Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Exploratory study on ctDNA-MRD-guided postoperative adjuvant therapy for patients with stage IB-IIIB non-small cell lung cancer

Daling Dong
Guiqian International Hospital
Wei Wei
Guiqian International Hospital
Chao Wang
Guiqian International Hospital
Qian Yang
Guiqian International Hospital
Tingzhi Yan
Guiqian International Hospital
Lingmin Shi
Guiqian International Hospital
Shixin Zhang
ggyyxxwkzsx@126.com
Guiqian International Hospital

Research Article

Keywords:

Posted Date: November 11th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-11101509/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Additional Declarations:
No competing interests reported.

Tables 1 and 2 are available in the supplementary files section

Exploratory study on ctDNA-MRD-guided postoperative adjuvant therapy for patients with stage IB-IIIB non-small cell lung cancer

Authors

Daling Dong[1], Wei Wei[1], Chao Wang[1], Qian Yang[1], Tingzhi Yan[1], Lingmin Shi[1], Shixin Zhang[1*]

Affiliations

1. Department of Cardiothoracic Surgery, Guiqian International Hospital, Guiyang, 550024, China.

***Corresponding author:**

Shixin Zhang, Department of Cardiothoracic Surgery, Guiqian International Hospital. No. 1 Dongfeng Avenue, Wudang District, Guiyang, 550024, China. E-mail: ggyyxxwkzsx@126.com.

Abstract

Background Molecular residual disease (MRD) is a well-established prognostic biomarker in non-small cell lung cancer (NSCLC) following radical resection. This study aimed to explore the applicability of the circulating tumor DNA (ctDNA)-derived MRD status in determining adjuvant therapy in patients with stage IB-IIIB NSCLC.

Methods Patients with stage IB-IIIB NSCLC were retrospectively enrolled into standard management group or to the MRD-guided group. The standard management group received standard treatment according to pathological type, stage, and molecular subtype. The MRD guidance group have additionally incorporated postoperative MRD results. The primary endpoint of this study was the 2-year recurrence-free survival (RFS) rate.

Results A total of 80 patients were included in this study. The 2-year RFS rate was 78% (95% CI, 66–92) in the MRD-guided group versus 53% (95% CI, 40–72) in the standard management group (hazard ratio [HR], 0.289; 95% CI, 0.144-0.579; $P < 0.001$). The 2-year overall survival (OS) rate of two groups was 95% (95% CI, 88–100) and 76% (95% CI, 63–92), respectively, and the corresponding HR was 0.104 (95% CI, 0.039–0.281; $P <0.001$).

Conclusions Our study provides ctDNA-MRD-guided adjuvant therapy significantly improved RFS in patients with stage IB-IIIB NSCLC.

Key words molecular residual disease, non-small cell lung cancer, adjuvant therapy, recurrence-free survival, circulating tumor DNA.

Introduction

In solid tumors, molecular residual disease (MRD) refers to tumor-derived molecular abnormalities that remain after treatment but are undetectable by conventional imaging or laboratory methods. Liquid biopsy techniques, such as circulating tumor DNA (ctDNA) analysis, can identify MRD, which serves as a biomarker for assessing tumor persistence and predicting clinical progression [1]. Many studies have shown that high-sensitivity methods such as next-generation sequencing (NGS) can be used to evaluate the MRD status of lung cancer patients by detecting circulating tumor DNA (ctDNA) in blood samples [2-6]. The postoperative ctDNA-MRD status can be used to effectively identify patients at high risk of recurrence, while persistently negative MRD results may reflect potential curing [5, 7, 8]. In addition, observational studies have shown that for postoperative MRD-positive patients, adjuvant therapy can improve the recurrence-free survival (RFS) of patients, whereas patients with negative postoperative MRD do not benefit from adjuvant therapy [5, 7-9]. The results of the LUNGCA study [10] revealed that for patients without targetable mutations in their tumor tissues, adjuvant chemotherapy failed to significantly prolong the RFS among MRD-positive patients before they underwent adjuvant therapy. Conversely, among postoperative MRD-positive patients whose primary tumors harbored targetable mutations, tyrosine kinase inhibitor (TKI) therapy significantly improved RFS, whereas chemotherapy failed to provide a benefit. These results indicate that for MRD-positive patients, the treatment regimen should be selected according to tumor gene mutation status to ultimately improve survival.

Currently, no published prospective interventional studies have utilized MRD to guide

postoperative adjuvant therapy in early-stage lung cancer. Therefore, prospective studies using MRD to guide postoperative treatment decisions in patients with early-stage NSCLC are urgently needed. The LUNGCA study [5, 10], initiated in 2017, has demonstrated the technical reliability of the MinerVa NGS platform for MRD detection. In this retrospective observational study, the postoperative and dynamic ctDNA-MRD status of patients was detected using the MinerVa technology platform. MRD status was combined with the pathological type, stage, and molecular subtype of the patients to explore the application value of a comprehensively assessed treatment strategy in guiding adjuvant therapy decisions for patients following radical resection for stage IB-IIIB NSCLC.

Materials and Methods

Study design and patient cohort

This retrospective, observational, single-center, and exploratory study aimed to investigate whether comprehensive treatment regimens designed on the basis of postoperative MRD status combined with pathological type, stage, and molecular subtype could increase RFS in patients with early-stage NSCLC. This study enrolled 80 patients with stage IB-IIIB NSCLC who underwent radical surgery in the Department of Cardiothoracic Surgery of Guiqian International Hospital from December 2019 to February 2023. The main inclusion criteria were an age between 18 and 80 years, a pathological diagnosis of stage IB-IIIB NSCLC (according to the 8th edition of the American Joint Committee for Cancer (AJCC) staging system), and an Eastern Cooperative Oncology Group (ECOG) performance-status score of 0–2. Patients were required to have complete medical records, medication histories and

follow-up data. Patients in the MRD group were additionally required to have serial MRD testing results. The main exclusion criteria were a history of other known primary tumors or the presence of other tumors; severe organ dysfunction (such as of the heart, liver, or kidney); prior treatment with therapies relevant to the investigational drugs (such as osimertinib or immune checkpoint inhibitors); severe immune-related diseases (such as autoimmune diseases); pregnancy or lactation; and other serious diseases or conditions that did not conform with the objectives or protocols of the study.

Sample collection

Surgically resected (and subsequently paraffin-embedded) tumor tissue specimens and control blood samples were collected from all enrolled patients for tumor molecular mutation detection. Tumor tissues were also used for immunohistochemical detection of PD-L1 expression. Blood samples from patients in the MRD-guided group were collected at landmark timepoints (approximately 1 month after surgery to before adjuvant therapy), and 20 ml of blood was collected for MRD detection approximately every 3 months or 6 months after surgery until recurrence/metastasis or up to 24 months after surgery.

Next-generation sequencing and ctDNA analysis

DNA extraction from tumor tissues and blood, library construction, and next-generation sequencing (NGS) were performed by Genecast Biotechnology Co., Ltd. (a CAP-certified laboratory). Hybridization capture was performed using a 2.4-MB NGS panel covering 769

cancer-related genes. The average sequencing depth for core region tissues was 6500×, that for blood cells was ≥4400×, and that for plasma was 58000×. ctDNA MRD detection was performed using the MinerVA platform from Genecast Biotechnology Co., Ltd. Detailed experimental procedures and data analysis methods can be found in publications related to the LUNGCA study [5, 10].

A tumor-informed analysis strategy was employed for MRD detection. For single-nucleotide variants and insertion/deletion mutations (SNVs/InDels), each candidate ctDNA mutation was statistically tested against the internal noise baseline constructed from negative data to exclude technical artifacts. A mutation was considered positive if the P value was ≤0.05. The combined P values of the sample levels were further calculated. A plasma sample was considered positive when the combined P value was ≤0.01. For gene fusion, if at least one crossing point completely matched the reference sequence of the fusion gene in the paired tissue sample, the plasma sample was determined to be positive.

Immunohistochemical staining

PD-L1 expression was measured using a Dako 22C3 kit (Dako; Agilent Technologies, Inc.). The tumor proportion score (TPS), which refers to the ratio of PD-L1-positive tumor cells to the total number of visible tumor cells, was calculated. The combined positive score (CPS) was calculated as the ratio of PD-L1-positive cells (the sum of the numbers of tumor cells, lymphocytes, and macrophages) to the total number of visible tumor cells multiplied by 100.

Procedure

Patients were enrolled to either the MRD-guided group or the standard management group, with stratification based on pathological type and stage. The standard management group received standard treatment designed on the basis of the pathological type, stage, and molecular subtype of the patients; the medication regimens (including cycles) of the patients were developed with reference to the National Comprehensive Cancer Network (NCCN) and Chinese Society of Clinical Oncology (CSCO) guidelines. Patients in the MRD-guided group underwent blood MRD detection after surgery (landmark timepoint) and approximately every 3 or 6 months after surgery until recurrence/metastasis or up to 24 months after surgery. The treatment plan was developed on the basis of a comprehensive assessment of the patient's landmark ctDNA-MRD test results and pathological type, stage, and molecular subtype. If the plasma of landmark ctDNA-MRD test result was positive, the patient received appropriate targeted therapy or immunotherapy based on the tumor genetic profile. That is, patients with ALK fusions receive second-generation ALK-TKIs, those with EGFR driver mutations receive third-generation EGFR-TKIs, and those without driver gene alterations receive immunotherapy combined with chemotherapy. The treatment duration was planned for a predefined period (e.g., up to 2-3 years for targeted therapy) or until disease recurrence/unacceptable toxicity, with the MRD status being one of the considerations for treatment continuation. If the results of the landmark plasma ctDNA-MRD test were negative, the patients receive conventional chemotherapy. However, if the patient requests targeted therapy or immunotherapy, after shared decision-making, those with EGFR driver

alterations may receive third-generation EGFR-TKIs, while those who are EGFR-negative or ALK fusion-negative may receive immunotherapy combined with chemotherapy.

The chemotherapy regimen for lung adenocarcinoma (AP regimen) was pemetrexed combined with platinum (cisplatin/carboplatin). The specific dosages were 500 mg/m² for pemetrexed, 200–400 mg/m^2 for carboplatin, and 50–100 mg/m^2 for cisplatin administered by intravenous (IV) infusion every 3 weeks (q3w) for 4–6 consecutive cycles. The chemotherapy regimen for lung squamous cell carcinoma (nab-PP) was albumin-paclitaxel combined with platinum (cisplatin/carboplatin). The specific dosages were 100 mg/m² for albumin-paclitaxel, 200–400 mg/m^2 for carboplatin, and 50–100 mg/m² for cisplatin, administered by IV infusion q3w for 4–6 consecutive cycles. For patients with wild-type EGFR and ALK, the immune checkpoint inhibitor treatment regimen included atezolizumab (1200 mg) or pembrolizumab (200 mg), administered by IV infusion q3w. For EGFR mutation-positive patients (19del/L858R/T790M), the third-generation EGFR-TKI osimertinib was used in targeted therapy at a dose of 80 mg administered orally once daily. Patients with ALK fusion were treated with alectinib (600 mg) administered orally twice daily with meals.

Endpoints

The primary endpoint was the 2-year RFS rate. In this study, RFS was defined as the time from the start of surgical resection to the first recorded occurrence of disease recurrence or death due to any cause, whichever occurred first. Secondary endpoints included the 2-year overall survival (OS) rate, and median RFS, as well as the 2-year RFS rates and 2-year OS

rates of the ctDNA-MRD-positive and ctDNA-MRD-negative patients in the MRD-guided group. The overall OS data will be reported in future analyses.

Statistical analysis

In this study, the R language (maftools package) was used to plot the mutation landscapes of the patients to determine the distribution of high-frequency mutated genes and mutation types. Kaplan–Meier survival curves were plotted on the basis of the RFS and OS of the patients using the survival and survminer packages in R language, and the log-rank test was used to assess the survival differences between different groups. A pooled graph was generated to show the MRD detection time and status, treatment method, recurrence time, and survival status of each patient. Appropriate statistical methods were used to compare the distributions of different clinical characteristics (such as age, ECOG PS score, and pathological type) between the groups: continuous variables were compared with the t test, and categorical variables were compared with the chi-square test or Fisher's exact test, as appropriate. The time between MRD positivity detection and recurrence according to patient imaging was calculated. In the subgroup analysis, the paired t test was used to assess differences between the patient subgroups. For all tests, $P < 0.05$ was considered to indicate statistical significance.

Ethics Statement

This clinical trial was approved by the Ethics Committee of Guiqian International Hospital (Approval Number: 2023 Ethics Review No. 01). Clinical trial number: not applicable. All procedures were performed in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants or their legal representatives.

Results

Patient characteristics

A total of 91 patients with stage IB-IIIB NSCLC who underwent radical surgery between December 2019 and February 2023 were enrolled. Three patients with multiple primary lung cancers, two patients with compound cancers, two patients with a previous history of malignant tumors, and four patients who refused chemotherapy were excluded. A total of 80 patients were therefore ultimately included into the MRD-guided group (n=40 patients) and the standard management group (n=40 patients) according to pathological type and stage (Fig 1).

The median age of the patients in the MRD-guided group was 58 years. This group consisted of 16 female patients (40%) and 24 male patients (60%), and 23 patients (57.5%) had a history of smoking. A majority of patients in the MRD-guided group (87.5%) had an ECOG PS score of 0 or 1; 8 patients had stage IB disease, 1 had stage IIA disease, 9 had stage IIB disease, 20 had stage IIIA disease, and 2 patients had stage IIIB disease. A total of 31 patients had lung adenocarcinoma, accounting for 77.5%; the remaining 9 patients had lung squamous cell carcinoma (22.5%; Table 1).

The average age of the patients in the standard treatment group was 61 years. This group consisted of 18 females (45%) and 22 males (55%) were included; among them, 18 patients (45.0%) had a history of smoking. A total of 92.5% of the patients in this group had an ECOG PS score of 0 or 1; 8 patients had stage IB disease, 2 had stage IIA disease, 11 had stage IIB disease, 16 had stage IIIA disease, and 3 patients had stage IIIB disease. Thirty patients had lung adenocarcinoma, accounting for 75%; 9 patients had lung squamous cell carcinoma (22.5%), and 1 patient had adenosquamous carcinoma (Table 1). The baseline characteristics were well-balanced between the two groups, with no significant differences identified (Table 1 and Table S1).

Tissue specimen and blood sample analysis

The details of the gene mutations in the tumor tissues of the patients are shown in Fig 2. The most commonly mutated genes were EGFR (58%), TP53 (56%), KRAS (16%), CDKN2A (14%), NTRK3 (12%), BRAF (11%), RB1 (11%), and RBM10 (11%). The tumor gene mutation spectra of the two groups were similar. The details of PD-L1 expression in the two groups of patients are shown in Table S1. In the MRD-guided group, 9 patients (22.5%) had positive landmark MRD results, and 31 patients (77.5%) had negative landmark MRD results (Table S1).

Patient treatment details

All patients in this study received adjuvant therapy. The median time from surgery to

the initiation of adjuvant therapy was 30 days (IQR, 22-40) in the MRD-guided group and 32 days (IQR, 22-39) in the standard management group. The mean treatment duration was 205 days in the MRD-guided group and 214 days in the standard management group (P=0.384). Within the MRD-guided group, the mean treatment duration was significantly longer in the landmark MRD-positive subgroup (466 days) than in the landmark MRD-negative subgroup (193 days; P=0.00018) (Table 2 and Table S2).

As mentioned above, the MRD-guided group included 9 landmark MRD-positive patients. Among them, 4 patients had EGFR p.L858R mutations in their tumor tissues and received third-generation EGFR-TKI treatment after chemotherapy; 2 patients exhibited ALK fusion and received second-generation ALK-TKI targeted treatment after chemotherapy. The remaining 3 patients had no targetable driver mutations and low expression levels of PD-L1 (CPS: 2, 5, and 8; TPS: 1% in two patients and <1% in the other patient). Treatment for these patients involved adjuvant chemotherapy combined with an immune checkpoint inhibitor. Among the 31 patients with negative landmark MRD status, 15 (15/31, 48.4%) received chemotherapy alone, 4 received chemotherapy followed by third-generation EGFR-TKI targeted therapy, and 12 received chemotherapy combined with immunotherapy (Table S1). In the standard management group, 15 patients (37.5%) received chemotherapy alone, 15 patients (37.5%) received chemotherapy combined with immunotherapy, 1 patient (2.5%) received sequential chemotherapy followed by ALK-TKI targeted therapy, and 9 patients (22.5%) received third-generation EGFR-TKI targeted therapy after chemotherapy (Table S1).

The treatment-related adverse events (AEs) in both groups were mainly grade 1–2. In the MRD-guided group, grade 1-2 AEs occurred in 39 patients (97.5%), and one patient (2.5%) experienced a grade 5 AE (immune-mediated myocarditis) resulting in death. In the standard management group, 36 patients (90%) had grade 1–2 AEs, 3 (7.5%) had a grade 3 AE, and 1 patient (2.5%) had a grade 5 AE, immune-related pneumonia, which resulted in the patient's death. There was no difference in the incidence of AEs between the two groups (P = 0.304) (Table 2). In the MRD-guided group, the AEs in the positive and negative landmark MRD subgroups were also mainly grades 1–2. In the positive landmark MRD subgroup, 5 patients (55.6%) had grade 1 AEs, and 4 patients (44.4%) had grade 2 AEs; in the negative landmark MRD subgroup, 11 patients (35.5%) had grade 1 AEs, and 20 (64.5%) had grade 2 AEs. There was no difference in the incidence of AEs between the two subgroups (P = 0.413) (Table S2).

Efficacy according to treatment group

The median follow-up time was 1048 (192–1529) days in the MRD-guided group and 730 (237–1528) days in the standard management group (Table 2). In the MRD-guided group, 10 patients experienced radiologic recurrence/metastasis or death. The sites of recurrence/metastasis were the lung in 1 patient, the brain in 1 patient, the bone in 1 patient, the lymph nodes (metastasis) in 2 patients, and the thoracic cavity in 1 patient; three patients experienced multiple organs recurrences/metastases, while the location of recurrence/metastasis could not be defined in one patient who died. In the standard

management group, 23 patients experienced radiologic recurrence/metastasis or death. The locations of recurrence/metastasis were the lung in 8 patients, the brain in 1 patient, the bone in 1 patient, the lymph nodes (metastasis) in 1 patient, and the pleura in 1 patient; seven patients experienced multiple organs recurrences/metastases, and four patients died without having their sites of recurrence/metastasis defined (Table 2). The median RFS in the MRD-guided group and the standard management group were not reached and 26.3 (21.7–could not be calculated [NC]) months, respectively. The 2-year RFS rate in the MRD-guided group and the standard management group were 78% (95% CI, 66–92) and 53% (95% CI, 40–72), respectively. These data indicate that compared with standard management, MRD guidance significantly reduced the risk of disease recurrence by approximately 71% (total hazard ratio [HR] 0.289, 95% CI, 0.144–0.579; $P < 0.001$) (Fig 3A and Table 2). The median OS of the MRD-guided group was not reached, and that of the standard management group was 34.7 (29.3–-[NC]) months. The 2-year OS rate of the MRD-guided group and the standard management group was 95% (95% CI, 88–100) and 76% (95% CI, 63–92), respectively, and the overall HR was 0.104 (95% CI, 0.039–0.281; $P <0.001$), corresponding to a reduction in the risk of death with MRD guidance by approximately 89.6% (Fig 3B and Table 2).

Efficacy in the MRD-guided subgroups

Among the patients with landmark MRD positivity, 5 experienced relapse/metastasis according to their imaging results or death. The sites of recurrence/metastasis were the lymph nodes (metastasis) in 1 patient; three patients experienced multiple organs

recurrences/metastases, and 1 patient died before their site(s) of recurrence/metastasis could be identified. Five landmark MRD-negative patients experienced recurrence/metastasis on the basis of their imaging results or death. The locations of recurrence/metastasis were the lung in 1 patient, the brain in 1 patient, the bone in 1 patient, the lymph nodes (metastasis) in 1 patient, and the thoracic cavity in 1 patient; 1 patient died due to the original tumor (Table S2). The 2-year RFS rates of patients with negative and positive landmark ctDNA-MRD results were 84% (95% CI, 72–98) and 56% (95% CI, 31–100), respectively. The median RFS of the landmark ctDNA-MRD-negative subgroup was not reached, and that of the landmark ctDNA-MRD-positive subgroup was 31.7 (95% CI, 18.9–NC) months; the total HR for RFS between the two groups was 0.247 (95% CI, 0.052–1.170; P = 0.016) (Fig 3C and Table S2). The median OS was not reached in either the ctDNA-MRD-negative or ctDNA-MRD-positive subgroups, while the 2-year OS rates were 97% (95% CI, 91–100) and 86% (95% CI, 63–100), respectively. The corresponding HR was 0.261 (95% CI, 0.009–7.985; P = 0.31) (Fig 3D and Table S2).

For MRD-negative patients at the landmark time point in the MRD-guided group, we compared the additional RFS benefit of EGFR-TKI versus chemotherapy in those with EGFR mutations and found no difference between the two groups (Fig S1 A) (HR (95% CI) = 0.69 [0.06, 7.59]; P = 0.758). Likewise, in MRD-negative patients without EGFR mutations or ALK fusions, we compared immunotherapy versus chemotherapy and also observed no RFS benefit (Fig S1 B) (P = 0.999). These findings indicate that in landmark MRD-negative patients, neither targeted therapy nor immunotherapy provides additional RFS benefit,

regardless of the presence of actionable driver mutations, which is consistent with previously reported observational studies.

In the MRD-guided group, among the 10 patients who experienced tumor metastasis/recurrence or death, MRD was positively detected at least once before recurrence, metastasis, or death in 7 patients, while for 2 patients (P24 and P29), MRD was positively detected immediately after recurrence. For both of these patients, the time between MRD testing prior to recurrence and recurrence confirmation was 4 months, which may have led to underdetection of MRD. The median lead time in which MRD detected recurrence earlier than imaging was 76 days (Fig 4A). Among the 9 MRD-positive patients in the MRD-guided group at the landmark time point, 3 received adjuvant immunotherapy and 6 received adjuvant targeted therapy. We present the changes in ctDNA concentration during treatment for the two groups. CtDNA concentration (hGE/ml) fluctuated markedly in patients receiving immunotherapy, whereas they remained generally low in those receiving targeted therapy (Fig 4B).

In the landmark MRD-positive subgroup, 6 patients were positive for MRD during follow-up dynamic monitoring, among whom 3 experienced recurrence, metastasis or death, indicating that the MRD-positive patients had a high risk of recurrence and that any patients exhibiting MRD positivity should be closely monitored and followed up even after intensive treatment. Seven of the landmark MRD-negative patients were MRD-positive on follow-up dynamic monitoring, four of whom experienced recurrence, metastasis or death. This finding indicates that even in patients with landmark MRD negativity, dynamic ctDNA-MRD

monitoring can identify those at potential risk of recurrence in a timely manner (Fig 4C and Table S3). In two patients who initially tested MRD-negative but later converted to positive before recurrence, we compared the plasma ctDNA mutation profile at recurrence with that of the original tumor (Fig S2). No plasma-naive mutations were detected, and all mutations identified in plasma originated from the tumor tissue.

Discussion

Currently, the choice of postoperative adjuvant therapy for NSCLC mainly involves consideration of pathological type, stage, molecular subtype, and clinical risk factors; however, some patients still experience recurrence or death. The utility of MRD-guided therapy is increasingly recognized in other malignancies. For instance, in stage II colorectal cancer, downgrading treatment based on negative postoperative ctDNA-MRD test results reduced the administration of unnecessary adjuvant chemotherapy for approximately half of the patients while unaffecting the 5-year RFS or OS rates [11, 12]. However, to date, no large-scale prospective interventional studies have investigated MRD-guided adjuvant therapy for lung cancer patients. Despite its exploratory nature, this study addresses a critical evidence gap in lung cancer and provides a foundation for designing larger prospective trials and potentially informing clinical practice.

At the time of this study, adjuvant immunotherapy and targeted therapy for NSCLC were not included in the guidelines for treating NSCLC; nevertheless, targeted therapy and immunotherapy have shown good efficacy in the treatment of advanced lung cancer. In this

study, targeted and immunotherapeutic interventions were retrospective reviewed. For patients negative for tumor driver genes, the treatment regimen could be chemotherapy combined with immunotherapy, while for those with positive tumor driver genes, sequential targeted therapy, which may include chemotherapy. Compared with the standard treatment group, the MRD-guided group showed significant improvements in RFS and OS, indicating that comprehensive treatment involving comprehensive clinical information and molecular mutations combined with dynamic ctDNA-MRD data indeed improved patient survival. In the MRD-guided group, 9 MRD-positive patients received adjuvant targeted therapy or immunotherapy, while in the MRD-negative group, nearly half of the patients received only chemotherapy; nevertheless, the RFS of the MRD-negative patients was significantly better than that of the MRD-positive patients, suggesting that high -risk patients identified by MRD screening indeed have a very high risk of recurrence. For MRD-positive patients, intensive treatment was performed mainly through dynamic monitoring of MRD status to determine whether the treatment time should be extended. More treatment regimen optimization or exploration is needed to reduce recurrence in MRD-positive patients.

Many studies have confirmed that the postoperative, dynamic monitoring of ctDNA in NSCLC can effectively predict the risk of tumor recurrence [2-8]. In this study, although some patients in the MRD-guided group were positive for MRD during the monitoring process, none experienced recurrence, reflecting the value of the dynamic monitoring of MRD status. Meanwhile, in the standard treatment group, 23 patients experienced recurrence or metastasis, and 14 patients died. Although more intense treatment and extended treatment

cycles were associated with an increased risk of treatment-related adverse events, the overall number of treatment-related adverse events was not significantly different. As postoperative adjuvant immunotherapy and its targeted application are standardized in treatment guidelines, the grades of AEs faced by intensive treatment are expected to decrease.

Among the MRD-negative patients who recurred, two tested MRD-positive prior to radiological recurrence, while three remained MRD-negative until recurrence was detected. This suggests that the 6-month monitoring interval for MRD-negative patients might have been too long to reliably detect MRD lead-time in all cases.

This study has several limitations. It is a retrospective analysis with a non-prospective, non-interventional design. The sample size is moderate, and this is a single-center exploratory study. Furthermore, although a statistically significant overall survival (OS) benefit was observed, the median OS in the MRD-guided group was not reached, indicating that longer follow-up is required to fully characterize its long-term survival benefit.

Conclusions

Although this study has several limitations, our findings demonstrate that ctDNA-MRD-guided adjuvant therapy significantly improves RFS in patients with stage IB–IIIB NSCLC, which may providing implications for the design of future large interventional MRD studies.

Abbreviations

NSCLC non-small cell lung cancer

392	MRD	molecular residual disease
393	ctDNA	circulating tumor DNA
394	RFS	recurrence-free survival
395	OS	overall survival
396	IQR	Interquartile Range
397	HR	hazard ratio
398	NC	not calculated
399	NR	not reached
400	CI	denotes confidence interval
401	NGS	next-generation sequencing
402	AJCC	the American Joint Committee for Cancer
403	ECOG	Eastern Cooperative Oncology Group
404	PD-L1	programmed death-ligand 1
405	SNVs	single-nucleotide variants
406	InDels	insertion/deletion mutations
407	TPS	the tumor proportion score
408	CPS	the combined positive score
409	NCCN	the National Comprehensive Cancer Network
410	CSCO	Chinese Society of Clinical Oncology
411	ALK	Anaplastic Lymphoma Kinase
412	EGFR	Epidermal Growth Factor Receptor

CT scan	computed tomography scan
AEs	adverse events
hGE	haploid genome equivalent
CT	chemotherapy
TKI	tyrosine kinase inhibitor
ICB	immune checkpoint blockade
NA	not available

Authors' contributions

Daling Dong: Conceptualization; visualization; validation; methodology; investigation; formal analysis; data curation; writing—original draft; writing—review and editing; software; project administration. **Wei Wei:** Visualization; methodology; investigation; formal analysis; validation; software. **Chao Wang:** Conceptualization; validation; data curation; resources. **Qian Yang:** Data curation; writing—review and editing; conceptualization; and investigation. **Tingzhi Yan:** Conceptualization; investigation; writing—review and editing; and data curation. **Lingmin Shi**: Investigation; and data curation. **Shixin Zhang:** Conceptualization; methodology; writing—review and editing; project administration; supervision; resources.

Acknowledgments

No

Data Availability

The datasets generated and/or analysed during the current study are not publicly available due to institutional policy and patient privacy restrictions, but are available from the corresponding author on reasonable request and subject to approval by the institutional ethics committee.

Funding

None declared.

Conflict of interest statement

No

Consent for publication

Not applicable.

References

1. Chinese Society of Pathology, National Pathology Quality Control Center: **Consensus on the molecular residual disease testing in solid tumor**. *Zhonghua Bing Li Xue Za Zhi* 2024, **53**(11):1088–1096.

2. Abbosh C, Birkbak NJ, Wilson GA, Jamal-Hanjani M, Constantin T, Salari R, Le Quesne J, Moore DA, Veeriah S, Rosenthal R *et al*: **Phylogenetic ctDNA analysis depicts early-stage lung cancer evolution**. *Nature* 2017, **545**(7655):446–451.

3. Chaudhuri AA, Chabon JJ, Lovejoy AF, Newman AM, Stehr H, Azad TD, Khodadoust MS, Esfahani MS, Liu CL, Zhou L *et al*: **Early detection of molecular residual disease in localized lung cancer by circulating tumor DNA profiling**. *Cancer Discov* 2017, **7**(12):1394–1403.

4. Chabon JJ, Hamilton EG, Kurtz DM, Esfahani MS, Moding EJ, Stehr H, Schroers-Martin J, Nabet BY, Chen B, Chaudhuri AA *et al*: **Integrating genomic features for non-invasive early lung cancer detection**. *Nature* 2020, **580**(7802):245–251.

5. Xia L, Mei J, Kang R, Deng S, Chen Y, Yang Y, Feng G, Deng Y, Gan F, Lin Y *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* 2022, **28**(15):3308–3317.

6. Gale D, Heider K, Ruiz-Valdepenas A, Hackinger S, Perry M, Marsico G, Rundell V, Wulff J, Sharma G, Knock H *et al*: **Residual ctDNA after treatment predicts early relapse in patients with early-stage non-small cell lung cancer**. *Ann Oncol* 2022,

33(5):500–510.

7. Zhang JT, Liu SY, Gao W, Liu SM, Yan HH, Ji L, Chen Y, Gong Y, Lu HL, Lin JT *et al*: **Longitudinal undetectable molecular residual disease defines potentially cured population in localized non-small cell lung cancer**. *Cancer Discov* 2022, **12**(7):1690–1701.

8. Zhang JT, Liu SY, Gao X, Liu SM, Yan B, Huang C, Jiao Z, Yan HH, Pan Y, Dong S *et al*: **Follow-up analysis enhances understanding of molecular residual disease in localized non-small cell lung cancer**. *Clin Cancer Res* 2025, **31**(7):1305–1314.

9. Qiu B, Guo W, Zhang F, Lv F, Ji Y, Peng Y, Chen X, Bao H, Xu Y, Shao Y *et al*: **Dynamic recurrence risk and adjuvant chemotherapy benefit prediction by ctDNA in resected NSCLC**. *Nat Commun* 2021, **12**(1):6770.

10. Xia L, Pu Q, Kang R, Mei J, Li L, Yang Y, Deng S, Feng G, Deng Y, Gan F *et al*: **Dynamic ctDNA informs whole-course postoperative precise management of NSCLC (LUNGCA study)**. *J Natl Cancer Inst* 2025, **117**(7):1474–1484.

11. Tie J, Cohen JD, Lahouel K, Lo SN, Wang Y, Kosmider S, Wong R, Shapiro J, Lee M, Harris S *et al*: **Circulating tumor DNA analysis guiding adjuvant therapy in stage II colon cancer**. *N Engl J Med* 2022, **386**(24):2261–2272.

12. Tie J, Wang Y, Lo SN, Lahouel K, Cohen JD, Wong R, Shapiro JD, Harris SJ, Khattak A, Burge ME *et al*: **Circulating tumor DNA analysis guiding adjuvant therapy in stage II colon cancer: 5-year outcomes of the randomized DYNAMIC trial**. *Nat Med* 2025, **31**(5):1509–1518.

493

494

495 **Tables**

496 **TABLE 1 |** Patient characteristics at baseline.

497 Abbreviation: MRD, molecular residual disease; ALK, anaplastic lymphoma kinase; EGFR,

498 epidermal growth factor receptor; CT, chemotherapy; TKI, tyrosine kinase inhibitor; ICB,

499 immune checkpoint blockade.

500 **TABLE 2 |** Tumor recurrence and death events.

501 Abbreviation: MRD, molecular residual disease.NR, not reached.NC, could not be

502 calculated.CI, denotes confidence interval. RFS, recurrence-free survival. OS, overall

503 survival.

504

505 **Figures**

506 Fig. 1 Study flow chart and patient enrollment. NSCLC, non-small cell lung cancer; MRD,

507 molecular residual disease; ctDNA, circulating tumor DNA; NGS, next-generation

508 sequencing; CT, chemotherapy; TKI, tyrosine kinase inhibitor; ICB, immune checkpoint

509 blockade.

510 Fig. 2 Heatmap of the top 30 mutated genes from the tumor tissues of the patients. MRD,

511 molecular residual disease.

512 Fig. 3 Kaplan–Meier analysis of survival outcomes in the MRD-guided and standard

513 management groups. (A) Kaplan–Meier curves of RFS in the MRD-guided and standard

514 management groups. (B) Kaplan–Meier curves of OS in the MRD-guided and standard

515 management groups. (C) Kaplan–Meier curves of RFS stratified by landmark ctDNA status.

516 (D) Kaplan–Meier curves of OS stratified by landmark ctDNA status. MRD, molecular

517 residual disease; RFS, recurrence-free survival; OS, overall survival.

518 Fig. 4 Longitudinal analysis of MRD and survival outcome in the MRD-guided cohort. (A)

519 Analysis of the median time from MRD detection of re-positive to imaging relapse for 9

520 patients. Each point on the plot represents a single patient. (B) The changes in ctDNA

521 concentration during treatment for the adjuvant immunotherapy and targeted therapy patients

of the 9 MRD-positive patients in the MRD-guided group. (C) Longitudinal analysis of MRD status (positive or negative) and survival outcome (relapse or death) in the MRD-guided cohort. MRD, molecular residual disease; ALK, anaplastic lymphoma kinase; EGFR, epidermal growth factor receptor; CT, chemotherapy; TKI, tyrosine kinase inhibitor; ICB, immune checkpoint blockade.

Supporting information

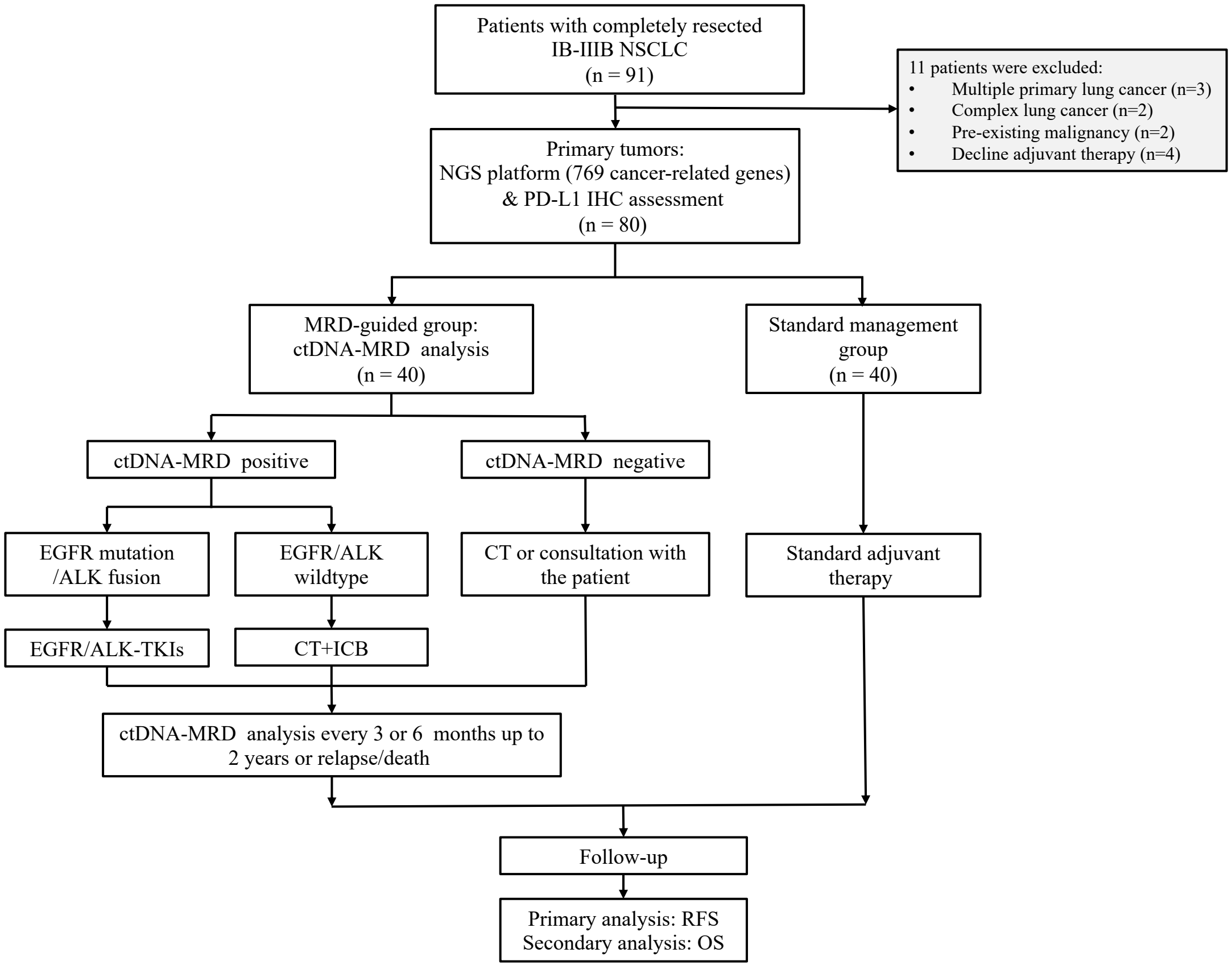
Figure S1. Kaplan–Meier analysis of survival outcomes in the MRD-guided and MRD-negative patients at the landmark time point. a Kaplan–Meier curves of RFS in the EGFR mutations patients. b Kaplan–Meier curves of RFS in the EGFR wild type and no ALK fusion patients. MRD, molecular residual disease; RFS, recurrence-free survival.

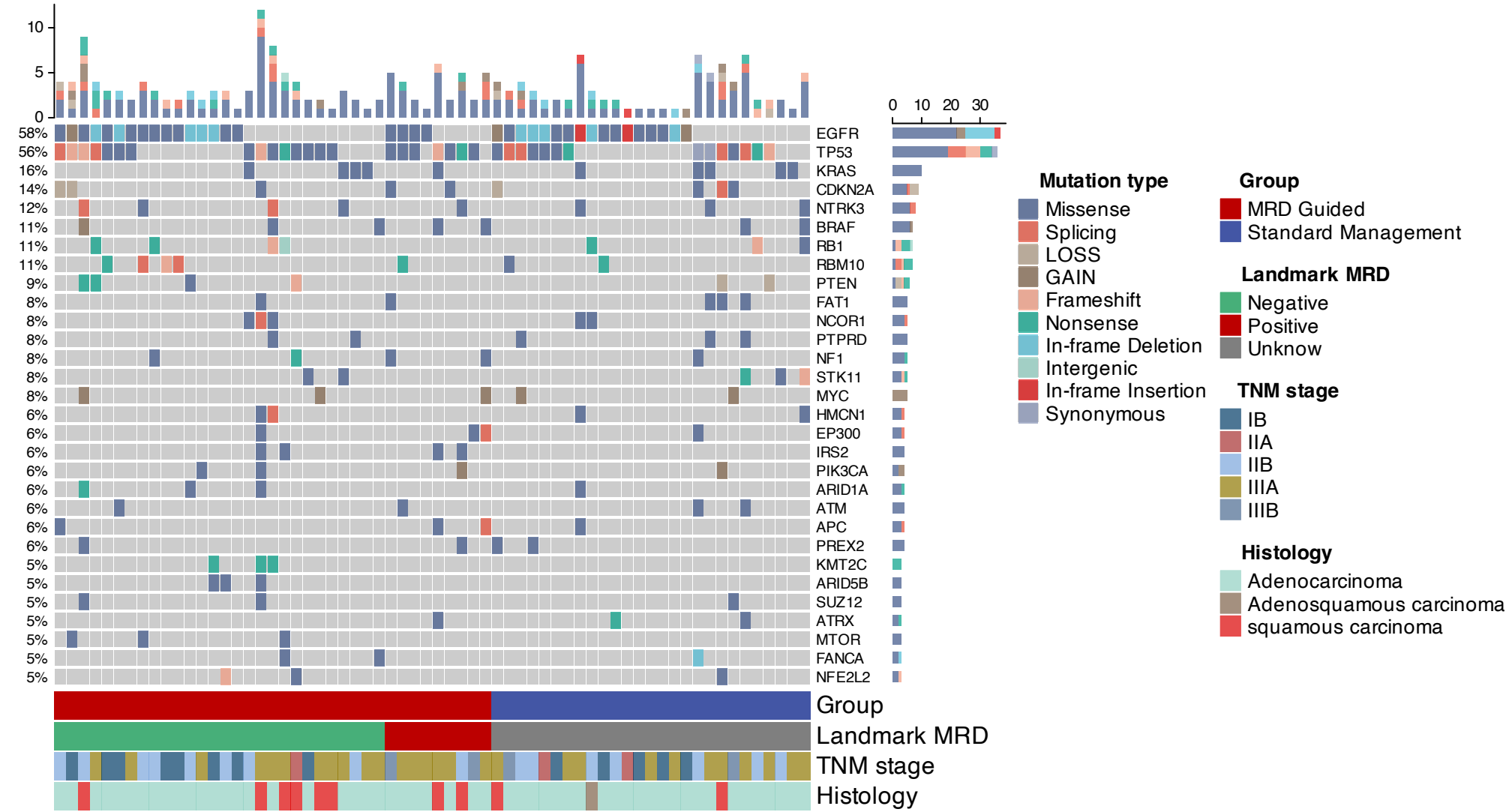
Figure S2. Analysis of two patients' plasma ctDNA mutation profile at recurrence with the original tumor.

Table S1. Details of patient characteristics.

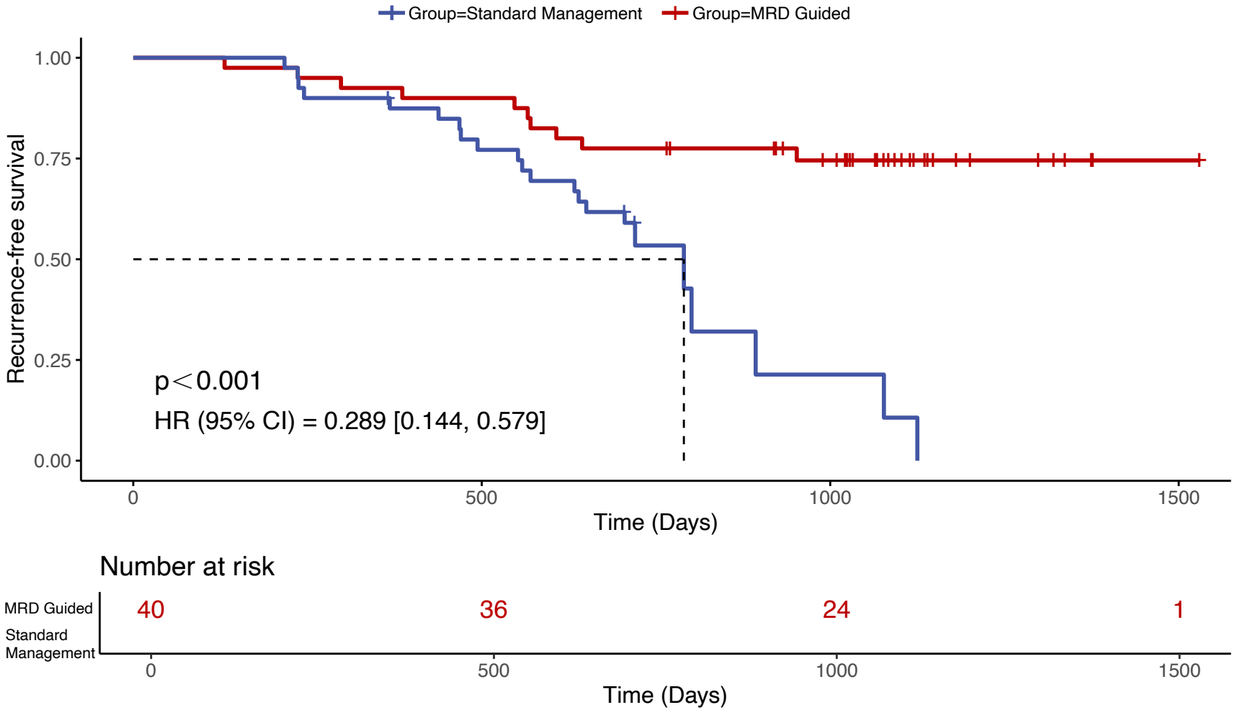
Table S2. Tumor recurrence and death events in the MRD-guided subgroup.

Table S3. Postoperative MRD status and hGE.

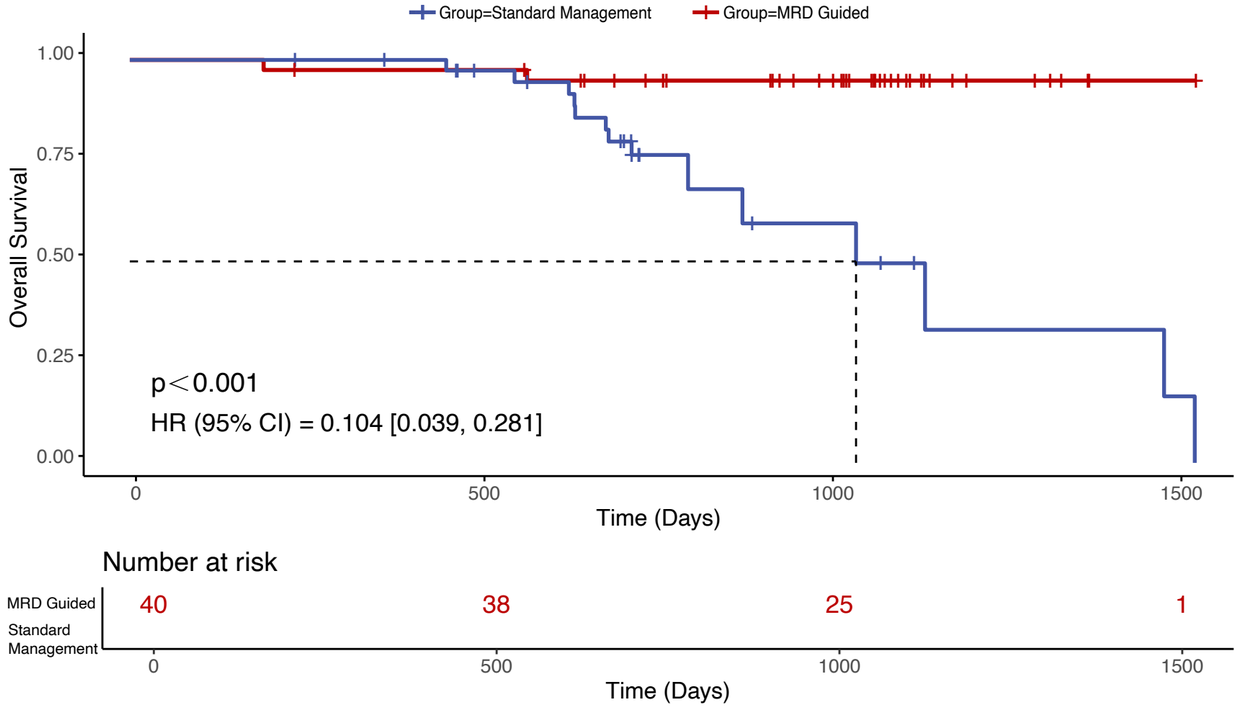

Patients with completely resected IB-IIIB NSCLC (n = 91)
11 patients were excluded:
• Multiple primary lung cancer (n=3)
• Complex lung cancer (n=2)
• Pre-existing malignancy (n=2)
• Decline adjuvant therapy (n=4)
Primary tumors: NGS platform (769 cancer-related genes) & PD-L1 IHC assessment (n = 80)
MRD-guided group: ctDNA-MRD analysis (n = 40)
Standard management group (n = 40)
ctDNA-MRD positive
ctDNA-MRD negative
EGFR mutation /ALK fusion
EGFR/ALK wildtype
CT or consultation with the patient
Standard adjuvant therapy
EGFR/ALK-TKIs
CT+ICB
ctDNA-MRD analysis every 3 or 6 months up to 2 years or relapse/death
Follow-up
Primary analysis: RFS
Secondary analysis: OS


10
5
0
0 10 20 30
58% EGFR
56% TP53
16% KRAS
14% CDKN2A
12% NTRK3
11% BRAF
11% RB1
11% RBM10
9% PTEN
8% FAT1
8% NCOR1
8% PTPRD
8% NF1
8% STK11
8% MYC
6% HMCN1
6% EP300
6% IRS2
6% PIK3CA
6% ARID1A
6% ATM
6% APC
6% PREX2
5% KMT2C
5% ARID5B
5% SUZ12
5% ATRX
5% MTOR
5% FANCA
5% NFE2L2
Group
Landmark MRD
TNM stage
Histology
Mutation type
Missense
Splicing
LOSS
GAIN
Frameshift
Nonsense
In-frame Deletion
Intergenic
In-frame Insertion
Synonymous
Group
MRD Guided
Standard Management
Landmark MRD
Negative
Positive
Unknow
TNM stage
IB
IIA
IIB
IIIA
IIIB
Histology
Adenocarcinoma
Adenosquamous carcinoma
squamous carcinoma

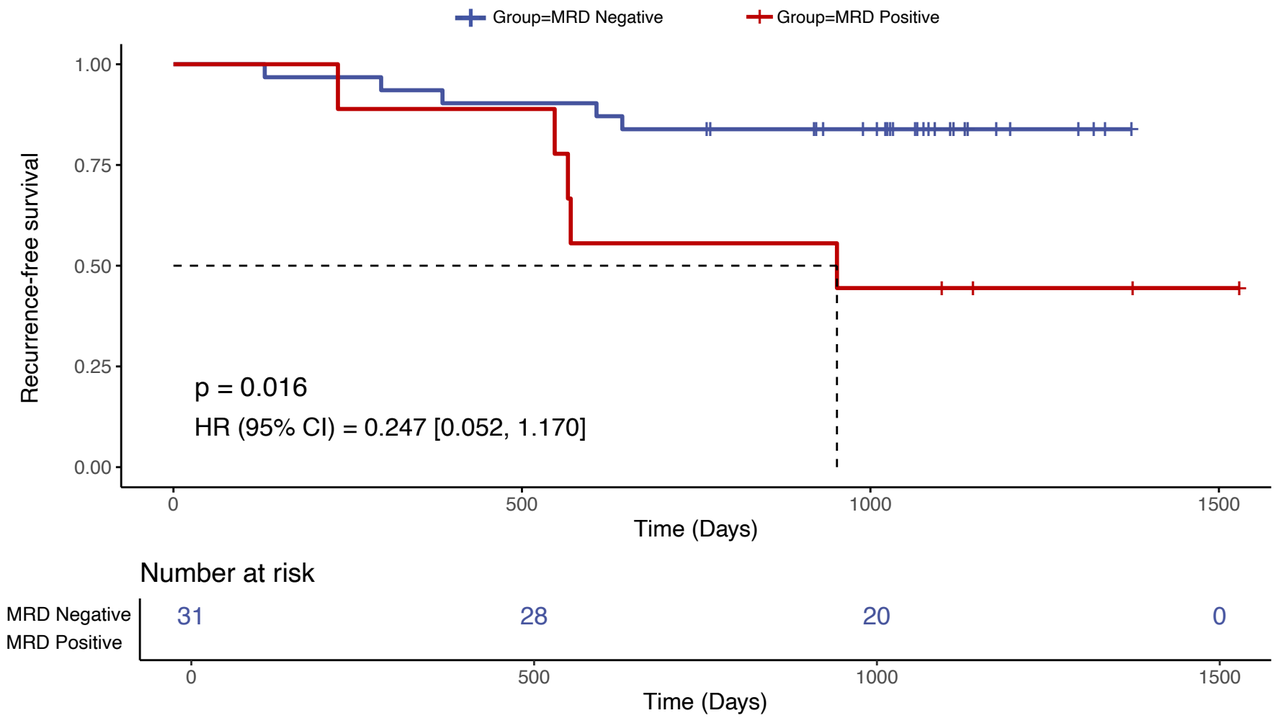
(A)


Group=Standard Management
Group=MRD Guided
Recurrence-free survival
1.00
0.75
0.50
0.25
0.00
p＜0.001
HR (95% CI) = 0.289 [0.144, 0.579]
0
500
1000
1500
Time (Days)
Number at risk
MRD Guided
Standard Management
40
36
24
1
0
500
1000
1500
Time (Days)

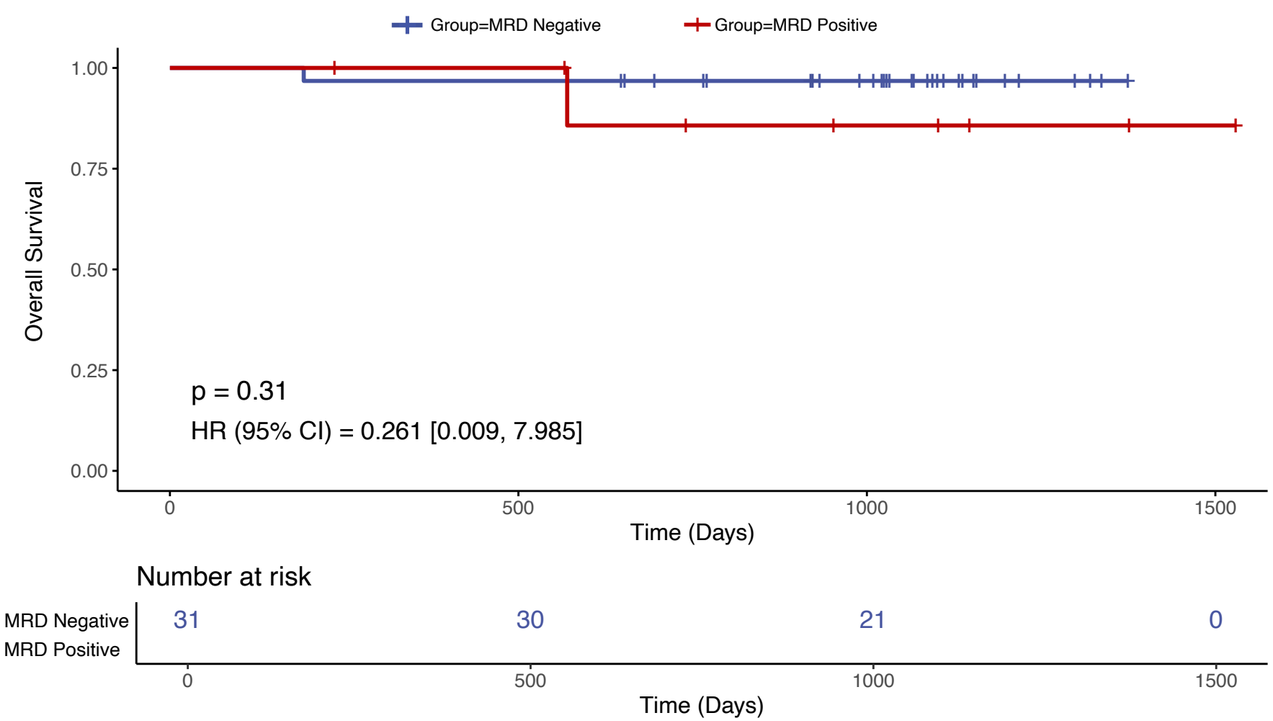
(B)


Group=Standard Management
Group=MRD Guided
Overall Survival
1.00
0.75
0.50
0.25
0.00
p＜0.001
HR (95% CI) = 0.104 [0.039, 0.281]
0
500
1000
1500
Time (Days)
Number at risk
MRD Guided
Standard Management
40
38
25
1
0
500
1000
1500
Time (Days)

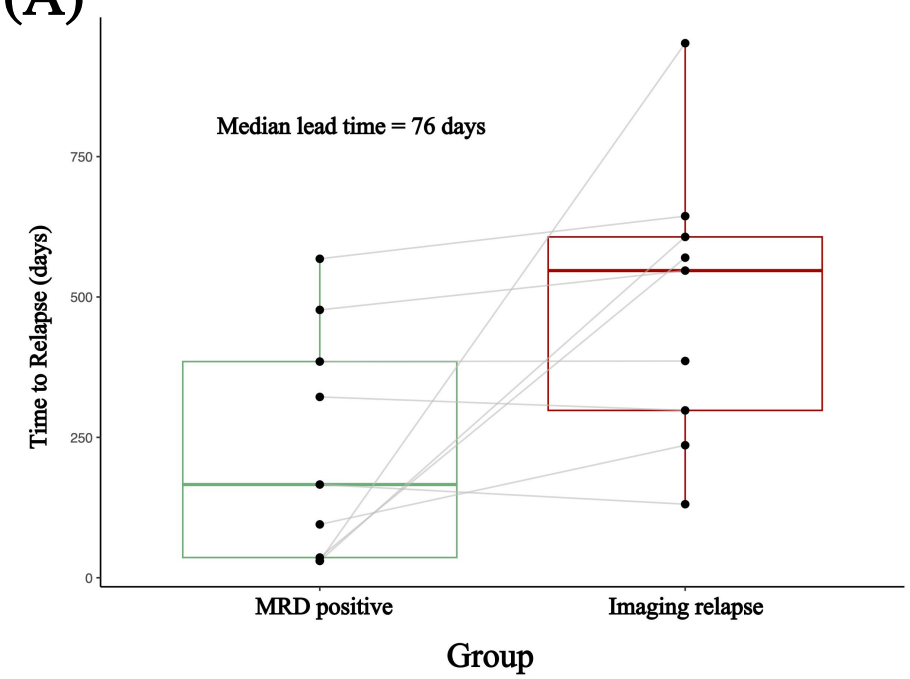
(C)


Group=MRD Negative
Group=MRD Positive
Recurrence-free survival
1.00
0.75
0.50
0.25
0.00
p = 0.016
HR (95% CI) = 0.247 [0.052, 1.170]
0
500
1000
1500
Time (Days)
Number at risk
MRD Negative
MRD Positive
31
28
20
0
0
500
1000
1500
Time (Days)

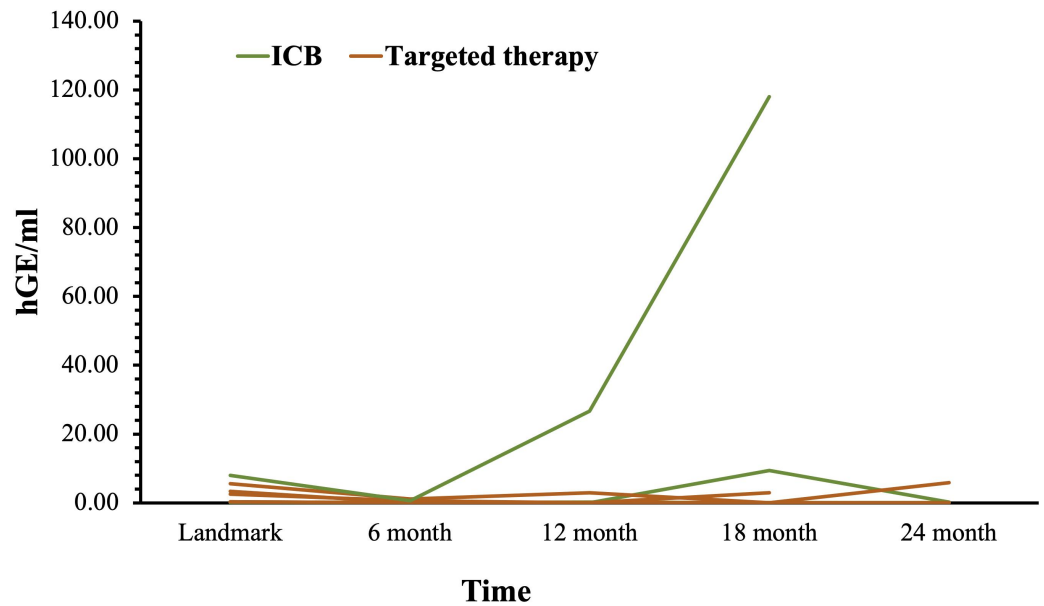
(D)


Group=MRD Negative
Group=MRD Positive
Overall Survival
1.00
0.75
0.50
0.25
0.00
p = 0.31
HR (95% CI) = 0.261 [0.009, 7.985]
0
500
1000
1500
Time (Days)
Number at risk
MRD Negative
MRD Positive
31
30
21
0
0
500
1000
1500
Time (Days)

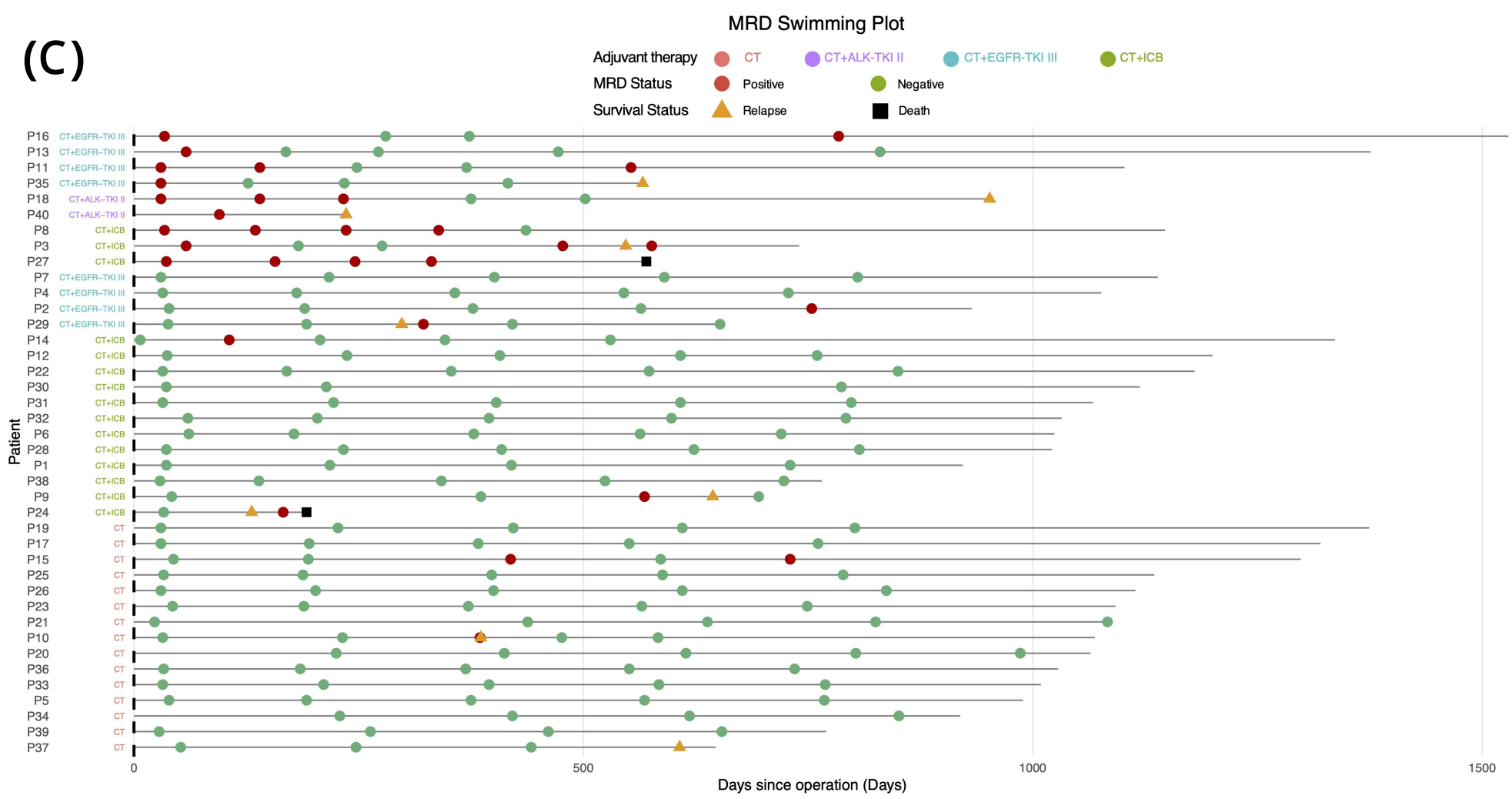
(A)


Median lead time = 76 days
Time to Relapse (days)
750
500
250
0
MRD positive
Imaging relapse
Group

(B)


ICB
Targeted therapy
hGE/ml
140.00
120.00
100.00
80.00
60.00
40.00
20.00
0.00
Landmark
6 month
12 month
18 month
24 month
Time

(C)


MRD Swimming Plot
Adjuvant therapy
CT
CT+ALK-TKI II
CT+EGFR-TKI III
CT+ICB
MRD Status
Positive
Negative
Survival Status
Relapse
Death
Patient
P16 CT+EGFR-TKI III
P13 CT+EGFR-TKI III
P11 CT+EGFR-TKI III
P35 CT+EGFR-TKI III
P18 CT+ALK-TKI II
P40 CT+ALK-TKI II
P8 CT+ICB
P3 CT+ICB
P27 CT+ICB
P7 CT+EGFR-TKI III
P4 CT+EGFR-TKI III
P2 CT+EGFR-TKI III
P29 CT+EGFR-TKI III
P14 CT+ICB
P12 CT+ICB
P22 CT+ICB
P30 CT+ICB
P31 CT+ICB
P32 CT+ICB
P6 CT+ICB
P28 CT+ICB
P1 CT+ICB
P38 CT+ICB
P9 CT+ICB
P24 CT+ICB
P19 CT
P17 CT
P15 CT
P25 CT
P26 CT
P23 CT
P21 CT
P10 CT
P20 CT
P36 CT
P33 CT
P5 CT
P34 CT
P39 CT
P37 CT
0
500
1000
1500
Days since operation (Days)

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Table1.xlsx
- Table2.xlsx
- FigureS1.pdf
- FigureS2.pdf
- TableS2.xlsx
- TableS1.xlsx
- TableS3.xlsx