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

Weihua Li, MD, a Yan Li, MD, a Lei Guo, MS, a Yutao Liu, MD, b Lin Yang, MD, a Jianming Ying, MD, PhD a, *

a Department of Pathology, National Cancer Center/National Clinical Research Center for Cancer/Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, People’s Republic of China
b Department of Medical Oncology, National Cancer Center/National Clinical Research Center for Cancer/Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, People’s Republic of China

Received 19 December 2020; revised 9 March 2021; accepted 12 March 2021
Available online - 24 March 2021

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.

© 2021 The Authors. Published by Elsevier Inc. on behalf of the International Association for the Study of Lung Cancer. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

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.)1, and targeted therapies have provided considerable improvements in the survival and quality of patients with metastatic NSCLC (mNSCLC) whose tumors...
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. 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. 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, 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. 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. 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). 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. In 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. 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. 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 part signals were deemed positive. The Vysis MET with more than 15% of tumor cells that revealed break-apart rearrangement probes specific to the ROS1 and RET genes (Abbott Molecular, Abbott Park, IL) were used to detect ALK expression in the NSCLC samples, as previously described. 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. 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.

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. 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%).
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.
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
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, though this was not assessed in our study.

The importance of NGS in the molecular profiling of NSCLC has already been well established. 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, 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 analysis of hotspot mutations and is able to be carried out with less abundant DNA input. 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. 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

| 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 L858R           | 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.
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.\(^{24,25}\) 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.\(^{26,27}\) 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.\(^{28}\) 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.\(^{29,30}\) 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.

**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_YV | 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.
These results confirm the notion that a positive finding of targetable alterations in plasma can immediately guide targeted therapy. 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. 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, especially for detection of...
ROS1 fusions and MET exon 14 skipping mutations. 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.

Acknowledgments
This work was supported by grants from the Beijing Hope Run Special Fund of Cancer Foundation of China (LC2019L04) and the National Key Research and Development Program (2017YFC1311005).

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 and at https://doi.org/10.1016/j.jtocrr.2021.100167.

References
1. Zhang S, Sun K, Zheng R, et al. Cancer incidence and mortality in China, 2015. J Natl Cancer Center. 2021;1:2-11.
2. Miller KD, Siegel RL, Lin CC, et al. Cancer treatment and survivorship statistics, 2016. CA Cancer J Clin. 2016;66:271-289.
3. Ettinger DS, Aisner DL, Wood DE, et al. NCCN guidelines insights: non-small cell lung cancer, version 5.2018. J Natl Compr Canc. 2018;16:807-821.
4. Lindeman NI, Cagle PT, Aisner DL, et al. Updated molecular testing guideline for the selection of lung cancer patients for treatment with targeted tyrosine kinase inhibitors: guideline from the College of American Pathologists, the International Association for the Study of Lung Cancer, and the Association for Molecular Pathology. J Thorac Oncol. 2018;13:323-358.
5. K alemerian GP, Narula N, Kennedy EB, et al. Molecular testing guideline for the selection of patients with lung cancer for treatment with targeted tyrosine kinase inhibitors: American Society of Clinical Oncology endorsement of the College of American Pathologists/International Association for the Study of Lung Cancer/Association for Molecular Pathology Clinical Practice guideline update. J Clin Oncol. 2018;36:911-919.
6. Drilon A, Wang L, Arcila ME, et al. Broad, hybrid capture-based next-generation sequencing identifies actionable genomic alterations in lung adenocarcinomas otherwise negative for such alterations by other genomic testing approaches. Clin Cancer Res. 2015;21:3631-3639.
7. Rozenblum AB, Ilouze M, Dudnik E, et al. Clinical impact of hybrid capture-based next-generation sequencing on changes in treatment decisions in lung cancer. J Thorac Oncol. 2017;12:258-268.
8. Al-Kateh H, Nguyen TT, Steeger-May K, Pfeifer JD. Identification of major factors associated with failed clinical molecular oncology testing performed by next-generation sequencing (NGS). Mol Oncol. 2015;9:1737-1743.
9. Zehir A, Benayed R, Shah RH, et al. Mutational landscape of metastatic cancer revealed from prospective clinical sequencing of 10,000 patients. Nat Med. 2017;23:703-713.
10. Li W, Qiu T, Guo L, Ying J. Major challenges related to tumor biological characteristics in accurate mutation detection of colorectal cancer by next-generation sequencing. Cancer Lett. 2017;410:92-99.
11. Li W, Liu Y, Li W, Chen L, Ying J. Intergenic breakpoints identified by DNA sequencing confound targetable kinase fusion detection in NSCLC. J Thorac Oncol. 2020;15:1223-1231.
12. Li W, Qiu T, Guo L, et al. Primary and acquired EGFR T790M-mutant NSCLC patients identified by routine mutation testing show different characteristics but may both respond to osimertinib treatment. Cancer Lett. 2018;423:9-15.
13. Ying J, Guo L, Qiu T, et al. Diagnostic value of a novel fully automated immunohistochemistry assay for detection of ALK rearrangement in primary lung adenocarcinoma. Ann Oncol. 2013;24:2589-2593.
14. Li W, Zhang J, Guo L, Chuai S, Shan L, Ying J. Combinational analysis of FISH and immunohistochemistry reveals rare genomic events in ALK fusion patterns in NSCLC that responds to crizotinib treatment. J Thorac Oncol. 2017;12:94-101.
15. Schildhaus HU, Schultheis AM, Ruschoff J, et al. MET amplification status in therapy-naive adenocarcinomas of squamous cell carcinomas of the lung. Clin Cancer Res. 2015;21:907-915.
16. Mao X, Zhang Z, Zheng X, et al. Capture-based targeted ultradeep sequencing in paired tissue and plasma samples demonstrates differential subclonal ctDNA-releasing capability in advanced lung cancer. J Thorac Oncol. 2017;12:663-672.
17. Yu H, Boyle TA, Zhou C, Rimm DL, Hirsch FR. PD-L1 expression in lung cancer. J Thorac Oncol. 2016;11:964-975.
18. Thomas A, Rajan A, Lopez-Chavez A, Wang Y, Giaccone G. From targets to targeted therapies and molecular profiling in non-small cell lung carcinoma. Ann Oncol. 2013;24:577-585.
19. Moreira AL, Eng J. Personalized therapy for lung cancer. Chest. 2014;146:1649-1657.
20. Turner SR, Buonocore D, Desmeules P, et al. Feasibility of endobronchial ultrasound transbronchial needle aspiration for massively parallel next-generation sequencing in thoracic cancer patients. Lung Cancer. 2018;119:85-90.
21. Pepe F, De Luca C, Smeraglio R, et al. Performance analysis of SiRe next-generation sequencing panel in diagnostic setting: focus on NSCLC routine samples. *J Clin Pathol.* 2019;72:38–45.

22. Zugazagoitia J, Rueda D, Carrizo N, et al. Prospective clinical integration of an amplicon-based next-generation sequencing method to select advanced non-small-cell lung cancer patients for genotype-tailored treatments. *Clin Lung Cancer.* 2018;19:65–73. e7.

23. Copur MS, Crockett D, Gauchan D, Ramaekers R, Mleczko K. Molecular testing guideline for the selection of patients with lung cancer for targeted therapy. *J Clin Oncol.* 2018;36:2006.

24. Li W, Qiu T, Ling Y, Gao S, Ying J. Subjecting appropriate lung adenocarcinoma samples to next-generation sequencing-based molecular testing: challenges and possible solutions. *Mol Oncol.* 2018;12:677–689.

25. Yang L, Ling Y, Guo L, et al. Detection of ALK translocation in non-small cell lung carcinoma (NSCLC) and its clinicopathological significance using the Ventana immunohistochemical staining method: a single-center large-scale investigation of 1,504 Chinese Han patients. *Chin J Cancer Res.* 2016;28:495–502.

26. Schwaederle MC, Patel SP, Husain H, et al. Utility of genomic assessment of blood-derived circulating tumor DNA (ctDNA) in patients with advanced lung adenocarcinoma. *Clin Cancer Res.* 2017;23:5101–5111.

27. Zhou C, Yuan Z, Ma W, et al. Clinical utility of tumor genomic profiling in patients with high plasma circulating tumor DNA burden or metabolically active tumors. *J Hematol Oncol.* 2018;11:129.

28. Liu L, Liu H, Shao D, et al. Development and clinical validation of a circulating tumor DNA test for the identification of clinically actionable mutations in nonsmall cell lung cancer. *Genes Chromosomes Cancer.* 2018;57:211–220.

29. Thompson JC, Yee SS, Troxel AB, et al. Detection of therapeutically targetable driver and resistance mutations in lung cancer patients by next-generation sequencing of cell-free circulating tumor DNA. *Clin Cancer Res.* 2016;22:5772–5782.

30. Li BT, Janku F, Jung B, et al. Ultra-deep next-generation sequencing of plasma cell-free DNA in patients with advanced lung cancers: results from the Actionable Genome Consortium. *Ann Oncol.* 2019;30:597–603.

31. Zhang YC, Zhou Q, Wu YL. The emerging roles of NGS-based liquid biopsy in non-small cell lung cancer. *J Hematol Oncol.* 2017;10:167.

32. Dagogo-Jack I, Robinson H, Mino-Kenudson M, et al. Expediting comprehensive molecular analysis to optimize initial treatment of lung cancer patients with minimal smoking history. *J Thorac Oncol.* 2019;14:835–843.

33. Benayed R, Offin M, Mullaney K, et al. High yield of RNA sequencing for targetable kinase fusions in lung adenocarcinomas with no mitogenic driver alteration detected by DNA sequencing and low tumor mutation burden. *Clin Cancer Res.* 2019;25:4712–4722.

34. Cohen D, Hondelink LM, Solleveld-Westerink N, et al. Optimizing mutation and fusion detection in NSCLC by sequential DNA and RNA sequencing. *J Thorac Oncol.* 2020;15:1000–1014.

35. Li W, Guo L, Liu Y, et al. Potential unreliability of uncommon ALK, ROS1, and RET genomic breakpoints in predicting the efficacy of targeted therapy in NSCLC. *J Thorac Oncol.* 2021;16:404–418.