

# Metastatic NSCLCs With Limited Tissues: How to Effectively Identify Driver Alterations to Guide Targeted Therapy in Chinese Patients



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## ABSTRACT

**Introduction:** Molecular diagnostics of newly diagnosed patients with metastatic NSCLC (mNSCLC) with limited tissue samples often face several obstacles in routine practice using next-generation sequencing (NGS), mainly owing to insufficient tissue or DNA; thus, how to effectively identify the molecular profiling of these cases to accurately guide targeted therapy remains elusive. We evaluated whether an optimized workflow with the combined use of multiple technologies could be helpful.

**Methods:** Tissue NGS was used as the frontline method. Amplification refractory mutation system polymerase chain reaction, immunohistochemistry, fluorescence in situ hybridization, and plasma NGS were used as supplements.

**Results:** Among 208 mNSCLC cases with limited tissue (cohort 1), molecular genotyping using single-tissue NGS failed in 42 (20.2%) and actionable alterations were identified in only 112 of 208 cases (53.8%). In comparison, the optimized workflow in 1184 additional mNSCLC cases with limited tissue (cohort 2) increased the discovery rate of actionable alterations from 59.7% detected by tissue NGS to 70.4%. It was because that driver alterations were identified using amplification refractory mutation system polymerase chain reaction plus immunohistochemistry or fluorescence in situ hybridization in 53 of 78 (67.9%) tissue NGS-failed cases, and using plasma NGS in 73 of 143 (51.0%) tissue NGS-failed cases, which led to matched targeted therapies in 57 cases with clinical response. Moreover, the median turnaround time of the optimized workflow was significantly shorter than that of repeated biopsy for tissue NGS ( $p < 0.001$ ).

**Conclusions:** The optimized workflow can improve mutation detection and may avoid repeated biopsy, thus allowing

the timely initiation of targeted therapies for patients with newly diagnosed mNSCLC.

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**Keywords:** Non-small cell lung cancer; Limited tissue sample; Next-generation sequencing; Actionable alteration; Targeted therapy

## Introduction

Lung cancer is the most common cancer in China (Zhang et al.)<sup>1</sup>, and targeted therapies have provided considerable improvements in the survival and quality of patients with metastatic NSCLC (mNSCLC) whose tumors

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harbor certain molecular alterations.<sup>2</sup> Several specific alterations, including *EGFR*, *ALK*, *ROS1*, *BRAF*, *HER2* (*ERBB2*), *RET*, *MET*, and *NTRK*, are recommended to be assessed in all patients with newly diagnosed mNSCLC by several clinical practice guidelines.<sup>3-5</sup> To fully assess patients with mNSCLC for these targetable alterations, next-generation sequencing (NGS) has been widely applied in routine molecular genotyping, as it can detect multiple genetic alterations in a single assay.<sup>6</sup> It is reported that NGS can provide a broad detection range of potential genomic alterations and allow the assessment of single-nucleotide variants (SNVs), fusions, and copy number variations (CNVs) from DNA or RNA,<sup>7</sup> thus being preferable for the initial screening of NSCLC samples. Nonetheless, a large amount of high-quality DNA or RNA is needed for NGS and the failure rate for tissue genotyping using this approach in routine clinical practice is approximately 20%, mainly owing to insufficient tissue or DNA.<sup>8,9</sup> For these NGS-failed cases, repeated biopsies are generally not feasible in most patients, which may hinder the potential for patients to ultimately benefit from targeted therapy.

To address this issue, amplification refractory mutation system (ARMS) polymerase chain reaction (PCR) plus immunohistochemistry (IHC) or fluorescence in situ hybridization (FISH) and plasma NGS were introduced as supplements for tissue NGS in our laboratory. Here, we describe an optimized workflow with the integrated use of multiple methods (tissue NGS, ARMS-PCR plus IHC/FISH, and plasma NGS) for newly diagnosed patients with mNSCLC with limited tissue sample. Accordingly, we aim to determine the feasibility of the optimized strategy as part of routine clinical care to efficiently select patients with mNSCLC for targeted therapy.

## Materials and Methods

### Study Design and Patients

A total of 1392 newly diagnosed, treatment-naive patients with mNSCLC with limited tissue biopsy sample who requested molecular testing in our laboratory between May 2017 and June 2019 were enrolled. The types of biopsy samples included samples from core biopsy, fine-needle aspirate, bronchoscopic biopsy, pleural effusion (cytology specimen), and excisional biopsy. Clinical data, including clinicopathologic features, turnaround time (TAT) for molecular testing results, and treatment histories, were obtained in clinical records. The study was approved by the Institute Review Board of the National Cancer Center/National Clinical Research Center for Cancer/Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College. The methods were carried out in accordance with the approved guidelines. Informed consent was obtained from all patients.

### Tumor Cellularity Assessment and DNA Extraction

Pathologic assessment was done by the surgical pathology group. The samples were retrieved when molecular testing was requested, and tumor cellularity was evaluated by two independent pathologists, as previously described.<sup>10</sup> Genomic DNA was extracted from formalin-fixed, paraffin-embedded (FFPE) tissue using QIAamp DNA FFPE Tissue Kits (Qiagen, Duesseldorf, Germany). DNA quantity was assessed with the use of a Qubit 3.0 Fluorometer (Thermo Fisher Scientific, Carlsbad, CA), whereas DNA quality was checked by 1% agarose gel electrophoresis.

### Tissue NGS

Tissue NGS was performed with a panel designed against 56 cancer-related genes ([Supplementary Table 1](#)) (Burning Rock Biotech, Guangzhou, People's Republic of China).<sup>11</sup> Briefly, 50 to 100 ng of genomic DNA was used, and sequencing libraries were generated through DNA fragmentation and PCR amplification, hybridization, and capture. Indexed successful libraries were mixed at a proper concentration and sequenced on the NextSeq N500 platform (Illumina, San Diego, CA). Sequencing data were analyzed using an in-house Molecular Diagnostics Management System provided by Burning Rock Biotech, and variants (SNVs, indels, and fusions) were identified and reported when the coverage was greater than or equal to 1000 and the variant allele frequency was greater than or equal to 2%. CNVs were detected by normalizing the sequence coverage in targeted genes. Amplifications were called at segments with greater than or equal to 6 copies.

### Identification of Tissue NGS-Failed Cases

Samples were considered successfully tested by tissue NGS when high-quality DNA sequence results were obtained. Otherwise, samples that failed to be tested by tissue NGS were defined as tissue NGS-failed samples, which were further classified into the following four subgroups: (1) failed samples owing to insufficient tissue: samples with scant tissue (tissue < 2 mm in greatest dimension) or less than 10% tumor cell content; (2) failed samples owing to insufficient DNA: samples with a poor quantity (<50 ng measured by Qubit) or a poor quality of DNA (fragment < 500 base pair assessed by 1% agarose gel electrophoresis); (3) failed library: poor PCR product (size < 280 or >400 base pair or quantity < 4.5 ng measured by Qubit); and (4) failed samples owing to low-quality sequences: sequencing data that did not meet the laboratory quality control metrics.

### Amplification Refractory Mutation System

The Human *EGFR/KRAS/BRAF* Gene Mutation Detection Kit (ACCB, Beijing, P. R. China) was used to confirm *EGFR*, *KRAS*, and *BRAF* mutations in tissue biopsy samples. ARMS-PCR was performed as previously reported.<sup>12</sup> Briefly, genomic DNA (15 ng) and PCR master mixture were mixed in PCR tubes. Real-time PCR was performed as follows: 5 minutes at 95°C, followed by 40 cycles at 95°C for 15 seconds and 60°C for 1 minute. Hotspot mutations were identified in *EGFR*, *KRAS*, and *BRAF*, according to the threshold count following the manufacturer's instructions (Supplementary Table 2).

### Immunohistochemistry

A fully automated Ventana Benchmark XT stainer (Ventana Medical Systems, Tucson, AZ) was used to detect ALK expression in the NSCLC samples, as previously described.<sup>13,14</sup> In brief, each slide was stained with the primary antibody Ventana anti-ALK (D5F3) and then incubated with an OptiView DAB IHC Detection Kit and an OptiView Amplification Kit (Ventana Medical Systems). Negative and positive controls were also stained in each sample. Samples with strong granular cytoplasmic staining in tumor cells were considered ALK positive.

### Fluorescence In Situ Hybridization

FISH analysis was performed as previously described.<sup>13</sup> Briefly, Vysis LSI Dual Color and break-apart rearrangement probes specific to the *ROS1* and *RET* genes (Abbott Molecular, Abbott Park, IL) were used to detect *ROS1* and *RET* fusions, respectively. Samples with more than 15% of tumor cells that revealed break-apart signals were deemed positive. The Vysis MET SpectrumRed FISH probe and the CEP7 SpectrumGreen probe (Abbott Molecular) were used to identify *MET* amplifications. Tumors with a ratio of *MET*-to-*CEP7* greater than or equal to 2.0 or an average gene copy number per nucleus greater than or equal to 6.0 were considered amplification.<sup>15</sup>

### Plasma NGS

Peripheral venous blood was obtained from patients, and circulating tumor DNA (ctDNA) was isolated from plasma. Plasma NGS was performed with a panel designed against 168 genes (Supplementary Table 3) (Burning Rock Biotech), which had been validated in a clinical cohort of advanced lung cancer.<sup>16</sup> Briefly, 30 to 50 ng of ctDNA was used, and sequence libraries were constructed as described in the tissue NGS assay. Successful libraries were then sequenced on the NextSeq N500 platform (Illumina). Variants (SNVs, indels, and fusions) were identified and reported when the coverage

was greater than or equal to 10,000, and the variant allele frequency was greater than or equal to 0.2%. CNVs were detected by normalizing the sequence coverage in target genes. Amplifications were called at segments with greater than or equal to 3 copies. For 28 cases with concurrent ctDNA and tissue NGS results, plasma NGS was performed within 8 weeks of tissue NGS, with no intervening therapy.

### Assessment of Clinical Outcomes

For cases who had received targeted therapies, clinical responses were assessed on the basis of radiographic imaging, such as computed tomography or magnetic resonance imaging, by the oncologists. The objective response rate (ORR) was defined as the percentage of patients with a complete response or a partial response, following the Response Evaluation Criteria in Solid Tumors version 1.1. Progression-free survival (PFS) was determined from the date of treatment to progressive disease.

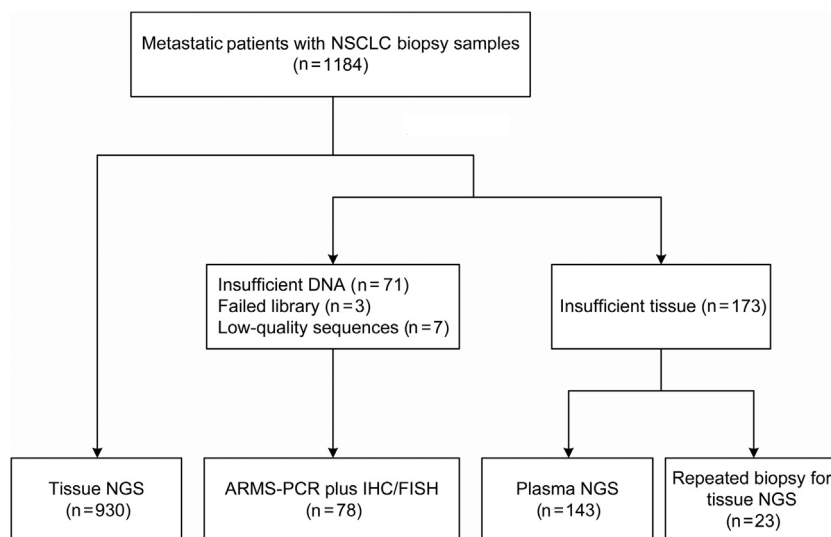
### Statistical Analysis

All analyses were performed using the software SPSS 22.0 (Chicago, IL). Differences in clinicopathologic variables between cohorts 1 and 2 were investigated by the chi-square test. TAT was measured from the time of biopsy to the time of receipt of molecular testing results, in business days. The TATs of different molecular assays were compared by the Wilcoxon signed rank test. A two-sided  $p < 0.05$  was considered statistically significant.

## Results

### Challenges for Routine Molecular Testing Using Single-Tissue NGS

Between May 2017 and December 2017, a total of 208 newly diagnosed mNSCLC cases with limited tissue sample were enrolled (cohort 1). Patient characteristics are provided in Supplementary Table 4. Actionable alterations, including *EGFR* mutations, *ALK* fusions, *ROS1* fusions, *KRAS* mutations, *BRAF* V600E mutation, *HER2* (*ERBB2*) exon 20 insertions, *RET* fusions, *MET* exon 14 skipping mutations, *MET* amplification, and *NTRK1* fusions, were evaluated. In total, DNA from 166 samples (166 of 208, 79.8%) was successfully sequenced. Actionable alterations were detected in 112 samples (112 of 208, 53.8%) (Supplementary Table 5), including one case with concurrent *EGFR* mutation and *MET* amplification. However, tissue genotyping failed in 42 samples (42 of 208, 20.2%) owing to insufficient tissue (28 of 42, 66.7%), insufficient DNA (12 of 42, 28.6%), failed library (1 of 42, 2.4%), and low-quality sequences (1 of 42, 2.4%).



**Figure 1.** Flowchart of the molecular testing strategy using different approaches for 1184 untreated mNSCLC cases with limited tissue samples. ARMS, amplification refractory mutation system; FISH, fluorescence in situ hybridization; IHC, immunohistochemistry; mNSCLC, metastatic NSCLC; NGS, next-generation sequencing; PCR, polymerase chain reaction.

### Optimization of the Molecular Screening Strategy With Multiple Platforms

To optimize the molecular screening strategy, additional platforms (ARMS-PCR, IHC, FISH, and plasma NGS) were validated and used in our laboratory. Between January 2018 and June 2019, a total of 1184 cases with mNSCLC were enrolled (cohort 2). There was no statistically significant difference between cohorts 1 and 2 with regard to patient characteristics (Supplementary Table 5). Of the 1184 cases, tissue NGS was successfully performed in 930 (78.5%), and actionable alterations were identified in 707 (59.7%) (Supplementary Table 4), including three cases with concurrent *EGFR* mutation and *HER2* amplification and one case with concurrent *EGFR* mutation and *MET* amplification. Concordance rates for cases with results from tissue NGS and the conventional laboratory testing (ARMS, IHC, or FISH) were 100% for *EGFR/KRAS/BRAF* mutations (109 of 109), 99.1% for *ALK* fusions (567 of 572), 98.5% for *ROS1* fusions (260 of 264), and 97.2% for *MET* amplification (104 of 107) (Supplementary Table 6).

Tissue NGS was unsuccessful in 254 samples (254 of 1184, 21.5%) owing to insufficient tissue (173 of 254, 68.1%), insufficient DNA (71 of 254, 30.0%), failed library (3 of 254, 1.2%), and low-quality sequences (7 of 254, 2.8%) (Fig. 1).

Regarding tissue NGS-failed samples ( $n = 81$ ) owing to insufficient DNA, failed library, and low-quality sequences, ARMS-PCR was performed and successfully completed in 78 samples (96.3%). Actionable alterations detected by ARMS-PCR included *EGFR* ( $n = 40$ ), *BRAF* V600E ( $n = 3$ ), and *KRAS* ( $n = 8$ ) mutations (Fig. 2A and

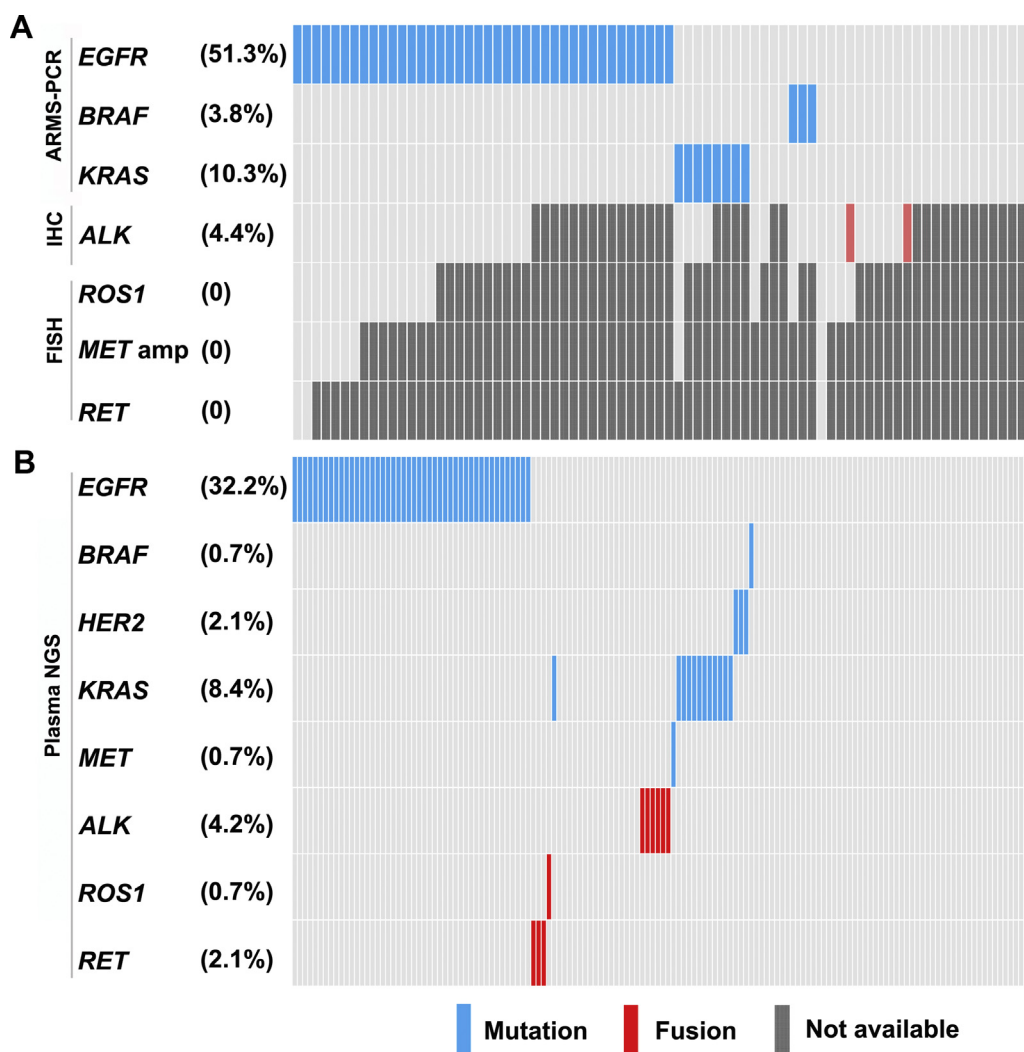
Supplementary Table 7). IHC or FISH (IHC/FISH) assays were performed in samples with available FFPE tumor slides to determine *ALK* ( $n = 43$ ), *ROS1* ( $n = 22$ ), and *RET* ( $n = 3$ ) fusions and *MET* amplification ( $n = 9$ ). *ALK* IHC positive was identified in two cases (Fig. 2A and Supplementary Table 7).

Regarding tissue NGS-failed cases owing to insufficient tissue, 143 cases (143 of 173, 82.7%) were analyzed using plasma NGS with ctDNA. In all cases, sequencing was successful, and the rate of detectable genomic variants in plasma ctDNA was 80.4% (115 of 143). Actionable alterations, including *EGFR*, *BRAF* V600E, *MET* exon 14 skipping, *HER2* exon 20 insertion, and *KRAS* mutations and fusions in *ALK*, *ROS1*, and *RET*, were identified in 73 cases (73 of 143, 51.0%) (Fig. 2B and Supplementary Table 8). Moreover, concurrent plasma NGS and tissue NGS were performed in an additional 28 mNSCLC cases. The concordance rate of targetable alterations between plasma NGS and tissue NGS was 71.4% (20 of 28) (Supplementary Table 9). Among cases with ctDNA NGS positive for actionable alterations, 93.8% (15 of 16) had identical tissue NGS results.

### Targeted Therapies and Clinical Outcomes

Targetable alterations were identified in 45 tissue NGS-failed cases using ARMS-PCR plus IHC/FISH assays. Of the 45 cases, 24 (53.3%) received a matched targeted therapy, including agents against *EGFR* mutations ( $n = 22$ ) and *ALK* fusions ( $n = 2$ ) (Table 1). A total of 23 cases were evaluated for clinical response. The ORR was 78.3%, and the median PFS was 10.3 (95% confidence interval: 7.0–11.6) months.





**Figure 2.** Molecular profiling of mNSCLC cases detected by ARMS-PCR plus IHC/FISH and plasma NGS. (A) A total of 78 tissue NGS-failed samples were successfully tested by ARMS-PCR plus IHC/FISH assays. (B) A total of 143 tissue NGS-failed samples were successfully sequenced by plasma NGS. amp, amplification; ARMS, amplification refractory mutation system; FISH, fluorescence in situ hybridization; IHC, immunohistochemistry; mNSCLC, metastatic NSCLC; NGS, next-generation sequencing; PCR, polymerase chain reaction.

Targetable alterations were identified in 61 tissue NGS-failed cases using plasma NGS, and 33 (54.1%) were treated with targeted therapies. Targets included *EGFR* (n = 25), *HER2* (n = 2), and *MET* (n = 1) driver mutations and *ALK* (n = 3), *ROS1* (n = 1), and *RET* (n = 1) fusions (Table 2). Among 30 assessable cases, the ORR was 70% and the median PFS was 9.6 (95% confidence interval: 6.8–12.6) months.

### Turnaround Time

We enrolled cases for whom the original biopsies were performed at our hospital to analyze TAT from the day of biopsy to the day of receipt of molecular testing results. The median TAT for tissue NGS performed in 884 samples from cohorts 1 and 2 was 12 business days

(range: 5–79 business d). The median TAT for ARMS-PCR plus IHC/FISH performed in 64 samples was 13 business days (range: 9–86 business d), and the median TAT for plasma NGS performed in 119 samples was also 13 business days (range: 6–115 business d). Of 72 cases with failed tissue NGS using original biopsy samples and who were not tested using ctDNA NGS (42 cases in cohort 1 and 30 cases in cohort 2), repeated biopsies were performed in 30 cases to complete genotyping. There were 29 cases whose original biopsies and repeated biopsies were both performed in our hospital. The median TAT for tissue NGS performed in these 29 cases was 24 business days (range: 14–94 business d). No statistically significant difference was found in TAT among results from tissue NGS, ARMS-PCR plus IHC/FISH, and plasma NGS ( $p = 0.196$ ). However, the median

**Table 1.** Targeted Therapies and Clinical Outcomes in Patients With Targetable Alterations Detected by ARMS-PCR Plus IHC/FISH

Patient ID	Targetable Alteration	Targeted Therapy	Optimal Response	PFS (mo)
1100	EGFR L858R	Icotinib	PD	1.5
1102	EGFR exon 19 del	Gefitinib	PR	11
1103	EGFR exon 19 del	Gefitinib	PR	16.3
1104	EGFR L858R	Gefitinib	SD	6.4
1105	EGFR exon 19 del	Gefitinib	PR	12
1106	ALK IHC positive	Crizotinib	PR	19.8
1107	EGFR L858R	Icotinib	PR	12.7
1108	EGFR exon 19 del	Gefitinib	PR	15.2
1118	EGFR exon 20 D770_N771insSVD	Afatinib	SD	5.4
1119	EGFR L858R	Afatinib	PR	7
1129	EGFR exon 19 del	Gefitinib	PR	10.3
1131	EGFR exon 19 del	Icotinib	PR	NR
1133	EGFR L858R	Osimertinib	PR	14.6
1135	EGFR L858R	Afatinib	PR	8
1136	EGFR L858R	Osimertinib	PR	11.3
1137	EGFR L858R	Icotinib	PR	11.6
1138	EGFR L858R	Gefitinib	SD	4.7
1140	EGFR G719C/S768I	Gefitinib	PR	7
1144	EGFR L858R	Gefitinib	PR	10
1148	EGFR L858R	Gefitinib	PR	11.2
1153	EGFR L858R	Gefitinib	Unknown	Unknown
1161	EGFR L858R	Erlotinib	SD	8
1167	EGFR exon 19 del	Erlotinib	PR	NR
1168	ALK IHC positive	Crizotinib	PR	NR

ARMS, amplification refractory mutation system; FISH, fluorescence in situ hybridization; ID, identification; del, deletion; IHC, immunohistochemistry; NR, not reached; PCR, polymerase chain reaction; PD, progression disease; PFS, progression-free survival; PR, partial response; SD, stable disease.

TAT of repeated biopsy for tissue NGS was significantly longer compared with the other approaches ( $p < 0.001$ ) (Fig. 3).

## Discussion

Here, we describe the feasibility and use of an optimized molecular diagnostic workflow for newly diagnosed patients with mNSCLC with limited tissue samples. Compared with molecular screening using a single-tissue NGS assay, the combined use of tissue NGS, ARMS-PCR plus IHC/FISH, and plasma NGS assays increased the discovery rate of actionable alterations from 707 of 1184 (59.7%) mNSCLC cases to 833 (70.4%). Therefore, implementation of our optimized workflow for routine molecular testing can improve mutation detection and thus alleviate the need for an additional invasive biopsy in real-world clinical practice (Fig. 4). The programmed death-ligand 1 IHC assay may also be performed concurrently to evaluate patients with mNSCLC who may benefit from immunotherapy,<sup>17</sup> though this was not assessed in our study.

The importance of NGS in the molecular profiling of NSCLC has already been well established.<sup>18,19</sup> Thus, tissue NGS was first introduced in our clinical routine for mNSCLC with limited tissue, as it offers the ability to

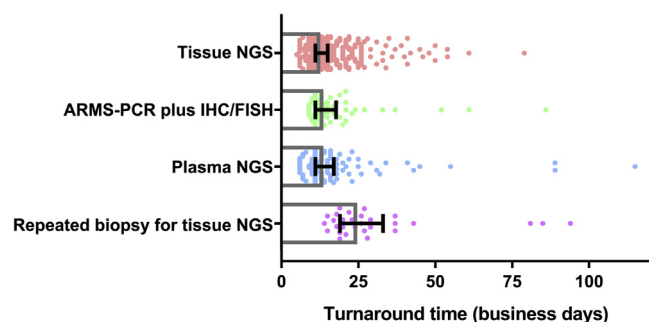
detect the full spectrum of known and unknown oncogenic alterations (SNVs, indels, fusions, and CNVs) in a single assay with high confidence. Despite its strength, NGS has several limitations, including the need to use large amounts of DNA. In accordance with previous studies,<sup>8,20</sup> the failure rate was 20.2% when only tissue NGS was used in cohort 1. Repeated biopsies are required for these failed patients to obtain additional tissue for genotyping. However, repeated biopsy was performed only in 7 of 42 cases (16.7%), possibly owing to anatomical difficulties, patient age, and comorbidities.

Within this context, the conventional laboratory testing or plasma NGS may be helpful. Compared with tissue NGS, the conventional laboratory testing, such as ARMS-PCR, focuses on the analysis of hotspot mutations and is able to be carried out with less abundant DNA input.<sup>21</sup> In this study, we performed ARMS-PCR on 81 tissue NGS-failed samples with relatively low DNA quality or quantity in cohort 2 and achieved successful sequencing in a major proportion of the samples (78 of 81, 96.3%). IHC and FISH assays are both quick and cost-effective methods for fusion detection, with only one to two slides.<sup>22,23</sup> Although ARMS-PCR plus IHC/FISH assays might offer a relatively narrow gene spectrum, actionable alterations were detected in 67.9% (53 of 78) of samples in our study, which is comparable to the 74.7% (819 of

**Table 2.** Targeted Therapies and Clinical Outcomes in Patients With Targetable Alterations Identified by Plasma NGS

Patient ID	Targetable Alteration	Targeted Therapy	Optimal Response	PFS (mo)
1183	EGFR L858R	Gefitinib	PR	13.2
1184	EGFR exon 19 del	Gefitinib	PR	13.4
1185	EGFR L858R	Gefitinib	PR	10.8
1186	EGFR L858R	Icotinib	PR	16.1
1187	EGFR exon 19 del	Gefitinib	PR	15.6
1188	ROS1 CD74-ROS1	Crizotinib	PR	10.8
1192	EGFR L858R	Icotinib	PR	15.1
1198	EGFR exon 19 del	Icotinib	PR	14.4
1199	EGFR L858R/V834L	Icotinib	PR	13.5
1200	EGFR exon 19 del	Icotinib	PR	11.7
1201	ALK EML4-ALK	Icotinib	Unknown	Unknown
1206	EGFR L858R	Gefitinib	PR	13
1208	RET CCDC6-RET	Cabozantinib	PD	1.2
1209	HER2 exon 20 G776>VV	Afatinib	PR	7.2
1210	EGFR L858R	Icotinib	PR	8.3
1219	EGFR L858R	Icotinib	SD	6
1222	EGFR exon 19 del	Icotinib	SD	2
1226	EGFR L858R	Icotinib	PR	12.2
1229	MET exon 14 c.3028+3A>T	Crizotinib	Unknown	Unknown
1231	EGFR L858R	Icotinib	SD	6.4
1232	EGFR L858R	Gefitinib	PD	2
1233	EGFR exon 19 del	Gefitinib	PR	12.1
1234	EGFR exon 19 del	Icotinib	Unknown	Unknown
1238	EGFR L858R/R776H	Icotinib	PR	7.1
1240	EGFR L858R	Icotinib	SD	5.4
1256	EGFR L858R	Icotinib	PR	14.3
1263	EGFR exon 19 del	Icotinib	PR	8
1264	EGFR L861Q	Osimertinib	SD	5.1
1266	EGFR L858R	Icotinib	SD	5.6
1273	ALK EML4-ALK	Crizotinib	PR	8
1276	HER2 exon 20 A775_G776insYVMA	Afatinib	PD	1.2
1291	ALK EML4-ALK	Crizotinib	PR	NR
1296	EGFR L858R	Icotinib	PR	NR

ID, identification; del, deletion; NGS, next-generation sequencing; NR, not reached; PD, progression disease; PFS, progression-free survival; PR, partial response; SD, stable disease.

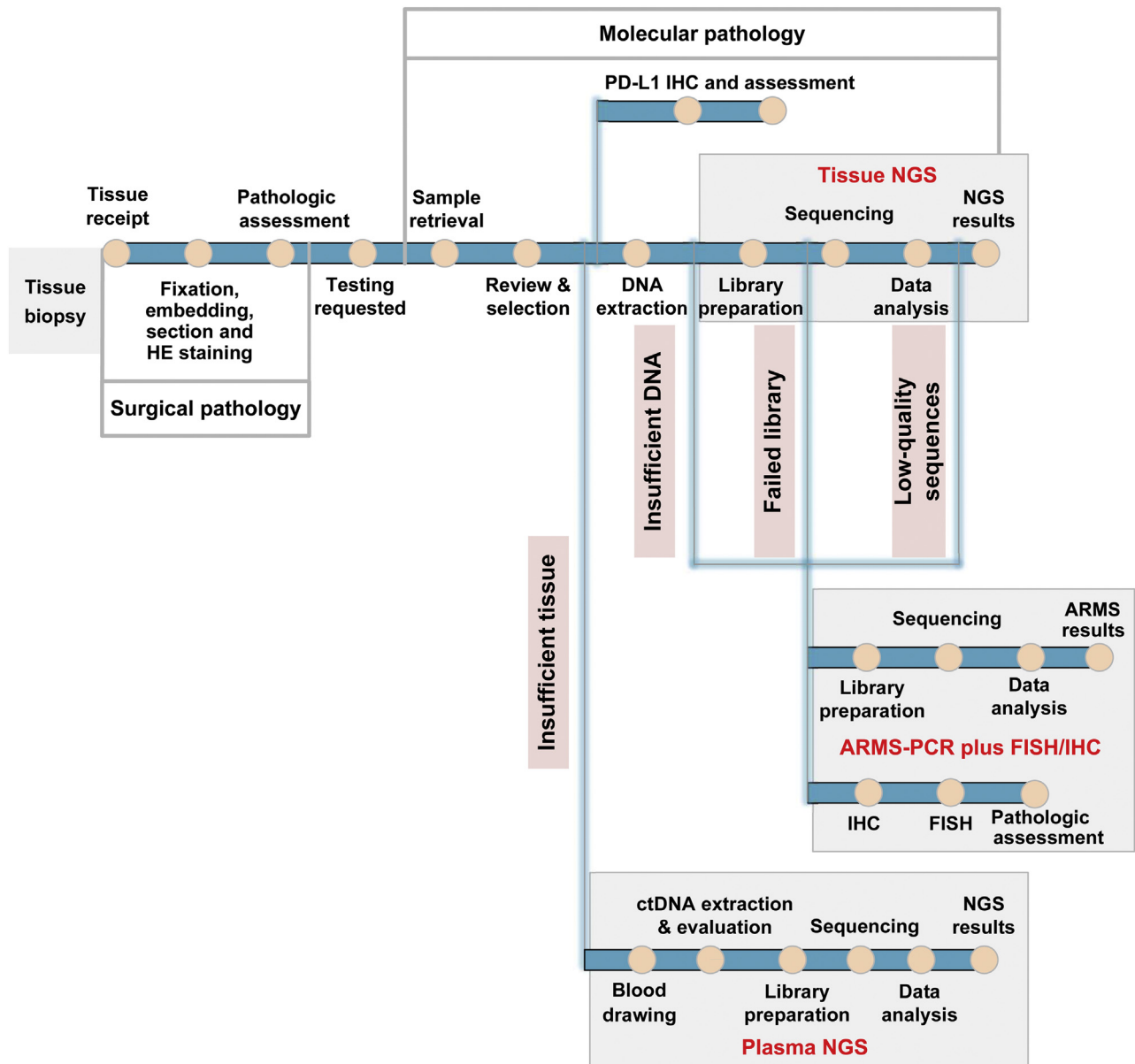


**Figure 3.** Turnaround time of tissue NGS, ARMS-PCR plus IHC/FISH, plasma NGS, and repeated biopsy for tissue NGS. ARMS, amplification refractory mutation system; FISH, fluorescence in situ hybridization; IHC, immunohistochemistry; NGS, next-generation sequencing; PCR, polymerase chain reaction.

1096) detection rate using tissue NGS ( $p = 0.746$ ), possibly owing to the high frequencies of *EGFR*, *ALK*, and *KRAS* alterations in Chinese patients with NSCLC.<sup>24,25</sup>

Moreover, 24 cases with targetable alterations identified by ARMS-PCR plus IHC/FISH received a matched therapy. The high ORR and long-term median PFS were also evidence of the high accuracy of conventional laboratory testing for these tissue NGS-failed samples.

Plasma NGS can detect the full spectrum of genomic alterations in ctDNA, which may serve as an alternative to tissue genotyping when tissue biopsy material is unavailable or insufficient.<sup>26,27</sup> Here, we identified actionable alterations in 51.0% (73 of 143) of tissue NGS-failed cases, which compared favorably with the 55.5% detection rate reported by Liu et al.<sup>28</sup> in Chinese patients with advanced NSCLC. Moreover, the concordance rate was 71.4% for an additional 28 cases for whom both ctDNA and tissue NGS results were available, which was in a range similar to those previously reported.<sup>29,30</sup> In addition, 33 cases with targetable alterations identified by plasma NGS received a matched therapy, with a high ORR and a long-term median PFS.



**Figure 4.** The molecular testing workflow with the combined use of multiple platforms. The major steps that occurred between the receipt of tissue samples and the issuing of molecular testing results were outlined in the molecular testing workflow for molecular screening of newly diagnosed mNSCLC cases with limited tissue samples. ARMS, amplification refractory mutation system; ctDNA, circulating tumor DNA; FISH, fluorescence in situ hybridization; HE, hematoxylin & eosin; IHC, immunohistochemistry; mNSCLC, metastatic NSCLC; NGS, next-generation sequencing; PCR, polymerase chain reaction; PD-L1, programmed death-ligand 1.

These results confirm the notion that a positive finding of targetable alterations in plasma can immediately guide targeted therapy.<sup>31</sup> Regardless, confirmatory repeated biopsy would be required in cases with a negative result.

NGS is a time-intensive process that may take several weeks from the time of biopsy to the time of receipt of molecular testing results.<sup>32</sup> Both ARMS-PCR plus IHC/FISH and plasma NGS can provide results generally faster than can tissue NGS. Thus, the TAT for results

from ARMS-PCR plus IHC/FISH or plasma NGS was comparable with that for tissue NGS but significantly shorter than repeated biopsy for tissue NGS. Therefore, our optimized workflow may potentially avoid treatment delays in patients with mNSCLC.

There were several limitations in our study. First, although tissue NGS was used as the frontline molecular screening method because of its technological advantage, some technical factors exist that may influence its ability for alteration detection,<sup>33,34</sup> especially for detection of



*ROS1* fusions and *MET* exon 14 skipping mutations.<sup>35</sup> Second, tissue for IHC/FISH assays was only available for a few tissue NGS-failed samples after DNA extraction, and the total number of targetable alterations detected by ARMS-PCR plus IHC/FISH would have been higher if enough FFPE tissue slides were obtained for all patients. Third, *ROS1* and *RET* fusions may be missed by FISH alone, and other druggable gene mutations such as *MET* exon 14 skipping, *BRAF* V600E, and *NTRK* fusions cannot be detected by ARMS-PCR plus IHC/FISH in our workflow. Thus, repeated biopsy or plasma NGS should be further considered for cases with negative results.

In summary, the optimized workflow for molecular testing is feasible, rapid, and useful in the real-world clinical practice setting, enabling improved routine identification of targetable alterations in patients with newly diagnosed mNSCLC and thus allowing the timely initiation of genotype-matched therapies.

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## Supplementary Data

Note: To access the supplementary material accompanying this article, visit the online version of the *JTO Clinical and Research Reports* at [www.jtocrr.org](http://www.jtocrr.org) and at <https://doi.org/10.1016/j.jtocrr.2021.100167>.

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