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Clinical Implications of Plasma-Based Genotyping With the Delivery of Personalized Therapy in Metastatic Non–Small Cell Lung Cancer

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IMPORTANCE The clinical implications of adding plasma-based circulating tumor DNA next-generation sequencing (NGS) to tissue NGS for targetable mutation detection in non–small cell lung cancer (NSCLC) have not been formally assessed.

OBJECTIVE To determine whether plasma NGS testing was associated with improved mutation detection and enhanced delivery of personalized therapy in a real-world clinical setting.

DESIGN, SETTING, AND PARTICIPANTS This prospective cohort study enrolled 323 patients with metastatic NSCLC who had plasma testing ordered as part of routine clinical management. Plasma NGS was performed using a 73-gene commercial platform. Patients were enrolled at the Hospital of the University of Pennsylvania from April 1, 2016, through January 2, 2018. The database was locked for follow-up and analyses on January 2, 2018, with a median follow-up of 7 months (range, 1-21 months).

MAIN OUTCOMES AND MEASURES The number of patients with targetable alterations detected with plasma and tissue NGS; the association between the allele fractions (AFs) of mutations detected in tissue and plasma; and the association of response rate with the plasma AF of the targeted mutations.

RESULTS Among the 323 patients with NSCLC (60.1% female; median age, 65 years [range, 33-93 years]), therapeutically targetable mutations were detected in *EGFR*, *ALK*, *MET*, *BRCA1*, *ROS1, RET, ERBB2,* or *BRAF* for 113 (35.0%) overall. Ninety-four patients (29.1%) had plasma testing only at the discretion of the treating physician or patient preference. Among the 94 patients with plasma testing alone, 31 (33.0%) had a therapeutically targetable mutation detected, thus obviating the need for an invasive biopsy. Among the remaining 229 patients who had concurrent plasma and tissue NGS or were unable to have tissue NGS, a therapeutically targetable mutation was detected in tissue alone for 47 patients (20.5%), whereas the addition of plasma testing increased this number to 82 (35.8%). Thirty-six of 42 patients (85.7%) who received a targeted therapy based on the plasma result achieved a complete or a partial response or stable disease. The plasma-based targeted mutation AF had no correlation with depth of Response Evaluation Criteria in Solid Tumors response (r = −0.121; P = .45).

CONCLUSIONS AND RELEVANCE Integration of plasma NGS testing into the routine management of stage IV NSCLC demonstrates a marked increase of the detection of therapeutically targetable mutations and improved delivery of molecularly guided therapy.

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Supplemental content

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The development of targeted therapies has changed the treatment paradigm for non–small cell lung cancer (NSCLC).[1] Molecularly targeted agents directed against driver and resistance mutations in *EGFR* [NG_007726.3], *ALK* [NG_009445.1], *ROS1* [NG_033929.1], and *BRAF* [NG_007873.3] have improved clinical outcomes in patients harboring these genetic alterations.[2,3] Indeed, for such patients, targeted therapy is the preferred treatment.[4-6] The National Comprehensive Cancer Network treatment guidelines advocate actionable mutation screening as standard of care,[7] but tumor tissue is often difficult to obtain or yields inadequate DNA, especially in the relapsed and metastatic settings.[8,9] Clinically relevant mutations may also change during the course of treatment,[10] an evolution that is difficult to monitor through tissue biopsy results alone. Spatial and temporal tumor heterogeneity make accurate assessment of resistance mutations based on biopsy of a single metastatic site challenging. A reliable method for noninvasive, clinically actionable mutation detection is, therefore, essential for the effective delivery of precision medicine for patients with NSCLC.

Liquid biopsy uses circulating tumor DNA (ctDNA) shed from tumors into the circulation as a substrate for molecular profiling.[11-18] Thompson et al[19] and Schwaederlé et al[20] have previously demonstrated the feasibility of mutation detection by clinical plasma-based ctDNA next-generation sequencing (NGS) for NSCLC. Recent retrospective and prospective studies[21] have used plasma for mutation detection in the focused setting of clinical trials of select targeted agents. Others have used research-based, nonclinical NGS platforms or polymerase chain reaction (PCR)–based tests for the detection of a limited number of mutations.[22-26] In 1 study,[26] patients with an *EGFR* T790M mutation detected in plasma using BEAMing (*b*eads, *e*mulsion, *a*mplification, and *m*agnetics) had a similar response rate to osimertinib mesylate as patients with the mutation detected in tissue, suggesting that *EGFR* T790M mutation detection might be achieved without tissue biopsy. To our knowledge, the implications of plasma NGS for a large panel of genes on clinical decision making as a part of routine care has not been formally assessed. We hypothesized that use of plasma NGS in addition to tissue NGS would improve the detection of actionable mutations in patients with NSCLC, thus aiding in prognostication and therapy selection. We report on the fully integrated use of clinical plasma and tissue NGS as part of routine clinical care for 323 patients with metastatic NSCLC enrolled during a 21-month period.

Key Points

Question Does adding plasma-based sequencing to tissue next-generation sequencing improve mutation detection for patients with non–small cell lung cancer?

Findings In this single-center cohort study of 323 patients with non–small cell lung cancer, 229 had concurrent plasma and tissue next-generation sequencing or were unable to complete tissue testing. Tissue alone detected targetable mutations for 47 patients (20.5%), whereas plasma sequencing increased targetable mutation detection to 82 (35.8%); 36 of 42 patients (85.7%) who received plasma next-generation sequencing–indicated therapy achieved a complete or a partial response or stable disease.

Meaning Adding plasma next-generation sequencing testing to the routine management of metastatic non–small cell lung cancer appears to increase targetable mutation detection and improve delivery of targeted therapy.

Methods

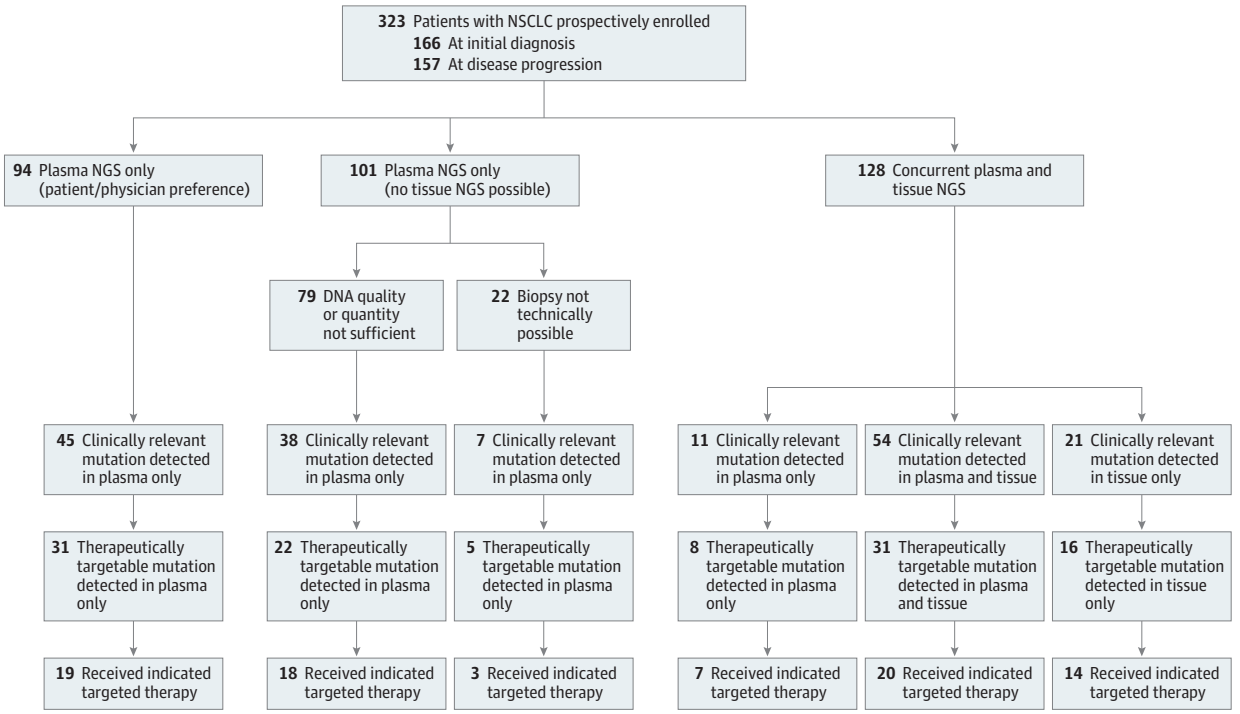
Study Design and Patients

This single-center prospective study was conducted at the Hospital of the University of Pennsylvania, Philadelphia, from April 1, 2016, through January 2, 2018. Eligible patients had histologically confirmed stage IV NSCLC, and plasma NGS was performed as part of routine clinical testing at diagnosis or at disease progression. Patients with a concurrent malignant neoplasm were excluded. Independent radiographic assessment using the Response Evaluation Criteria in Solid Tumors (RECIST), version 1.1 was performed for patients who received a targeted therapy based on plasma NGS results. Interval and frequency of radiographic assessments were based on standard of care clinical guidelines. Tumor response was assessed at the first follow-up imaging after initiation of a targeted agent. A clinically significant response was defined as a complete or partial response or stable disease by RECIST. We followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) reporting guideline statement to ensure the quality of data reported in this study.[27] The study was approved by the institutional review board of the University of Pennsylvania, which waived the need for informed consent.

Mutation Detection Using Plasma and Tissue DNA NGS

Plasma was analyzed by Guardant Health as previously described.[19] During our study, the Guardant360 panel expanded from 70 genes (116 patients) to 73 genes (207 patients). Tissue NGS results were considered concurrent if ordered within 24 weeks of plasma NGS testing, with no intervening therapy. Tissue NGS results from an outside hospital were obtained for 15 patients via electronic medical record abstraction. Concurrently obtained tissue samples from 113 patients were processed at our Clinical Laboratory Improvement Amendments–certified, College of American Pathologists–accredited Center for Personalized Diagnostics clinical laboratory. During our study, the Center for Personalized Diagnostics panel expanded from 47 genes[19] (17 patients) to a 153-gene panel (Comprehensive Solid Tumor HaloPlex^{HS}, version 2.0; Agilent Technology, Inc) (47 patients). The remaining 49 tissue samples yielded insufficient DNA for these panels, so a 20-gene panel (Penn Precision Panel; Perelman School of Medicine) was used (eTable 1 in the Supplement). Clinically relevant mutations included therapeutically targetable driver and resistance mutations in *EGFR*, *ALK*, *MET* [NG_008996.1], *BRCA1* [NG_007503.1], *ROS1, RET* [NG_007489.1], *ERBB2* [NG_007503.1], and *BRAF*. *KRAS* (NG_007524.1) mutations were also included because these are generally mutually exclusive with other

Figure 1. Patient Enrollment and Testing Flowchart



Flowchart summarizes patient enrollment, types of next-generation sequencing (NGS) tests conducted, and mutations detected. Concurrent plasma and tissue NGS was defined as tests ordered within 24 weeks of each other and no intervening systemic therapy. A clinically relevant mutation (in *EGFR*, *ALK*, *MET*, *BRCA1*, *ROS1*, *RET*, *ERBB2*, *BRAF*, and *KRAS*) was detected in 176 patients; a therapeutically targetable mutation (a subset of clinically relevant mutations that have targeted therapies available [eTable 2 in the Supplement]), in 113. Eighty-one patients received indicated targeted therapy. NSCLC indicates non–small cell lung cancer.

targetable variants and obviate further consideration of targeted therapy (eTable 2 in the Supplement).[25] A median of 3 mutations (range, 0-14) was detected per patient in plasma; however, no patient had more than 1 therapeutically targetable mutation detected (eTable 3 in the Supplement).

Statistical Analysis

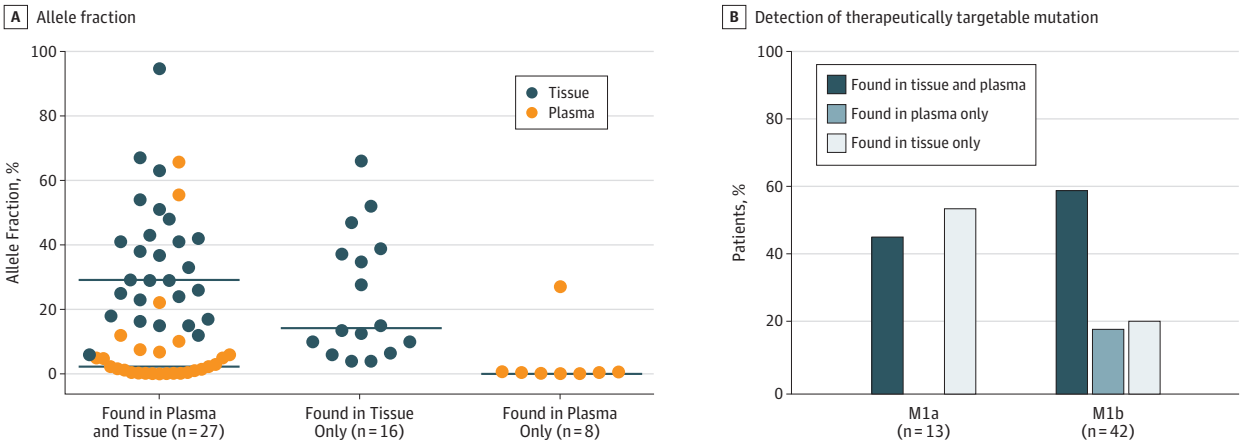
In patients with concurrent plasma and tissue NGS, Spearman rank correlation was used to quantify the association between the allelic fractions (AFs) of mutations detected in tissue and plasma. Concordance was calculated for 113 patients whose tissue NGS was performed at the University of Pennsylvania. Included were therapeutically targetable mutations in *EGFR*, *ALK*, *MET*, *BRCA1*, *ROS1, RET, ERBB2,* and *BRAF*. No changes in coverage for these 8 genes occurred between the 47- and 153-gene tissue NGS panels. The Pearson correlation coefficient was used to determine the association of percentage change in target lesions (determined by RECIST) with the targeted mutation plasma AF and the ratio of the AFs for the resistance and driver mutations. The Pearson χ^2 test was used to evaluate whether plasma-tissue NGS concordance differed by line of therapy. We used the nonparametric Wilcoxon rank sum test to assess differences in AF by line of therapy. Stata software (version 14; StataCorp) was used for all analyses.

Results

Patient Characteristics and Summary of NGS Test Results

A total of 323 patients (129 men [39.9%] and 194 women [60.1%]) with stage IV NSCLC underwent plasma-based NGS testing, with 166 enrolled at the time of initial diagnosis of stage IV disease and 157 at disease progression. Median age was 65 years (range, 33-93 years); 105 (32.5%) had never smoked; and 276 (85.4%) had adenocarcinoma (eTable 4 in the Supplement). Fifteen patients with tumors of squamous histologic origin were included; 5 of these patients had limited or no smoking history, and the National Comprehensive Cancer Network guidelines now recommend considering broad molecular profiling in this patient population.[7] For the 323 patients, 207 tissue NGS tests were ordered concurrently with plasma NGS tests at our hospital or the referring institution's hospital. Seventy-nine of these 207 patients had insufficient quantity or quality of tissue DNA for NGS, consistent with other published data.[19,28-30] For the 128 patients with concurrent tissue NGS results, 24 were discordant (therapeutically targetable mutation found in tissue or plasma but not both) (**Figure 1**), 31 had a therapeutically targetable mutation detected in tissue and plasma, and 73 had a wild-type report for targetable mutations in both tests, resulting in concordance of 81.3% (eFig-

Figure 2. Analysis of Mutation Detection by Type of Test and Disease Stage



A, Fifty-five patients had concurrent plasma and tissue next-generation sequencing (NGS) with a therapeutically targetable mutation detected. This subset included 4 patients with outside hospital testing for whom no allele fraction (AF) was reported. For the remaining 51 patients, a comparison of the AFs of therapeutically targetable mutations is shown. The horizontal black line indicates median AF for each group. For the 27 patients who had the mutation AF reported for plasma and tissue, the upper horizontal line corresponds to the median for the tissue AFs, and the lower horizontal line corresponds to the median for the plasma AFs. B, To assess the effect of disease location on detection of therapeutically targetable mutations in plasma and tissue, plasma and tissue testing results were compared for 55 patients with concurrent testing. Included are 13 with disease limited to the thoracic cavity (M1a) and 42 with extrathoracic metastases (M1b) as determined by imaging.

ure 1A-B in the Supplement). Concordance for 81 patients at diagnosis was significantly higher (88.9%) than for 47 patients at progression (70.2%; $P = .008$). Concordance for 46 patients who received therapy within 4 weeks of NGS (71.1%) was lower than for the remaining 82 patients (86.5%; $P = .04$). For 39 patients who had a therapeutically targetable mutation detected in plasma, we found no significant difference in median AF between those receiving active treatment (2.1%) vs those who were not (1.1%; $P = .76$).

Detection of Clinically Relevant and Therapeutically Targetable Mutations

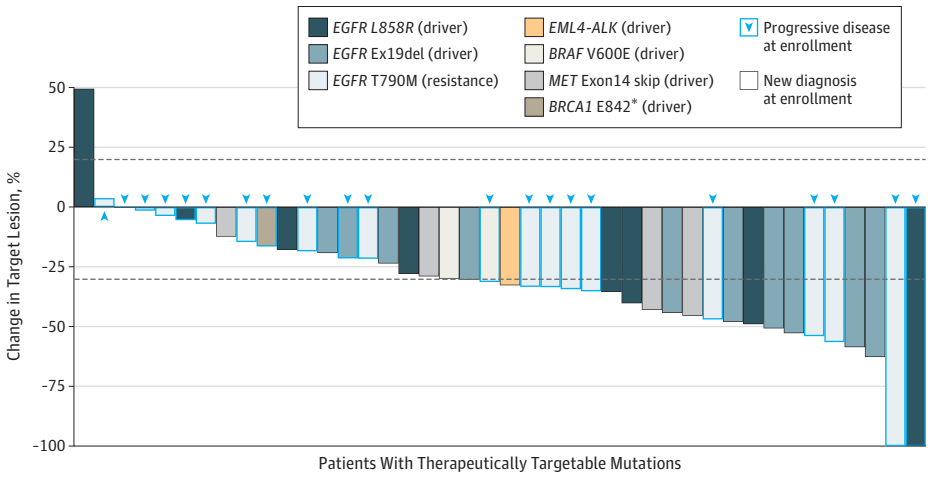
Clinically relevant mutations (eTable 2 in the Supplement) were detected in tissue and/or plasma of 176 of 323 patients (54.5%). Of the 176 patients, 101 (57.4%) had the mutation detected in plasma only, including 45 for whom it was the patient's or physician's preference to order plasma NGS only, and 11 patients who had a concurrent tissue test with a wild-type report. Fifty-four of 176 patients (30.7%) had the mutation detected in plasma and concurrent tissue, and 21 (11.9%) had the mutation detected in tissue only (Figure 1). Among 15 patients with squamous cell disease, 6 had clinically relevant mutations detected in plasma alone (n = 1) and in tissue and plasma (n = 5) (eTable 3 in the Supplement).

Therapeutically targetable mutations were detected in 113 patients (35.0%), among whom 66 (58.4%) had the mutation detected in plasma only, including 8 patients who had negative concurrent tissue test results. A targetable mutation was detected in plasma and concurrent tissue samples for 31 patients, among whom 27 completed their tissue NGS at our institution and had AFs reported. For these 27 patients, a significant correlation between the tissue and plasma mutation AFs was found ($\rho = 0.40$; $P = .02$) (eFigure 1C in the Supplement). Sixteen of 113 patients (14.2%) had a targetable mutation in tissue only (Figure 1). Among these 113 patients, 81 (71.7%) received the indicated targeted therapy, 2 were lost to follow-up or death, and 3 received an alternate therapy at their oncologist's discretion. Twenty-seven of 113 patients (23.9%) had a driver mutation detected in plasma that had first been detected before enrollment in our study. These patients were already receiving the indicated therapy.

To assess whether adding plasma NGS to tissue NGS improved mutation detection, we considered the 229 patients who had concurrent plasma and tissue NGS (n = 128) or for whom a tissue NGS test was not possible (n = 101). Among the 128 patients with concurrent plasma and tissue NGS testing, 8 had a therapeutically targetable mutation detected in plasma for which the tissue test result was wild-type, with plasma testing thus increasing mutation detection from 36.7% (47 of 128 patients) to 43.0% (55 of 128 patients). For the 101 patients for whom tissue NGS was not possible, 27 (26.7%) had a therapeutically targetable mutation detected. Therefore, for these 229 patients, mutation detection increased from 47 mutations (20.5%) to 82 (35.8%) when plasma testing was added to tissue NGS (Figure 1). For the remaining 94 patients, the physician recommended or the patient chose to perform plasma testing instead of concurrent tissue testing. These patients were not included in our calculation above because tissue testing could have been performed. Importantly, 31 of the 94 patients (33.0%) had a therapeutically targetable mutation detected in plasma and thus avoided an invasive biopsy.

For 16 patients for whom plasma NGS failed to detect a therapeutically targetable mutation concurrently found in tissue, we hypothesized that the tissue AFs were low, leading to a plasma AF below the test's level of detection. However, tissue mutation AFs were often quite high (median, 14.3%; range,

Figure 3. Response of Patients to Plasma-Indicated Targeted Therapy as Measured by Response Evaluation Criteria in Solid Tumors (RECIST)



Waterfall plot shows the percentage change in target lesion diameter as determined by RECIST for patients with therapeutically targetable mutations detected by plasma. Forty-two patients with driver or resistance mutations underwent analysis, including 21 undergoing plasma next-generation sequencing at diagnosis and 21 at disease progression. Thirty-six patients (85.7%) achieved a complete response, partial response, or stable disease. An increase in size of target lesions by more than 20% indicates progressive disease, while decrease in size of target lesion of more than 30% indicates disease response.

4.0%-66.0%) (**Figure 2**A). To assess whether disease stage might affect mutation detection, we compared mutation detection for plasma- and tissue-based NGS by metastatic stage. In the setting of M1b (extrathoracic) disease, mutations for 8 of 42 patients (19.0%) were detected in plasma only, and mutations for another 25 patients (59.5%) were detected in plasma and tissue. However, for patients with M1a (intrathoracic) disease, plasma NGS utility was lower, with mutations for 7 of 13 patients (53.8%) found in tissue only, and no mutations found in plasma alone (Figure 2B). We noted that for 13 patients with liver metastases, 100% of mutations were detected in plasma. These results suggest that adding plasma NGS to tissue NGS can enhance the detection of therapeutically targetable mutations but may vary by location of metastatic disease.

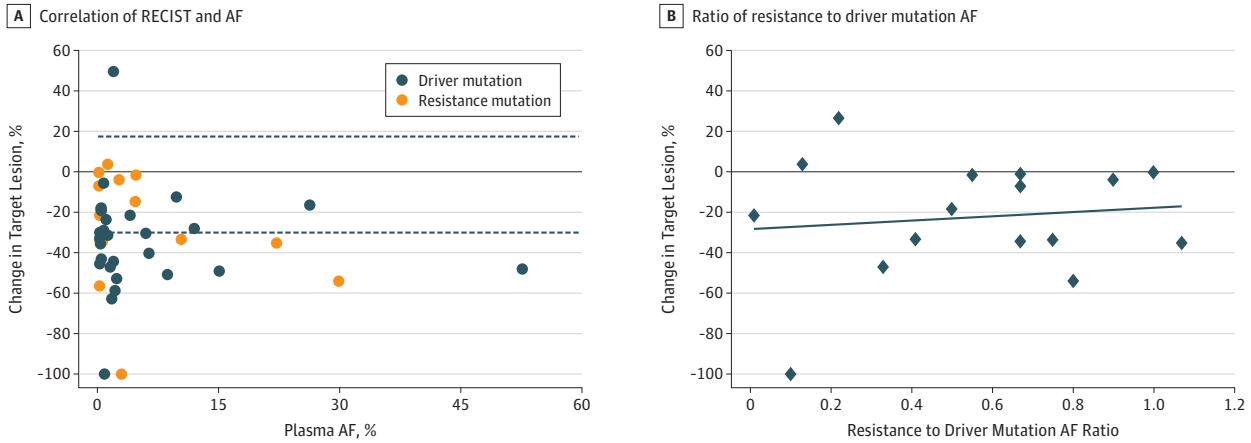
Response to Plasma NGS-Based Indicated Therapy

Sixty-seven patients received a targeted therapy indicated by plasma NGS alone (n = 47) or concurrent plasma and tissue NGS (n = 20) (Figure 1). Targets included *EGFR*, *ALK*, *BRAF*, *BRCA1*, and *MET* driver mutations (n = 41), *EGFR* T790M (n = 24), and *ALK* resistance mutations (n = 2). Among 42 patients who were evaluable by RECIST (30 with the mutation detected in plasma only plus an additional 12 patients with mutation detection in plasma and tissue) (eTable 5 in the Supplement), the percentage change in target lesions ranged from −100% to 49.6% (median, −32.0%) (**Figure 3**). Thirty-six of 42 patients with evaluable results (85.7%) achieved either a complete response (n = 1), a partial response (n = 19), or stable disease (n = 16). Eighteen of 21 patients (85.7%) achieved disease control after first-line therapy, and the disease control rate was the same for the 21 patients treated at disease progression. We next examined the correlation between the percentage change of the target lesion measured by RECIST and the AF of the plasma-based mutation for which a targeted therapy was indicated. We found no correlation between the 2 variables (r = −0.121; P = .45) (**Figure 4**A). Tissue AF was also not correlated with depth of RECIST response for the subset of 10 patients for whom tissue NGS results were available (r = 0.216; P = .18) (eFigure 2 in the Supplement). Among patients who achieved a RECIST response, the plasma AF of the targeted mutation ranged from 0.3% to 52.6% (median, 1.9%). Finally, for the 16 patients who received osimertinib targeting the *EGFR* T790M resistance mutation based on plasma test results, we assessed whether the plasma-based ratio of resistance to driver mutation AFs at the time of progression during treatment with a front-line *EGFR* inhibitor was indicative of depth of response to osimertinib. We found no significant correlation between the AF ratio and depth of response (r = 0.116; P = .67) (Figure 4B).

Discussion

This single-center prospective study assessed the real-world clinical utility of plasma-based genotyping in patients with metastatic NSCLC. We hypothesized that adding plasma NGS would increase detection of therapeutically targetable mutations and allow personalized therapy for more patients. Therapeutically targetable mutations were detected in 113 of 323 patients (35.0%) overall. Importantly, mutations for 35 of 113 patients (31.0%) were detected in plasma only when tissue DNA was insufficient or unavailable, or no mutation was detected in tissue. Targetable mutations were detected for 31 patients in plasma and tissue. In 16 patients, targetable mutations were found in tissue only. Sixty-seven of 97 patients (69.1%) with a targetable mutation detected in plasma subsequently underwent targeted therapy with clinically significant disease control (36 of 42 evaluable patients [85.7%]). This group includes 5 patients whose therapies targeted uncommon mutations in *MET* (n = 4) and *BRCA1* (n = 1), most of whom achieved a clinical response. Among 128 patients with concurrent tissue and plasma NGS, a therapeutically targetable mutation was detected for 55 (43.0%), whereas if tissue had been the only NGS test, a mutation would have been found for only 47 patients (36.7%). Among 101 patients for whom tissue NGS was impossible, 27 (26.7%) had a therapeutically targetable mutation detected. Altogether in our study, adding plasma NGS to tissue

Figure 4. Plasma-Based Indicators of Response to Plasma Next-Generation Sequencing (NGS)–Indicated Therapy



A, Correlation between depth of response to the targeted therapy indicated by plasma, and plasma allele fraction (AF) for the therapeutically targeted mutation (r = −0.121; P = .45) in the 42 patients for whom Response Evaluation Criteria in Solid Tumors (RECIST) analysis was completed. B, Correlation between depth of response to targeted therapy and the ratio of resistance to driver mutation AF (r = 0.116; P = .67). This analysis was conducted for the 16 patients who received osimertinib mesylate to target the *EGFR* T790M resistance mutation detected in plasma and for whom RECIST analysis was completed.

NGS increased detection of therapeutically targetable mutations from 47 of 229 patients (20.5%) to 82 (35.8%).

Although a tissue biopsy remains essential for initial cancer diagnosis, in the setting of inadequate tissue DNA, our results show that plasma NGS can be an adequate surrogate for molecular profiling. Plasma-based mutation detection methods, including the PCR-based cobas *EGFR* plasma test (Roche) and BEAMing, are sensitive tools but limited to detection of a restricted number of mutations.[26,28,31-33] Oxnard et al[26] showed in a clinical trial of patients with *EGFR*-mutant NSCLC whose disease had progressed during first-generation tyrosine-kinase inhibitor therapy that patients with the *EGFR* T790M mutation in plasma have similar outcomes when treated with osimertinib compared with patients with the mutation detected in tissue. Certainly, cobas or another PCR-based *EGFR* test could have been used to detect the T790M mutation found in the plasma of 24 of our patients who received osimertinib at progression after a front-line *EGFR* inhibitor. However, among all 67 patients who received a plasma NGS-indicated targeted therapy, PCR-based *EGFR* mutation testing would have missed therapeutically targetable mutations in *BRAF*, *MET*, and *BRCA1* for 11 patients, including 2 at progression. The use of plasma-based NGS for the management of NSCLC has only been explored in a few studies with small patient numbers.[21,26,28] To our knowledge, our study is the largest to report the detection and clinical utility of plasma-based NGS ordered as part of routine clinical care for patients with metastatic NSCLC.

As ctDNA sensitivity improves,[18] the question arises regarding whether therapeutic targeting of a low AF mutation will yield clinical benefit. We showed for 42 evaluable patients that depth of response to targeted therapy did not correlate with the mutation AF; even patients with very low AFs (as low as 0.3%, just above the test level of detection of 0.1%) sustained a significant clinical response. We also assessed whether the resistance to driver mutation AF ratio in 16 RECIST-evaluable patients with *EGFR* T790M–positive NSCLC correlated with response to osimertinib. Consistent with a larger study[26] using PCR-based plasma testing, no overall correlation was seen (P = .67). The previous study also reported that patients with a relative *EGFR* T790M AF greater than 10% had greater depth of response,[26] a result our study was insufficiently powered to assess.

We report an overall concordance of 81.3% and the novel observation that therapeutically targetable mutation detection was highest for patients with liver metastases (100% concordance with tissue [n = 13]) compared with patients with M1a disease (46.2% concordance). Sixteen patients had negative plasma test results despite having detectable mutations in tissue, some at high AF. Our results support recently reported findings, in which patients with intrathoracic metastases alone were less likely to have detectable ctDNA.[28] Larger studies are needed, but these findings suggest a decision metric whereby the order in which plasma or tissue NGS is requested could be guided by disease stage, with tissue biopsy preferred for patients with M1a disease, for example.

Limitations

Our results show that effective delivery of precision medicine requires the integration of plasma and tissue testing, which are potentially practice changing. Nevertheless, our study has limitations. This single-center study was conducted among physicians who were comfortable ordering and interpreting plasma NGS tests. This user bias probably enriched for patients who had plasma NGS only and were likely to have targetable mutations. A sizeable proportion of patients underwent testing after progression to detect resistance mutations, which likely increased the frequency of patients with *EGFR* T790M. Moreover, our study only considers plasma NGS testing at a single point. The clinical utility of longitudinal plasma NGS-based monitoring is an area of active study in our group.

Conclusions

This clinical study is, to our knowledge, one of the largest to measure the implications of plasma-based genotyping for the delivery of targeted therapy in NSCLC and clearly demonstrates that liquid biopsy can improve delivery of therapy and, consequently, outcomes. To keep up with rapid therapeutic progress in the molecular diagnosis and treatment of NSCLC, we must incorporate safe and facile noninvasive methods for sensitive, comprehensive tumor profiling to select patients for personalized therapy. Given the ease of obtaining plasma-based genotyping and the success observed with such a noninvasive approach, our results argue for incorporation of plasma-based genotyping into routine clinical management of patients with NSCLC.

ARTICLE INFORMATION

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Acquisition, analysis, or interpretation of data: Aggarwal, Thompson, Black, Katz, Fan, Yee, Chien, Evans, Bauml, Alley, Ciunci, Cohen, Lieberman, Majmundar, Savitch, Morrissette, Hwang, Langer, Carpenter.
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