Circulating tumor DNA- and cancer tissue-based next-generation sequencing reveals comparable consistency in targeted gene mutations for advanced or metastatic non-small cell lung cancer

Weijia Huang[1,2], Kai Xu[1,2], Zhenkun Liu[1,2], Yifeng Wang[1,2], Zijia Chen[1,2], Yanyun Gao[3,4], Renwang Peng[3,4], Qinghua Zhou[1,2]

[1]Lung Cancer Center/Lung Cancer Institute, West China Hospital, Sichuan University, Chengdu, Sichuan 610041, China;
[2]Department of Thoracic Surgery, West China Hospital, Sichuan University, Chengdu, Sichuan 610041, China;
[3]Department of General Thoracic Surgery, Inselspital, Bern University Hospital, University of Bern, Bern 3010, Switzerland;
[4]Department for BioMedical Research, University of Bern, Bern 3010, Switzerland.

Abstract

Background: Molecular subtyping is an essential complementarity after pathological analyses for targeted therapy. This study aimed to investigate the consistency of next-generation sequencing (NGS) results between circulating tumor DNA (ctDNA)-based and tissue-based in non-small cell lung cancer (NSCLC) and identify the patient characteristics that favor ctDNA testing.

Methods: Patients who diagnosed with NSCLC and received both ctDNA- and cancer tissue-based NGS before surgery or systemic treatment in Lung Cancer Center, Sichuan University West China Hospital between December 2017 and August 2022 were enrolled. A 425-cancer panel with a HiSeq 4000 NGS platform was used for NGS. The unweighted Cohen's kappa coefficient was employed to discriminate the high-concordance group from the low-concordance group with a cutoff value of 0.6. Six machine learning models were used to identify patient characteristics that relate to high concordance between ctDNA-based and tissue-based NGS.

Results: A total of 85 patients were enrolled, of which 22.4% (19/85) had stage III disease and 56.5% had stage IV disease. Forty-four patients (51.8%) showed consistent gene mutation types between ctDNA-based and tissue-based NGS, while one patient (1.2%) tested negative in both approaches. Advanced diseases and metastases to other organs would be fit for the ctDNA-based NGS, and the generalized linear model showed that T stage, M stage, and tumor mutation burden were the critical discriminators to predict the consistency of results between ctDNA-based and tissue-based NGS.

Conclusion: ctDNA-based NGS showed comparable detection performance in the targeted gene mutations compared with tissue-based NGS, and it could be considered in advanced or metastatic NSCLC.

Keywords: Circulating tumor DNA; Next-generation sequencing; Non-small cell lung cancer; Targeted gene mutations

Introduction

Lung cancer is the leading cause of cancer-related deaths in China, and surgical resection can improve the prognosis of early-stage non-small cell lung cancer (NSCLC).[1,2] Targeted therapy and immunotherapy have made notable strides in NSCLC treatment over the past decade, achieving durable responses in selected patients.[3,4] Despite next-generation sequencing (NGS)-based confirmation of the indication, only approximately a quarter of patients benefit from targeted therapy.[3] Molecular subtyping is an essential complement to pathological analyses for personalized cancer treatment. NGS is commonly performed on formalin-fixed paraffin-embedded (FFPE) samples for molecular genetic profiling to guide targeted therapy in clinical practice.[5–7] However, this might be inappropriate for patients who are intolerant or unsuitable for invasive biopsy, or when dynamic monitoring of genetic mutations is expected.

Recently, liquid biopsy-based cell-free DNA (cfDNA) analysis has emerged as a novel approach for malignancy detection, and genomic profiling of circulating tumor DNA (ctDNA) has demonstrated clinical utility, showing appreciable therapeutic efficacy.[8,9] Previous studies have shown that ctDNA testing may be unreliable owing to its

Access this article online

Quick Response Code:

Website: www.cmj.org

DOI: 10.1097/CM9.0000000000003117

Weijia Huang, Kai Xu, and Zhenkun Liu contributed equally to this work.

Correspondence to: Qinghua Zhou, Lung Cancer Center/Lung Cancer Institute; Department of Thoracic Surgery, West China Hospital, Sichuan University, Chengdu, Sichuan 610041, China
E-Mail: prof_qh_zhou@126.com;
Renwang Peng, Department of General Thoracic Surgery, Inselspital, Bern University Hospital; Department for BioMedical Research, University of Bern, Bern 3010, Switzerland
E-Mail: renwang.peng@insel.ch

Chinese Medical Journal 2024;XX(XX)
Received: 15-10-2023; **Online:** 06-05-2024 **Edited by:** Xiangxiang Pan

low sensitivity, especially in early-stage patients, which might result from low ctDNA levels *in vivo*.[10–12] However, other studies have revealed that ctDNA tests have high sensitivity and specificity for NSCLC, including early-stage disease.[8,13,14] Therefore, consistency between liquid biopsy and the gold standard remains controversial. In this study, we investigated the concordance between ctDNA- and cancer tissue-based NGS and identified the clinical features favored for ctDNA testing using machine learning.

Methods

Patient enrollment and sample acquisition

Patients who diagnosed with NSCLC at the Lung Cancer Center, West China Hospital, Sichuan University, China between December 2017 and August 2022 were enrolled in this study. Both plasma and tumor samples were collected from each patient. The present study adhered to ethical guidelines and was meticulously reviewed and approved by the Ethics Committee of West China Hospital, Sichuan University (No.2022-1663). Written informed consent was obtained from all enrolled patients. Chest and abdominal computed tomography (CT), cranial magnetic resonance imaging, single-photon emission CT, and fluorodeoxyglucose positron emission tomography/CT were performed for clinical diagnosis and staging. Cancer staging was performed according to the eighth edition of the American Joint Committee on Cancer Staging Manual. Clinical information, including age, sex, metastasis, pleural effusion, TNM stage, survival, and serum tumor biomarkers, was obtained from the medical records. Treatment strategies were implemented according to the current guidelines.[15,16] Tumor tissues were obtained from surgical specimens for resectable tumors and from percutaneous lung puncture biopsy or fiber bronchoscopy for unresectable or metastatic diseases. Plasma was collected before surgery for resectable tumors and prior to systemic treatment. NGS was used to sequence DNA from the tumor tissue and plasma samples.

Targeted sequencing and data curation

In the current study, a customized NGS panel of 425 cancer-relevant genes (Geneseeq Technology, Nanjing, China) with a HiSeq 4000 NGS platform (Illumina, San Diego, CA, USA) was used for targeted sequencing, the QIAamp DNA FFPE Tissue Kit (QIAGEN, Hilden, Germany) was used for DNA extraction, and an amplicon-based library was constructed from the extracted DNA and synthetic complementary DNA. The Genome Reference Consortium Human Build 37 was used for the reference of gene sequencing. A minimum DNA sequencing depth of 500× was considered sufficient, and 1% allele frequency (AF) was used as the cut-off value for variants.

In this paper, genes with a mutation frequency >5% in the cohorts were considered high-frequency mutations. The sensitivity of ctDNA-based NGS for targeted gene was defined as the proportion of ctDNA-based NGS indicated mutation among those with the mutation, whereas the specificity was the proportion of those who were detected negative by ctDNA-based NGS among those without the mutation.

DNA extraction and targeted NGS

Approximately, 10 mL of venous blood was collected in a cfDNA storage tube, which was gently inverted and mixed. Plasma was separated within 2 h post-collection by centrifugation at 1800 × *g* for 10 min and extracted using the QIAamp Circulating Nucleic Acid Kit (QIAGEN). DNA extraction was conducted using the Gentra Puregene Blood Kit (QIAGEN), and it was quantified using Qubit 3 Fluorometer (Thermo Fisher Scientific, Waltham, MA, USA). The sequencing library was constructed by KAPA HyperDNA Library Prep Kit (KAPA Biosystems, Wilmington, MA, USA), and hybridization enrichment was performed using a custom probe targeting 425 lung cancer-associated genes (Pulmocan, Geneseeq Technology, Nanjing, China). Target-enriched libraries were sequenced on an Illumina sequencing platform (Illumina, San Diego, CA, USA), and the depths of sequencing were averaged approximately 5000× and 150× for the plasma and white blood cell samples, respectively.

Fresh tissues were promptly placed in tissue preservation solution, and FFPE tissues were sectioned with a thickness of 10 μm. DNA was extracted from FFPE tissue samples using the DNeasy Blood & Tissue Kit (QIAGEN) and QIAampDNA FFPE Tissue Kit (QIAGEN), respectively. Quantification, library construction, and purification were described above. Customized probes targeting 425 lung cancer-related genes (Geneseeq Technology) were used for hybridization with sequencing platforms. The average sequencing depth was approximately 1000× for fresh tissue and FFPE samples.

Statistical analyses

Data were presented as medians and ranges or interquartile ranges (IQR) for quantitative variables and as numbers and percentages for categorical variables. Tumor mutation burdens were quantified as the number of mutations per million bases (muts/Mb). The chi-squared test was used to assess the variance between groups. Spearman's correlation was used to assess the association between gene mutations and clinical characteristics, and the log-rank test was used to assess survival differences. The unweighted Cohen's kappa coefficient was used to calculate the concordance between ctDNA- and cancer tissue-based NGS, with a kappa score cut-off of 0.6 to divide the cohort into high and low concordance groups.[17] Multiple imputations were performed to replace the missing values [Supplementary Figure 1, http://links.lww.com/CM9/B989].[18] Six machine learning models were developed to investigate the role of clinical characteristics on the kappa score, including a featureless classifier, decision-making tree, generalized linear model, XGBoost, random forest, and SVM model, which have been precisely described in previously published articles,[19,20] and 10-fold cross-validation was used for internal validation. A stepwise generalized linear regression model was used to visualize the prediction model. A two-tail *P*-value <0.05 was

identified as statistical significance, and all statistical analyses were performed using R software (version 4.2.1; The R Foundation for Statistical Computing, Vienna, Austria).

Results

Patient characteristics

A total of 85 patients were enrolled in this study, with a median age of 63 years (range, 31–84 years), of whom 48 were males (56.5%) and 66 (77.6%) patients were lung adenocarcinoma (LUAD). Overall, 18 (21.2%), 19 (22.4%), and 48 (56.5%) patients were diagnosed with stage I–II, III, and IV diseases, respectively, with 21 (24.7%) patients presenting with pleural effusion. The median carcinoembryonic antigen (CEA) level was 6.9 ng/mL (IQR, 2.3–14.3 ng/mL), and the median tumor mutation burden (TMB) was 4.1 (IQR, 3.1–10.3) muts/Mb for cancer tissue and 5.1 (IQR, 0.0–10.7) muts/Mb for ctDNA [Table 1].

Table 1: Demographics and clinical characteristics ($N = 85$).

Characteristics	Number (%)
Gender	
Male	48 (56.5)
Female	37 (43.5)
Age (years, median and range)	63 (31–84)
<65	47 (55.3)
≥65	38 (44.7)
Histology	
LUAD	66 (77.6)
LUSC	10 (11.8)
Others	9 (10.6)
Stages	
I	13 (15.2)
II	5 (5.9)
III	19 (22.4)
IV	48 (56.5)
T stages	
T1	16 (18.8)
T2	14 (16.5)
T3	18 (21.2)
T4	37 (43.5)
N stages	
N0	25 (29.4)
N1	9 (10.6)
N2	37 (43.5)
N3	14 (16.5)
M stages	
M0	37 (43.5)
M1a	14 (16.5)
M1b	14 (16.5)
M1c	20 (23.5)
Pleural effusion	
No	64 (75.3)
Yes	21 (24.7)
Bone metastasis	
No	63 (74.1)
Yes	22 (25.9)
Brain metastasis	
No	76 (89.4)
Yes	9 (10.6)
Other metastases	
No	55 (64.7)
Yes	30 (35.3)
CEA (ng/mL, median and IQR)	6.9 (2.3–14.3)
TMB for tissue (muts/Mb, median and IQR)	4.1 (3.1–10.3)
TMB for ctDNA (muts/Mb, median and IQR)	5.1 (0.0–10.7)

CEA: Carcinoembryonic antigen; ctDNA: Circulating tumor DNA; IQR: Interquartile range; LUAD: Lung adenocarcinoma; LUSC: Lung squamous cell carcinoma; muts/Mb: The number of mutations per million bases; TMB: Tumor mutation burden.

Genomic profiles of ctDNA- and cancer tissue-based NGS

Overall, genetic alterations based on cancer tissue-based NGS were identified in 70 patients (82.4%), and 77 patients (90.6%) had genetic mutations identified using ctDNA-based NGS. The most common gene alterations from cancer tissue-based NGS were mainly missense mutations, nonsense mutations, and frameshift mutations. *TP53* was the most frequently mutated gene, occurring in 49.4% (42/85) of patients [Figure 1A], followed by *EGFR* (36.5%, 31/85), *STK11* (14.1%, 12/85), *LRP1B* (14.1%, 12/85), *KRAS* (11.8%, 10/85). Compared with cancer tissue-based genomic profiles, ctDNA-based genomic profiles showed significant diversity in the gene alteration types and mainly distributed in single-nucleotide polymorphisms (SNPs). The most common SNPs were *NQO1* (61.2%, 52/85), *GSTM1* (54.1%, 46/85), *XRCC1* (54.1%, 46/85), and *MTHFR* (50.6%, 43/85), and the mutated genes were *TP53* (48.2%, 41/85), *EGFR* (28.2%, 24/85), *LRP1B* (10.6%, 9/85), and *PIK3CA* (10.6%, 9/85). Stage I lung cancer only detected SNPs using ctDNA-based NGS [Figure 1B].

Collectively, 44 patients (51.8%) had the same mutations detected in both ctDNA-based and cancer tissue-based NGS, with consistent mutation subtypes, and one patient (1.2%) was negative for both NGS. Seven patients (8.2%) had mutations only in the cancer tissue, and 15 patients (17.6%) had mutations in ctDNA among the 40 patients with discordant mutations between cancer tissue and ctDNA [Figure 2A]. Among all samples, 236 mutations were concordant between ctDNA- and cancer tissue-based NGS, 227 mutations were detected only in cancer tissue-based NGS, and 607 mutations were detected only in ctDNA-based NGS [Figure 2B].

Association of clinical characteristics with genomic profiles

In the genomic profiles of the cancer tissue-based NGS, M stage was associated with *EGFR* and *SMARCA4* mutations, pleural effusion was associated with *EGFR* mutations, and TMB was significantly correlated with *TP53*, *STK11*, *KEAP1*, and *LRP1B* mutations [Supplementary Figure 2A, http://links.lww.com/CM9/B989]. The genomic profiles of the ctDNAs-based NGS were comparable to those of cancer tissues-based NGS. SNP were mostly observed in genes with a high mutation frequency in ctDNA-based NGS, including *NQO1*, *GSTM1*, and *XRCC1*, which were also slightly associated with the TNM stage [Supplementary Figure 2B, http://links.lww.com/CM9/B989]. Eleven genes

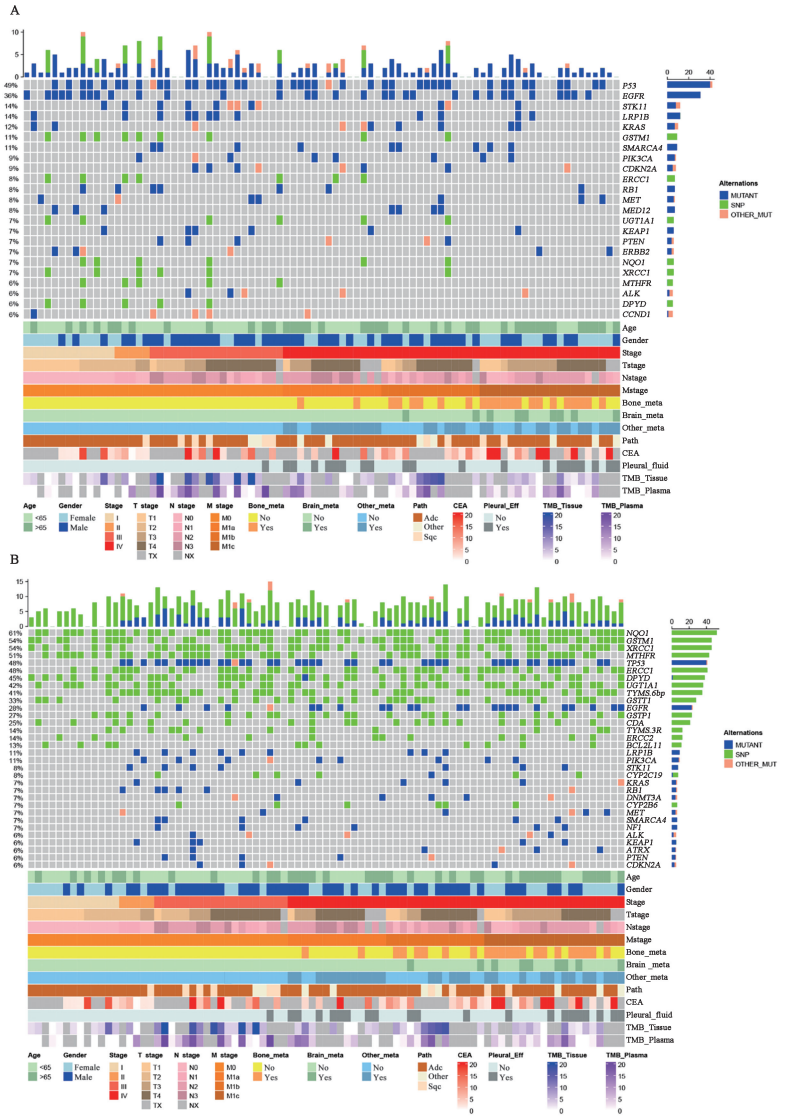

Figure 1: The genomic profiles of the clinical characteristics and gene mutations from the tissue- (A) and ctDNA-based (B) NGS. Bone_meta: Bone metastases; Brain_meta: Brain metastases; CEA: Carcinoembryonic antigen; ctDNA: Circulating tumor DNA; NGS: Next-generation sequencing; Other_meta: Other metastases; Other_mut: Other mutations; SNP: Single-nucleotide polymorphism; TMB: Tumor mutation burden.

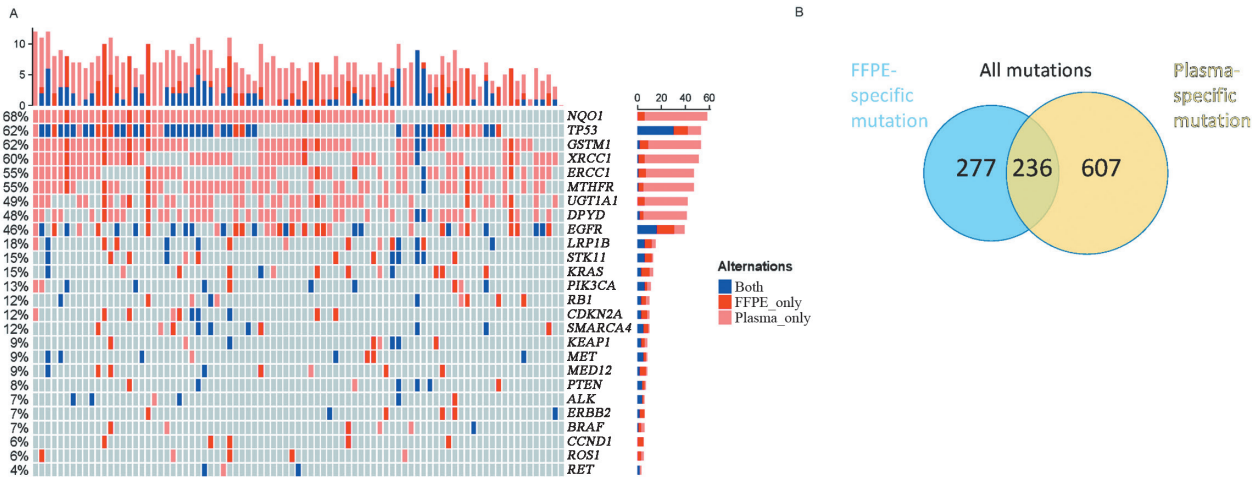

Figure 2: Comparison of the genomic profiles between the tissue- and ctDNA-based NGS with heatmap (A) and Veen plot (B). ctDNA: Circulating tumor DNA; FFPE: Formalin-fixed paraffin-embedded; NGS: Next-generation sequencing.

showed high concordance (correlation coefficient $r > 0.5$) between cancer tissues-based and ctDNA-based NGS, including *STK11*, *ALK*, *ERBB2*, *ATRX*, *KEAP1*, *PTEN*, *SMARCA4*, *NF1*, *LRP1B*, *PIK3CA*, and *MET* [Supplementary Figure 2C, http://links.lww.com/CM9/B989].

Discrimination ability of the targeted gene mutations between cancer tissues-based and ctDNA-based NGS

We first compared the mutation phenotypes of the nine targeted genes with clinical implications (*TP53*, *EGFR*, *KRAS*, *ALK*, *MET*, *ERBB2*, *BRAF*, *ROS1*, and *RET*) between the cancer tissues-based and ctDNA-based NGS, and the sensitivity, specificity, and accuracy are shown in Supplementary Table 1, http://links.lww.com/CM9/B989, which shows a high prediction accuracy among the nine mutated genes. The kappa test was used to explore the consistency of these nine targeted genes between the cancer tissues-based and ctDNA-based NGS within each patient, and the cohort was divided into low and high concordance groups according to the kappa score. The chi-squared test showed a significant difference between the two groups in stage, bone metastasis, and other organ metastases [Table 2]. The high concordance group was significantly associated with worse survival than the low concordance group ($P = 0.003$; Figure 3).

Table 2: Patient characteristics in the poorly agreement group and highly agreement group.

Characteristics	Poorly agreement group *N* = 38 (%)	Highly agreement group *N* = 47 (%)	Statistics values	*P*-value
Gender			0.210*	0.647
Male	23 (60.5)	25 (53.2)		
Female	15 (39.5)	22 (46.8)		
Age (years, median and range)	65 (31–84)	61 (37–83)	0.070†	0.793
Histology			0.277*	0.598
LUAD	28 (73.7)	38 (80.9)		
Other	10 (26.3)	9 (19.1)		
T stages			9.371*	0.025
T1	11 (28.9)	5 (10.6)		
T2	9 (23.7)	5 (10.6)		
T3	5 (13.2)	13 (27.7)		
T4	13 (34.2)	24 (51.1)		
N stages			7.787*	0.051
N0	17 (44.7)	8 (17.0)		
N1	3 (7.9)	6 (12.8)		
N2	13 (34.2)	24 (51.1)		
N3	5 (13.2)	9 (19.1)		
M stages			13.913*	0.003
M0	25 (65.8)	12 (25.5)		
M1a	4 (10.5)	10 (21.3)		
M1b	4 (10.5)	10 (21.3)		
M1c	5 (13.2)	15 (31.9)		
Bone metastasis			7.062*	0.008
No	34 (89.5)	29 (61.7)		
Yes	4 (10.5)	18 (38.3)		
Brain metastasis			<0.001*	>0.999
No	34 (89.5)	42 (89.4)		
Yes	4 (10.5)	5 (10.6)		
Other metastases			5.028*	0.025
No	30 (78.9)	25 (53.2)		
Yes	8 (21.1)	22 (46.8)		
Stages			–	<0.001‡
I	13 (34.2)	0 (0.0)		
II	1 (2.6)	4 (8.5)		
III	11 (28.9)	8 (17.0)		
IV	13 (34.2)	35 (74.5)		
Pleural effusion			0.202*	0.653
No	30 (78.9)	34 (72.3)		
Yes	8 (21.1)	13 (27.7)		
CEA (ng/mL, median and IQR)	5.1 (2.4–14.1)	7.3 (2.3–17.1)	0.654†	0.421
TMB for tissue (muts/Mb, median and IQR)	3.7 (2.1–10.3)	5.3 (3.1–10.3)	0.006†	0.935
TMB for ctDNA (muts/Mb, median and IQR)	1.0 (0.0–10.1)	6.3 (1.1–13.9)	3.288†	0.073

*χ^2 values; †Z values; ‡Fisher test. CEA: Carcinoembryonic antigen; ctDNA: Circulating tumor DNA; LUAD: Lung adenocarcinoma; IQR: Interquartile range; LUSC: Lung squamous cell carcinoma; muts/Mb: The number of mutations per million bases; TMB: Tumor mutation burden; –: Not available.

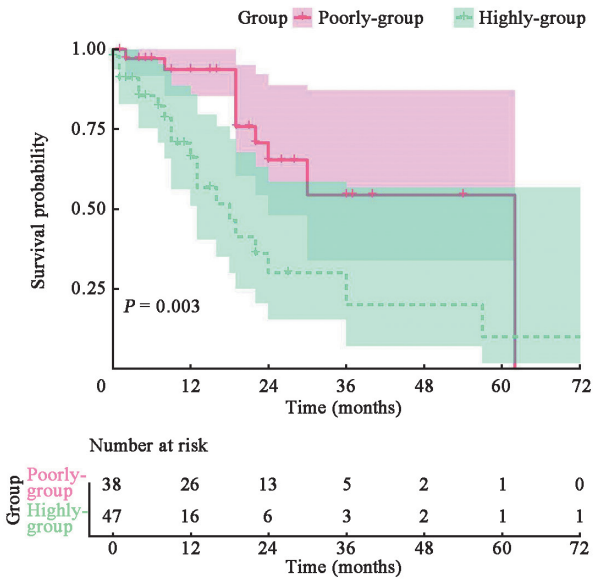

Figure 3: Kaplan-Meier curve compared the survival between the highly agreement group and poorly agreement groups.

Machine learning and prediction model visualization

Six machine learning algorithms were used to investigate the role of clinical characteristics on the kappa score, and the generalized linear model showed the best prediction accuracy among the six models [Figure 4A]. In the stepwise generalized linear model, T stage, M stage, pathological classification, and TMB in ctDNA made major contributions. The possibility of being in the high concordance group was 0.41 × T stage + 0.66 × M stage–1.26 × histology + 0.06×TMB_ctDNA – 0.60 [Figure 4B], and the area under the receiver operator characteristic curve was 0.79 [Figure 4D]. When the regression model was simplified by eliminating the pathological features, the likelihood of being in the high concordance group was 0.41 × T stage + 0.58 × M stage–1.64 with a satisfactory prediction ability that area under curve (AUC) was 0.74 [Figure 4C,E].

Discussion

Molecular genetic profiling for the malignancy would instruct clinical practice and personalized cancer treatment, including targeted therapy and immunotherapy, enabling patients to achieve more survival benefits. Previous studies revealed that the findings of ctDNA-based NGS are associated with high concordance with the results of tissue-based NGS in advanced NSCLC.[13] Considering the quantity, quality, and detection duration, ctDNA-based NGS can detect the current gene mutations among patients who are intolerant to biopsy, and dynamically surveil gene mutations during treatment that could help identify the mechanisms of resistance.[21–23] However, the precision of liquid biopsy remains unconvincing and it continues to be controversial regarding which patients with specific clinical characteristics are suitable for ctDNA-based NGS.[24,25]

The cancer tissue-based genomic profiles showed high mutation frequencies in *TP53*, *EGFR*, *KRAS*, and *STK11* in the study population, which is in agreement with previous studies.[14,26,27] We have comprehensively demonstrated the high concordance between ctDNA- and cancer tissue-based NGS findings in those with more advanced disease including larger tumor size and more extensive metastasis, consistent with previous studies. [13,28,29] It might be interpreted that ctDNA would be detected more easily with disease progression, and tumor staging might be associated with the favored performance of ct-DNA-based NGS. Hence, the T and M stages were critical discriminators in our prediction model, and a high TMB

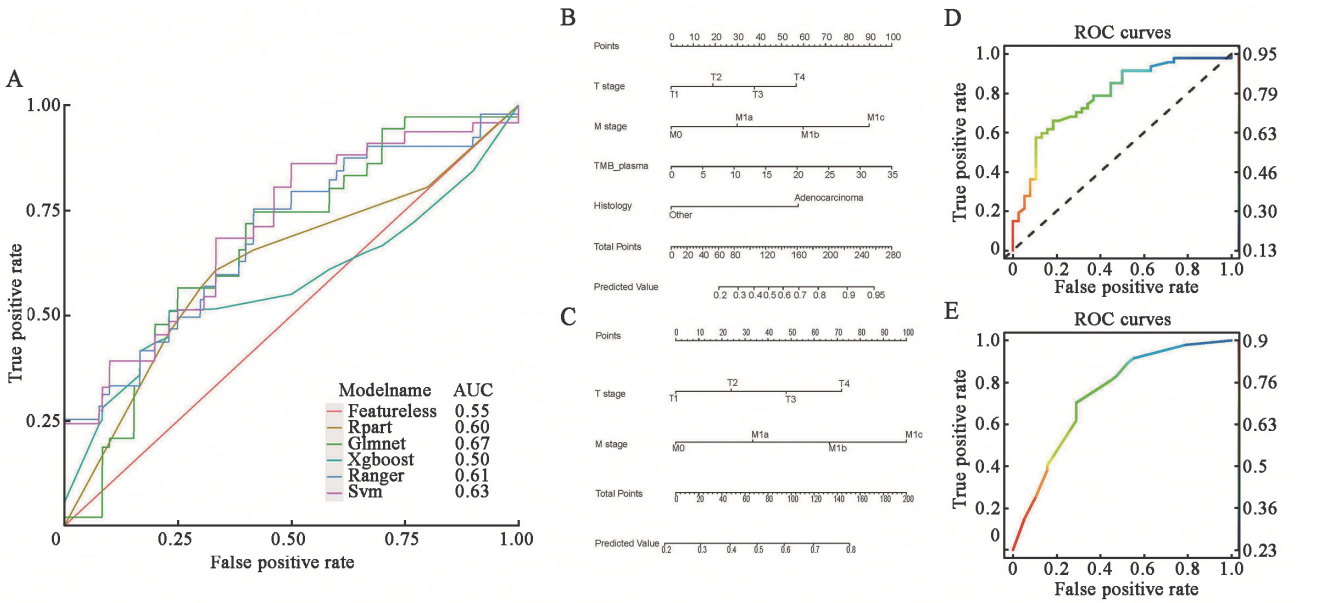

Figure 4: Six machine learning algorithms (a featureless classifier, decision-making tree, generalized linear model, XGBoost, random forest and SVM model) investigated the role of clinical characteristics in the Kappa score (A). Nomograms of the prediction model (B) and simplified model (C) predicted the possibility of being the highly agreement group, and the prediction performance of the prediction model (D) and simplified model (E) was visualized by the receiver operating characteristic curves. AUC: Area under curve; ROC: Receiver operating characteristic curve; TMB: Tumor mutation burden.

was also associated with favorable concordance, as previously reported.[30]

Our findings suggest that ctDNA-based NGS showed remarkable consistency for common driver genes with tissue-based NGS including *EGFR*, *ALK*, and *MET*, which could help targeted therapy and enable dynamic surveillance. We presumed that ctDNA-based NGS could instruct targeted therapy on conventional targeted genes, considering the high consistency, which is in line with prior findings.[31] While, the prediction accuracy showed favored consistency for rare mutation driver genes, including *RET*, *ALK*, and *MET*, which is inconsistent with a previous report.[32] This may be due to the small number of cases; therefore, our findings needed to be validated in a large cohort. We acquired a kappa score based on the consistency of nine oncogenes, and then developed a prediction model using clinicopathological characteristics by machine-learning algorithm that could effectively discriminate patients with high concordance between cancer tissue- and ctDNA-based NGS. Moreover, we proposed that disease staging and TMB are critical indicators when assessing the credibility of ctDNA-based NGS, and those with advanced disease and high TMB are referred to as potential target patients.

We noticed that there were few mutated genes other than SNP in ctDNA for stage I NSCLC, which might be explained by the limited circulating ctDNA detected in early-stage diseases.[33] Therefore, it might account that ctDNA-based NGS may not be practical for detecting early-stage diseases. Furthermore, the ctDNA-based genomic profile revealed oncogenes with a high frequency of SNP distributed in metabolizing enzyme modulation and DNA damage repair, which are potentially related to tumor development and progression.[34–37] Prior studies indicated that tumor staging, tumor volume, disease status, and cancer treatment may interfere with the detection and analysis of ctDNA, leading to false negativity; hence, improving the accuracy and standardization issues should be further investigated, which might help promote the feasibility of liquid biopsy in clinical practice.[28,38,39]

This study has several limitations. First, we merely enrolled the patients from a single center, and most of the enrolled patients had advanced or metastatic disease. The retrospective review of patients might have led to an inherent bias in the research findings, besides some important clinical indictors also missing, such as serum tumor biomarkers, which may also compromise the results. Although multiple imputations were performed to replenish the missing data, systematic errors were also introduced. Therefore, large prospective clinical trials are required to confirm these findings.

Even though ctDNA-based sequencing might not be practical or convincing for early-stage NSCLC, this study provided dependable insights into ctDNA-based NGS employed in advanced or metastatic diseases, and tumor staging and TMB were critical indicators when considering ctDNA-based NGS. We also revealed the favored detection consistency of ctDNA-based NGS in several specific driver genes, which could help instruct targeted therapy in clinical practice with less invasiveness and shorter duration before tissue biopsy. Thus, ctDNA-based NGS could be a complementary approach for the patients with challenge biopsy or required dynamic surveillance.

Acknowledgement

The targeted gene sequencing was supported by Nanjing Geneseeq Technology, Inc., China.

Funding

This study was partly supported by the grants from the Tianjin science and technology innovation system construction project (No. 07SYSYSF05000), the Tianjin Science and Technology Support Plan Key Project (No. 06YFSZSF05300), the National Natural Science Foundation of China (No. 81572288), and the Key Project of International Cooperation of Science and Technology Innovation between Governments, the National Key Research and Development Plan of China (No. 2016YEE0103400).

Conflicts of interest

None.

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How to cite this article: Huang WJ, Xu K, Liu ZK, Wang YF, Chen ZJ, Gao YY, Peng RW, Zhou QH. Circulating tumor DNA- and cancer tissue-based next-generation sequencing reveals comparable consistency in targeted gene mutations for advanced or metastatic non-small cell lung cancer. Chin Med J 2024;XXX:1–8. doi: 10.1097/CM9.0000000000003117