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CORRESPONDENCE

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Dynamically monitoring minimal residual disease using circulating tumour cells to predict the recurrence of early-stage lung adenocarcinoma

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Abstract

Lung adenocarcinoma (LUAD) is one of the leading causes of cancer-related deaths worldwide, with a 5-year survival rate of approximately 19%. With the advent of screening and diagnostic techniques such as low-dose spiral CT and liquid biopsy, the detection rate of early stage LUAD is increasing. Even in stage I LUAD, the cumulative 5-year recurrence rate after radical surgical resection is 17.9%. This may be related to the presence of microscopic residual disease (MRD), a potential source of recurrence and metastasis. Circulating tumour cells (CTCs) are key biomarkers in liquid biopsies, but the ability of dynamic CTC detection to monitor MRD and warn of recurrence in patients with early LUAD has not been validated. Here, we conducted a prospective study using the telomerase reverse transcriptase-based CTC detection method (TBCD) to evaluate perioperative and follow-up CTC levels for dynamic monitoring to evaluate its clinical efficacy in predicting postoperative recurrence in early-stage LUAD. By longitudinal dynamic monitoring of CTC, we accurately predicted recurrence within 2 years after surgery, with an AUC of 0.9786, demonstrating the clinical values of CTC in predicting recurrence. The median lead time from positive detection of CTC to radiological recurrence was 183 days, with the earliest CT recurrence predicted 354 days in advance. Taken together, our study demonstrates that longitudinal monitoring of CTC is effective in early warning of LUAD recurrence and provides valuable information on early detection and intervention strategies for the management of LUAD.

Keywords Lung adenocarcinoma, Circulating tumour cells, Recurrence, Microscopic residual disease

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To the Editor

Postoperative surveillance of early-stage lung adenocarcinoma (LUAD), especially in stage I patients, is easily overlooked, but it also carries a 5-year recurrence rate of 17.9% [1]. Previous studies have identified circulating tumour cells (CTCs) as a risk factor for a poor prognosis in LUAD, but these have been limited to single-point retrospective studies based on preoperative or postoperative stratification of patients, and its efficacy as a means of monitoring microscopic residual disease (MRD) is unknown [2–4]. In addition to CTC, ctDNA has also been widely used for monitoring MRD in LUAD, but due to its high correlation with tumour load, the value of ctDNA is primarily seen in advanced stage III-IV patients [5]. Therefore, there is an urgent need for more reliable monitoring of MRD in patients with early stage LUAD [6], and the clinical efficacy of CTC as one of the available options must be verified.

Based on the previous CTC study [7, 8], we further validated the assay using FlowSight (Suppl Fig. 1A). Single-cell whole genome sequencing was performed on flow-sorted 9 tumour cells from 7 LUAD patients. The results of the analysis showed the presence of CNVs to varying degrees in single cells, and lung cancer-associated mutations such as EGFR, ROS1, and KRAS were observed (Suppl Fig. 1B-D), which were largely consistent with the mutational profile of the primary tumours (Suppl Table 1). This suggests that CTCs can be effectively captured using TBCD technology.

A total of 180 patients with LUAD who were to undergo radical surgical resection at the Cancer Hospital of the Chinese Academy of Medical Sciences from 2021 to 2024 were included in this prospective study cohort, of which 21 were excluded due to loss of follow-up and other reasons. The clinical characteristics of the remaining 159 patients are shown in Table 1. Peripheral blood samples were collected before and after surgery in the enrolled patients to detect perioperative CTC levels. Subsequently, they underwent computed tomography (CT) scanning and CTC testing every 6 months until recurrence was determined based on CT scan results or a two-year follow-up period was reached (Suppl Fig. 2).

No correlation was found between preoperative baseline CTC and clinical features such as pathological subtype, tumour stage, tumour size or imaging features, suggesting that the biological behaviour of CTC may lag behind pathological progression in early LUAD. Interestingly, baseline TERT + leukocytes were significantly negatively correlated with tumour progression (Suppl Fig. 3).

In the 2-year prospective longitudinal surveillance follow-up, we observed that the majority of patients had CTCs that gradually cleared or fell below the detection threshold within 1 year after surgery (Suppl

Table 1 Clinical information on patients in the 2 years follow-up cohort

Characteristics	All patients(N = 159)
Tumour size (average, cm)	1.79
Neurological violation, n (%)	6 (3.8)
Vessel carcinoma embolus, n (%)	15 (9.4)
Median age (years, range)	60 (34–82)
Gender, n (%)	
Male	62 (39.0)
Female	97 (61.0)
Smoking history, n (%)	
Smoker	41 (25.8)
Nonsmoker	118 (74.2)
Pathological TNM stage, n (%)	
0-I	137 (86.2)
II	8 (5)
III	14 (8.8)
Pathology, n (%)	
AIS	10 (6.3)
MIA	28 (17.6)
IAC	121 (76.1)
Adjuvant therapy, n (%)	
Chemotherapy	12 (7.5)
Targeted therapy	6 (3.7)
Chemotherapy + Immunotherapy	6 (3.7)
Chemotherapy + Targeted therapy	6 (3.7)
Radiological features, n (%)	
Solid	60 (36.5)
Sub-Solid	60 (37.7)
GGO	39 (37.7)
Surgical procedure, n (%)	
Lobectomy	93 (58.5)
Sublobar resection	66 (41.5)
Histomorphology, n (%)	
SMC	53 (33.3)
nSMC	106 (66.7)
Tumour location, n (%)	
LUL	46 (28.9)
LLL	24 (15.1)
RUL	57 (35.9)
RML	5 (3.1)
RLL	27 (17.0)
Recurrence, n (%)	
Intrapulmonary	8 (5.0)
Brain	1 (0.6)
Lung + Lymph mode	1 (0.6)
No recurrence	149 (93.8)

AIS: adenocarcinoma in situ; MIA: minimally invasive adenocarcinoma; IAC: invasive subtype of lung adenocarcinoma; GGO: ground glass opacity; SMC: solid and micropapillary components; nSMC: non-solid and micropapillary components; LUL: left upper lobe; LLL: left lower lobe; RUL: right upper lobe; RML: right middle lobe; RLL: right lower lobe

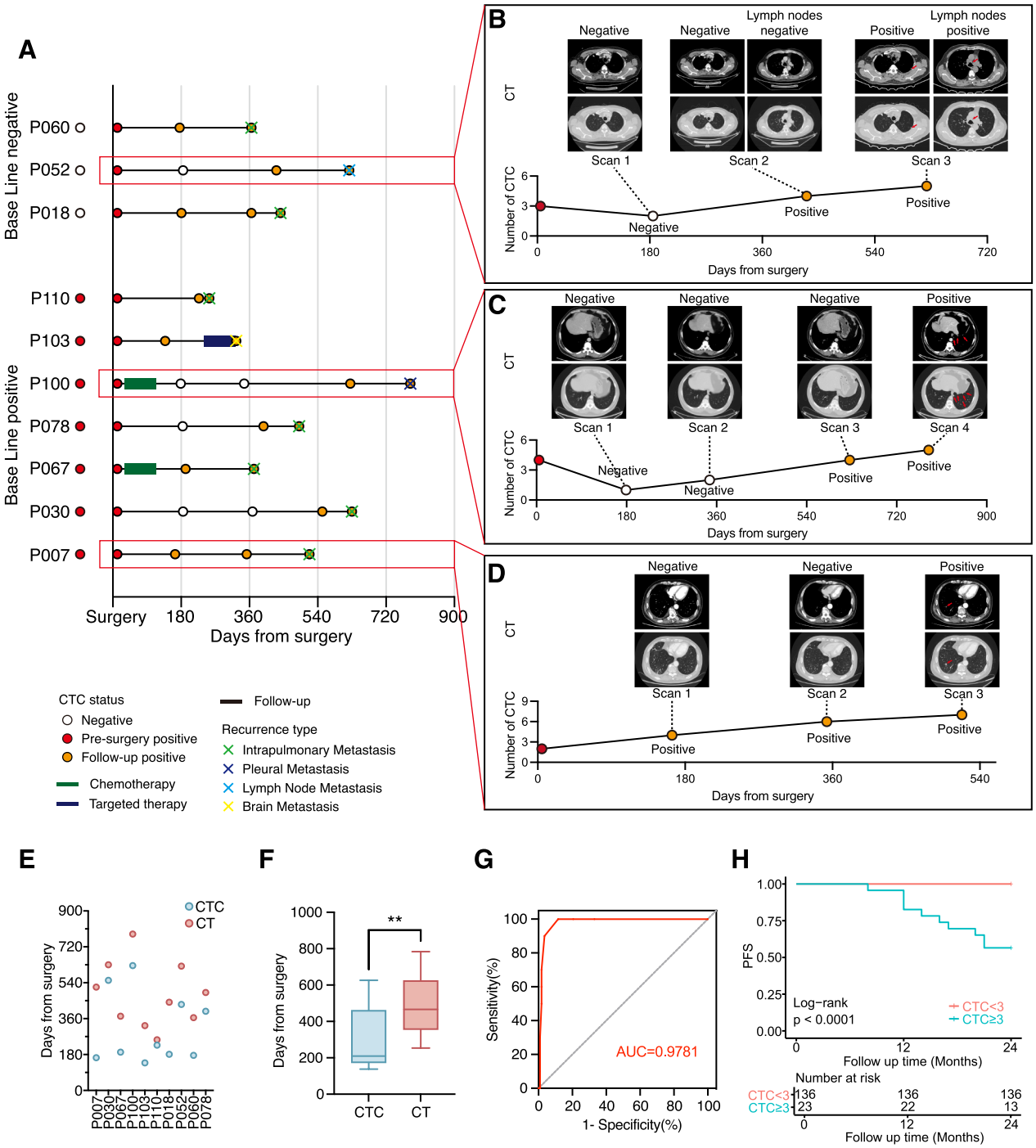

Fig. 1 CTCs can serve as reliable biomarkers to monitor the recurrence of early-stage LUAD. **A** Dynamic monitoring of CTC in patients with radiological recurrence. Circles represent CTC status. Patients are classified according to preoperative CTC status. Treatment and imaging information is indicated for each patient. **B**–**D** Dynamically followed up with CT scans and CTC detection for patients P052 (B, lymph node metastasis), P100 (**C**, pleural metastasis), and P007 (**D**, intrapulmonary metastasis). **E** Time to recurrence on CTC and CT scans for each patient. **E** The median time to diagnosis of CTC and CT in patients with recurrence. Data are shown as median ± IQR. Two-sided Wilcoxon two-sample paired signed rank test, $^{**}p < 0.01$. **G** ROC curve for recurrence within two years of CTC diagnosis, AUC-CTC = 0.9781. **H** Kaplan–Meier curves for progression-free survival in CTC follow-up positive vs negative patients. Cutoff values: ≥ 3 CTC per 4 mL PB. *P* values were calculated by the log-rank test

Fig. 4). Of the final 159 cases of early-stage LUAD, 10 patients with recurrences and metastases were identified, including lung metastases, brain metastases, and lymph node metastases. Clinical details are shown in Suppl Table 2. Among the patients with recurrences and metastases, 70% (7/10) were CTC-positive before surgery, while 30% (3/10) were CTC-negative, highlighting the need for dynamic monitoring. However, all patients had at least one point of CTC positivity during postoperative follow-up, occurring earlier than on CT scans (Fig. 1A). For example, patient P052 had a negative preoperative CTC, but turned positive at the second follow-up visit, and the patient's third CT scan revealed mediastinal lymph node enlargement and lung metastases with a delay of 192 days from CTC (Fig. 1B). Patient P100 had a positive preoperative CTC, and extensive pleural recurrence was confirmed on the fourth CT, with a positive CTC advance CT of 161 days (Fig. 1C). Patient P007 had positive and increasing CTC at all three postoperative follow-up visits, with a confirmed lung recurrence 354 days ahead of CT (Fig. 1D). This result is consistent with the findings of a pancreatic cancer study that CTC results at 9–15 months postoperatively were predictive of postoperative recurrence [9]. The median lead time from positive detection of CTC to radiological recurrence was 183 days (Fig. 1E and F), with the earliest CT recurrence predicted 354 days in advance, which was superior to the median lead times of 70 and 88 days in ctDNA-related studies [10, 11].

To further determine the efficacy of CTC in diagnosing recurrence and metastasis, ROC analysis of follow-up CTC in 159 patients showed an AUC of 0.9786 (95% CI = 0.9531 to 1.000) for diagnosing recurrence and metastasis within 2 years of follow-up (Fig. 1G). The Youden index indicated that the optimal cutoff value was 3 CTCs/4 ml PB, with diagnostic sensitivity and specificity of 100% and 88.39%, respectively. Survival analysis based on this cut-off value grouping showed a significant correlation between follow-up CTCs and progression-free survival (Fig. 1H). This result suggested that CTCs monitored during patient follow-up can effectively predict the possibility of short-term or long-term recurrence. And correlation analysis found that long-term survival of postoperative CTCs was significantly associated with reduced TERT + leukocyte levels (Suppl Fig. 4). Excluding pre-invasive and stage II + patients from the cohort, a cohort of 96 patients with stage I invasive LUAD was obtained, and CTC predicted recurrence significantly earlier than clinical imaging confirmation, with an AUC of 0.993 for predicting recurrence in patients with invasive stage I LUAD. Additionally histomorphology (solid and micropapillary components, SMC) can still be used as a complementary aid to improve the diagnostic efficacy of CTC (Suppl Fig. 5).

In conclusion, our prospective study suggests that CTC can be used as a reliable indicator for MRD dynamic monitoring of early LUAD, providing a potential new strategy for early detection and intervention of LUAD recurrence, which may lead to a paradigm shift in postoperative monitoring, provide more personalized and timely treatment opportunities for patients at high risk of recurrence, and improve patient prognosis. Given the low recurrence rate of early-stage LUAD, there are few recurrent cases in this study, necessitating further research to expand the cohort to confirm the reliability of the results.

Abbreviations

LUAD	Lung adenocarcinoma
MRD	Microscopic residual disease
CTC	Circulating tumour cells
TBCD	Telomerase reverse transcriptase-based CTC detection
CT	Computed tomography
EGFR	Epidermal growth factor receptor
PB	Peripheral blood
AIS	Adenocarcinoma in situ
GGO	Ground glass opacity
IAC	Invasive adenocarcinoma
LLL	Left lower lobe
LUL	Left upper lobe
MIA	Minimally invasive adenocarcinoma
nSMC	Non-solid and micropapillary components
pre-IAC	Pre-invasive adenocarcinoma
RUL	Right upper lobe
RLL	Right lower lobe
SMC	Solid and micropapillary
SN	Solid nodule
SSN	Subsolid nodule

Supplementary Information

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Additional file1

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Author contributions
Y. M., W.Z. and K.Z. conceived and designed the study. Q.Z. and Z.L. collected clinical samples. Q.Z. and X.Z. completed single cell and tissue sample collection, library construction and sequencing. Q.Z., X.Z. and L.F. performed genomic and transcriptomic data analysis. Q.Z., X.Z. and Z.L. completed clinical data collection and analysis. H.H., D.W., and P.X. assisted with data analysis. Q.Z., X.Z. and W.Z. wrote the original manuscript. S.C., K.Z. and Y.M. supervised the project and revised the manuscript, and all authors contributed and provided feedback.

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Availability of data and materials
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
The study complied with all relevant ethical regulations and was approved by the Ethics Committee of the National Cancer Center/Cancer Hospital of Peking Union Medical College, Chinese Academy of Medical Sciences (No. 21/093–2764). All participants provided written informed consent.

Consent for publication
Written informed consent was obtained from all patients or their guardians.

Competing interests
The authors declare no competing interests.

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