



Perioperative blood staging based on personalized ctDNA assay for recurrence prediction in NSCLC



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Circulating tumour DNA (ctDNA) is a valuable biomarker for relapse monitoring. However, current personalized approaches are unable to detect emerging clones, and perioperative ctDNA dynamics remain to be defined. We prospectively validated Hi-SECURE, a tumor-informed MRD assay, in 132 NSCLC patients (POEM cohort). Analytical performance was benchmarked against reference standards and a fixed 158-gene panel. A perioperative blood (PB) staging system was proposed and externally validated. Hi-SECURE achieves a limit of detection of 0.005% with a DNA input of 20 ng. At the landmark time point, ctDNA positivity predicted recurrence with a hazard ratio (HR) of 17.14 (95% CI: 3.06–96.11). Hi-SECURE outperformed the fixed panel, especially in stage IA (25.0% vs. 6.8% detection rate, $p = 0.020$). Pre- and postoperative ctDNA status enabled PB staging, which showed strong prognostic discrimination (log rank $p < 0.001$), was validated across external cohorts. Moreover, Hi-SECURE's core panel identified drug-resistant mutations (e.g., *EGFR*-T790M) during follow-up, enabling timely therapeutic adjustments. Hi-SECURE is a highly sensitive and specific personalized MRD assay. The proposed PB staging system provides a functional, dynamic complement to anatomical TNM staging, enabling precise prognostic stratification and offering potential guidance for adjuvant therapy, resistance monitoring, and immunotherapy candidate selection.

Circulating tumour DNA (ctDNA) is a promising biomarker with diverse applications in cancer detection and the assessment of treatment efficacy^{1,2}. The identification and analysis of somatic mutations in ctDNA extracted from plasma has demonstrated efficacy in identifying nonmetastatic cancer patients at an increased risk of relapse owing to minimal residual disease (MRD)³. An MRD assay should have a low limit of detection (LoD) to detect minute molecular signals precisely. As demonstrated in a previous study, a tumour burden of 1 cm³ yields a mean plasma variant allele frequency (VAF) of only 0.006%⁴, posing a significant challenge for MRD detection in

early-stage cancer patients. Personalized tumour-informed ctDNA assays have emerged as a potent technology for MRD surveillance, exhibiting improved sensitivity by focussing on patient-specific somatic mutations derived from tumour tissue sequencing^{5,6}. In the TRACERx study, 25 out of 27 patients with landmark-positive results experienced disease recurrence⁷.

Current personalized tumour-informed ctDNA assays are limited to monitoring mutations present in the primary tumour and lack the ability to detect newly emerging clones. Tumour cells exert evolutionary pressure through clonal selection, leading to the emergence of novel clones that can

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contribute to drug resistance and metastasis^{8,9}. Notably, over 50% of resistance mechanisms to osimertinib are attributable to genomic variants associated with tyrosine kinase inhibitors (TKIs) resistance⁸, among which the *EGFR* C797S mutation represents the most prominent *EGFR*-dependent resistance pathway¹⁰. For patients treated with first- or second-generation *EGFR* TKIs, *EGFR*-T790M remains the predominant resistance mechanism in non-small cell lung cancer (NSCLC)¹¹. Therefore, longitudinal monitoring of resistance-associated mutations could guide more precise therapeutic strategies and facilitate timely clinical decision-making.

Recent research has highlighted the prognostic value of dynamic MRD detection after surgery and during follow-up for predicting relapse. However, the role of preoperative ctDNA is increasingly recognized, raising the question of whether it can enhance prognostic stratification beyond postoperative ctDNA alone. Nonetheless, integrating MRD detection into routine clinical surveillance for relapse remains challenging. First, the extremely low ctDNA abundance in early-stage NSCLC demands assays with ultra-high sensitivity to reliably detect residual disease¹². Second, the lack of standardized methodologies across different platforms complicates cross-study comparison and hinders broad clinical adoption¹³. Addressing these challenges is essential to establish MRD detection as a robust tool for prognostic stratification and personalized management.

In this study, we developed and validated a highly sensitive personalized tumour-informed ctDNA assay named High-Sensitivity and Efficiency Capture-based ctDNA Tracer (Hi-SECURE). We conducted a comparative analysis between Hi-SECURE and a fixed panel comprising 158 genes, evaluating the clinical efficacy of Hi-SECURE in tracking relapsed and newly emerging clones in NSCLC patients and suggesting prognostic stratification and potential therapeutic approaches.

Results

Clinical assessment of Hi-SECURE

A total of 132 participants were enrolled in the POEM study (Fig. S1). Stage I patients accounted for 53.8% of the cohort, with adenocarcinoma being predominant (77.3%, Table S1). Overall, 68.9% of patients received adjuvant therapy (ADT): 65 received chemotherapy or combination therapy, and 26 received targeted therapy alone. The median follow-up period was 19.6 months as of May 2024, 19 patients had experienced recurrence (5 local, 14 distant). The recurrence group had higher TNM and N stages at baseline (Table S1).

Hi-SECURE was applied for residual disease detection in patients with NSCLC and demonstrated a LoD of 0.005% with a 20-ng DNA input (Fig. S2–3). Customized probes were successfully designed for 99.2% (131/132) of enrolled participants. Clinical analysis based on these patients demonstrated that preoperative ctDNA positivity has a significantly poorer prognosis [hazard ratio (HR): 4.93, 95% confidence interval (CI): 1.99–12.23, Fig. 1A]. Hi-SECURE detected positive preoperative ctDNA in 78.9% of relapsed patients, and these patients had a significantly higher recurrence rate compared with ctDNA-negative patients (23.8% vs. 5.9%, $p = 0.004$). In postoperative plasma samples, 110 of 131 patients (84.0%) were ctDNA-negative, of whom 101 remained recurrence-free, yielding a negative predictive value (NPV) of 91.8%. ctDNA positivity at 1 month after surgery predicted relapse (HR: 6.26, 95% CI: 1.78–21.99, Fig. 1B) and remained an independent predictor of relapse in multivariate Cox regression analysis ($p < 0.001$, Table S2). In landmark analysis, Hi-SECURE demonstrated better predictive value for recurrence-free survival (RFS) than postoperative analysis (HR: 17.14, 95% CI: 3.06–96.11, Fig. 1C).

In patients who did not receive adjuvant therapy, postoperative ctDNA status was strongly prognostic (Fig. S4A). Among those receiving adjuvant chemotherapy, landmark ctDNA outperformed postoperative assessment for RFS prediction (HR: 13.99 vs 2.76, Fig. S4B, C). Notably, patients who remained ctDNA-positive at the landmark time point exhibited the poorest prognosis, significantly worse than those who remained negative or converted to negative (log rank $p < 0.001$, Fig. 1D). Furthermore, serial monitoring further improved predictive prediction (HR: 30.66, Fig. S4D). In the context of recurrence monitoring, ctDNA positivity preceded radiographic

confirmation of relapse in 77.8% (14/18) of patients who had both landmark and subsequent time-point ctDNA (Fig. 1E), with a median lead time of 152 days (range, 3–724 days; Wilcoxon test; $p < 0.001$; Fig. 1F).

Comparison of Hi-SECURE with fixed panel

Hi-SECURE detected ctDNA in 48.1% (63/131) preoperative plasma samples, with positivity rates of 25.0%, 42.3%, 71.4%, and 63.6% for stage IA, IB, II, and III, respectively (Fig. 2A). Overall, Hi-SECURE significantly outperformed this fixed-panel assay (48.1% vs. 35.1%, $p = 0.033$), with the most notable difference observed in stage IA patients (25.0% vs. 6.8%, $p = 0.020$). In this subgroup, the mean VAF detected by Hi-SECURE was significantly lower than that of the fixed panel ($p = 0.037$, Fig. 2B). Furthermore, among the 63 patients with positive Hi-SECURE results, the 21 cases that were positive only by Hi-SECURE had a significantly lower mean VAF compared with the 42 cases that were positive by both assays ($p = 0.004$, Fig. 2C), indicating that the personalized tumour-informed assay enables detection of mutations at lower VAFs, thereby enhancing sensitivity. In *EGFR*-wild-type (*EGFR*-wt) patients, Hi-SECURE also achieved a significantly higher preoperative ctDNA positivity rate than the fixed panel (Fig. 2D, 70.4% vs. 50.0%, $p = 0.049$), with an even more pronounced difference in the non-LUAD subgroup (Fig. 2E, $p = 0.024$).

Beyond detection sensitivity, Hi-SECURE showed superior predictive value for RFS over the fixed panel across all evaluated time points (pre-operation, HR: 4.93 vs 2.94; postoperation, HR: 6.26 vs. 5.23; landmark, HR: 17.14 vs 6.26, Fig. 1A–C, Fig. S5A–C). At the landmark time point, Hi-SECURE also achieved better detection performance [positive predictive value (PPV): 76.9% vs 57.1%; NPV: 94.0% vs 87.6%; Fig. 2F]. Among patients who experienced recurrence, the two assays showed concordant ctDNA results in 80.8% (59/73) of measurements across all time points, while 78.6% (11/14) of discordant cases were positive only by Hi-SECURE (Fig. S5D). In terms of lead time, Hi-SECURE detected recurrence earlier than radiographic imaging in two patients (M98 and M157), in whom the fixed panel remained negative. Moreover, in two additional patients (M122 and M160), Hi-SECURE preceded the fixed panel in predicting recurrence, with lead times of 119 and 66 days, respectively (Fig. S5E, F).

Prognostic value of perioperative blood staging

Beyond individual prognosis prediction, the combination of preoperative and postoperative ctDNA status as determined by Hi-SECURE further enabled risk stratification among patients who were ctDNA-negative after surgery ($p = 0.044$, Fig. 3A). On the basis of these observations, we propose a novel perioperative blood (PB) staging system that integrates both pre- and postoperative ctDNA status. According to this system, patients with detectable postoperative ctDNA were classified as PB stage III. Among those with undetectable postoperative ctDNA, PB stage I was assigned to patients also negative preoperatively, and PB stage II to those positive preoperatively (Fig. 3B).

In the POEM cohort, baseline characteristics differed significantly across PB stages with respect to TNM stage ($p < 0.001$) and pathological subtype ($p < 0.001$, Table S3). Notably, 73% of PB stage I patients had TNM stage I, whereas 56% of PB stage III patients had TNM stage III. Due to differences in preoperative ctDNA detection rates between lung adenocarcinoma (LUAD) and non-LUAD, all PB stage I patients were patients with LUAD. In terms of prognostic prediction, PB staging outperformed TNM staging, as reflected by the log-rank test (PB: $p < 0.001$; TNM: $p = 0.017$; Fig. S6A). Furthermore, the recurrence rate was substantially lower in PB stage I patients than in stage III patients, suggesting that PB stage I may identify a subset of patients approaching clinical cure (Fig. S7A).

Validation of perioperative blood staging

The prognostic value of PB staging was further validated in multiple external NSCLC cohorts^{14–16}, consistently showing its ability to predict recurrence (MEDAL cohort, $n = 141$, log rank $p < 0.001$; LUNGCA cohort, $n = 325$, log rank $p < 0.001$; NeXT cohort, $n = 223$, log rank $p < 0.001$; Fig. 3C–E). In the NeXT cohort, PB staging also significantly stratified patients for overall

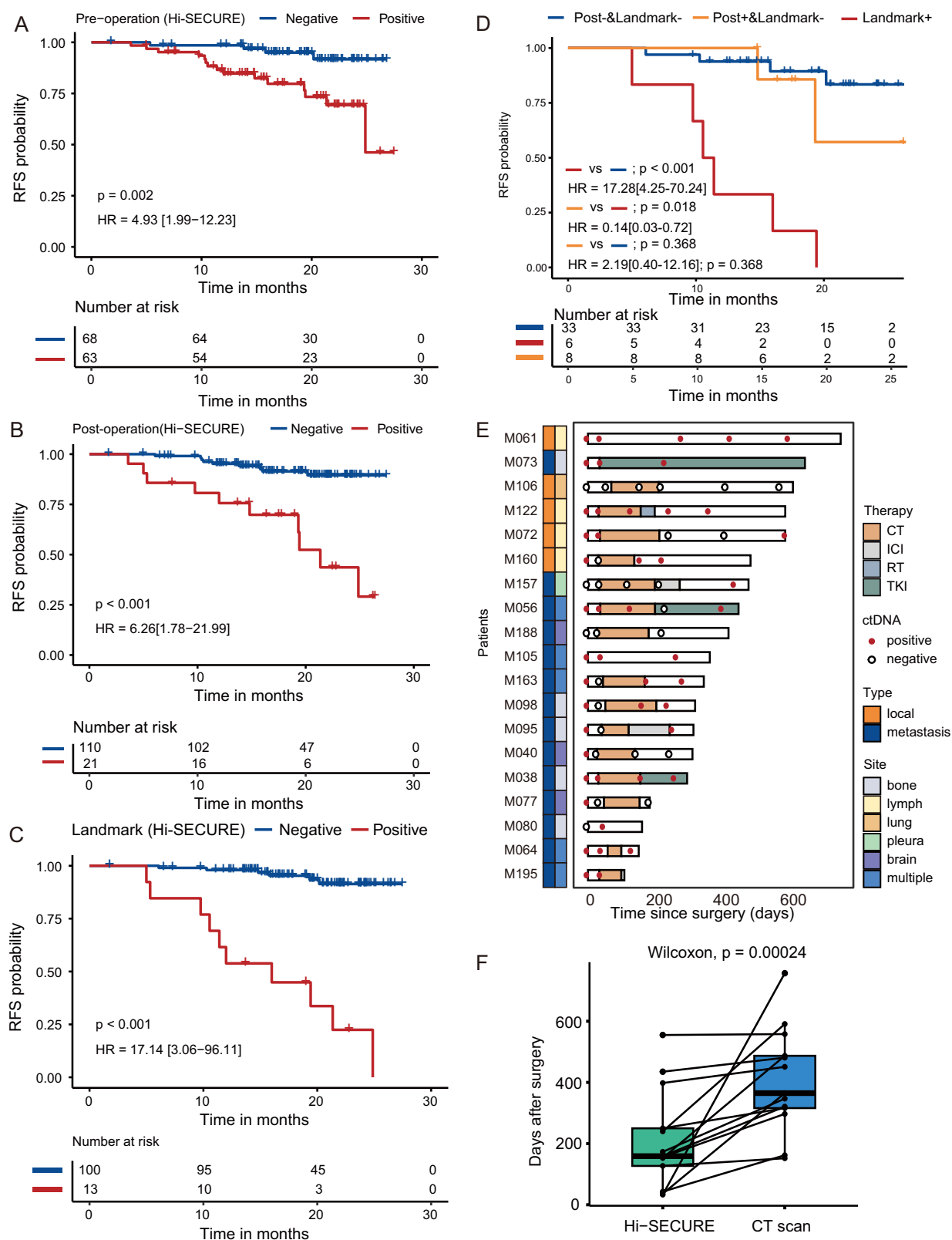


Fig. 1 | Clinical assessment of Hi-SECURE. A–C Kaplan-Meier (KM) curve for recurrence free survival (RFS) analysis on preoperative ctDNA (A), postoperative ctDNA (B), and landmark ctDNA (C). D KM curve for RFS analysis based on postoperative and landmark ctDNA status detected by Hi-SECURE. E Summary of treatment and Hi-SECURE MRD detection for all 19 patients with recurrence. CT

chemotherapy, ICI immune checkpoint inhibitor, RT radiotherapy, TKI tyrosine kinase inhibitor. F Comparison of postoperative time intervals between positive Hi-SECURE MRD results and radiographically confirmed recurrence. Postoperative ctDNA, ctDNA at 1 month after surgery. CT scan, computed tomography scan.

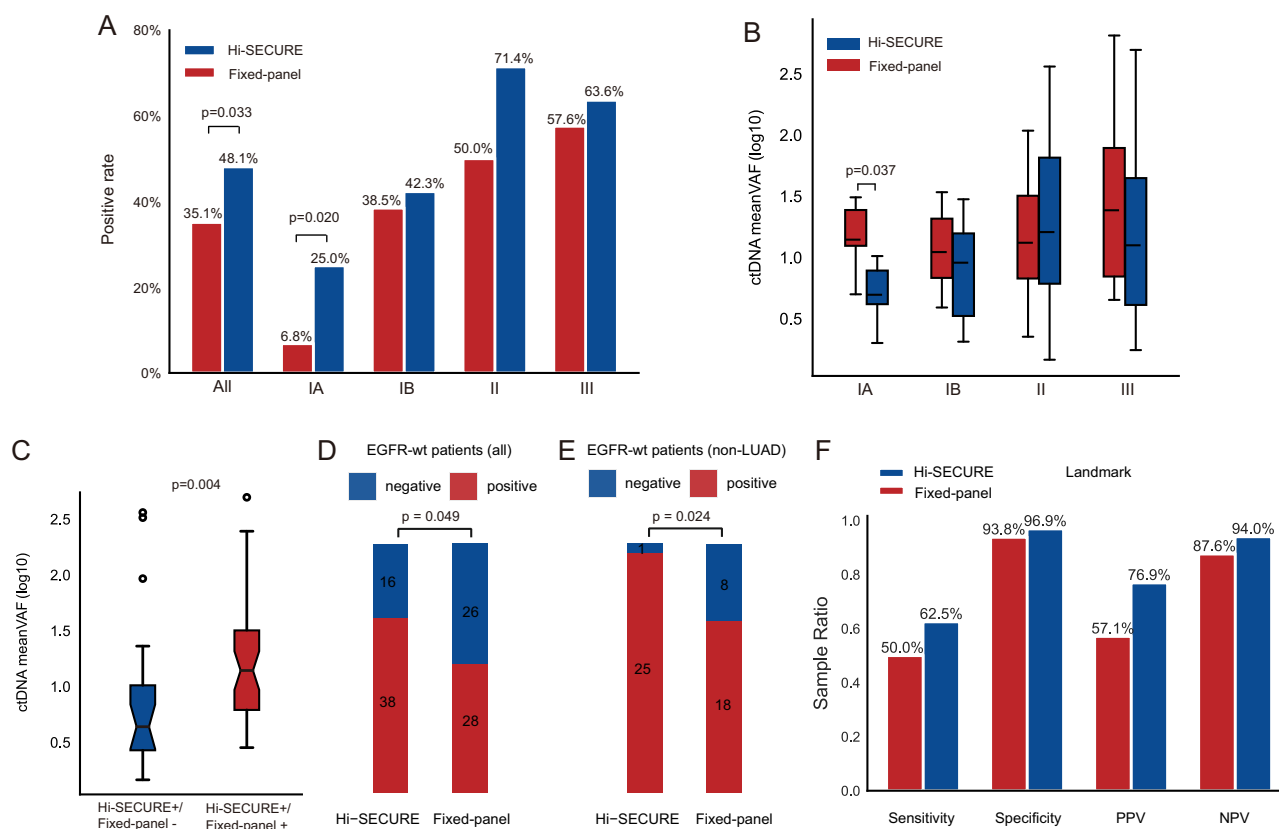


Fig. 2 | Performance comparison of Hi-SECURE with fixed panel. **A** Preoperative ctDNA positive detection rate of Hi-SECURE and fixed panel at different stages. **B** Comparison of preoperative ctDNA mean Variant Allele Frequency (VAF) at different stages. **C** Comparison of preoperative ctDNA meanVAF between positive samples detected only by Hi-SECURE or by Hi-SECURE and fixed panel

simultaneously. **D, E** Preoperative ctDNA detection rates of Hi-SECURE versus the fixed panel in EGFR-wild-type NSCLC patients: **(D)** overall cohort and **(E)** non-LUAD subgroup. **F** Statistical analysis of PPV and NPV for recurrence prediction at the landmark time point.

survival (OS; log rank $p < 0.001$, Fig. 3F). When compared with the TNM staging, PB staging demonstrated more pronounced prognostic separation between stage I and stage III patients (Fig. S6A–D). Furthermore, based on continuous concordance index (C-index), PB staging outperformed TNM staging for both disease-free survival (DFS) and OS in the NeXT cohort (Fig. S6E, F).

Notably, the classification performance of PB staging was closely associated with the sensitivity of the ctDNA assay used. Personalized assays achieved superior PB stage classification accuracy compared with fixed-panel assays (Fig. S7B–C), and improved detection performance correlated strongly with lower recurrence rates in PB stage I patients ($R^2 = 0.897$, Fig. S7D).

Potential clinical treatment applications

Previous personalized tumor-informed panels have faced challenges in effectively monitoring drug resistance and emerging clonal variations. Here, we explored whether the core panel could address these limitations.

Patient M56, diagnosed with stage IIIA LUAD, carried a sensitive EGFR 19del mutation identified by tissue whole exome sequencing (WES). After six cycles of chemotherapy followed by first-generation targeted therapy (icotinib), the patient's MRD status turned positive 397 days after surgery. The core panel detected the EGFR-T790M resistance mutation (Fig. 4A), and shortly thereafter, the patient developed multiple metastases. These findings suggest that the core panel may help monitor drug resistance and raise the question of whether early intervention or medication adjustment upon detection of resistance mutations is feasible—an issue warranting further investigation.

For patients with stage I LUAD, particularly those with EGFR-wt, postoperative observation is considered a standard-of-care approach.

Among such patients, ctDNA-positive status was associated with a worse prognosis (log-rank test, $p < 0.001$; Fig. 4B). Notably, EGFR-wt ctDNA-positive patients had significantly higher TMB ($p < 0.001$, Fig. 4C), suggesting that immunotherapy may be a viable treatment option for this subgroup.

Discussion

This prospective POEM cohort study confirms that the tumor-informed Hi-SECURE assay achieves ultra-high sensitivity for MRD detection in NSCLC. More importantly, the proposed perioperative blood (PB) staging system—integrating dynamic pre- and postoperative ctDNA information—demonstrated superior prognostic value across multiple external cohorts, offering a new functional dimension for individualized diagnosis and treatment of early-stage NSCLC.

Patients with negative ctDNA status have a significantly better prognosis than those with positive ctDNA status^{5,15–19}. Among postoperative ctDNA-positive patients, we observed two distinct evolutionary trajectories during subsequent ADT. A subset achieved ctDNA clearance by the landmark time point, suggesting that residual tumor clones retained sensitivity to standard adjuvant chemotherapy or targeted therapy, and the treatment successfully eradicated MRD. Clinically, this clearance supports continuing planned adjuvant regimens without unnecessary escalation. Another subset maintained or even increased ctDNA positivity at the landmark time point, a pattern we attribute to “therapy-resistant clonal persistence.” This reflects that persistent ctDNA during therapy is not merely residual disease but the outgrowth of intrinsically resistant clones that have survived therapeutic pressure.

We compared Hi-SECURE with fixed-panel approaches. Our finding demonstrates that personalized MRD detection achieves superior

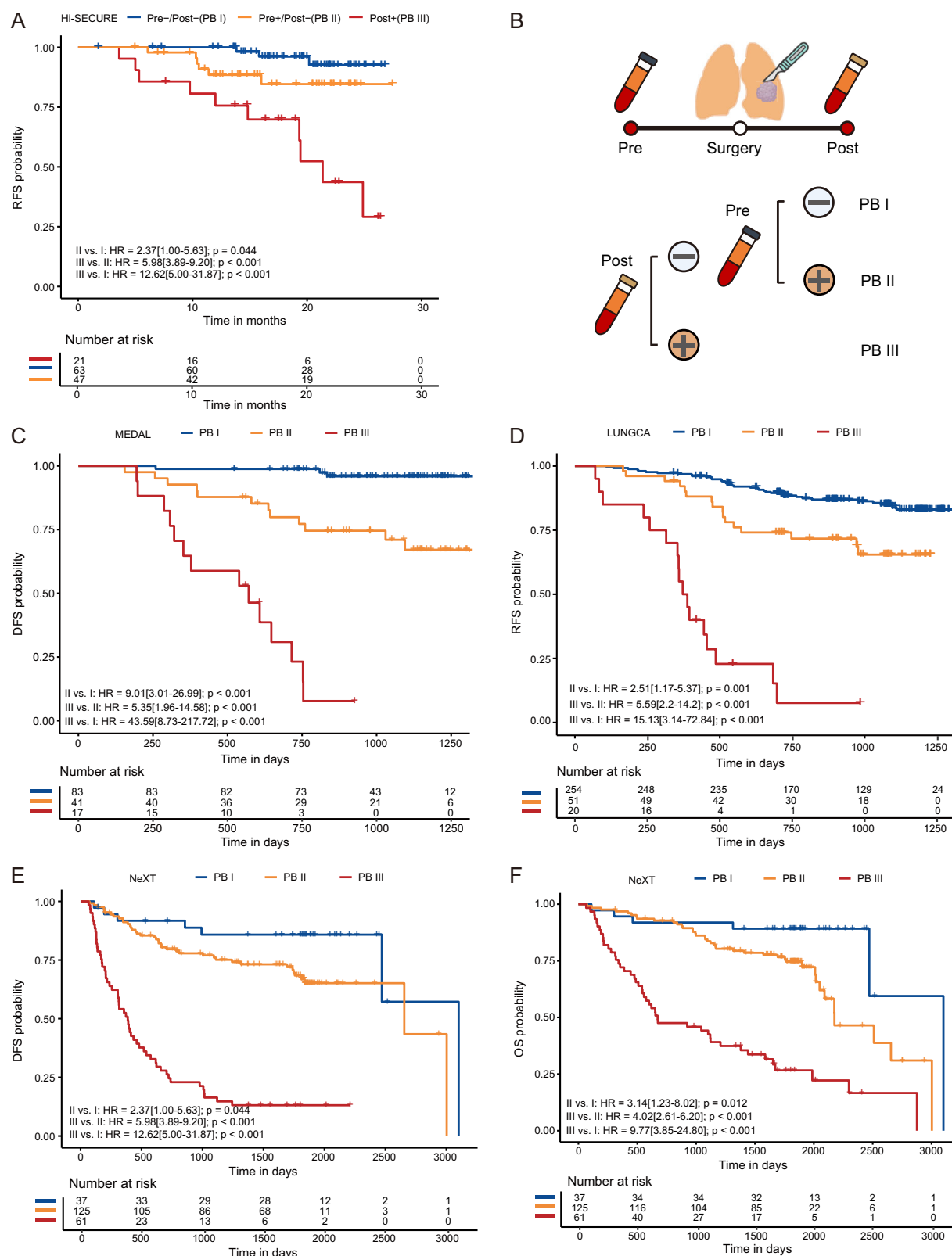


Fig. 3 | Preoperative ctDNA stratification and perioperative blood (PB) stage. **A** Kaplan-Meier (KM) curve presents recurrence free survival (RFS) analysis based on perioperative blood (PB) stage. The prognosis of postoperative ctDNA-negative patients can be categorized according to preoperative ctDNA status. **B** The

schematic diagram of PB staging. **C** KM curve for disease-free survival (DFS) analysis based on PB stage from MEDAL cohort. **D** KM curve for RFS analysis based on PB stage from LUNGCA cohort. **E**, **F** KM curve for DFS (**E**) and OS(**F**) analysis based on PB stage from NeXT cohort.

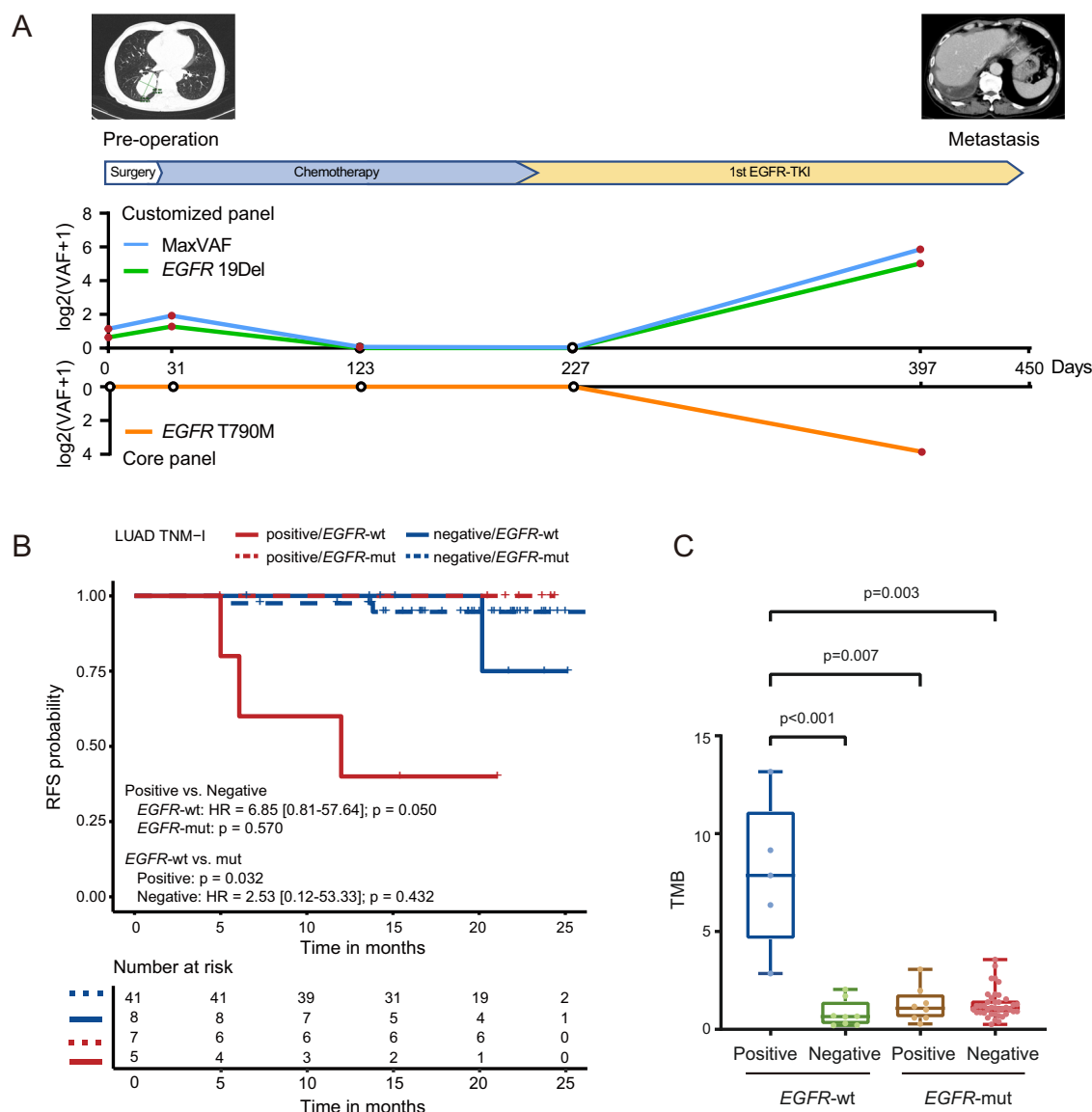


Fig. 4 | The potential clinical application of Hi-SECURE MRD monitoring.

A Longitudinal MRD detections and imaging scans are presented for patient M056 across various follow-up time points. **B** Kaplan-Meier (KM) curve indicates the influence of preoperative ctDNA status and EGFR mutation status on recurrence

free survival (RFS) in patients with stage I adenocarcinoma (LUAD). **C** TMB differences among stage I LUAD patients across different groups based on preoperative ctDNA status and EGFR mutation status.

sensitivity, particularly in stage I patients. Although WGS-based platforms such as the NeXT Personal claim enhanced sensitivity through the analysis of ~1800 mutation sites²⁰, they incur substantially higher costs. An optimal MRD assay must balance sensitivity, cost-effectiveness, turnaround time²¹, quality control, and regulatory compliance. Fixed-panel assays remain a viable alternative due to their standardized protocols, lower costs, and rapid processing, especially in settings with limited tissue availability, urgent clinical needs, or when testing for known oncogenes such as *EGFR*.

In our study, 8.2% of postoperative ctDNA-negative patients relapsed. Preoperative ctDNA status further stratified these patients. We therefore established a novel tumour staging system, the PB staging, based entirely on blood ctDNA results. Our data, along with external data^{15,16}, revealed that the PB stage clearly separated patient outcomes. PB stage III indicates that patients are unlikely to fully benefit from standard treatment and may require intensified therapy. PB stage II suggests that the tumor has been completely removed by surgery, but molecular metastasis via other routes may still exist. PB stage I indicates relatively indolent tumor biology and achieves outcomes approaching clinical cure. However, the predictive ability

of PB staging is intrinsically linked to ctDNA assay performance, emphasizing the need for highly sensitive assays in clinical practice. In addition, pathological subtype influences PB staging. Non-LUAD has a higher preoperative positive detection rate^{4,22,23}, which reduces the proportion of PB stage I cases.

Existing personalized ctDNA MRD methods based on WES cannot track emerging clonal variants^{7,15,16,19}, as treatment pressure selects for resistant subclones^{8,9}. Hi-SECURE addresses this limitation through an off-the-shelf core panel that provides enhanced monitoring capabilities. In the POEM study, we detected EGFR-T790M resistance before radiographic recurrence, enabling preemptive drug monitoring and timely treatment adjustments. When combined with a drug-holiday strategy²⁴, Hi-SECURE may optimize guidance and resource allocation. Beyond resistance monitoring, Hi-SECURE may expand to other applications. For stage I *EGFR*-wt LUAD patients with preoperative ctDNA positivity, ADT may improve prognosis. These patients also have high TMB; persistent plasma clones suggest immune evasion, making them ideal candidates for trials of immune checkpoint inhibitors as ADT.

There were some limitations in our study. Firstly, the follow-up period was relatively short. Secondly, while the prognostic value of PB staging has been validated in NSCLC patients undergoing direct surgical resection, its applicability to those receiving neoadjuvant therapy remains to be established. Further validation in this specific population is warranted to systematically assess the clinical utility of PB staging in the context of contemporary perioperative systemic treatment paradigms. Additionally, the sample size for each subgroup analysis was limited. Especially, the discovery of T790M resistance was only based on individual cases and requires large-scale sample validation.

The considerable heterogeneity among existing MRD detection platforms poses substantial challenges to clinical translation, underscoring the urgent need for standardized analytical procedures and reporting systems²⁵. Moreover, evidence on optimal management following MRD-positive or -negative results remains inconclusive, and findings from interventional studies in other tumor types have yielded inconsistent outcomes^{26–29}. This uncertainty complicates the integration of MRD detection into routine decision-making. Therefore, prospective evidence is urgently needed to establish MRD-guided management strategies and to define the clinical utility of MRD testing in routine lung cancer practice.

In conclusion, Hi-SECURE is a highly sensitive and specific assay for MRD detection. The PB staging system proposed herein serves as an effective complement to the TNM staging framework, enabling more precise prognostic stratification. These findings, however, require further validation within the context of contemporary multimodal management strategies for resectable NSCLC. This approach holds promise for guiding adjuvant therapy, monitoring treatment resistance, and identifying patients who may benefit from immunotherapy. Nonetheless, its clinical implementation warrants prospective interventional validation.

Methods

Participants and sample collection

We conducted a prospective multicentre study on MRD in patients with lung cancer. All participants provided informed consent. A total of 132 participants with resectable stage I–III NSCLC were enrolled in the POEM study from December 2021 to December 2022. This study adhered to the Declaration of Helsinki and its current amendments. Approval was obtained from the Ethics Committee of Jiangsu Province Hospital (No. 2019-SR-156), the Second Affiliated Hospital of Nanjing Medical University, and Nanjing Chest Hospital.

Tumour tissue was collected during surgery for variant screening. Peripheral blood was collected for ctDNA MRD monitoring before surgery, one month after surgery, and every 3–6 months thereafter. All peripheral blood samples were collected in Apostle MiniMaxTM cell-free DNA (cfDNA) collection tubes and processed within 12 hours for ctDNA extraction.

External validation cohort data from the MEDAL¹⁵, LUNGCA¹⁶ and NeXT¹⁴ were obtained from open-access databases.

Performance assessment

We established a system for the number of monitored variants and positive determination rules of plasma for use with the Hi-SECURE method and determined the associated LoD. Using an input volume of 20–80 ng, a panel integrating 60 tumor specific variants from the Onco gDNA reference standard HD827 was diluted to 7 concentrations, with ctDNA fractions ranging from 0.001% to 0.075%, prepared using enzyme-fragmented DNA from HD827 and diluted into NA12878 as the background. We randomly selected subsets of 10, 20, ... 55 variants up to 500 times for mutations contained in diluted HD827 samples and used them as monitored variants sets. At an input of 20 ng and with 40 monitored variants, Hi-SECURE achieved an LoD of 0.005%; inputs below 20 ng precluded attainment of this LoD. When the input was increased to 40 ng and the number of monitored variants to 45, the LoD improved to 0.003%. With a 60-ng input and 40 monitored variants, the assay achieved 95% detection sensitivity at 20 parts per million (PPM). Although the assay exhibited higher sensitivity when

positivity was defined by a single monitored variant, the specificity required careful evaluation; it reached 100% when concurrent detection of ≥ 2 predefined variants was required as the positivity criterion.

To methodologically validate the Hi-SECURE-derived conclusions, orthogonal verification was performed using: (1) the LUAD cell line NCI-H2009 and (2) the SeraseqTM ctDNA MRD Reference Material. A panel integrating 65 tumor specific variants from NCI-H2009 was diluted to ctDNA fractions with 0.005%, prepared using enzyme-fragmented DNA from NCI-H2009 and diluted into A290T as the background. The performance was also validated using Seraseq MRD standards with a panel integrating 72 tumor specific variants, encompassing 4 concentrations with ctDNA fraction of 0%, 0.5%, 0.05%, and 0.005%.

WES library preparation

WES library preparation was performed using KAPA HyperPlus Kits (KAPA Biosystems, Cat. #KK8514) according to the manufacturer's instructions. Briefly, formalin-fixed paraffin embedded (FFPE) DNA samples and paired white blood cell genomic DNA samples were sheared to a peak size of 200 bp with an M220 Focused ultrasonicator (Covaris, Woburn, MA, US). Sheared DNA samples were end-repaired and dA-tailed, and ligation with Universal Adapters was subsequently performed. After post-ligation cleanup, the ligated products were amplified by polymerase chain reaction (PCR) with index primers. The number of amplification cycles used during library preparation varied according to the manufacturer's recommendations.

Exome capture was performed with KAPA HyperExome Probes (KAPA Biosystems, Cat. 09062572001) using HyperCap Workflow v3.0. Up to 8 libraries were multiplexed in one capture reaction, and 400 ng of each library was used as input. The final WES libraries were quantified using a Qubit dsDNA HS assay (Thermo Fisher, Cat. Q32854). After library size distribution determination using the Agilent 4200 TapeStation system, the libraries were sequenced on a NovaSeq 6000 sequencer (Illumina, San Diego, CA, US) with 2×150 bp paired-end reads with a unique dual index, with a mean target coverage of 500x for tumour samples and 150x for paired normal samples.

WES data analysis

The analysis of primary tumor and matched white blood cell WES data was performed using the next-generation sequencing (NGS) analysis process developed by Simceredx. Fastp software (v0.19.5)³⁰ was used to trim adapter sequences and low-quality bases, and reads with lengths < 50 bp were discarded. BWA software (v0.7.17)³¹ was used to align the clean reads to the hg19 human reference genome (NCBI GRCh37; hg19)³² to generate bam files. PCR duplicates were marked and removed with Sambamba software (v0.6.8)³³. Abra2 software (v2.21)³⁴ was used to realign specific regions to improve the alignment quality of insertion/deletion (INDEL) and complex mutations. Variations such as single-nucleotide variants, INDELs and complex mutations were called by VarDict software (v1.7.0)³⁵ and annotated by SnpEff (v4.3)³⁶ and ANNOVAR³⁷ using multiple databases such as COSMIC (v98), gnomAD (v2), ExAC, 1000 Genomes, and dbSNP. Finally, any tumour-derived variants with < 5 supporting reads or a mutation VAF $< 1\%$ were excluded. The TMB calculation followed predefined criteria: (1) only nonsynonymous mutations located within protein coding regions were included; (2) somatic variants with an allele frequency $> 5\%$ were retained to ensure detection reliability; and (3) known driver mutations and COSMIC annotated hotspot variants were excluded. The final TMB value was derived by dividing the total number of eligible mutations by the effective coding region coverage length.

Personalized panel design

The personalized panel for Hi-SECURE ctDNA detection includes both a customized panel and a core panel. The customized panel is determined based on the somatic mutations identified by WES of each patient's primary tumour and paired samples. All somatic mutations are ranked according to the in-house sorting criteria, and the top 40 mutations are selected. The core

panel includes 12 genes, which designed based on the common hotspot mutations and actionable sensitive or resistant mutations recommended by the National Comprehensive Cancer Network, Food and Drug Administration, and China Food and Drug Administration.

Fixed panel design

Building upon the CAPP-seq paradigm, we developed a 158-gene fixed panel spanning 118 kb of genomic territory specifically optimized for lung, gastrointestinal, and breast cancers. Briefly, driver mutations and actionable sensitive or resistant mutations across all three cancer types were considered.

cfDNA library preparation

Peripheral blood samples were collected in Cell-Free DNA Storage Tubes (CWBIO) and transported to the central laboratory at ambient temperature within 48 h of collection. Upon arrival, blood samples were centrifuged at $1800 \times g$ for 15 min at 4°C to separate plasma. The plasma fraction was then subjected to a second centrifugation at $16,000 \times g$ for 10 min at 4°C to eliminate residual cellular debris. The resulting plasma samples were aliquoted and stored at -80°C until further processing. cfDNA was extracted from plasma using the Magbead Free-Circulating DNA Maxi Kit (CWBIO) following the manufacturer's instructions. The concentration of extracted cfDNA was measured using the Qubit dsDNA HS Assay Kit (Thermo Fisher, Cat. Q32854), and fragment size distribution was assessed using Agilent 4200 TapeStation system.

Library preparation was performed using an xGen[™] cfDNA & FFPE DNA Library Preparation Kit (Integrated DNA Technologies, Cat. 10010207) according to the manufacturer's instructions. Briefly, up to 80 ng of extracted cfDNA were end-repaired, followed by ligation with adapters containing UMIs. Post-ligation cleanup was performed using AMPure XP Beads (Beckman Coulter, Cat. A63882), after which the ligated products were amplified by PCR with dual-index primers. A minimum of 500 ng of each library was hybridized and captured by a personalized biotinylated probe panel with a proprietary hybridization enhancer or the fixed panel for 12–16 hours. Target enrichment was performed using the xGen[™] Hybridization and Wash Kit according to the manufacturer's instructions. The final cfDNA libraries were cleaned and quantified using the Qubit dsDNA HS assay (Thermo Fisher, Cat. Q32854). For the personalized panel, sequencing was performed on a NovaSeq 6000 sequencer (Illumina, San Diego, CA, US) with 2×150 bp paired-end reads with a unique dual index, with a mean target coverage of 100,000x for the personalized library and 60,000X for paired normal samples. For the fixed panel, sequencing was performed on a NovaSeq 6000 sequencer (Illumina, San Diego, CA, US) with 2×150 bp paired-end reads with a unique dual index, with a mean target coverage of 60,000x for the personalized library and 30,000X for paired normal samples.

Hi-SECURE site-level variant calling

Data analysis of plasma and peripheral blood cell sequencing was performed using the NGS analysis process developed by Simceredx. The pre-processing procedure was consistent with the WES analysis process. Furthermore, a self-developed filtering module was used to perform a series of filtering measures for cfDNA mutations. In short, for a mutation pattern such as A[A > C]A, in the cfDNA sequencing data of the healthy population group, its cumulative error rate for 1, 2, ... 10 supporting reads was calculated, and a regression model relating the cumulative error rate of the mutation pattern to the number of supporting reads was constructed. Next, clonal hematopoietic (CH) mutations were filtered out using buffy-coat informed approach, with control gDNA subjected to UMI-based ultra-deep sequencing at a depth of 60,000X. In parallel, a distribution model was constructed based on the in-house CH mutation database and the characteristic molecular features of CH mutations, enabling hierarchical filtering of CH and other background mutations. This combined strategy effectively removed CH-derived variants and minimized analytical noise. When two or more monitored somatic variants were detected, the plasma sample was defined as

MRD positive. The ctDNA level of each ctDNA-positive plasma sample was calculated directly from ctDNA sequencing data as the mean VAF of all detected somatic variants³⁸.

ctDNA monitoring and definition

During the analysis, we focused primarily on preoperative, postoperative, landmark, and longitudinal ctDNA status. The postoperative time point was defined as one month after surgery. The landmark time point was defined as one month after surgery or chemotherapy, depending on whether patients received adjuvant chemotherapy. Patients with a landmark assessment and at least one subsequent ctDNA test, or at least two ctDNA tests after the landmark time point were included in the longitudinal analysis. Longitudinal ctDNA positivity was defined as the positive detection of ctDNA at any time during the monitoring period.

Statistical analysis

For the clinical cohort, RFS was investigated. For patients who experienced recurrence, RFS was calculated from the date of radical surgical resection to the date of confirmed recurrence or death. For nonrecurrent patients, RFS was calculated from the date of radical surgical resection to the date of the last imaging examination. The sensitivity, specificity, PPV, and NPV were used to assess the predictive prognostic power.

Survival analysis was performed using the Kaplan-Meier (KM) method and the log-rank test. Multivariate analysis was conducted using the Cox proportional hazards regression model to calculate HR with corresponding 95% CI. The chi-square test or Fisher's exact test was used to compare categorical variables between groups, whereas the Mann-Whitney U test was used to compare numerical variables between groups. P values < 0.05 were considered statistically significant.

Data availability

The datasets generated and/or analysed during the current study are not publicly available due data privacy laws but are available from the corresponding author on reasonable request.

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References

1. Cohen, S. A., Liu, M. C. & Aleshin, A. Practical recommendations for using ctDNA in clinical decision making. *Nature* **619**, 259–268 <https://doi.org/10.1038/s41586-023-06225-y> (2023).
2. Gouda, M. A. et al. Liquid Biopsy Response Evaluation Criteria in Solid Tumors (LB-RECIST). *Ann. Oncol.* **35**, 267–275 <https://doi.org/10.1016/j.annonc.2023.12.007> (2024).
3. Wan, J. C. M. et al. Liquid biopsies come of age: towards implementation of circulating tumour DNA. *Nat. Rev. Cancer* **17**, 223–238 <https://doi.org/10.1038/nrc.2017.7> (2017).
4. Abbosh, C. et al. Phylogenetic ctDNA analysis depicts early-stage lung cancer evolution. *Nature* **545**, 446–451 <https://doi.org/10.1038/nature22364> (2017).
5. Moding, E. J., Nabat, B. Y., Alizadeh, A. A. & Diehn, M. Detecting Liquid Remnants of Solid Tumors: Circulating Tumor DNA Minimal Residual Disease. *Cancer Discov.* **11**, 2968–2986 <https://doi.org/10.1158/2159-8290.CD-21-0634> (2021).
6. Wan, J. C. M. et al. ctDNA monitoring using patient-specific sequencing and integration of variant reads. *Sci. Transl. Med.* **12** <https://doi.org/10.1126/scitranslmed.aaz8084> (2020).
7. Abbosh, C. et al. Tracking early lung cancer metastatic dissemination in TRACERx using ctDNA. *Nature* **616**, 553–562 <https://doi.org/10.1038/s41586-023-05776-4> (2023).
8. Passaro, A., Janne, P. A., Mok, T. & Peters, S. Overcoming therapy resistance in EGFR-mutant lung cancer. *Nat. Cancer* **2**, 377–391 <https://doi.org/10.1038/s43018-021-00195-8> (2021).

9. Jamal-Hanjani, M. et al. Tracking the Evolution of Non-Small-Cell Lung Cancer. *N. Engl. J. Med* **376**, 2109–2121 <https://doi.org/10.1056/NEJMoa1616288> (2017).
10. Chmielecki, J. et al. Candidate mechanisms of acquired resistance to first-line osimertinib in EGFR-mutated advanced non-small cell lung cancer. *Nat. Commun.* **14**, 1070 <https://doi.org/10.1038/s41467-023-35961-y> (2023).
11. Cross, D. A. et al. AZD9291, an irreversible EGFR TKI, overcomes T790M-mediated resistance to EGFR inhibitors in lung cancer. *Cancer Discov.* **4**, 1046–1061 <https://doi.org/10.1158/2159-8290.CD-14-0337> (2014).
12. Tan, A. C. et al. Consensus Statement on ctDNA minimal residual disease testing in early stage nscl: a delphi study by the asian thoracic oncology research group. *J. Thorac. Oncol.* 103696 <https://doi.org/10.1016/j.jtho.2026.103696> (2026).
13. Pantel, K. & Alix-Panabieres, C. Minimal residual disease as a target for liquid biopsy in patients with solid tumours. *Nat. Rev. Clin. Oncol.* **22**, 65–77 <https://doi.org/10.1038/s41571-024-00967-y> (2025).
14. Black, J. R. M. et al. Longitudinal ultrasensitive ctDNA monitoring for high-resolution lung cancer risk prediction. *Cell* **188**, 7083–7098 e7018 <https://doi.org/10.1016/j.cell.2025.10.020> (2025).
15. Chen, K. et al. Individualized tumor-informed circulating tumor DNA analysis for postoperative monitoring of non-small cell lung cancer. *Cancer Cell* **41**, 1749–1762 e1746 <https://doi.org/10.1016/j.ccell.2023.08.010> (2023).
16. Xia, L. et al. Perioperative ctDNA-based molecular residual disease detection for non-small cell lung cancer: a prospective multicenter cohort study (LUNGCA-1). *Clin. Cancer Res* **28**, 3308–3317 <https://doi.org/10.1158/1078-0432.CCR-21-3044> (2022).
17. Abbosh, C. et al. Implementing circulating tumor DNA as a prognostic biomarker in resectable non-small cell lung cancer. *Trends Cancer* **10**, 643–654 <https://doi.org/10.1016/j.trecan.2024.04.004> (2024).
18. Zhang, J. T. et al. Longitudinal undetectable molecular residual disease defines potentially cured population in localized non-small cell lung cancer. *Cancer Discov.* **12**, 1690–1701 <https://doi.org/10.1158/2159-8290.CD-21-1486> (2022).
19. Gale, D. et al. Residual ctDNA after treatment predicts early relapse in patients with early-stage non-small cell lung cancer. *Ann. Oncol.* **33**, 500–510 <https://doi.org/10.1016/j.annonc.2022.02.007> (2022).
20. Black, J. R. M. et al. Ultrasensitive ctDNA detection for preoperative disease stratification in early-stage lung adenocarcinoma. *Nat. Med* **31**, 70–76 <https://doi.org/10.1038/s41591-024-03216-y> (2025).
21. Abbosh, C., Birkbak, N. J. & Swanton, C. Early stage NSCLC - challenges to implementing ctDNA-based screening and MRD detection. *Nat. Rev. Clin. Oncol.* **15**, 577–586 <https://doi.org/10.1038/s41571-018-0058-3> (2018).
22. Hong, T. H. et al. Clinical utility of tumor-naïve presurgical circulating tumor DNA detection in early-stage NSCLC. *J. Thorac. Oncol.* **19**, 1512–1524 <https://doi.org/10.1016/j.jtho.2024.07.002> (2024).
23. Boukouris, A. E. et al. A comprehensive overview of minimal residual disease in the management of early-stage and locally advanced non-small cell lung cancer. *NPJ Precis Oncol.* **9**, 178 <https://doi.org/10.1038/s41698-025-00984-9> (2025).
24. Dong, S. et al. Circulating Tumor DNA-Guided De-Escalation Targeted Therapy for Advanced Non-Small Cell Lung Cancer: A Nonrandomized Controlled Trial. *JAMA Oncol.* **10**, 932–940 <https://doi.org/10.1001/jamaoncol.2024.1779> (2024).
25. Normanno, N. et al. Circulating tumour DNA in early stage and locally advanced NSCLC: ready for clinical implementation?. *Nat. Rev. Clin. Oncol.* **22**, 215–231 <https://doi.org/10.1038/s41571-024-00985-w> (2025).
26. Tie, J. et al. Circulating Tumor DNA Analysis Guiding Adjuvant Therapy in Stage II Colon Cancer. *N. Engl. J. Med* **386**, 2261–2272 <https://doi.org/10.1056/NEJMoa2200075> (2022).
27. Powles, T. et al. ctDNA-Guided Adjuvant Atezolizumab in Muscle-Invasive Bladder Cancer. *N. Engl. J. Med* **393**, 2395–2408 <https://doi.org/10.1056/NEJMoa2511885> (2025).
28. Tie, J. et al. Circulating tumor DNA-guided adjuvant therapy in locally advanced colon cancer: the randomized phase 2/3 DYNAMIC-III trial. *Nat. Med* **31**, 4291–4300 <https://doi.org/10.1038/s41591-025-04030-w> (2025).
29. Bando, H. et al. Post-adjuvant chemotherapy in ctDNA-positive patients with resected colorectal cancer: a randomized phase 3 trial. *Nat. Med* <https://doi.org/10.1038/s41591-026-04428-0> (2026).
30. Chen, S., Zhou, Y., Chen, Y. & Gu, J. Fastp: an ultra-fast all-in-one FASTQ preprocessor. *Bioinformatics* **34**, i884–i890 <https://doi.org/10.1093/bioinformatics/bty560> (2018).
31. Li, H. & Durbin, R. Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics* **25**, 1754–1760 <https://doi.org/10.1093/bioinformatics/btp324> (2009).
32. Lander, E. S. et al. Initial sequencing and analysis of the human genome. *Nature* **409**, 860–921 <https://doi.org/10.1038/35057062> (2001).
33. Tarasov, A., Vilella, A. J., Cuppen, E., Nijman, I. J. & Prins, P. Sambamba: fast processing of NGS alignment formats. *Bioinformatics* **31**, 2032–2034 <https://doi.org/10.1093/bioinformatics/btv098> (2015).
34. Mose, L. E., Perou, C. M. & Parker, J. S. Improved indel detection in DNA and RNA via realignment with ABRA2. *Bioinformatics* **35**, 2966–2973 <https://doi.org/10.1093/bioinformatics/btz033> (2019).
35. Lai, Z. et al. VarDict: a novel and versatile variant caller for next-generation sequencing in cancer research. *Nucleic Acids Res.* **44**, e108 <https://doi.org/10.1093/nar/gkw227> (2016).
36. Cingolani, P. et al. A program for annotating and predicting the effects of single nucleotide polymorphisms, SnpEff: SNPs in the genome of *Drosophila melanogaster* strain w1118; iso-2; iso-3. *Fly. (Austin)* **6**, 80–92 <https://doi.org/10.4161/fly.19695> (2012).
37. Wang, K., Li, M. & Hakonarson, H. ANNOVAR: functional annotation of genetic variants from high-throughput sequencing data. *Nucleic Acids Res* **38**, e164 <https://doi.org/10.1093/nar/gkq603> (2010).
38. Chabon, J. J. et al. Integrating genomic features for non-invasive early lung cancer detection. *Nature* **580**, 245–251 <https://doi.org/10.1038/s41586-020-2140-0> (2020).

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Author contributions

W.W., L.C., Y.Y. and D.C. conceptualized the study. Z.G., J.L. and P.P.Z. conducted the investigation. Z.G., Y.Z., W.S. and Y.H. curated the data. Z.C., W.D. and L.Y. developed the software and methodology. W.D. provided software support. X.X.W., Z.C. and X.Z. analysed the data. X.Q.W., C.Z. and R.D. provided technical or material support. Z.G., X.X.W. and Z.C. conducted the data visualization. W.W. and D.C. supervised the study. L.C., Y.Y. and Y.H. obtained funding. Z.G., X.X.W., Z.C., W.D., L.Y., Y.Z. and W.S. wrote the draft preparation. All authors contributed to the review and revision of the manuscript.

Competing interests

XX.W., Z.C., W.D., L.Y., XQ.W., X.Z., C.Z., R.D., and D.C. were employed by Jiangsu Simcere Diagnostic Technology Co., Ltd.; The other authors declare no competing financial or non-financial interests.

Additional information

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