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RESEARCH ARTICLE

Evaluation of molecular residual disease in operable non-small cell lung cancer with gene fusions, *MET* exon skipping or *de novo MET* amplification

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Abstract **Gene fusions and *MET* alterations are rare and difficult to detect in plasma samples. The clinical detection efficacy of molecular residual disease (MRD) based on circulating tumor DNA (ctDNA) in patients with non-small cell lung cancer (NSCLC) with these mutations remains unknown. This prospective, non-intervention study recruited 49 patients with operable NSCLC with actionable gene fusions (*ALK*, *ROS1*, *RET*, and *FGFR1*), *MET* exon 14 skipping or *de novo MET* amplification. We analyzed 43 tumor tissues and 111 serial perioperative plasma samples using 1021- and 338-gene panels, respectively. Detectable MRD correlated with a significantly higher recurrence rate ($P < 0.001$), yielding positive predictive values of 100% and 90.9%, and negative predictive values of 82.4% and 86.4% at landmark and longitudinal time points, respectively. Patients with detectable MRD showed reduced disease-free survival (DFS) compared to those with undetectable MRD ($P < 0.001$). Patients who harbored tissue-derived fusion/*MET* alterations in their MRD had reduced DFS compared to those who did not ($P = 0.05$). To our knowledge, this is the first comprehensive study on ctDNA-MRD clinical detection efficacy in operable NSCLC patients with gene fusions and *MET* alterations. Patients with detectable tissue-derived fusion/*MET* alterations in postoperative MRD had worse clinical outcomes.**

Keywords ctDNA; molecular residual disease; operable NSCLC; gene fusion; *MET* exon skipping; *MET* amplification

Introduction

In resectable non-small cell lung cancer (NSCLC), some patients experience disease recurrence post-radical surgical resection [1,2], highlighting the need for enhanced risk stratification and personalized monitoring strategies. Molecular residual disease (MRD) detection via circulating tumor DNA (ctDNA) has emerged as a promising approach to identify potentially cured populations [3] and individuals at high-risk of disease recurrence [4,5] as well as potentially guide adjuvant therapy decisions [6,7]. Although ctDNA assays are extensively used for detecting various gene variations, they often exhibit reduced sensitivity to structural variations, copy number alterations (CNA), and splice site variants [8]. Given ctDNA's extremely low presence in plasma samples, especially post-curative treatment, and its potential origin in multiple tumors or clones, the detection of structural variations, CNA and splice site variants relies on sufficient read coverage [9]. Studies that reported the clinical potential of perioperative ctDNA in informing disease recurrence of lung cancer included a small number of patients, 15 patients in Zhang *et al*.'s [3] and 16 patients in Fu *et al*.'s study [4], with gene fusions, *MET* exon 14 skipping, or *MET* amplification. Some studies excluded structural variations or CNA from ctDNA detection during MRD testing [5,10]. As such, no studies have described the clinical detection efficacy of ctDNA-based MRD testing in operable NSCLC with oncogenic gene fusions, *MET* exon skipping, or *MET* amplification, separately and comprehensively. To this end, we conducted a prospective observational study to elucidate the role of MRD detection in patients with operable NSCLC harboring oncogenic fusions, *MET* exon skipping, or *MET* amplification following surgical

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resection and explore the association between postoperative MRD mutations and clinical outcomes.

Materials and methods

Study design and patients

This prospective observational study was designed to evaluate the clinical utility of MRD testing in operable NSCLC with oncogenic actionable gene fusions, *MET* exon 14 skipping, or *de novo MET* amplification without other driver mutations. A total of 49 NSCLC patients who harbored next-generation sequencing (NGS)-confirmed actionable gene fusions, *MET* exon 14 skipping, or *de novo MET* amplification and underwent surgery from January 2018 to March 2023 at Guangdong Provincial People's Hospital, China. Six patients were excluded because of a lack of perioperative blood samples (n = 4) or loss of follow-up (n = 2). Ultimately, 43 patients were included in this study. Of these, 37 early-stage patients underwent radical surgery, and six patients with oligopersistent NSCLC underwent systemic treatment and received surgery as a local consolidative therapy. Lung cancer and disease stages were classified according to the World Health Organization criteria and the American Joint Committee on Cancer staging system (eighth edition), respectively.

Sample collection

Tumor tissues were obtained via needle biopsy or surgery. Peripheral blood samples (20 mL) were collected in Streck tubes before and/or after surgery and during follow-up. The landmark time points were samples collected in the time window of 3–37 days after surgery. Patients were then scheduled to be followed up every 3 or 6 months with CT scans and ctDNA tests until disease recurrence was determined by CT scan results. The longitudinal time point was defined as the serial postoperative time points after surgery until disease recurrence (including the disease recurrence time point). The surveillance time point was defined as within 6 months before clinical recurrence (including the disease recurrence time point).

Sample processing and DNA extraction

Tumor DNA was extracted from formalin-fixed, paraffin-embedded tumor tissue specimens using the ReliaPrep™ FFPE gDNA Miniprep System (Promega, Madison, WI, USA). Peripheral blood samples were separated by concentration at 1600× g for 10 min, and the supernatant was transferred to microcentrifuge tubes and centrifuged at 16 000× g for 10 min to remove cell debris. cfDNA was isolated using the QIAamp Circulating Nucleic Acid Kit (Qiagen, Hilden, Germany). Peripheral blood leukocytes (PBLs) were collected in the first centrifugation step to extract germline genomic DNA. To avoid contamination by ctDNA, the junction between the plasma and PBLs was first discarded and then the PBLs were carefully transferred. The QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany) was used to extract the germline genomic DNA from the PBLs. The concentration and fragment distribution of cfDNA were assessed using an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA).

Targeted next-generation sequencing

Genomic DNA (400–800 ng) extracted from PBLs and tumor specimens was sheared into fragments at a 200–250 bp peak using a Covaris S2 ultrasonicator (Covaris, Woburn, MA, USA). A volume of 20–80 ng DNA from the plasma was used for library construction, and unique identifiers were tagged onto each double-stranded DNA to distinguish authentic somatic mutations from artifacts. Indexed Illumina NGS libraries were prepared from PBLs, tumors, and plasma DNA using a KAPA Library Preparation Kit (Kapa Biosystems, Wilmington, MA, USA).

DNA libraries of the tumor and its paired germline were hybridized to a previously reported custom-designed panel, which covers ~1.5 Mbp of the genome and targets 1021 cancer-related genes [3,4]. DNA libraries of plasma and paired germline DNA were hybridized to a custom-designed biotinylated oligonucleotide probe covering 550 kbp of the genome, and targeting 338 cancer-related genes [3,4]. The hybridized libraries were sequenced using a 100-bp paired-end configuration on a Gene^{+}Seq-2000 sequencing system (GenePlus-Suzhou, Suzhou, China). The mean depth of coverage for tissue DNA and ctDNA were 1808× and 38 756× respectively.

Tumor somatic variant calling

After removing terminal adaptor sequences and low-quality reads using fastp [11], the remaining reads were mapped to the reference human genome (hg19) and aligned using Burrows-Wheel Aligner (v0.7.12-r1039) with default parameters. Duplicate reads were removed using the MarkDuplicates tool in Picard (v4.0.4.0; Broad Institute, Cambridge, MA, USA). RealDcaller (v1.8.1, Geneplus-Beijing, Beijing, China, in-house) [3] and GATK (v3.6-0-g89b7209, Broad Institute) were employed to call the tumor somatic single nucleotide variants (SNVs) and small insertions and deletions (indels). CNVkit was used to identify copy number variations [12]. Copy number alterations of *MET* with copy number $\geqslant$ 2.6 were considered the potential amplification, alterations were manually confirmed with a CNA plot. NCsv (v0.2.3, Geneplus-Beijing, Beijing, China, in-house) was applied to detect structural variants

[3,13]. All the candidate variants were manually confirmed using an integrative genomics viewer browser. Variants were filtered to exclude germline mutations in dbSNP and those that occur at a population frequency > 1% in ExAc (v0.3.1) or 1000 Genomes Project. Variants captured by PBL sequencing that are canonically associated with hematologic malignancies but do not meet the leukemia diagnostic criteria are considered to have clonal hematopoiesis of indeterminate potential. An in-house database of clonal hematopoiesis variants > 10 000 pan-cancer patients and healthy individuals was used to filter clonal hematopoiesis-related variants [14].

ctDNA-based MRD detection

The ctDNA-based MRD detection method has been previously reported [3,4] and is detailed below. Duplicated reads were removed, and sequencing errors were polished using unique identifiers and realSeq (v3.1.0, Geneplus-Beijing, in-house) [3]. SNV and indels were called using realDcaller and GATK. TNscope (Sentieon Inc., San Jose, CA, USA) was used as an auxiliary software to improve the detection of long indels. Variants meeting the following criteria were filtered out: (1) variants present in matched germline DNA, (2) variants occurring at a population frequency > 1% in ExAc (v0.3.1) or the 1000 Genomes Project, (3) variants with positional depth less than 300×, and (4) for sequencing error removal, a set of ~500 healthy plasma samples was sequenced to construct a sequencing background for each targeted SNV.

Depending on whether the plasma variants occurred in the matched tumor tissue, the resulting plasma variants were classified as tissue-derived and ctDNA-private. In other words, tissue-derived ctDNA mutations occurred in matched tumor tissues, whereas ctDNA-private mutations did not. The tissue-derived variants showed statistically significant differences in background characteristics and were considered reliable. When the following conditions were met, true somatic mutations were identified as follows: (1) for tissue-specific driver mutations, ⩾ 2 high-quality support reads; and (2) for other tissue-specific non-recurrent mutations, ⩾ 4 high-quality support reads. For ctDNA-private variants, the reliable somatic mutations were identified if they met the following stringent conditions: (1) for hotspot mutations, ⩾ 4 high-quality support reads; (2) for non-hotspots, ⩾ 8 high-quality support reads; and (3) clonal hematopoiesis were filtered through deep sequencing of PBLs. Positive ctDNA was defined as the detection of at least one variant [3,4].

Statistical analysis

The primary outcome measure was disease-free survival (DFS), which was assessed using standard radiological criteria. DFS was defined as the time from the date of surgery to the first radiological recurrence and was censored at the last follow-up. Statistical analyses were performed using the R statistical software (v4.1.3 for Windows). Analysis of the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) was conducted in patients with disease recurrence and patients without disease recurrence who were followed up for at least 6 months after the first MRD test. Survival analysis was performed using the Kaplan–Meier method and compared using the log-rank test. The Mann–Whiney U test and Student's *t*-test were used for non-normally and normally distributed continuous variables, respectively. A comparison of categorical variables was conducted with Pearson's χ^2 test or Fisher's exact tests. A two-sided P value < 0.05 was considered significant. Univariate and multivariate analyses were performed using the Cox proportional hazards regression analysis and included all factors that were significant in the univariate analysis.

Results

Patient characteristics

Forty-nine NSCLC patients with actionable gene fusions, *MET* exon 14 skipping, or *de novo MET* amplification who underwent surgery were included in this study. Four patients had no perioperative ctDNA test and two patients lost to follow-up were excluded; the remaining 43 patients were included for analysis of the clinical performance of perioperative ctDNA, including 22 patients with *ALK* fusion, 9 with *ROS1* fusion, 6 with *RET* fusion, 1 with *FGFR1* fusion, 3 with *MET* exon 14 skipping and 2 with *de novo MET* amplification (Fig. 1). Notably, patients with *de novo MET* amplification did not harbor any other driver mutations. The median age at diagnosis was 53 years (range, 32–73), with 55.8% females and 16.3% smokers. Most patients had adenocarcinoma (n = 38, 88.4%). Nineteen patients had stage III disease (44.2%), followed by stage I (n = 14, 32.5%) and stage II (n = 4, 9.3%). Six patients (14.0%) with oligopersistent NSCLC who underwent systemic therapy and received surgery as a local consolidative therapy were also included. Lymph node metastasis was in 28 (65.1%) patients. With a median follow-up of 9.9 months (range, 0.1–54.2), 13 patients experienced disease recurrence (Tables S1 and S2).

MRD predicts prognosis in operable NSCLC with fusions, *MET* exon 14 skipping or *MET* amplification

A total of 111 perioperative blood samples (including four preoperative and 107 postoperative samples) were collected in our study (Fig. 2A). Four patients had

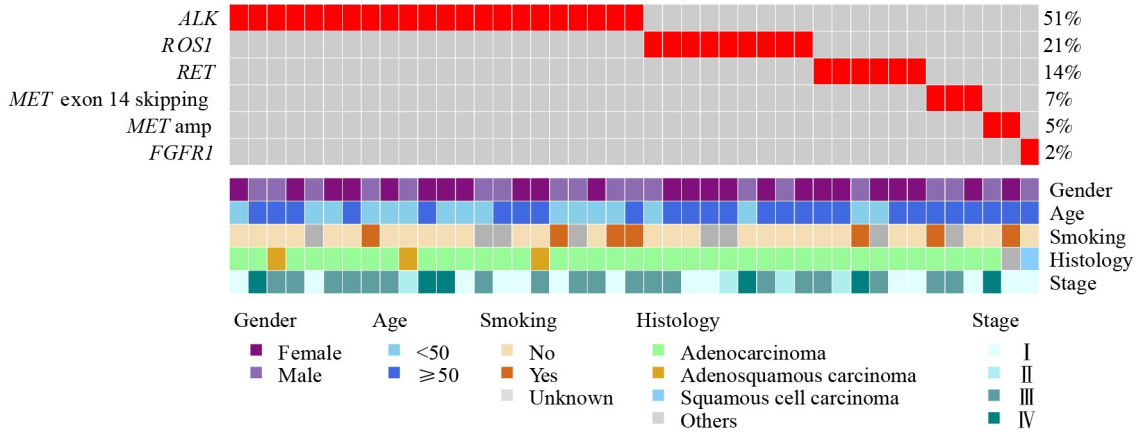

Fig. 1 Baseline characteristics of our cohort. Heat map plot based on each patient's baseline clinical and molecular characteristics. Amp, amplification.

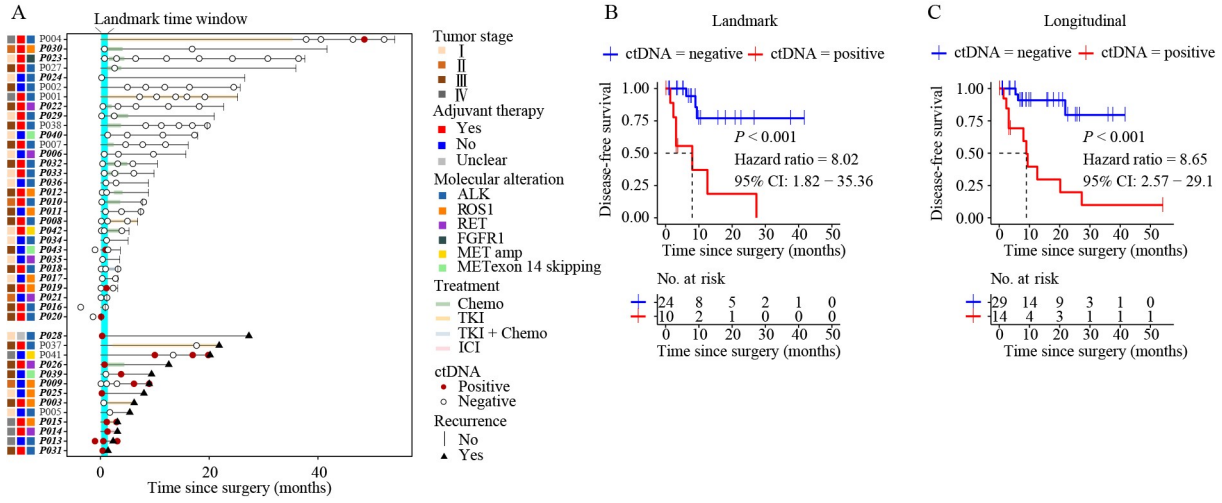

Fig. 2 Disease-free survival stratified by postoperative MRD status. (A) Event chart showing clinical characteristics, preoperative and postoperative longitudinal ctDNA status and recurrence status in the overall cohort. Patients were separated by their radiographic outcome. Patients included in the analysis of landmark time points were marked with bold italics, and the landmark time window was marked with cyan. Chemo, chemotherapy; TKI, tyrosine kinase inhibitor; ICI, immune checkpoint inhibitor. (B, C) Kaplan–Meier estimates of disease-free survival in patients stratified by ctDNA status at landmark (B) and longitudinal (C) time points. *P* value was calculated by the log-rank test and the hazard ratio by the Cox exp(β) method.

preoperative blood samples, and all patients had postoperative samples. Thirty-four patients and 41 blood samples were collected 3–37 days after surgery and used for landmark MRD analysis (Table S3). Three patients (P008, P014, and P040) had landmark ctDNA samples collected 37 days after surgery, and one of them (P008) also had landmark ctDNA sample collected 5 days after surgery. Two of these three patients had negative landmark MRD (P008 and P040) and remained disease-free at 6.9 and 17.9 months of follow-up, respectively, while one patient (P014) had a positive landmark MRD and experienced disease recurrence 1.8 months after the landmark MRD test (Fig. 2A). These results indicated that clinical outcomes in patients with NSCLC could be predicted 37 days post-surgery. Among the 34 patients who underwent a landmark MRD test, 10 had a detectable landmark MRD, and its proportion was similar across patients with different fusion/*MET* alterations (Fig. S1A). Importantly, patients with positive landmark MRD had significantly reduced DFS compared with those with negative landmark MRD (HR, 8.02; 95% CI, 1.82–35.36; $P < 0.001$; Fig. 2B). Univariate Cox proportional hazards regression analysis showed that advanced tumor stage (III/IV), lymph node involvement, and positive landmark MRD were associated with worse DFS, while positive landmark MRD was the only independent risk factor in the multivariate analysis (Fig. S2A).

All 43 patients and 107 blood samples were enrolled for longitudinal MRD analysis. Fourteen patients had positive longitudinal MRD, and their proportions were

comparable across patients with different fusion/*MET* alterations (Fig. S1B). Expectably, patients with positive longitudinal MRD had markedly worse DFS than those with negative longitudinal MRD (HR, 8.65; 95% CI, 2.57–29.1; $P < 0.001$; Fig. 2C), and positive longitudinal MRD was independently associated with worse DFS (Fig. S2B).

Clinical efficiency of MRD in operable NSCLC with fusions, *MET* exon 14 skipping or *MET* amplification

To eliminate the effect of a short follow-up time on the performance of MRD in predicting disease recurrence, we excluded recurrence-free patients < 6 months after the first postoperative MRD test. Patients with a positive landmark MRD had a higher recurrence rate (100% (7/7)) than those with a negative landmark MRD (17.7% (3/17), $P < 0.001$), with a sensitivity of 70%, specificity of 100%, PPV of 100% and NPV of 82.4% (Fig. S3A and S3B). Moreover, the recurrence rate was significantly higher in patients with positive longitudinal MRD (positive vs. negative, 90.9% (10/11) vs. 13.6% (3/22), $P < 0.001$), the sensitivity and NPV increased to 76.9% and 86.4%, respectively, and the specificity and PPV were 95% and 90.9%, respectively (Fig. S3A and S3B). Of the patients in the surveillance MRD test, seven had positive ctDNA, producing a sensitivity of 70% (Fig. S3A).

Of the 13 patients with radiological recurrence, positive postoperative MRD was found in 10 patients, and detectable ctDNA was seen on the median of 4.2 months (range, 1.0–27.0 months) before radiological recurrence (Fig. 3A). Three patients (P003, P005, and P037) showed undetectable ctDNA before recurrence (Fig. 2A). Two of them (P003 and P037) had brain-only recurrence, and patient P005 had pleural recurrence, supporting the low sensitivity of the MRD test for brain-only and intrathoracic-only metastases [3,8,10].

Clinical surveillance after curative-intent surgery using diagnostic blood biomarkers such as carcinoembryonic antigen (CEA) is recommended in current treatment regimens to inform recurrence early. However, their sensitivity is limited [15,16]. Indeed, four patients with disease recurrence in our cohort had successive CEA detection during follow-up; none of them had positive CEA (< 5 ng/mL) before recurrence, while positive longitudinal ctDNA identified 10 out of 13 (76.9%) disease recurrence ($P = 0.01$). For example, patient P009 with stage IIB adenocarcinoma underwent postoperative MRD and CEA monitoring. CEA was negative before recurrence and turned positive at relapse, whereas ctDNA was detected 2.8 months before imaging recurrence (Fig. 3B). Our data suggested that ctDNA-MRD is superior to CEA for predicting disease recurrence.

ctDNA-MRD mutation types are associated with patient outcome

We then explored the relationship between the mutation types in postoperative MRD and prognosis. Nineteen positive postoperative samples from 14 patients were included in this exploration. *ROS1* and *TP53* were the two most commonly altered genes in detectable longitudinal ctDNA (Fig. 4A). ctDNA variants were grouped into tissue-derived and ctDNA-private variants according to whether they occurred in their matched tumor tissues; the former occurred in their matched tumor tissues, whereas the latter did not. Eleven and three patients had tissue-derived and ctDNA-private mutations, respectively. Fusion/*MET* alterations detected in the tumor tissues were found in the longitudinal ctDNA of the six patients (Fig. 4A). The detection rates of

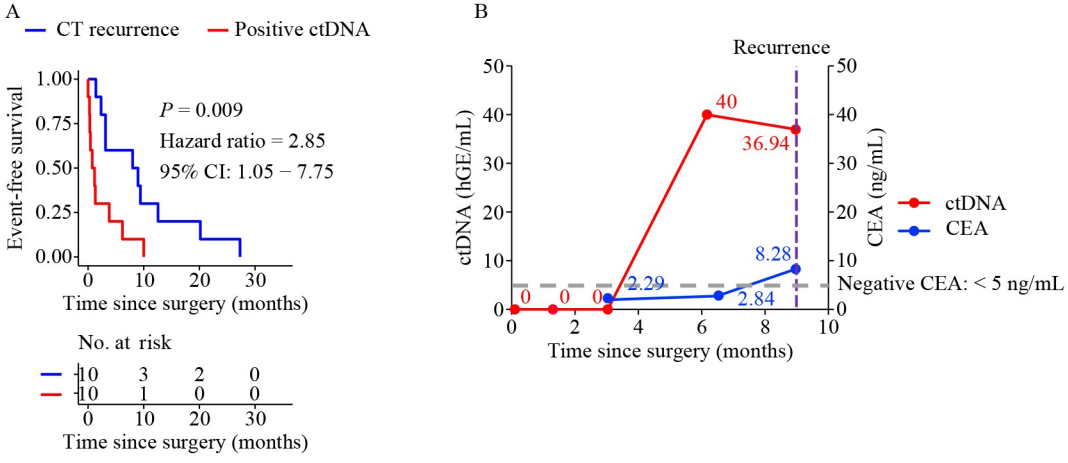

Fig. 3 MRD is superior to CEA in predicting disease recurrence. (A) Analysis of recurrence time measured by the first ctDNA detection and CT. *P* value was calculated by the log-rank test and the hazard ratio by the Cox exp(β) method. (B) Example of patient P009 with positive ctDNA and negative CEA before recurrence.

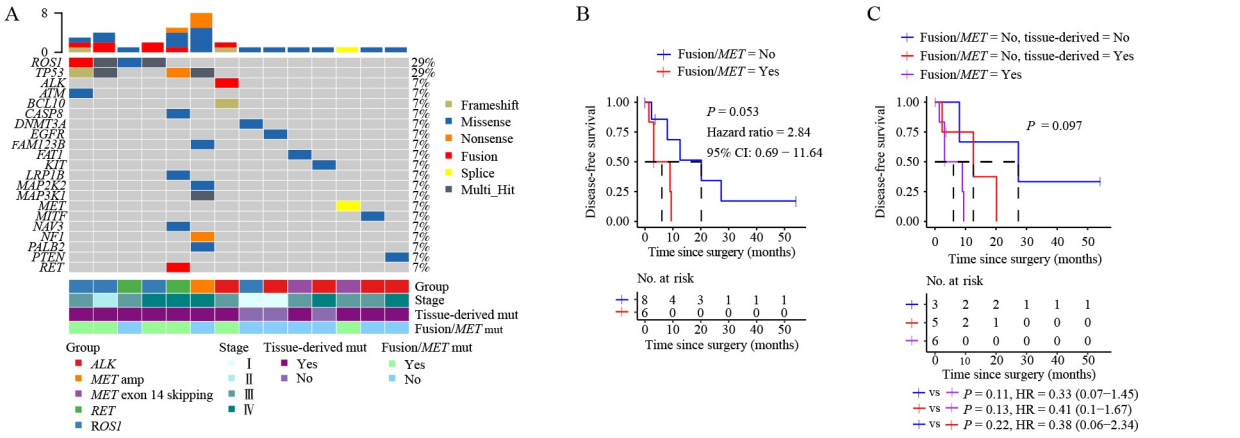

Fig. 4 Association of postoperative ctDNA mutation types with patient outcome. (A) Mutational landscape of longitudinal detectable ctDNA in 14 patients with operable NSCLC. (B) Kaplan–Meier estimates of disease-free survival in patients with and without fusion/*MET* mutations of longitudinal ctDNA. (C) Kaplan–Meier estimates of disease-free survival in patients stratified by whether the longitudinal ctDNA mutations are tissue-derived and fusion/*MET* mutations. The detectable fusion/*MET* group comprised patients with at least one fusion/*MET* mutation detected in at least one postoperative plasma sample. The detectable tissue-derived and undetectable fusion/*MET* group comprised patients with at least one tissue-derived mutation detected in at least one postoperative plasma sample and no fusion/*MET* mutation detected in any postoperative samples. *P* value was calculated by the log-rank test and the hazard ratio by the Cox exp(β) method.

fusion/*MET* alterations at the landmark and longitudinal points were comparable across patients with different fusion/*MET* alterations (Fig. S4A and S4B). Patients with fusion/*MET* alterations in longitudinal ctDNA had clinical features similar to those of patients without fusion/*MET* alterations (Table S4). Intriguingly, patients with fusion/*MET* alterations of longitudinal ctDNA had reduced DFS, although the difference is at the very edge of significance (HR, 2.84; 95% CI, 0.69–11.64; P = 0.053; Fig. 4B). Moreover, we grouped patients into those with fusion/*MET* alterations in their longitudinal ctDNA, those with other tissue-derived alterations except fusion/*MET* alterations and those with ctDNA-private alterations. We observed that patients with fusion/*MET* alterations in their longitudinal ctDNA tended to have worse DFS than those in the other two groups and that patients with ctDNA-private alterations tended to have better DFS than those in the other two groups, although this difference was not statistically significant ($P > 0.05$, Fig. 4C).

Discussion

Oncogenic driver gene fusions comprised ~10% of Chinese patients with NSCLC [17]. *MET* exon skipping and *de novo MET* amplification occurred in 2% [18] and 1%–5% [19] NSCLC patients, respectively. However, owing to the limited sensitivity of ctDNA assays for detecting gene fusions, copy number changes, and splicing variations in plasma samples, expert consensus recommends prioritizing tissue-based testing for these tumor types [8,20]. Nevertheless, the potent prognostic value of ctDNA-based MRD detection in operable lung cancer cannot be discounted. Hence, in this prospective observational study, we examined the clinical performance of MRD testing in operable NSCLC patients with actionable gene fusions, *MET* exon 14 skipping, and *de novo MET* amplification for the first time. Our results demonstrated that detectable postoperative MRD is significantly associated with a high risk of recurrence and poor clinical outcomes. Moreover, besides postoperative MRD status, the mutation types of postoperative plasma samples, classified as whether they are tissue-derived gene fusions or *MET* alterations, also inform prognosis.

Our results suggest that MRD serves as a reliable predictor of disease recurrence in operable NSCLC harboring oncogenic actionable fusion mutations, *MET* exon skipping, or *de novo MET* amplification, with a PPV of 100% and 90.9% at the landmark and longitudinal time points, respectively, comparable with previous NSCLC cohorts [3,21]. However, longitudinal ctDNA detection produced an NPV of 86.4%, which was lower than those reported by Zhang (96.8%) [3] and Chen (96.6%) [22]. This disparity might be due to our smaller cohort size (n = 43) compared with 261 in Zhang's study and 181 in Chen's study, as well as a shorter median follow-up time (9.9 months) versus 19.7 in Zhang's study and 1071 days in Chen's study. Postoperative longitudinal MRD detection demonstrated the potential to identify recurrence earlier than imaging modalities with a median lead time of 4.2 months, similar to that in previously reported cohorts [3,23]. Collectively, this study provides evidence for the use of ctDNA-based MRD detection in postoperative recurrence risk stratification in operable NSCLC with actionable fusion mutations, *MET* exon skipping or *de novo MET* amplification.

The comparable clinical performance between our cohort with actionable gene fusions, *MET* exon skipping, or *de novo MET* amplification-driven NSCLC patients and other cohorts with SNV/indels mutation-driven NSCLC patients may be due to our tumor-informed MRD assay. Having prior knowledge of tumor mutations can maximize ctDNA detection sensitivity [24]. Notably, among patients with undetectable MRD but experienced recurrence, the primary sites of recurrence were identified as the brain or small-volume intrathoracic tumors. This finding aligns with previous reports and highlights the limitations of ctDNA-based MRD, presenting false negatives [3,8,10]. Thus, it emphasizes the necessity of imaging examinations such as brain magnetic resonance imaging in follow-up surveillance to address this specific limitation.

Diseases with fusion variants exhibit unique tumor biological behavior characterized by a potentially brief window of early-stage residence and rapid progression to advanced stages [25]. Therefore, the detection of fusion or non-fusion mutations in the blood may indicate different prognoses. We observed worse prognosis in patients with detectable driver fusions, splices, and amplification mutations in longitudinal ctDNA than in those with other tissue-derived mutations or ctDNA-private mutations. The absence of statistical significance in DFS between them may be explained by the limited number of patients enrolled in this analysis (n = 14). We speculate that a worse prognosis in patients with detectable driver fusions, splices, and amplification mutations in longitudinal plasma samples may be attributed to the sustained oncogenic effects of driver mutations on promoting tumor growth.

This study has several limitations. First, due to the relatively low incidence of fusion mutations, *MET* splicing variants and *de novo MET* amplification without other driver mutations in the NSCLC population, our cohort had a limited sample size, primarily comprising adenocarcinoma (88.4%). Second, the correlation between postoperative MRD status and the treatment course was not explored in this study. Therefore, the generalizability of our findings to larger NSCLC populations with comprehensive histology, treatment data and long-term follow-up should be confirmed in future prospective studies with larger sample sizes.

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Compliance with ethics guidelines

Conflicts of interest Wenzhao Zhong declares speaker fees from AstraZeneca, BeiGene, Boehringer Ingelheim, Bristol-Myers Squibb, Eli Lilly, Hengrui, Merck Sharp & Dohme, Pfizer, Roche, Sanofi. Yilong Wu declares advisory services for AstraZeneca, Boehringer Ingelheim, Novartis, and Takeda; speaker fees from AstraZeneca, BeiGene, Boehringer Ingelheim, Bristol-Myers Squibb, Eli Lilly, Hengrui, Merck Sharp & Dohme, Pfizer, Roche, Sanofi; and grants from AstraZeneca, Boehringer Ingelheim, BMS, Pfizer and Roche outside the submitted work. Yuanyuan Xiong, Miao Cai, Fang Li, and Rongrong Chen are current employees of Geneplus-Beijing Ltd. All financial interests are unrelated to this study. Rui Fu declares that he has no conflict of interest. This study was approved by the Institutional Review Board of the Guangdong Provincial People's Hospital (No. GDREC2018115H) and the study was performed in accordance with the ethical standards as laid down in the 1975 *Declaration of Helsinki* and its later amendments. Informed consent was obtained from all patients for being included in the study.

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References

1. Pignon JP, Tribodet H, Scagliotti GV, Douillard JY, Shepherd FA, Stephens RJ, Dunant A, Torri V, Rosell R, Seymour L, Spiro SG, Rolland E, Fossati R, Aubert D, Ding K, Waller D, Le Chevalier T; LACE Collaborative Group. Lung adjuvant cisplatin evaluation: a pooled analysis by the LACE Collaborative Group. J Clin Oncol 2008; 26(21): 3552–3559
2. NSCLC Meta-analysis Collaborative Group. Preoperative chemotherapy for non-small-cell lung cancer: a systematic review and meta-analysis of individual participant data. Lancet 2014; 383(9928): 1561–1571
3. Zhang JT, Liu SY, Gao W, Liu SM, Yan HH, Ji L, Chen Y, Gong Y, Lu HL, Lin JT, Yin K, Jiang BY, Nie Q, Liao RQ, Dong S, Guan Y, Dai P, Zhang XC, Yang JJ, Tu HY, Xia X, Yi X, Zhou Q, Zhong WZ, Yang XN, Wu YL. Longitudinal undetectable molecular residual disease defines potentially cured population in localized non-small cell lung cancer. Cancer Discov 2022; 12(7): 1690–1701
4. Fu R, Huang J, Tian X, Liang C, Xiong Y, Zhang JT, Jiang B, Dong S, Gong Y, Gao W, Li F, Shi Y, Liu Z, Gao X, Chen R, Zhong W, Zhang Y. Postoperative circulating tumor DNA can refine risk stratification in resectable lung cancer: results from a multicenter study. Mol Oncol 2023; 17(5): 825–838
5. Abbosh C, Birkbak NJ, Wilson GA, Jamal-Hanjani M, Constantin

T, Salari R, Le Quesne J, Moore DA, Veeriah S, Rosenthal R, Marafioti T, Kirkizlar E, Watkins TBK, McGranahan N, Ward S, Martinson L, Riley J, Fraioli F, Al Bakir M, Grönroos E, Zambrana F, Endozo R, Bi WL, Fennessy FM, Sponer N, Johnson D, Laycock J, Shafi S, Czyzewska-Khan J, Rowan A, Chambers T, Matthews N, Turajlic S, Hiley C, Lee SM, Forster MD, Ahmad T, Falzon M, Borg E, Lawrence D, Hayward M, Kolvekar S, Panagiotopoulos N, Janes SM, Thakrar R, Ahmed A, Blackhall F, Summers Y, Hafez D, Naik A, Ganguly A, Kareht S, Shah R, Joseph L, Marie Quinn A, Crosbie PA, Naidu B, Middleton G, Langman G, Trotter S, Nicolson M, Remmen H, Kerr K, Chetty M, Gomersall L, Fennell DA, Nakas A, Rathinam S, Anand G, Khan S, Russell P, Ezhil V, Ismail B, Irvin-Sellers M, Prakash V, Lester JF, Kornaszewska M, Attanoos R, Adams H, Davies H, Oukrif D, Akarca AU, Hartley JA, Lowe HL, Lock S, Iles N, Bell H, Ngai Y, Elgar G, Szallasi Z, Schwarz RF, Herrero J, Stewart A, Quezada SA, Peggs KS, Van Loo P, Dive C, Lin CJ, Rabinowitz M, Aerts HJWL, Hackshaw A, Shaw JA, Zimmermann BG; TRACERx consortium; PEACE consortium; Swanton C. Phylogenetic ctDNA analysis depicts early-stage lung cancer evolution. Nature 2017; 545(7655): 446–451

6. Xia L, Mei J, Kang R, Deng S, Chen Y, Yang Y, Feng G, Deng Y, Gan F, Lin Y, Pu Q, Ma L, Lin F, Yuan Y, Hu Y, Guo C, Liao H, Liu C, Zhu Y, Wang W, Liu Z, Xu Y, Li K, Li C, Li Q, He J, Chen W, Zhang X, Kou Y, Wang Y, Wu Z, Che G, Chen L, Liu L. Perioperative ctDNA-based molecular residual disease detection for non-small cell lung cancer: A prospective multicenter cohort study (LUNGCA-1). Clin Cancer Res 2022; 28(15): 3308–3317
7. Tie J, Cohen JD, Lahouel K, Lo SN, Wang Y, Kosmider S, Wong R, Shapiro J, Lee M, Harris S, Khattak A, Burge M, Harris M, Lynam J, Nott L, Day F, Hayes T, McLachlan SA, Lee B, Ptak J, Silliman N, Dobbyn L, Popoli M, Hruban R, Lennon AM, Papadopoulos N, Kinzler KW, Vogelstein B, Tomasetti C, Gibbs P; DYNAMIC Investigators. Circulating tumor DNA analysis guiding adjuvant therapy in stage II colon cancer. N Engl J Med 2022; 386(24): 2261–2272
8. Pascual J, Attard G, Bidard FC, Curigliano G, De Mattos-Arruda L, Diehn M, Italiano A, Lindberg J, Merker JD, Montagut C, Normanno N, Pantel K, Pentheroudakis G, Popat S, Reis-Filho JS, Tie J, Seoane J, Tarazona N, Yoshino T, Turner NC. ESMO recommendations on the use of circulating tumour DNA assays for patients with cancer: a report from the ESMO Precision Medicine Working Group. Ann Oncol 2022; 33(8): 750–768
9. Gawroński AR, Lin YY, McConeghy B, LeBihan S, Asghari H, Koçkan C, Orabi B, Adra N, Pili R, Collins CC, Sahinalp SC, Hach F. Structural variation and fusion detection using targeted sequencing data from circulating cell free DNA. Nucleic Acids Res 2019; 47(7): e38
10. Abbosh C, Frankell AM, Harrison T, Kisistok J, Garnett A, Johnson L, Veeriah S, Moreau M, Chesh A, Chaunzwa TL, Weiss J, Schroeder MR, Ward S, Grigoriadis K, Shahpurwalla A, Litchfield K, Puttick C, Biswas D, Karasaki T, Black JRM, Martínez-Ruiz C, Bakir MA, Pich O, Watkins TBK, Lim EL, Huebner A, Moore DA, Godin-Heymann N, L'Hernault A, Bye H, Odell A, Roberts P, Gomes F, Patel AJ, Manzano E, Hiley CT, Carey N, Riley J, Cook DE, Hodgson D, Stetson D, Barrett JC, Kortlever RM, Evan GI, Hackshaw A, Daber RD, Shaw JA, Aerts HJWL, Licon A, Stahl J, Jamal-Hanjani M; TRACERx Consortium; Birkbak NJ, McGranahan N, Swanton C. Tracking early lung cancer metastatic dissemination in TRACERx using ctDNA. Nature 2023; 616(7957): 553–562
11. Chen S, Zhou Y, Chen Y, Gu J. fastp: an ultra-fast all-in-one FASTQ preprocessor. Bioinformatics 2018; 34(17): i884–i890
12. Li J, Lupat R, Amarasinghe KC, Thompson ER, Doyle MA, Ryland GL, Tothill RW, Halgamuge SK, Campbell IG, Gorringe KL. CONTRA: copy number analysis for targeted resequencing. Bioinformatics 2012; 28(10): 1307–1313
13. Ai X, Cui J, Zhang J, Chen R, Lin W, Xie C, Liu A, Zhang J, Yang W, Hu X, Hu X, Zhao Q, Rao C, Zang YS, Ning R, Li P, Chang L, Yi X, Lu S. Clonal architecture of EGFR mutation predicts the efficacy of EGFR-tyrosine kinase inhibitors in advanced NSCLC: a prospective multicenter study (NCT03059641). Clin Cancer Res 2021; 27(3): 704–712
14. Zhang Y, Yao Y, Xu Y, Li L, Gong Y, Zhang K, Zhang M, Guan Y, Chang L, Xia X, Li L, Jia S, Zeng Q. Pan-cancer circulating tumor DNA detection in over 10 000 Chinese patients. Nat Commun 2021; 12(1): 11
15. Reinert T, Henriksen TV, Christensen E, Sharma S, Salari R, Sethi H, Knudsen M, Nordentoft I, Wu HT, Tin AS, Heilskov Rasmussen M, Vang S, Shchegrova S, Frydendahl Boll Johansen A, Srinivasan R, Assaf Z, Balcioglu M, Olson A, Dashner S, Hafez D, Navarro S, Goel S, Rabinowitz M, Billings P, Sigurjonsson S, Dyrskjøt L, Swenerton R, Aleshin A, Laurberg S, Husted Madsen A, Kannerup AS, Stribolt K, Palmelund Krag S, Iversen LH, Gotschalck Sunesen K, Lin CJ, Zimmermann BG, Lindbjerg Andersen C. 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
16. Tie J, Cohen JD, Wang Y, Christie M, Simons K, Lee M, Wong R, Kosmider S, Ananda S, McKendrick J, Lee B, Cho JH, Faragher I, Jones IT, Ptak J, Schaeffer MJ, Silliman N, Dobbyn L, Li L, Tomasetti C, Papadopoulos N, Kinzler KW, Vogelstein B, Gibbs P. Circulating tumor DNA analyses as markers of recurrence risk and benefit of adjuvant therapy for stage III colon cancer. JAMA Oncol 2019; 5(12): 1710–1717
17. Gou LY, Wu YL. Prevalence of driver mutations in non-small-cell lung cancers in the People's Republic of China. Lung Cancer (Auckl) 2014; 5: 1–9
18. Vuong HG, Ho ATN, Altibi AMA, Nakazawa T, Katoh R, Kondo T. Clinicopathological implications of MET exon 14 mutations in non-small cell lung cancer—a systematic review and meta-analysis. Lung Cancer 2018; 123: 76–82
19. Drilon A, Cappuzzo F, Ou SI, Camidge DR. Targeting MET in lung cancer: will expectations finally be MET? J Thorac Oncol 2017; 12(1): 15–26
20. Rolfo C, Mack P, Scagliotti GV, Aggarwal C, Arcila ME, Barlesi F, Bivona T, Diehn M, Dive C, Dziadziuszko R, Leighl N, Malapelle U, Mok T, Peled N, Raez LE, Sequist L, Sholl L, Swanton C, Abbosh C, Tan D, Wakelee H, Wistuba I, Bunn R, Freeman-Daily J, Wynes M, Belani C, Mitsudomi T, Gandara D. Liquid biopsy for advanced NSCLC: a consensus statement from the international association for the study of lung cancer. J Thorac Oncol 2021; 16(10): 1647–1662
21. Gale D, Heider K, Ruiz-Valdepenas A, Hackinger S, Perry M, Marsico G, Rundell V, Wulff J, Sharma G, Knock H, Castedo J, Cooper W, Zhao H, Smith CG, Garg S, Anand S, Howarth K,

Gilligan D, Harden SV, Rassl DM, Rintoul RC, Rosenfeld N. Residual ctDNA after treatment predicts early relapse in patients with early-stage non-small cell lung cancer. Ann Oncol 2022; 33(5): 500–510

22. Chen K, Yang F, Shen H, Wang C, Li X, Chervova O, Wu S, Qiu F, Peng D, Zhu X, Chuai S, Beck S, Kanu N, Carbone D, Zhang Z, Wang J. Individualized tumor-informed circulating tumor DNA analysis for postoperative monitoring of non-small cell lung cancer. Cancer Cell 2023; 41(10): 1749–1762.e6
23. Qiu B, Guo W, Zhang F, Lv F, Ji Y, Peng Y, Chen X, Bao H, Xu Y, Shao Y, Tan F, Xue Q, Gao S, He J. Dynamic recurrence risk and adjuvant chemotherapy benefit prediction by ctDNA in resected NSCLC. Nat Commun 2021; 12(1): 6770
24. Hellmann MD, Nabet BY, Rizvi H, Chaudhuri AA, Wells DK, Dunphy MPS, Chabon JJ, Liu CL, Hui AB, Arbour KC, Luo J, Preeshagul IR, Moding EJ, Almanza D, Bonilla RF, Sauter JL, Choi H, Tenet M, Abu-Akeel M, Plodkowski AJ, Perez Johnston R, Yoo CH, Ko RB, Stehr H, Gojenola L, Wakelee HA, Padda SK, Neal JW, Chaft JE, Kris MG, Rudin CM, Merghoub T, Li BT, Alizadeh AA, Diehn M. Circulating tumor DNA analysis to assess risk of progression after long-term response to PD-(L)1 blockade in NSCLC. Clin Cancer Res 2020; 26(12): 2849–2858
25. Tang W, Lei Y, Su J, Zhang C, Fu R, Kang J, Yan H, Yang X, Tu H, Wu Y, Zhong W. TNM stages inversely correlate with the age at diagnosis in *ALK*-positive lung cancer. Transl Lung Cancer Res 2019; 8(2): 144–154