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Dynamic Surveillance of Minimal Residual Disease via a Tumor-Informed Circulating Tumor DNA Assay for Outcome Prediction in Small-Cell Lung Cancer: A Prospective Pilot Study

Qiuyi Zhang [1], Die Dai [2], Yikun Yang [1], Lihong Guo [1], Jiesheng Su [1], Shiqi Lyu [1], Suni Huang [1], Meng Zhang [2] and Jianhua Chang [1,*]

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Abstract

Background: Small-cell lung cancer (SCLC) represents an aggressive malignancy associated with a poor prognosis, underscoring the critical demand for enhanced monitoring methodologies. Circulating tumor DNA (ctDNA) constitutes a promising non-invasive biomarker; however, reports employing highly sensitive, tumor-informed assays in SCLC remain scarce. This investigation aimed to assess the clinical utility of a personalized ctDNA monitoring strategy for predicting therapeutic outcomes and resistance in SCLC patients. **Methods**: This prospective, observational study enrolled patients diagnosed with unresectable SCLC. Whole exome sequencing was conducted on baseline tumor specimens to design customized 16-plex multiplex PCR panels. Serial blood samples were obtained at baseline, at six-week intervals during treatment, and upon disease progression. Detection of ctDNA-based minimal residual disease (MRD) was performed using a tumor-informed assay (Huajianwei® bespoke MRD) with ultra-deep sequencing. **Results**: Among seven evaluable patients, the baseline ctDNA-MRD positivity rate was 100%. A significant positive correlation was observed between baseline ctDNA levels and radiographic tumor burden ($r = 0.821$, $P = 0.034$). Longitudinal analysis indicated that patients exhibiting an early decline in MRD levels (n=5) demonstrated a trend toward superior progression-free survival (PFS) compared to those with an MRD increase (n=2) ($P = 0.0665$, hazard ratio (HR) = 0.24 (95% CI: 0.02 - 3.19)). Notably, elevation in MRD preceded radiographic progression by as much as 135 days in certain instances. **Conclusions**: Tumor-informed ctDNA-MRD monitoring effectively mirrors tumor burden and offers early prediction of treatment response and clinical outcomes in SCLC. ctDNA kinetics may serve as a crucial prognostic indicator, presenting the potential to inform personalized management approaches and facilitate earlier therapeutic interventions compared to conventional imaging techniques.

Keywords: small-cell lung cancer; circulating tumor DNA; minimal residual disease; liquid biopsy; tumor-informed

1. Introduction

Lung cancer remains the foremost cause of cancer-associated mortality globally [1]. Primary lung cancer is primarily categorized into two major pathological subtypes: non-small cell lung cancer (NSCLC) and small-cell lung cancer (SCLC). Although SCLC constitutes only 15% to 20% of all primary lung cancer cases [2], it is distinguished by a high degree of malignancy, poor differentiation, and rapid proliferation, contributing to an exceptionally poor prognosis with a five-year survival rate below 5% [3,4]. Given its aggressive characteristics, there exists a pressing and substantial clinical

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requirement for effective strategies for early disease detection, real-time assessment of therapeutic response, and accurate prognostic forecasting.

Circulating tumor DNA (ctDNA) has emerged as a precise, non-invasive predictive biomarker with considerable potential across the spectrum of cancer management, encompassing early screening, diagnosis, prognostic assessment, and treatment monitoring [5–7]. In SCLC, dynamic alterations in ctDNA levels function as a sensitive metric for evaluating therapeutic response and forecasting survival outcomes. For example, an early reduction in ctDNA levels exceeding two-fold during chemotherapy is significantly correlated with extended progression-free survival (PFS) and overall survival (OS) [8]. Similarly, patients who achieve clearance of the molecular tumor burden index (mTBI) following induction chemotherapy demonstrate prolonged OS [9]. Moreover, ctDNA status can aid in identifying patients most likely to respond to immunotherapy. An integrated algorithm that incorporates serial ctDNA measurements during chemoradiotherapy, prophylactic cranial irradiation, and radiographic regression has been demonstrated to effectively predict progression risk and pinpoint high-risk limited-stage (LS)-SCLC populations who would derive maximal benefit from consolidation immunotherapy [10].

Research has established that the molecular response of ctDNA, characterized by the clearance of mutations and copy number variations, can predict clinical outcomes approximately four weeks earlier than conventional imaging modalities on average; patients exhibiting a molecular response demonstrate significantly improved PFS and OS [11]. Conversely, elevated baseline ctDNA levels and the persistence of ctDNA positivity following consolidation immunotherapy are associated with an unfavorable prognosis [10,12,13]. Extensive evidence underscores the critical role of minimal residual disease (MRD) in predicting outcomes for patients with locally advanced or advanced lung cancer [14,15]. Next-generation sequencing (NGS)-based ctDNA detection serves as the primary methodology for MRD monitoring [16]. In solid tumors, two principal strategies are employed for MRD detection: tumor-informed and tumor-agnostic analysis. The tumor-informed approach represents a highly sensitive and specific technique [17]. It involves performing NGS on blood samples using a predefined panel of oncogenic mutations or employing machine learning algorithms to identify potential tumor-derived ctDNA variants. Furthermore, by conducting comparative analyses between tumor and normal tissues and utilizing small, targeted panels (typically spanning a few kilobases) sequenced to ultra-high depth (often exceeding 100,000x coverage) [18], the tumor-informed method effectively distinguishes ctDNA signals of tumor origin from background noise, such as clonal hematopoiesis or germline variants. This significantly enhances both the sensitivity and specificity of detection.

Overall, the tumor-informed approach is supported by more robust and extensive clinical validation, whereas the tumor-agnostic method requires further evidentiary support. Although prior studies have demonstrated correlations between ctDNA levels and treatment efficacy or prognosis in SCLC patients [9,11,13], reports utilizing the highly sensitive tumor-informed methodology remain limited. In this study, we performed whole exome sequencing (WES) on SCLC tissue samples to design and synthesize customized panels. This enabled personalized and dynamic monitoring of MRD in peripheral blood samples throughout the treatment course. The objective was to investigate the clinical utility of this customized plasma ctDNA-based MRD monitoring approach in SCLC patients and its significance in predicting therapeutic outcomes and the emergence of resistance.

2. Materials and Methods

2.1. Study Design and Patients

This was a prospective, observational, single-cohort study. The research flowchart is shown in Figure 1 (Created with BioGDP.com [19]). Inclusion criteria were: (1) age ≥ 18 years; (2) histopathologically confirmed unresectable SCLC; (3) a life expectancy exceeding three months; (4) availability of sufficient tumor tissue for WES; (5) ability to provide adequate peripheral blood samples for ctDNA-based MRD detection during treatment; and (6) presence of at least one

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measurable lesion according to Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1. The primary endpoint was the correlation between ctDNA-based MRD status and radiologically assessed PFS.

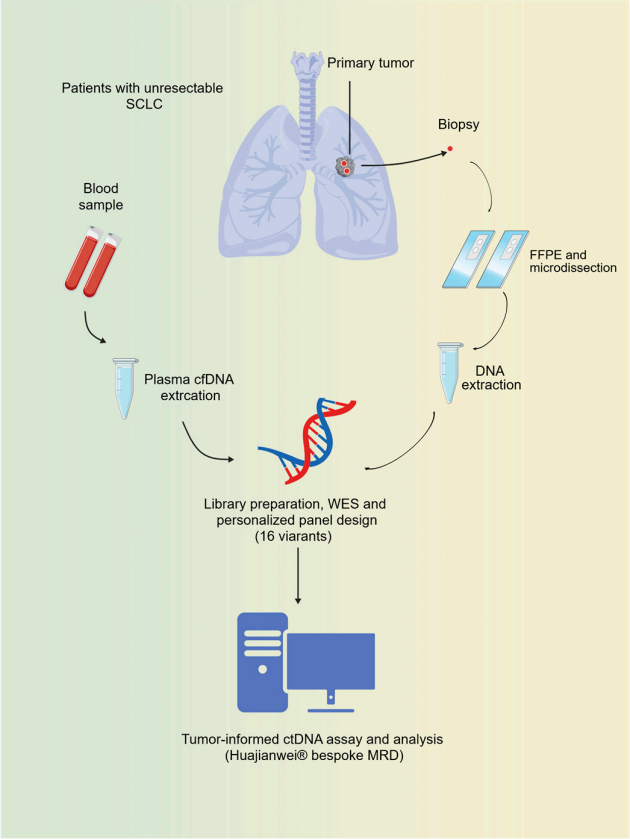

Figure 1. The flowchart of this study. Whole exome sequencing from 20 participants was conducted on baseline tumor specimens. Ultimately, personalized panels were successfully customized for seven patients. Serial peripheral blood samples from these patients were collected at baseline, every six weeks, and upon disease progression. Detection of ctDNA-based MRD was performed via a tumor-informed assay with ultra-deep sequencing. The trends in these measurements were then analyzed in relation to clinical efficacy and outcomes. SCLC, small-cell lung cancer; cfDNA, cell-free DNA; FFPE, formalin-fixed paraffin-embedded; WES, whole exome sequencing. MRD, minimal residual disease.

2.2. Sample Collection

Baseline tumor formalin-fixed paraffin-embedded (FFPE) samples were collected (consisting of 15-20 sections, each 5 μm thick). A total of 20 mL of peripheral venous blood was collected in Streck tubes at baseline, every six weeks during treatment, and at the time of disease progression. These samples were transported to the BGI Tianjin Specimen Center within 72 hours of collection.

2.3. Efficacy Evaluation

Computed tomography (CT) and/or magnetic resonance imaging (MRI) was performed every six weeks (± seven days) during treatment. Therapeutic efficacy was evaluated according to RECIST version 1.1. PFS was defined as the time from treatment initiation to the first documentation of objective tumor progression or death from any cause, whichever occurred first. OS was defined as the time from pathological diagnosis to death from any cause. Patients who were lost to follow-up were censored at the date of their last known contact. For those who remained alive at the study cutoff date, survival data were censored on that date.

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2.4. Extraction of Cell-free DNA (cfDNA)

CfDNA, including ctDNA, was isolated from human plasma using the QIAamp Circulating Nucleic Acid Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. Briefly, the plasma samples were subjected to enzymatic lysis with QIAGEN Proteinase K and Buffer ACL (supplemented with carrier RNA) at 60°C for 30 minutes to ensure the complete release of nucleic acids from proteins and vesicles. Binding conditions were adjusted by the addition of Buffer ACB, and the resulting lysate was drawn through a QIAamp Mini column using the QIAvac 24 Plus vacuum manifold. The silica membrane was sequentially washed with Buffers ACW1, ACW2, and 96 - 100% ethanol to efficiently remove residual contaminants and inhibitors. Finally, the purified nucleic acids were eluted in the Buffer AVE. The extracted DNA was either used immediately for downstream applications or stored at -20°C for long-term stability.

2.5. WES at Baseline

Fresh tumor tissue samples were FFPE, stained with hematoxylin and eosin (H&E), and subjected to pathological review to confirm a tumor content of at least 30%. Genomic DNA (gDNA) was extracted from tumor tissue using the QIAamp DNA FFPE Tissue Kit and from matched whole blood samples using the MagPure Buffy Coat DNA Midi KF Kit, followed by quantification with a Qubit 3.0 fluorometer.

For library preparation, approximately 400 ng of tumor gDNA and 200 ng of germline DNA underwent fragmentation, end repair, 3'-adenylation, and adapter ligation. The resulting libraries were pooled and hybridized to the Quanxi® pan-cancer whole exome panel. Sequencing was performed on the MGISEQ-2000 platform, achieving a mean coverage depth of 500× for tumor samples and 200× for normal controls. In addition to identifying single nucleotide variants (SNVs), insertions/deletions (indels), copy number variations (CNVs), and rearrangements, the analysis included evaluations of tumor mutational burden (TMB) and microsatellite instability (MSI). To facilitate personalized monitoring, sequencing data were processed through the Signatera™ WES pipeline to select 16 prioritized SNVs per patient. Based on these variants, 16 specific multiplex PCR primer pairs were optimized, designed, and synthesized at BGI Tech Solutions for subsequent ctDNA detection.

2.6. Personalized Tumor-Informed ctDNA Detection

At each monitoring time point, 20 mL of peripheral blood was collected in cfDNA BCT® tubes (Streck) and processed using the Huajianwei® bespoke MRD assay (BGI Genomics), a tumor-informed approach based on the Signatera™ platform as previously described [20,21]. cfDNA was extracted from a median plasma volume of 8 mL using the QIAamp Circulating Nucleic Acid Kit (Qiagen). Subsequently, 10 - 66 ng of cfDNA was used for library preparation with patient-specific primer sets. In this method, the 16 prioritized SNVs previously identified via WES were targeted. Personalized 16-plex primer pairs were employed to amplify universal cfDNA libraries, which were then sequenced on the MGISEQ-2000 platform to a median depth exceeding 110,000× per amplicon. Data analysis was performed using the Signatera™ plasma pipeline (Natera), and MRD positivity was defined by the detection of at least two patient-specific variants. ctDNA burden was quantified as mean tumor molecules per milliliter of plasma (MTM/mL). The assay demonstrates a sensitivity of >95% for detecting variants at a 0.03% variant allele frequency (VAF).

2.7. Statistic Analysis

Survival analysis was conducted using the Kaplan-Meier method, and differences between groups were compared with the log-rank test. The correlation between ctDNA levels and the sum of diameters of target lesions was analyzed using Spearman's rank correlation coefficient. All statistical tests were two-sided, and a P-value < 0.05 was considered statistically significant. Data analysis was performed using R software (version 4.1).

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3. Results

3.1. Patient Characteristics

A total of 20 patients were enrolled in this study. However, personalized panels were successfully designed from WES data for only seven participants, whose clinical data are summarized in Table 1. The cohort was predominantly male, with a median age of 60 years (range: 58 to 74 years). All participants had a documented smoking history, with substantial cumulative exposure ranging from 15 to 122 pack-years. Regarding disease severity at baseline, two patients presented with limited-stage disease, while the remaining five had extensive-stage disease. The majority of the cohort was diagnosed with pure SCLC; one patient was diagnosed with combined SCLC (C-SCLC).

Table 1. Patient characteristics.

Patient	Age	Sex	Smoking, pack-years	Pathological diagnosis	Tumor stage	Treatment	Lines	PFS (months)	Tumor response	TMB (Muts/Mb)	PD-L1 (TPS)
LTJ	58	Male	80	SCLC	LS	platinum-etoposide with thoracic radiotherapy	1st	10.0	SD	7.97	NA
MCJ	60	Male	40	SCLC	ES	platinum-etoposide with PD-L1 inhibitor	1st	5.8	SD	5.19	0%
ZDL	59	Male	40	SCLC	ES	platinum-etoposide with thoracic radiotherapy	2nd	8.3	PR	5.01	NA
HSP	60	Male	122	SCLC	ES	platinum-etoposide with PD-1 inhibitor	3rd	5.2	SD	61.8	NA
YLQ	61	Male	60	SCLC	ES	anlotinib	5th	3.6	PD	4.81	<1%
HCJ	60	Male	15	C-SCLC	ES	platinum-etoposide	3rd	1.0	PD	8.25	<1%
SZ	74	Male	30	SCLC	LS	platinum-etoposide with thoracic radiotherapy	1st	19.3	PR	11.17	NA

Abbreviations: SCLC, small-cell lung cancer; C-SCLC, combined small cell lung cancer; LS, limited stage; ES, extensive stage; PFS, progression-free survival; SD, stable disease; PD, progressive disease; PR, partial response. TMB, tumor mutational burden; PD- (L) 1, programmed cell death (ligand) -1; TPS, Tumor Proportion Scores; NR, not reached. NA, not available.

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The treatment administered varied across different lines of therapy. Six patients received platinum-based combination chemotherapy, frequently combined with thoracic radiotherapy, programmed cell death ligand-1 (PD-L1) inhibitors, or PD-1 inhibitors. One patient was administered anti-angiogenesis monotherapy as a fifth-line treatment. Clinical outcomes exhibited variability, with PFS durations spanning from 1.0 to 19.3 months. The best overall responses comprised partial response (PR) in two patients, stable disease (SD) in three patients, and progressive disease (PD) in two patients. TMB also demonstrated considerable heterogeneity, ranging from 4.81 to 61.8 mutations per megabase. PD-L1 expression levels were predominantly low or not assessed, with documented Tumor Proportion Scores (TPS) of either 0% or less than 1%.

3.2. Clinical Relevance of Baseline WES and ctDNA Analysis

WES was conducted to delineate the baseline genomic profile of the enrolled patients. Evaluation of mutation frequency identified *TP53* and *RB1* as the most commonly altered genes, present in 100% and 85.7% of the cohort, respectively (Figure 2). Other frequently observed mutations included *RYR2* (approximately 80%), *LRP1B* (approximately 70%), and *ZFHX4* (approximately 60%). The gene mutation waterfall plot revealed a predominance of missense mutations among the detected genes, accompanied by frameshift, nonsense, and splice-site variants. Notably, the baseline positivity rate for ctDNA-based MRD detection was 100%, as ctDNA was successfully detected in all seven patients prior to the commencement of study treatment.

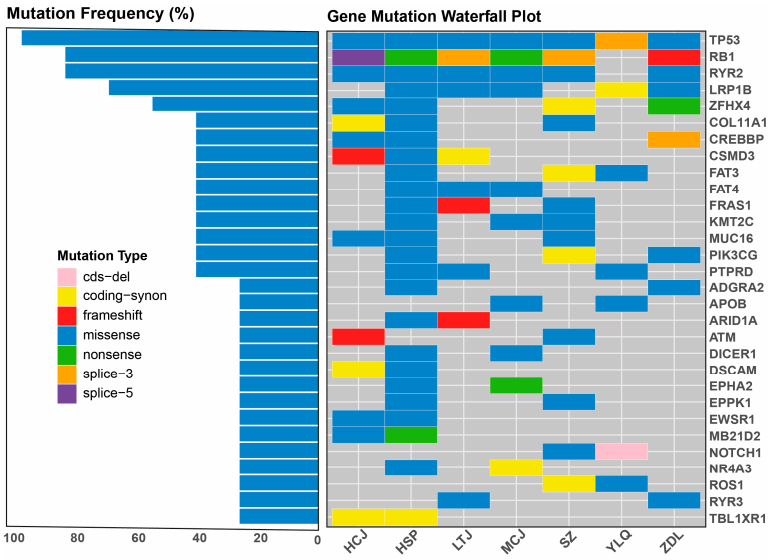

Figure 2. Genomic profile of the enrolled patients by WES (n=7). *TP53* and *RB1* were the most commonly altered genes, present in 100% and 85.7% of the cohort, respectively. Other frequently observed mutations included *RYR2, LRP1B*, and *ZFHX4*. WES, whole exome sequencing.

Beyond genomic characterization, we further examined the association between ctDNA levels and physical tumor burden. Spearman's rank correlation analysis indicated a significant positive linear relationship between baseline ctDNA quantification and the sum of the longest diameters of target lesions, producing a correlation coefficient of 0.821 (P = 0.034). These results, depicted in a scatter plot (Figure 3), imply that ctDNA can effectively function as a molecular proxy for overall tumor volume.

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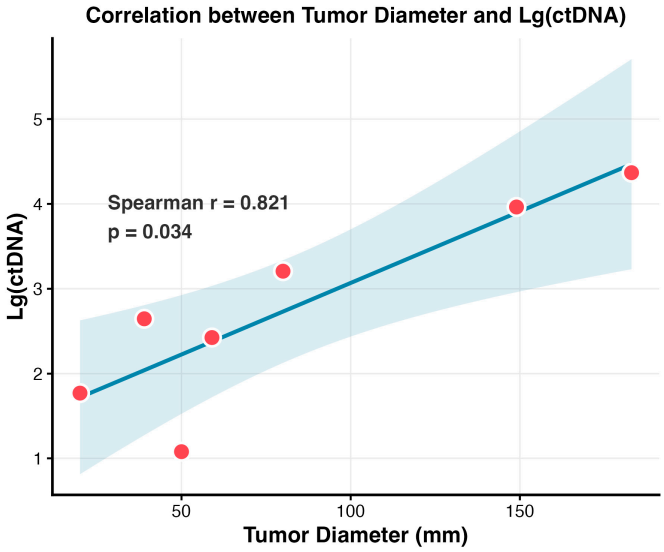

Figure 3. Spearman's rank correlation between baseline ctDNA levels and the longest diameters of target lesions.

3.3. Longitudinal Assessment of ctDNA-MRD During Treatment

Longitudinal monitoring of MRD was performed for all seven patients to assess the predictive utility of ctDNA dynamics. A total of 23 blood samples were collected for analysis. Based on MRD trends observed at the second monitoring time point (the first on-treatment evaluation), patients were categorized into two distinct groups: the MRD-decrease group (n=5) and the MRD-increase group (n=2), as shown in the swimmer plot (Figure 4). Patients in the MRD-decrease group achieved either PR or SD as their best response, with a tendency toward longer PFS, extending up to 19.3 months. Although the limited cohort size yielded a P-value of 0.0665, the hazard ratio (HR) of 0.24 (95% confidence interval: 0.02 - 3.19) suggests a pronounced trend toward a reduced risk of disease progression in patients with declining ctDNA levels (Figure 5). Remarkably, one patient attained sustained MRD clearance after three cycles of sequential chemoradiotherapy; this individual remains alive with an OS currently surpassing 30 months. In contrast, the two patients demonstrating an early increase in MRD experienced rapid disease progression, with PFS durations of only 5.2 and 1.0 months, respectively. These findings underscore that early on-treatment ctDNA-MRD kinetics may constitute a crucial indicator of therapeutic response and long-term prognosis in SCLC patients. Moreover, our longitudinal data suggest that MRD elevation can act as an early warning signal, preceding radiographic disease progression by up to 135 days.

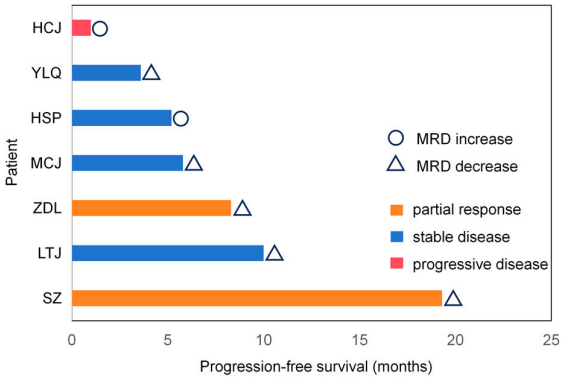

Figure 4. Swimmer plot of the participants. MRD, minimal residual disease.

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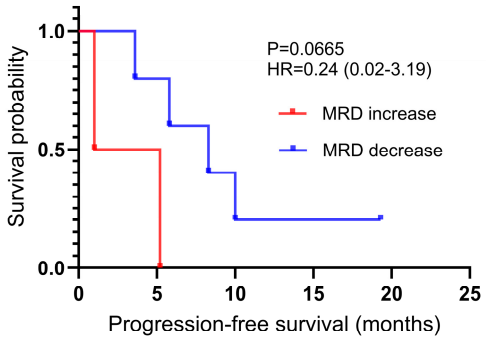

Figure 5. Kaplan – Meier survival curves of the MRD increase and decrease cohorts. Patients in the MRD-decrease exhibited a tendency toward longer PFS. MRD, minimal residual disease; PFS, progression-free survival.

4. Discussion

This study represents an innovative application of a tumor-informed methodology for dynamic MRD monitoring in SCLC patients, offering a preliminary investigation into the relationship between ctDNA-MRD kinetics and clinical efficacy. Despite the inherent constraints of a limited sample size, our findings reveal a distinct clinical signal: patients achieving a significant reduction or clearance of MRD during treatment demonstrated a marked trend toward superior PFS. This was exemplified most strikingly by a single patient who achieved MRD clearance after only three cycles of chemotherapy and subsequently attained an OS exceeding 30 months. This case strongly suggests that MRD clearance could serve as a surrogate marker for "molecular complete response", potentially offering prognostic insights as significant as radiographic complete response. Furthermore, the positive correlation identified between ctDNA and tumor burden provides empirical support for ctDNA as a quantitative surrogate in SCLC management.

From a mechanistic perspective, the dynamic fluctuations of MRD likely reflect the differential sensitivity of specific tumor clones to cytotoxic agents. Given that SCLC is characterized by high proliferative activity and a propensity for early systemic dissemination, traditional imaging often fails to capture micro-residual disease or occult metastases. As genetic fragments released into the bloodstream via apoptosis or necrosis, ctDNA may serve as a more sensitive indicator, capable of reflecting the evolution of total tumor burden earlier and more accurately than conventional methods. The observed correlation between ctDNA levels and the sum of target lesion diameters validates this biological rationale and provides a theoretical foundation for future trials exploring whether ctDNA-MRD status could justify extending intervals between radiographic evaluations.

When compared with existing literature, our results align with several prospective cohorts in both non-small-cell lung cancer (NSCLC) and SCLC. For instance, previous research by Chaudhuri et al. noted that post-treatment ctDNA status was highly associated with recurrence risk in LS-SCLC [6]. Similarly, research by Moding et al. confirmed that long-term survival rates for patients achieving MRD clearance were significantly higher than for those who do not [22]. By replicating these findings, our study suggests that the prognostic stratification value of dynamic MRD monitoring may be a universal feature that transcends specific disease stages and is applicable across the SCLC spectrum.

We must acknowledge that the small sample size is the primary limitation of this study. Additionally, the optimal timing and thresholds for MRD monitoring remain unstandardized; the trend-based classification at the second monitoring point used in this study remains exploratory. However, despite these limitations, the high degree of data integrity and the biological consistency of our results underscore the value of this work as early exploratory evidence.

Future research should pivot toward several key directions to build upon these preliminary findings. First, the initiation of prospective, multicenter cohorts is necessary to systematically

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evaluate the predictive efficacy of MRD kinetics at various clinical milestones. Second, there is a clear need to explore MRD-driven adaptive therapeutic strategies, such as treatment de-escalation for those with durable MRD clearance or treatment intensification for those with persistent molecular positivity. Finally, researchers should focus on integrated modeling that combines genomic features with MRD dynamics to enhance personalized prognostic precision. It is worth noting that while the prospects are broad, ctDNA detection in SCLC still faces challenges. For example, *TP53* mutations can be detected in the cfDNA of non-cancerous populations due to clonal hematopoiesis of indeterminate potential [23], limiting its use in early screening. Additionally, in heavily pre-treated patients, ctDNA dynamics may show a poor correlation with radiographic responses under certain combination therapies [23], suggesting its value may vary by treatment regimen and clinical context. Future studies are needed to further standardize detection protocols and validate clinical utility in large-scale trials across diverse therapeutic scenarios.

5. Conclusions

This investigation demonstrates that dynamic MRD monitoring via ctDNA effectively mirrors fluctuations in tumor burden and is associated with clinical outcomes in SCLC patients. As a convenient and efficient liquid biopsy approach, ctDNA analysis offers significant utility for real-time disease surveillance and prognostic stratification. These findings support the potential of ctDNA to enhance precision and personalized treatment strategies for SCLC.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org, Figure S1: Raw data of the Spearman's rank correlation coefficient; Figure S2: Data of the PFS and ctDNA-MRD levels. Figure S3: Raw data of WES.

Author Contributions: Conceptualization, J.C.; methodology, Q.Z and D.D.; software, Q.Z., J.S. and D.D; validation, J.C.; formal analysis, M.Z.; investigation, X.X.; data curation, Y.Y., L.G, S.H. and S.L.; writing—original draft preparation, Q.Z.; writing—review and editing, D.D.; supervision, J.C.; project administration, J.C. and Q.Z.; funding acquisition, J.C and Q.Z. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki, and approved by the Ethics Committee of Cancer Hospital Chinese Academy of Medical Sciense, Shenzhen (YLQX2023-1-2; October 23, 2023).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The original contributions presented in this study are included in the supplementary material. Further inquiries can be directed to the corresponding author.

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

SCLC	Small-cell lung cancer
ctDNA	circulating tumor DNA
PFS	progression-free survival
OS	overall survival
mTBI	molecular tumor burden index
LS-SCLC	limited-stage small-cell lung cancer
MRD	minimal residual disease

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NGS	next-generation sequencing
WES	whole exome sequencing
RECIST	Response Evaluation Criteria in Solid Tumors
FFPE	formalin-fixed paraffin-embedded
CT	computed tomography
MRI	magnetic resonance imaging
cfDNA	cell-free DNA
H&E	hematoxylin and eosin
gDNA	genomic DNA
SNVs	single nucleotide variants
indels	insertions/deletions
CNVs	copy number variations
TMB	tumor mutational burden
MSI	microsatellite instability
MTM/mL	mean tumor molecules per milliliter
VAF	variant allele frequency
PD-(L)1	programmed cell death (ligand) -1
PR	partial response
SD	stable disease
PD	progressive disease
TPS	Tumor Proportion Scores
HR	hazard ratio
NSCLC	non-small-cell lung cancer

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