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ORIGINAL RESEARCH

Exploratory Identification of Gene Copy Number Cut-Off for NGS-Based *MET* Amplification Assessment and Clinical Relevance to MET Inhibitor Outcomes in Non-Small-Cell Lung Cancer

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Introduction: *MET* amplification is a critical oncogenic driver and a major mechanism of acquired resistance to epidermal growth factor receptor tyrosine kinase inhibitors (EGFR-TKIs) in non-small-cell lung cancer (NSCLC). Currently, next-generation sequencing (NGS)-based assays are increasingly used in clinical practice. However, they lack a unified standard for defining MET amplification across multiple NGS platforms with a potential actionable threshold.

Methods: This was a multiple-site joint study to optimize the cut-off value for *MET* amplification using tumor tissue and NGS analysis. In the training cohort, five NGS panels were used to detect *MET* gene copy number (GCN), and fluorescence in situ hybridization (FISH) was used as a reference. Receiver operating characteristic (ROC) curve analysis was used to identify the optimal cutoff with 100% specificity and 66.7% sensitivity. The results were validated in an independent cohort to explore the correlation between *MET* amplification and efficacy of MET inhibitors in patients with NSCLC.

Results: In the training cohort (n=21), the optimal *MET* GCN cut-off was determined to be 6.55, yielding an area under the curve of 0.9, corresponding to an accuracy of 85.7%. *MET* polysomy, identified using FISH (n=2), was also detected using NGS. All five NGS panels demonstrated good concordance with FISH, with accuracies ranging from 75.0% to 85.7%. In the validation cohort (n=29), NGS analysis demonstrated a good performance, with an accuracy rate of 79.3%. Patients with *MET* GCN $\geq$ 6.55 had a significantly longer median progression free survival than those with *MET* GCN < 6.55 (hazard ratio = 0.42; p = 0.03).

Conclusion: In this study, an exploratory cut-off of 6.55 for *MET* GCN by NGS was associated with *MET* amplification and longer progression-free survival in patients receiving targeted therapy. However, given the retrospective design and limited sample size, these findings should be regarded as exploratory and require validation in larger, prospective cohorts before broader clinical application.

Keywords: *MET* amplification, next-generation of sequencing, gene copy number, non-small-cell lung cancer, targeted therapy

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Introduction

The mesenchymal-epithelial transition (*MET*/c-*MET*) gene encodes a transmembrane tyrosine kinase receptor for hepatocyte growth factor (HGF). Aberrant activation of the HGF/*MET* pathway promotes malignant behaviors of cancer cells, such as cell proliferation, migration, survival, and invasion-metastasis.[1] *MET* activation involves *MET* exon 14 skipping mutations, *MET* gene amplification, c-*MET* overexpression, and *MET* fusion. *MET* amplification occurs de novo or is acquired. De novo *MET* amplification has been reported as a primary driver in only 1%-5% of non-small cell lung cancer (NSCLC) cases. In contrast, 5%-50% of patients with *EGFR*-mutated NSCLC acquire *MET* amplification as a resistance mechanism to epidermal growth factor receptor tyrosine kinase inhibitors (EGFR-TKIs).[2–4]

Currently, *MET* amplification is performed using various methods, including Fluorescence in situ hybridization (FISH), immunohistochemistry (IHC), and next-generation sequencing (NGS).[1,5] FISH remains the gold standard for detecting *MET* amplification in clinical practice.[1] Two definitions of *MET* amplification, according to *MET* copy number, are commonly used. One method calculates the signal ratio of *MET* to the chromosome enumerating probe against chromosome 7 (*MET/CEP7*), distinguishing between *MET* focal amplification and polysomy. Another method scores the mean *MET* gene copy number (GCN) per cell.[1] Several clinical trials have suggested that the efficacy of *MET* inhibitors in patients with NSCLC varies with the *MET* gene copy number.[5–9] *MET* amplification represents a resistance mechanism in *EGFR*-mutated NSCLC treated with EGFR-TKIs. In a phase Ib/II study, the combination of capmatinib with gefitinib showed promising efficacy and tolerable safety profile in post-first/second-generation EGFR-TKI-resistant EGFRm NSCLC patients with high *MET*-amplified disease (GCN≥6). The objective response rate (ORR) was 47% and the median progression-free survival (mPFS) was 5.5 months.[10] Yang et al also reported the results of another phase Ib study showing that savolitinib plus gefitinib had an acceptable safety profile and promising antitumor activity in EGFRm-*MET*-amplified advanced NSCLC patients who had disease progression on EGFR-TKIs. The ORR for the EGFR T790M negative patients was 52%.[11] In the SAVANNAH study, patients with *EGFR*-mutant NSCLC that progressed to osimertinib were treated with savolitinib and osimertinib. The ORR for high *MET* amplification or overexpression (defined as GCN ≥10 and/or IHC 3+ in ≥ 90% of tumor cells) was 49%, the mPFS was 7.1 months, and the ORR for increased *MET* amplification or overexpression (GCN≥ 5 and/or MET/CEP ≥ 2 or IHC 3+ in ≥ 50% of tumor cells) was 32%.[5] In the TATTON study, in previously treated and untreated with 3rd generation EGFR-TKIs, for FISH-positive patients, the ORR was 30% and 65%; for FISH GCN≥10 patients, the ORR was 34% and 79%, respectively.[12] *MET* amplification, particularly at high levels, has also been shown to play a key role in advanced NSCLC in several trials.[6–9] Thus, the detection of *MET* amplification has a significant clinical value.

Next-generation sequencing (NGS) enables simultaneous detection of diverse clinically relevant genomic alterations. With the advantages of saving biological specimens, improving turnaround time, and high cost-effectiveness, NGS has become an integral part of routine molecular diagnostics for patients with cancer, especially non-small cell lung cancer.[13,14] Thus, NGS-based assays are a good surrogate for single-gene *MET* detection using FISH.

Several studies have explored the concordance between NGS and FISH in the detection *MET* amplification. Lai et al reported that among 18 patients identified as *MET*-high by FISH, only eight (44.4%) were *MET* amplifications identified by NGS in tissue samples.[15] Concordance was only 33.3% in patients with FISH and a copy number (CN) > 8 (n=3). The results from the TATTON study also showed low concordance between NGS and FISH for the detection *MET* amplification. In the FISH-positive group, only 47.9% of the patients (23/48) were identified to have *MET* amplification using tissue NGS.[16] Notably, there are several independent central laboratories for NGS testing with different testing platforms or panels, data analysis pipelines, and bioinformatics algorithms in China that lack a unified standard for cut-off selection to define *MET* amplification.[17] In clinical practice, patients with negative FISH results but with high CNV under NGS testing are treated with MET-TKIs.

Despite the increasing use of tissue NGS for *MET* amplification in NSCLC, the definition of *MET* amplification by NGS remains inconsistent across assays, panels, and bioinformatic pipelines.[17,18] Prior studies comparing NGS and FISH have shown variable concordance depending on the analytical method and clinical setting, highlighted the lack of a universally accepted NGS-based threshold for *MET* amplification. Moreover, concordance with FISH alone does not fully address the clinically relevant question of whether an NGS-derived MET copy number threshold can help identify

patients more likely to benefit from MET-targeted therapy. Therefore, in the present study, a multisite joining study was conducted to explore the unified criteria for tissue *MET* amplification detection using different NGS panels.[19] The cutoff value of *MET* GCN with tissue NGS for *MET* amplification was explored, and the association between the optimal cutoff value and the clinical efficacy of MET-targeted therapies was presented.

Materials and Methods

The study was divided into three stages. In the first stage, the five NGS laboratories in China, including Burning Rock Biotech (Guangzhou), AmoyDx (Xiamen), Geneseeq Technology (Nanjing), GenePlus (Beijing) and Berry Oncology (Beijing). Five laboratories independently established methods for copy number evaluation of *MET* amplification. In the second stage, 21 samples were collected and sent to the central laboratory for FISH. Subsequently, the DNA was distributed to five different laboratories for parallel single-blind NGS testing, and the FISH results were used to train the optimal NGS detection cutoff. In the third stage, a clinical cohort with information on MET TKI treatment was used to validate the optimal cut-off for *MET* amplification detection obtained in the first stage.

Patients

The study included two cohorts for training and validation.

Twenty-one patients were included in the training cohort once they met the eligibility criteria pre-defined: (i) written informed consent for specimen reuse. (ii) Histological or cytological confirmation of locally advanced or metastatic NSCLC (iii) Sufficient tissue samples for FISH testing and five parallel NGS sample testing. This study was conducted in accordance with the Declaration of Helsinki, and approved by the Research Ethics Committee of Guangdong Provincial People's Hospital (approval number: 2016175H[R2]). All patients provided written informed consent for the use of their tumor specimens.

The validation cohort was independent, and the results have already been published. Forty patients had FISH and NGS results, which were conducted prior to MET inhibitor treatment. After confirmation, 11 patients were excluded because their NGS results could not be determined for focal amplification or polysomes. Ultimately, 29 patients were included in this study.

Tissue NGS Testing

Tumor DNA was extracted from formalin-fixed paraffin-embedded (FFPE) tissue samples.100 ng of DNA from each sample with tumor content ≥30% was sent to five laboratories for NGS testing. The five laboratories used their respective commonly used NGS panels, with an effective sequencing depth of ≥500x to detect *MET* amplification.

The results were collected, including whether the samples were positive or negative for *MET* amplification, whether there was focal amplification or polysomy, and the value of *MET* GCN. *MET* GCN ≥ 5 was defined as *MET* amplification criteria from the TATTON trial in these NGS panels.

Algorithms for CNV Calling

Initially, coverage data were corrected for sequencing biases related to GC content and probe design and then normalized using a reference region or a genome-wide baseline. Next, the depth ratio was calculated by comparing the depth of coverage of the detected samples with that of a normal pool (NP). The normalized depth for each targeted region in the tumor was compared to the median depth of the corresponding region in the NP to identify the true CNV signal. The median log2 ratio for each gene region was considered the log2 ratio and was subsequently converted into a Copy Number (CN) value using the following formula: CN= 2×2 log2 ratio. For the absolute Gene Copy Number (GCN) analysis, we relied on the CN value and tumor cell content, which were calculated using the ABSOLUTE method.[20] The GCN of MET in tumor cells was derived from the CN value using the following formula: GCN=(CN-2)/P + 2. where GCN represents the absolute gene copy number of MET in tumor cells, CN refers to the copy number derived from sequencing data, and P is the tumor cell content. All participating laboratories used validated panel-specific workflows and routine laboratory quality control procedures for DNA extraction, library preparation, sequencing, and copy number analysis. Only samples meeting the respective laboratory acceptance criteria were included for downstream analysis. To

improve comparability across the five panels, MET GCN values were converted to absolute tumor-cell GCN using a unified formula and integrated for subsequent analyses.

FISH Testing

FISH analysis of FFPE tissue sections was performed using a commercial *MET* probe kit. The FISH assays were performed according to the criteria recommended by the manufacturer. A tumor was considered positive for *MET* amplification if it had an MET/centromere ratio of ≥2 or a *MET* gene copy number (GCN) of ≥5, which included MET/CEP7≥2 regarded as focal amplification and MET GCN≥5 and MET/CEP7<2 regarded as polysome, using the criteria established by Cappuzzo. FISH interpretation was performed independently by two pathologists (Zhi Xie and Li-Xu Yan). When discrepant results occurred, adjudication was performed by a third senior pathologist (Qing-Ling Zhang), and the final result was established by consensus.

Clinical Outcome

Clinical outcome data, including response to therapy and survival, were collected from the patients' medical records and analyzed in relation to *MET* amplification status determined by both tissue NGS and FISH testing. The objective response rate (ORR) was defined as the proportion of patients with an unconfirmed complete response (CR) or part response (PR). Progression-free survival (PFS) was defined as the time from the date of the first dose of anti-tumor treatment until the date of disease progression or death in the absence of disease progression.

Exploratory MET NGS GCN Cut–Off Discovery

In the training cohort, the average GCN value of the five laboratories' measurements was calculated, and the FISH results were used as a reference to train the exploratory tissue NGS GCN cut-off for determining *MET* amplification. Because false-positive classification of MET amplification may have direct therapeutic implications, the threshold-selection strategy prioritized specificity. Candidate cut-offs were evaluated by ROC analysis using FISH as the reference. To minimize false-positive classification, thresholds achieving 100% specificity in this pooled analysis were prioritized, and among these candidates, the cut-off with the highest sensitivity was selected for further evaluation. The ability of the exploratory cut-off to predict *MET* amplification and stratify PFS was validated in the validation cohort.

Statistical Analysis

Fisher's exact test was used to compare unordered categorical variables. Survival curves were constructed using the Kaplan-Meier product limit method and compared using the Log rank test. Cox proportional hazards regression was performed with or without adjustment for available prognostic clinical covariates to calculate hazard ratios (HRs) and 95% confidence intervals. All analyses were conducted using R version 4.1.2 (https://cran.r-project.org/; accessed May 31, 2024) and its associated packages. This was an exploratory retrospective study based on case availability. The target population consisted largely of patients with advanced NSCLC, for whom available tumor material was often limited. Only cases with sufficient remaining specimen to support both FISH analysis and parallel testing across five NGS panels were eligible for inclusion. Therefore, no formal sample size calculation was performed. Multivariable analyses were adjusted for clinical covariates.

Results

Clinical Characteristics

The molecular diagnostic workflow is shown in Figure 1. Fifty patients with advanced NSCLC were enrolled in the study. In the training cohort (n=21), *MET* amplification was identified by matching the tumor tissue to compare the FISH and NGS results, and the optimal cut-off for *MET* amplification was calculated. The performance of the optimal cutoff was validated in the validation cohort (n=29), and its relevance to the clinical outcome of MET-targeted therapies was evaluated. No RTK/RAS/RAF driver genes were identified in either of the cohorts.

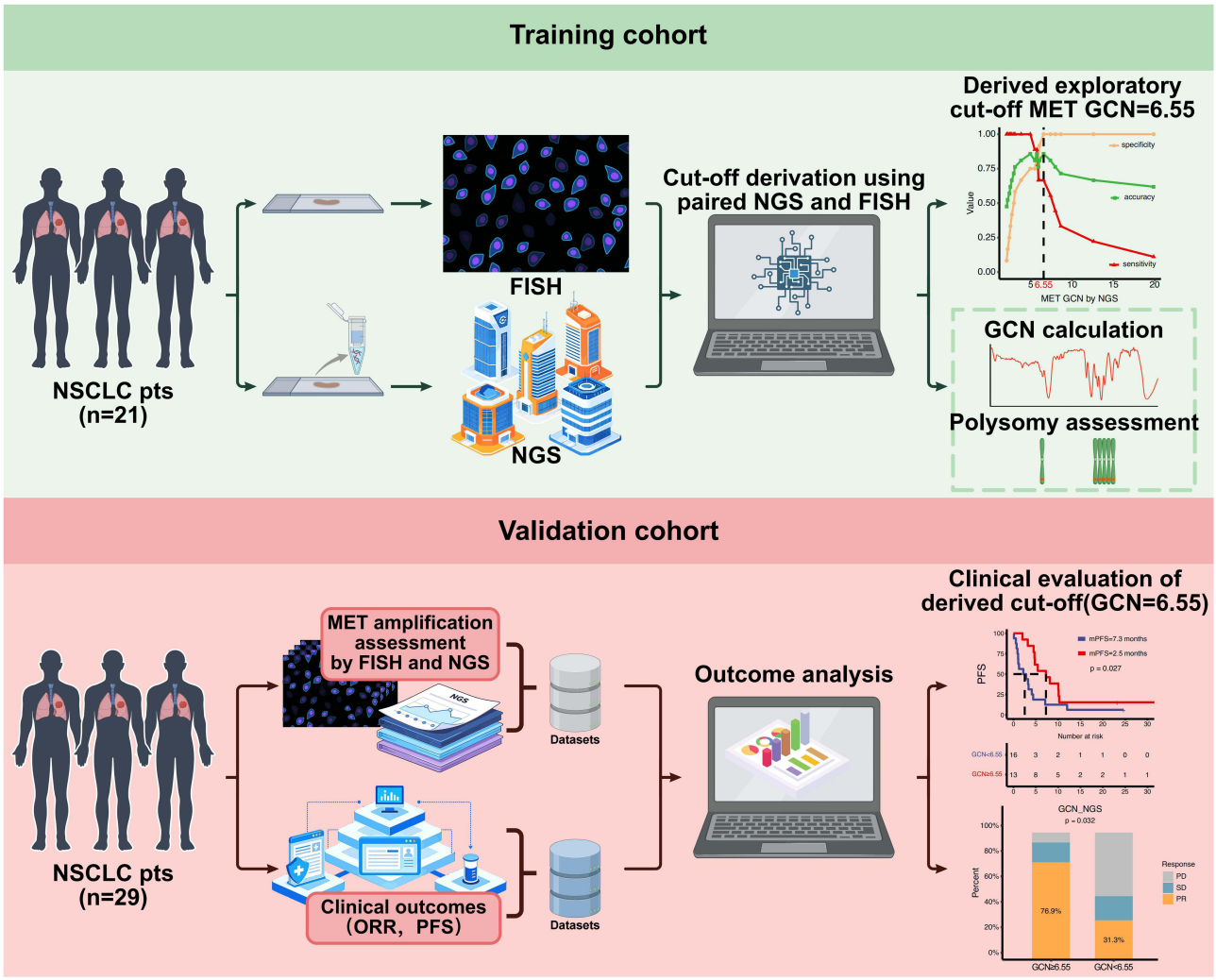

Figure 1 Study workflow. In the training cohort (n=21), paired tissue FISH and NGS data were used to derive an exploratory MET GCN cut-off of 6.55 (the data in red font) by ROC analysis. In the validation cohort (n=29), the derived cut-off (GCN 6.55) was applied to evaluate its association with objective response rate (ORR) and progression-free survival (PFS) in patients receiving MET-directed therapy.

The clinical and pathological characteristics of the patients are summarized in Table 1. In the training cohort, the median age of the patients was 63 years old (range: 47–79 years old); 61.9% were men, and 66.7% were adenocarcinomas. The basic characteristics of patients in the validation cohort were comparable to those in the training cohort. The median age of the patients was 58 years (range: 32–76 years); 61.9% were men and 66.7% were adenocarcinomas. 96.6 Of the patients, 96.6% had stage IV disease; 58.6% were EGFR mutation-positive and treated with EGFR-TKIs and MET-TKIs; 41.2% were EGFR mutation-negative and treated with MET-TKIs; 82.8% of the treatment was second and beyond the second-line setting.

Using the gold standard FISH method, nine (42.9%, 9/21) patients in the training cohort were classified as *MET* amplification-positive, which included seven patients with focal *MET* amplification and two patients with *MET* polysomy. Twelve (57.1%) patients tested negative for *MET* amplification. In the validation cohort, 17 (58.6%, 17/29) patients were *MET* amplification and 15 patients had focal *MET* amplification. 12 (41.4%) tested negative for *MET* amplification.

Identify the Exploratory MET GCN Cut-Off Using Different NGS Panels

Tumor tissues from all patients were tested using five different NGS panels with adjusted bioinformatic methods to identify the optimal cut-off value of *MET* GCN. Using FISH as the gold standard, NGS performance was evaluated by

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Table 1 Clinical and Pathological Characteristics of Patients in Two Cohorts

	Training Cohort (n=21)	Validation Cohort (n=29)	P values
Age			0.20 *
Median (range), years	63 (47–79)	58 (32–76)	
< 65 years	12 (57.1%)	22 (75.9%)	
≥ 65 years	9 (42.9%)	7 (24.1%)	
Sex			0.84 #
Male	13 (61.9%)	19 (65.5%)	
Female	8 (38.1%)	10 (34.5%)	
Pathological types			0.09 #
Adenocarcinoma	14 (66.7%)	28 (96.6%)	
Others$	7 (33.3%)	1 (3.5%)	
Clinical stages			
IIIB	/	1 (3.4%)	
IV	/	28 (96.6%)	
Brain metastasis			
Yes	/	6 (20.7%)	
No	/	23 (79.3%)	
EGFR mutation status			
Positive	/	17 (58.6%)	
Negative	/	12 (41.4%)	
Treatment types			
Monotherapy	/	12 (41.4%)	
Combination therapy	/	17 (58.6%)	
Treatment lines			
1st line	/	5 (17.2%)	
2nd line and beyond lines	/	24 (82.8%)	
***MET* Amplification (FISH)**			0.29 #
Positive	9 (42.9%)	17 (58.6%)	
Negative	12 (57.1%)	12 (41.4%)	

Notes: *Wilcoxon test; # Fisher test; $ undefined NSCLC.

integrating the *MET* GCN data from the five NGS panels (Supplementary Table 1). The area under the receiver operating characteristic curve (AUC) was 0.94 (95% confidence interval (CI) = 0.84–1.00) (Figure 2A and Supplementary Table 1). With an increase in the cut-off value, the sensitivity was stable at 100% and then gradually decreased, accompanied by increased specificity and then stabilized at 100%. (Supplementary Table 1). To derive a more clinical applicable threshold while minimizing false-positive classification, a specificity higher than 90% and optimal accuracy were set as the standards to identify the cut-off. 6.55 was selected as the exploratory cutoff value for NGS *MET* GCN, with a specificity of 100%, sensitivity of 66.7%, and accuracy of 85.7%. The negative and positive predictive values (NPV) was 80.0% and the positive predictive value (PPV) was 100%, respectively (Figure 2B and Supplementary Table 1). Furthermore, all polysomy identified using FISH (n = 2) were identified using NGS.

Next, the performance of the cut-off value of 5, which is often used in clinics, and the cut-off value of 6.55 to assess *MET* amplification status, was compared (Table 2). When the cut-off of *MET* GCN was 5, three patients were FISH-negative but were evaluated as positive by NGS. When the cutoff was 6.55, the three FISH-negative patients were correctly defined as negative by NGS, but three patients were FISH-positive but incorrectly defined as negative by NGS. Generally, when the specificity of the cut-off value was 6.55, NGS performed better than when it was 5, and both cut-off values had an accuracy of 85.7%.

In addition, the performance of *MET* GCN with 5 and 6.55 using NGS in different NGS panels to assess *MET* amplification status is presented in Table 2. Compared with the GCN cut-off of 5, the cut-off of 6.55 showed improved specificity (GCN = 6.55, 81.8%-100.0%; GCN = 5:63.6%-83.3%), reduced sensitivity (GCN = 6.55:33.3%–88.9%; GCN

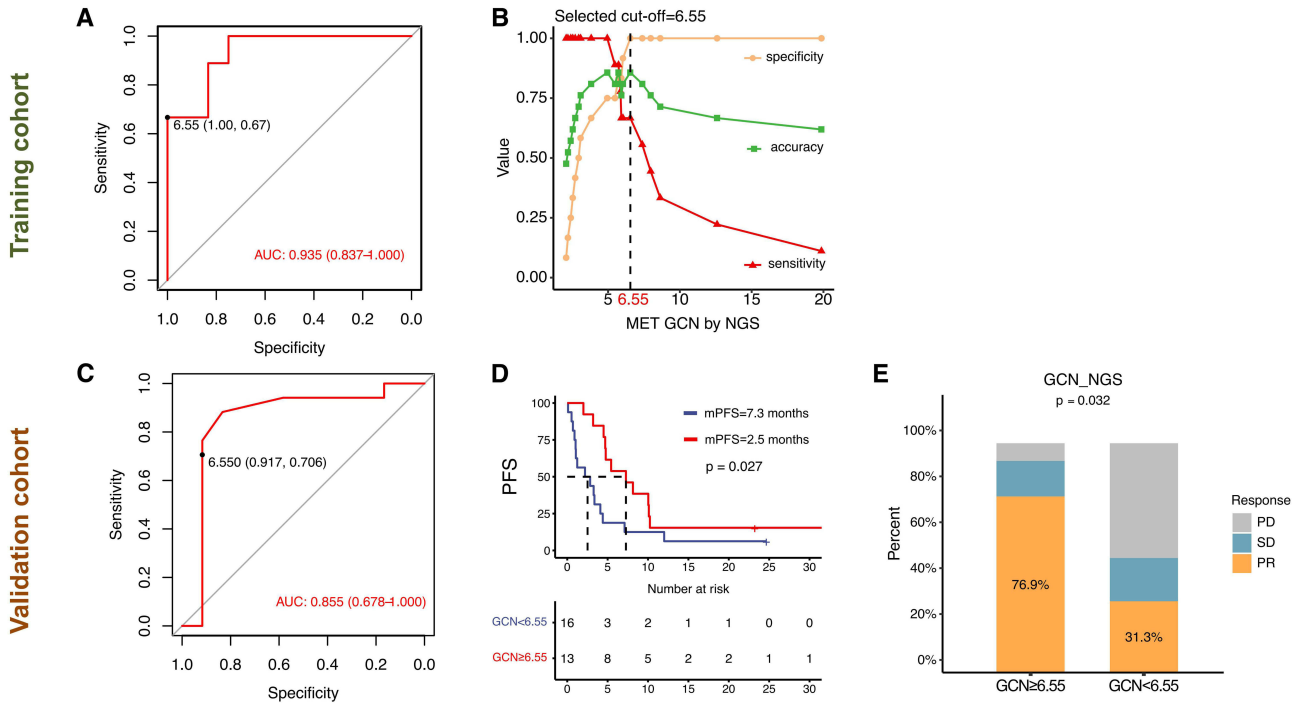

Figure 2 Performance and clinical association of the exploratory MET GCN cut-off. In the training cohort. (**A**) ROC analysis was performed using FISH as the reference for MET amplification, and (**B**) sensitivity, specificity, and accuracy across candidate NGS-derived MET GCN thresholds were evaluated to derive the exploratory cut-off of 6.55 (the data in red font). In the validation cohort, (**C**) ROC analysis assessed the classification performance of the derived cut-off, (**D**) Kaplan–Meier analysis compared progression-free survival according to MET GCN ≥6.55 versus <6.55, and (**E**) best overall response distributions were compared between the two groups.
Abbreviations: AUC, area under curve; p, P value; PD, progressive disease; SD, stable disease; PR, partial response.

= 5:55.6%-100.0%), and similar accuracy (GCN = 6.55:71.4%-85.7%; GCN = 5:71.4%-85.7%) across the five different panels. Additional panel details are provided in Supplementary Figure 1 and Supplementary Table 2.

In this study, *MET* GCN of 10 by FISH was used as the reference, and the optimal cut-off value of *MET* GCN by NGS was calculated under the criteria of 100% specificity and optimal sensitivity. As shown in Supplementary Table 3 and Supplementary Figure 2, the cutoff value of *MET* GCN with NGS was 8.63, with an AUC of 88.7%, specificity of 100%, sensitivity of 60.0%, accuracy of 90.5%, NPV of 88.9%, PPV of 100%, precision of 100%, recall of 60.0%, and Youden index of 60.0%, according to the pre-defined criteria.

Table 2 The Confusion Matrix with New Optimal Cutoff Value and *MET* GCN of 5 by Integrating Five Different Panels

		Training Cohort				Validation Cohort			
		Cutoff Value of 5 by NGS (n=21)		Cutoff Value of 6.55 by NGS (n=21)		Cutoff Value of 5 by NGS (n=29)		Cutoff Value of 6.55 by NGS (n=29)	
	***MET* amp Status**	**Positive**	**Negative**	**Positive**	**Negative**	**Positive**	**Negative**	**Positive**	**Negative**
FISH	Positive	9	0	6	3	15	2	12	5
	Negative	3	9	0	12	2	10	1	11
Sensitivity		100.0%		66.7%		88.2%		70.6%	
Specificity		75.0%		100.0%		83.3%		91.7%	
Accuracy		85.7%		85.7%		86.2%		79.3%	
NPV		100.0%.		80.0%		83.3%		68.8%	
PPV		75.0%		100.0%		88.2%		92.3%	

Abbreviations: Amp, amplification; NPV, negative predictive value; PPV, positive predictive value.

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Validation of the MET GCN Optimal Cutoff and Relation with Efficacy of MET-Targeted Therapy

In the validation cohort, when using FISH results as standard, *MET* amplification detected with tumor tissue by NGS analysis showed a high AUC of 0.86 (95% CI = 0.68–1.00) (Figure 2C). The optimal cutoff of 6.55 for NGS *MET* GCN also exhibited good performance in identifying *MET* amplification, with a specificity of 91.7%, sensitivity of 70.6%, and accuracy of 79.3% (Table 2). When the cutoff was set to 5, the specificity decreased to 83.3%, with a sensitivity of 88.2% and an accuracy of 86.2%. This suggests that the cutoff value of 6.55 improved the specificity for *MET* amplification detection when compared with that of the cutoff value of 5.

All 29 patients in the validation cohort were treated with MET inhibitors alone or in combination with EGFR-TKIs. Thus, to evaluate the performance of the cutoff value of 6.55, *MET* GCN ≥ 6.55 was defined as *MET* amplification positive and those < 6.55 as *MET* amplification negative, and the objective response rate (ORR) and survival benefit of MET-directed therapy. As shown in Figure 2D, patients with *MET* amp positivity (n = 13) had a significantly longer median PFS than those with *MET* amp-negative (7.3 months vs. 2.5 months; HR = 0.42 (95% CI: 0.20–0.96); p = 0.027). The ORR was 2.5-fold higher in *MET* amp-positive than in *MET* amp-negative patients (76.9% vs. 31.3%; p = 0.032) (Figure 2E). The survival benefit analysis of MET-directed therapy under FISH testing (A), with a cut-off value of 5 in MET GCN under NGS (B), and with a cut-off value of 10 under NGS (C) are provided in Supplementary Figure 3A–C.

Univariate and multivariate Cox regression analyses were used to explore the association of clinical variables with PFS of MET-directed therapy, *MET* amplification status by NGS (cutoff of 6.55), age, sex, *EGFR* mutation status, treatment line, and brain metastasis status (Figure 3). *MET* amplification positive identified by NGS with *MET* GCN ≥ 6.55 was also associated with a significant PFS benefit (HR = 0.42, p = 0.031) in univariate and multivariate analyses. Other clinical variables were not significantly associated with PFS after MET-targeted therapy.

Discussion

MET amplification is regarded as an oncogenic driver and mechanism of acquired resistance to first-, second-, and third-generation EGFR TKIs.[6,9,21–23] Currently, different testing methods are available, and the threshold for *MET* amplification using NGS varies in prospective clinical trials.[5–9,12] This may cause confusion in the interpretation of the test results and clinical applications. Although FISH has been used as the gold standard for identifying patients with *MET* amplification in previous studies,[18,24,25] with the increased adoption of comprehensive NGS as routine testing for patients with lung cancer, NGS can concurrently derive copy number alteration (CNA) data and provides a cost-effective and less labor-intensive alternative. The identification of the exploratory cutoff value for *MET* amplification under NGS testing in this study is consistent with the current reality of the widespread use of NGS for oncogenic driver detection, as well as the harmonization of standards for *MET* amplification, which is of great clinical importance.

In the current study, the optimization algorithm and *MET* GCN cutoff value for *MET* amplification were applied to distinguish *MET* amplification status using tumor tissue and NGS.[18,26] However, FISH and NGS detection results for *MET* amplification status were inconsistent due to the pathological dependence of FISH results and methodological problems in NGS assays. In this study, a training cohort and a validation NSCLC cohort were used to establish the exploratory cut-off for detecting *MET* amplification and to validate the clinical association of MET-targeted therapies. The optimal cutoff for *MET* GCN of 6.55 by NGS showed good performance with a specificity of 100% and an acceptable optimal sensitivity of 66.7% in identifying *MET* amplification in tissue samples. The AUC was 0.94. These results were replicated in the validation cohort study. Additionally, patients with *MET* GCN ≥ 6.55 had a significantly longer median PFS than those with *MET* GCN < 6.55 who received MET-targeted therapies. Overall, this study integrated results from five different NGS panels to explore an NGS-based MET GCN cut-off and evaluated its association with MET-targeted therapy outcomes in this cohort, suggesting potential clinical relevance. In the current clinical setting, MET copy number assessment is performed across multiple NGS platforms and reporting standards remain variable between laboratories; therefore, a potentially actionable threshold for interpreting NGS-derived MET GCN is still clinically meaningful. In our validation cohort, the percentage of *MET* amplification positive based on the

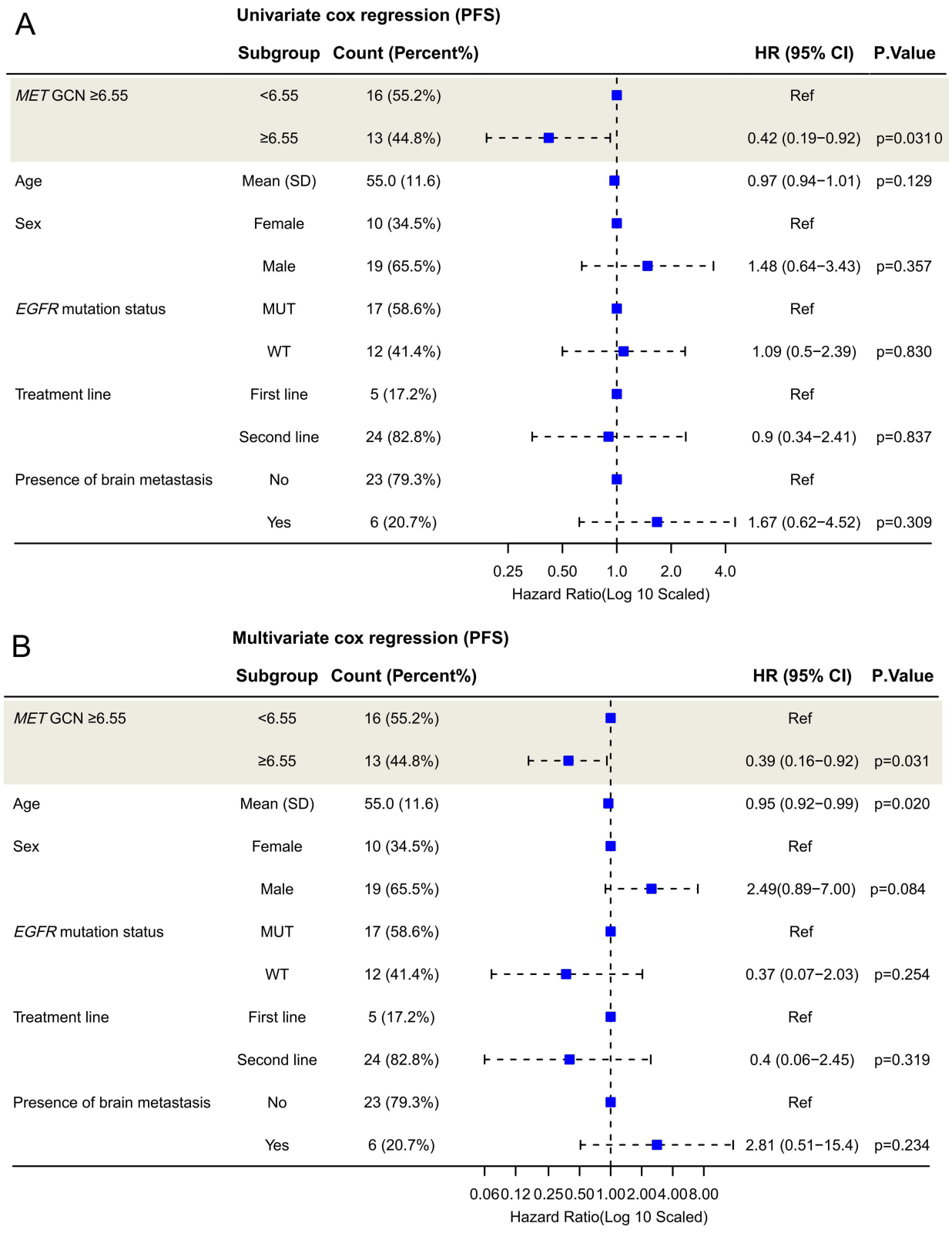

Figure 3 Univariate and multivariate cox regression analysis. Univariate (**A**) and multivariate cox regression (**B**) showed that the association between MET GCN 6.55 by NGS (in light grey background) or clinical characteristics and PFS in patients treated with MET-directed therapy.

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MET GCN cut-off of 6.55 by tissue NGS was 45%, which is very similar to the prevalence data of *MET* GCN cutoff of 5 by FISH in previous studies.[5,12,27]

In addition, when compared with *MET* GCN cut-off of 5, which is a currently used criterion to identify *MET* amplification, the *MET* GCN cut-off of 6.55 showed improved specificity in the training cohort and among these five different NGS panels. Using the criteria established by Cappuzzo, *MET* amplification by tissue NGS was defined as a mean GCN of ≥ 5. However, our previous study reported a concordance of 62.50% (25/40) between FISH and NGS (*MET* GCN ≥ 5) for determining *MET* amplification, with a sensitivity of 40% and specificity of 100%, respectively.[18] Lin et al also reported a prospective study comparing NGS and FISH platforms to test *MET* amplification in Chinese lung cancer patients. The results showed that the sensitivity and specificity of tissue NGS (*MET* GCN ≥ 5 by NGS) were 66.7% (14/21) and 91.3% (42/46), respectively, compared to FISH.[17] The results showed high specificity and acceptable sensitivity.

From a clinical perspective, specificity could be more important than sensitivity in *MET* amplification detection because reducing false-positive classification may help avoid inappropriate assignment to MET-targeted therapy. The standard of 100% specificity and optimal sensitivity was selected to optimize the cut-off value. Here, *MET* GCN cut-off of 6.55 met the pre-defined standard and correctly classified three *MET*-FISH negative cases as *MET*-NGS negative cases, all of which were defined as positive using a *MET*-NGS GCN cut-off of 5.

Patients with *MET* GCN≥ 10 by FISH who received savolitinib plus osimertinib showed more promising efficacy in the SAVANNAH study.[5] In this study, *MET* GCN of 10 by FISH was used as the reference, and the identified cut-off value of *MET* GCN was 8.63. However, whether the *MET* GCN of 8.63 is an optimal cut-off to distinguish patient benefits from MET inhibitors warrants further prospective exploration. In the future, MET-targeted PROTAC drugs represent a promising novel therapeutic strategy for MET-altered NSCLC, which may offer an alternative approach for patients with MET amplification or other MET alterations who have progressed on conventional MET-TKIs.[28]

In previous clinical studies, such as SAVANNAH, INSIGHT, and INSIGHT 2, *MET* FISH test results have been commonly used as the inclusion criteria.[5,9,11,27] However, exploratory analysis of tissue NGS in TATTON showed that *MET* GCN ≥ 5 had a preliminary efficacy trend; however, the optimal threshold could not be defined.[16] Our previous study also reported no significant difference in the clinical efficacy of MET-targeted therapies between patients with *MET* GCN ≥ 5 and *MET* GCN < 5 using NGS.[18] In the NSCLC patients who received MET-targeted therapies in our study, using the exploratory *MET* GCN cutoff, there was better efficacy in patients with *MET* GCN ≥ 6.55 compared than in those with *MET* GCN < 6.55. This suggests that the exploratory *MET* GCN cutoff with tissue NGS for *MET* amplification testing may help enrich for patients more likely to benefit from MET-targeted therapies.

It is worth noting that there are several limitations in our study. First, this was a retrospective study with a limited sample size, although the cut-off value was further evaluated in an independent cohort. Second, *MET* testing strategies remain variable across clinical settings, and technical variability between platforms may affect the comparability of amplification assessment. Third, the treatment regimens were not uniform, including both MET-TKI monotherapy and combination therapy, and some patients received MET-TKIs under negative FISH results owing to the complexity of real-world clinical practice, possibly because all other options were exhausted or based on high CNV under NGS. Finally, biological heterogeneity and unmeasured clinical confounders may also have influenced the observed associations. Thus, we are unable to further analyze whether the predictive value of the cutoff value of 6.55 varied according to line to therapy.

In conclusion, this study suggests that an exploratory cut-off of 6.55 for *MET* GCN by tissue NGS was associated with *MET* amplification and longer progression-free survival in patients receiving MET-targeted therapy. However, given the retrospective design, limited sample size, and lack of independent external validation, these findings are required further validation in larger prospective mult-center cohorts before broader clinical implementation. In addition, integration into routine clinical decision-making would require further standardization and reproducibility testing.

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