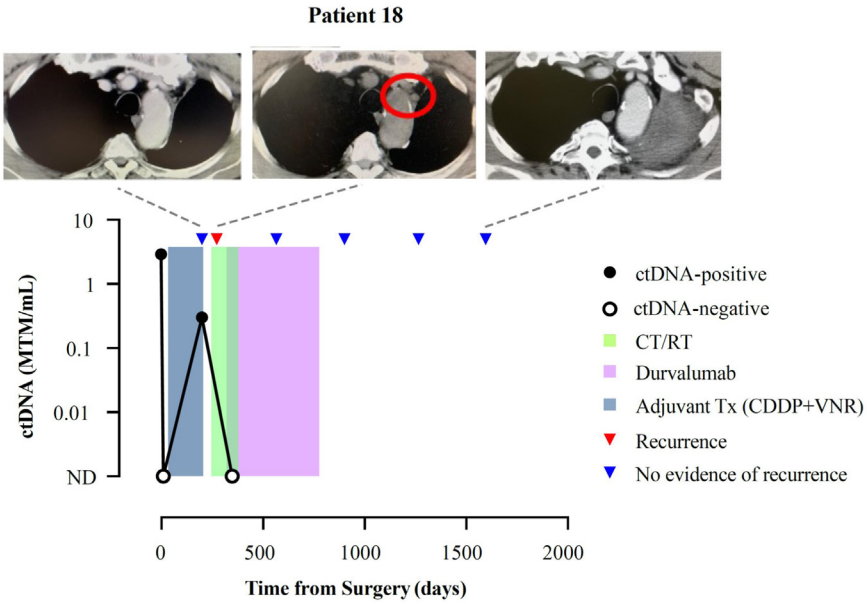

Figure 4. Patient-specific changes in ctDNA levels in response to treatment and disease status by imaging. The red triangle indicates lymph node recurrence. CDDP, cisplatin; ctDNA, circulating tumor DNA; CT/RT, chemotherapy/radiotherapy; MTM, mean tumor molecules, Tx, treatment, VNR, vinorelbine.

cell-free RNA[29] or tumor educated platelets,[30–32] and or multiomics approach[33] may be the solutions to improve the sensitivity in the near future.

In the longitudinal analysis, ctDNA-positivity preceded radiological recurrence by a median of 3.2 months in 13 patients. At this time, it is unclear whether the earlier start of treatment for molecular recurrence will lead to improved outcomes. In the exploratory analysis of our phase III study that compared gefitinib with platinum doublet chemotherapy for patients with NSCLC with *EGFR* mutation (WJTOG3405), patients with postoperative recurrence (N = 74) survived for a significantly longer time (median 44.5 months) compared with patients at stage IIB to IV (N = 101, 27.5 months) with a hazard ratio of 0.43 ($p = 0.0014$), even though both groups had metastatic disease.[34] This was potentially attributed to lower tumor burden in patients with postoperative recurrences compared with those with a metastatic diagnosis.[34] Therefore, earlier detection of postoperative recurrences and early intervention can potentially result in improved survival outcomes. Ongoing prospective studies, such as ctDNA Lung RCT (NCT04966663) for patients with T1-2N0M0 NSCLC or T3/T4 multifocal NSCLC, may shed light on the benefit of treatment in patients with detectable plasma ctDNA before or after complete surgical resection.

In conclusion, our analyses with a long follow-up of the patients reported that all the patients with positive ctDNA at the landmark and during longitudinal surveillance analyses had disease recurrence and a significantly worse prognosis. This study also reports the value of serial ctDNA monitoring over a single time point for detecting postsurgical recurrences. These patients present a compelling opportunity to utilize ctDNA for escalating adjuvant therapy, both post-operatively and in the surveillance setting on molecular recurrence. Further studies investigating a ctDNA-guided approach to inform systemic therapy in NSCLC, both in the adjuvant and surveillance settings, are warranted.

Ethics Statement

The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. This study was approved by the Institutional Review Board of Kindai University Faculty of Medicine (30-009). Written informed consent was obtained from each patient. All procedures performed in this study were in accordance with the Declaration of Helsinki (as revised in 2013).

CRediT Authorship Contribution Statement

Shuta Ohara: Conceptualization, Investigation, Methodology, Data curation, Formal analysis, Writing - original draft.

Kenichi Suda: Conceptualization, Data curation, Formal analysis, Writing - review & editing, Funding acquisition.

Sumedha Sudhaman: Data curation, Formal analysis.

Akira Hamada: Investigation, Methodology.
Masato Chiba: Investigation, Methodology.
Masaki Shimoji: Investigation, Methodolog.y
Toshiki Takemoto: Investigation, Methodology.
Ekaterina Kalashnikova: Data curation, Formal analysis.
Samantha K. Cheung: Data curation, Formal analysis.
Michael Krainock: Data curation, Formal analysis.
Jordan Feeney: Data curation, Formal analysis.
Himanshu Sethi: Data curation, Formal analysi.s
Minetta C. Liu: Data curation, Formal analysis.
Junichi Soh: Investigation, Methodology.
Yasuhiro Tsutani: Investigation, Methodology.
Tetsuya Mitsudomi: Conceptualization, Writing - original draft, Writing - review & editing, Funding acquisition, Project administration.

Disclosure

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Supplementary Data

Note: To access the supplementary material accompanying this article, visit the online version of the *JTO Clinical and Research Reports* at www.jtocrr.org and at https://doi.org/10.1016/j.jtocrr.2024.100762.

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