

Complementary Roles for Tissue- and Blood-Based Comprehensive Genomic Profiling for Detection of Actionable Driver Alterations in Advanced NSCLC



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Received 27 April 2022; revised 19 July 2022; accepted 23 July 2022
Available online - 2 August 2022

ABSTRACT

Introduction: Whereas tumor biopsy is the reference standard for genomic profiling of advanced NSCLC, there are now multiple assays approved by the Food and Drug Administration for liquid biopsy testing of circulating tumor DNA. Here, we study the incremental value that liquid biopsy comprehensive genomic profiling (CGP) adds to tissue molecular testing.

Methods: Patients with metastatic NSCLC were enrolled in a prospective diagnostic study to receive circulating tumor DNA CGP; tissue CGP was optional in addition to their standard tissue testing. Focusing on nine genes listed per the National Comprehensive Cancer Network (NCCN) guidelines, liquid CGP was compared with available tissue testing results across three subcohorts: tissue CGP, standard-of-care testing of up to five biomarkers, or no tissue testing.

Results: A total of 515 patients with advanced nonsquamous NSCLC received liquid CGP. Among 131 with tissue CGP results, NCCN biomarkers were detected in 86 (66%) with tissue CGP and 56 (43%) with liquid CGP ($p < 0.001$). Adding liquid CGP to tissue CGP detected no additional patients with NCCN biomarkers, whereas tissue CGP detected NCCN biomarkers in 30 patients (23%) missed by liquid CGP. Studying 264 patients receiving tissue testing of up to five genes, 102 (39%) had NCCN biomarkers detected in tissue, with an additional 48 (18%) detected using liquid CGP, including 18 with *RET*, *MET*, or *ERBB2* drivers not studied in tissue.

Conclusions: For the detection of patients with advanced nonsquamous NSCLC harboring 9 NCCN biomarkers, liquid CGP increases detection in patients with limited tissue results, but does not increase detection in patients with tissue CGP results available. In contrast, tissue CGP can add

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Disclosure: Dr. Li, Dr. Tolba, Dr. Fine, Dr. Lofgren, Ms. Gadgil, Dr. Graf, and Dr. Oxnard are employees of Foundation Medicine, a wholly owned subsidiary of Roche, and have equity interest in Roche. Dr. Bourla reports employment in Flatiron Health, Inc., an independent subsidiary of Roche, and reports stock ownership in Roche and Flatiron Health equity. Dr. Schulze is an employee of Genentech and has equity interest in Roche. Schwartzberg reports being a consultant for Merck, Pfizer, AstraZeneca, Myriad, Odonate, Amgen, Eli Lilly, Bristol-Myers Squibb, Napo, Helsinn, Bayer, Beyond Spring, Seagen, Sanofi, Coherus, Genentech, and Spectrum Pharmaceuticals, honoraria payment from Coherus, PUMA, Merck, Seagen, Pfizer, receiving research funding from Amgen within the past 36 months, and he also reports being on the speaker's bureau for Puma Biotechnology. Dr. Daniel reports receiving institutional research support from AstraZeneca, Genentech, Guardant Health, Janssen Research and Development, Bristol-Myers Squibb, G1 Therapeutics, Merck & Co, Inc., Novartis, AbbVie, ARMO Biosciences, Immunomedics, Eli Lilly, Merus NV, and Daiichi Sankyo.

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Cite this article as: Schwartzberg LS, Li G, Tolba K, et al. Complementary roles for tissue- and blood-based comprehensive genomic profiling for detection of actionable driver alterations in advanced NSCLC. *JTO Clin Res Rep.* 2022;3:100386.

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ISSN: 2666-3643

<https://doi.org/10.1016/j.jtocr.2022.100386>

meaningfully to liquid CGP for detection of NCCN biomarkers and should be considered as a follow-up when an oncogenic driver is not identified by liquid biopsy.

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Keywords: Comprehensive genomic profiling; Liquid biopsy; Biomarkers; Tissue-based testing; NSCLC

Introduction

Precision oncology has changed the way cancer patients are cared for, and nowhere is this more true than in the care of advanced NSCLC.¹⁻³ Wide adoption of molecular profiling paved the way for the rapid development of targeted therapies for patients with advanced NSCLC, with Food and Drug Administration (FDA)-approved targeted therapies for driver alterations in eight different genes: *EGFR* sensitizing mutations and exon 20 insertions, fusions in *ALK*, *ROS1*, *RET*, and *NTRK*, *BRAF* V600E, *KRAS* G12C, *MET* exon 14 splicing mutations.⁴ The NCCN guidelines also recommend testing for *ERBB2* mutations and *MET* amplification as these emerging biomarkers are associated with the established activity from available targeted agents.^{5,6} Finally, genomic testing is also useful for anticipating the effectiveness of immunotherapy in NSCLC, with *EGFR* and *ALK* driver variants established as negative predictors for benefit from immunotherapy,^{4,7} and excluded from the FDA label of some regimens.⁸

Advances in precision oncology are premised on the availability of tumor-derived genomic material suitable for molecular profiling. Traditionally tumor biopsy has been considered the accepted standard for genomic profiling; however, recent advances in liquid biopsy have created an additional diagnostic approach to increase access to personalized cancer therapy.⁹⁻¹¹ There are now multiple FDA-approved multigene assays both for tumor tissue testing and for liquid biopsy testing of circulating tumor DNA (ctDNA).¹² Liquid biopsy is a compelling complement to tissue testing given its convenience, both owing to ease of sampling (blood versus biopsy) and because tissue quantity can be limited, especially when iterative tissue testing can exhaust a sample.¹³ However, all liquid biopsy assays come with a key caveat: assay sensitivity is dependent on the variable shed of ctDNA.¹⁴ For this reason, the FDA-approved liquid biopsy assays include, on their labels, language indicating that negative results should be confirmed with tissue testing owing to the risk of false negatives.^{15,16}

With such a diversity of diagnostic tools, a key clinical question is how to piece these diagnostics together to optimally identify precision treatment options for patients with advanced NSCLC. Clearly, liquid biopsy is a compelling pragmatic alternative when tissue is not available.¹⁷ But it is less clear how liquid biopsy adds value when comprehensive genomic profiling (CGP) of tumor tissue is available. Although it has been proposed that liquid biopsy can capture heterogeneous variants not detected in the tumor,¹⁸ a recognized value of liquid biopsy for patients with treatment resistance,¹⁹ it is unclear whether this adds value for the initial profiling of advanced NSCLC in the detection of driver alterations. Here, we study a prospective cohort of patients with advanced NSCLC, in which patients received liquid biopsy CGP and also are offered tissue CGP as a complement to their standard-of-care (SOC) testing. With a focus on guideline-recommended actionable driver alterations, we hypothesized that the added value of liquid biopsy for detecting driver alterations depends on the extent of complementary tissue testing available.

Materials and Methods

Patient Cohort

Patients were identified who underwent liquid biopsy profiling at the time of enrollment in the Prospective Clinicogenomic Program clinical trial (ClinicalTrials.gov identifier NCT04180176).²⁰ In this ongoing multicenter study, up to 1000 adult patients with metastatic NSCLC or SCLC enroll before initiating SOC systemic anticancer therapy. All patients sign consent to participate in the study, which must be approved by the institutional review board at the participating site. In the study, each patient has peripheral blood submitted for CGP of ctDNA with FoundationOne Liquid (Foundation Medicine, Cambridge, Massachusetts) or FoundationOne Liquid CDx (Foundation Medicine, Cambridge, Massachusetts). In addition, tumor tissue specimens available at enrollment may be optionally submitted for CGP with FoundationOne CDx. Liquid biopsy is then repeated on treatment and at progression or end of treatment. Longitudinal EHR-derived data are linked to CGP results. As follow-up is ongoing, for this initial analysis, we only studied pretreatment data including clinical features and molecular testing results, leveraging a data cut with recency of June 30, 2021. Patients were included in this analysis if they had a chart abstraction-confirmed diagnosis of metastatic nonsquamous NSCLC.

CGP on Study

Blood was collected at enrollment for CGP of ctDNA. Tissue CGP was offered in the study as a complement to SOC biomarker testing and was, therefore, optional.

Samples were assayed with hybrid capture-based CGP performed in a Clinical Laboratory Improvement Amendments–certified, College of American Pathologists–accredited, New York State–regulated reference laboratory (Foundation Medicine, Inc.). For liquid biopsy specimens, cell-free DNA was extracted to create adapted sequencing libraries before hybrid capture and sample-multiplexed sequencing using either a 70-gene (FoundationOne Liquid) or 324-gene (FoundationOne Liquid CDx) panel.^{21,22} The level of ctDNA shed for each specimen was quantified using a composite measure considering an aneuploidy-based measure of tumor fraction (TF) and a variant-based measure of variant allelic frequency, as described previously¹⁹; elevated ctDNA shed was considered present when a sample had tumor DNA levels comparable to a tissue biopsy (>10% TF). For tissue specimens collected as part of the study, at least 50 ng of DNA was isolated from formalin-fixed, paraffin-embedded tumor specimens and sequenced to high, uniform coverage of at least 500×, with a 324-gene panel (FoundationOne CDx).

NCCN Biomarkers of Interest

This analysis focused on the detection of actionable driver alterations in eight oncogenes recommended for testing per the NCCN guidelines (*EGFR*, *KRAS*, *ALK*, *ROS1*, *RET*, *BRAF*, *MET* exon 14, and *NTRK*) and two emerging biomarkers (*ERBB2* mutations and *MET* amplification), totaling nine oncogenes. The analysis was limited to the following driver alterations: (1) *KRAS* G12X, G13X, Q61X; (2) *EGFR* L858R, exon 19 deletions, exon 20 insertions, G719X, L861Q, and S768I; (3) *ALK*, *ROS1*, *RET*, and *NTRK* rearrangements; (4) *BRAF* V600E; (5) *MET* exon 14 skipping mutations and amplifications; and (6) *ERBB2* mutations.

Comparison of Liquid Biopsy Results to Tissue Results

Liquid biopsy CGP was compared with tissue-based biomarker testing by dividing the patients who completed liquid biopsy CGP testing into three subcohorts on the basis of the type of tissue-based biomarker testing they received. For patients completing FoundationOne CDx testing in the study, tissue CGP was the comparator. For patients undergoing any SOC tissue testing before enrollment, results for the five SOC biomarkers at the time of study launch were manually abstracted according to protocol specifications: (1) *EGFR* mutations (L858R, exon 19 deletions, exon 20 insertions, and other pathogenic *EGFR* mutations); (2) pathogenic *KRAS* mutations; (3) *ALK* rearrangements; (4) *ROS1* rearrangements; and (5) *BRAF* V600E. The results from the testing of these five biomarkers were

used as a comparator representing limited SOC testing. For patients in this group who were tested for at least one but not all the biomarkers of interest, the untested biomarkers were considered not detected. The final subcohort includes patients not undergoing tissue testing.

Statistical Considerations

R version 4.1.1 software was used for all statistical analyses. 95% binomial confidence intervals reported for proportions were calculated using the Wilson score method with continuity correction (`prop.test` in R). The positive percent agreement (PPA) for liquid biopsy was calculated from the percentage of patients who were biomarker positive by any tissue-based test who were also biomarker positive by liquid biopsy CGP. For the PPA analysis, we included any known pathogenic substitution or indel. For the analyses of NCCN actionability, across each subgroup, we calculated the proportion positive for one (or more) actionable biomarkers in the nine NCCN genes of interest. The rate of biomarker detection was tested using McNemar's test of difference in paired observations.

Results

Of 768 NSCLC patients enrolled in the study, 702 underwent liquid biopsy at study enrollment, and 515 of these were of nonsquamous histologic subtype and were included in the analysis (261 FoundationOne Liquid, 254 FoundationOne Liquid CDx) (Fig. 1A). Most of the cohort (n = 364, 71%) were previously untreated for their advanced lung cancer, whereas a minority (n = 151, 29%) were previously treated, and a small number (n = 20) had resistance after previous targeted therapy. A total of 131 patients (25%) received tissue CGP; 264 patients (51%) had up to five genes analyzed (more often for *EGFR* and *ALK*, less often for *KRAS*) (Supplementary Table 1); and 120 patients (23%) did not have tissue testing. These three subcohorts were clinically comparable, with the exception that the no-tissue-testing group was enriched for first-line patients (81% versus 64% among patients who received some form of tissue testing) (Table 1). Biomarker prevalence across the three subcohorts was comparable in ctDNA, with expected differences in tissue (Supplementary Tables 2 and 3). As expected, the biomarkers were generally mutually exclusive, with drivers in multiple genes detected in fewer than 1% of patients (Supplementary Table 2). Median ctDNA shed was comparable across the three subcohorts (Kruskal-Wallis $p = 0.7$) (Fig. 1B). The median number of days from order to report was 12 (interquartile range: 10–15, n = 515) for liquid CGP and 28 (interquartile range:

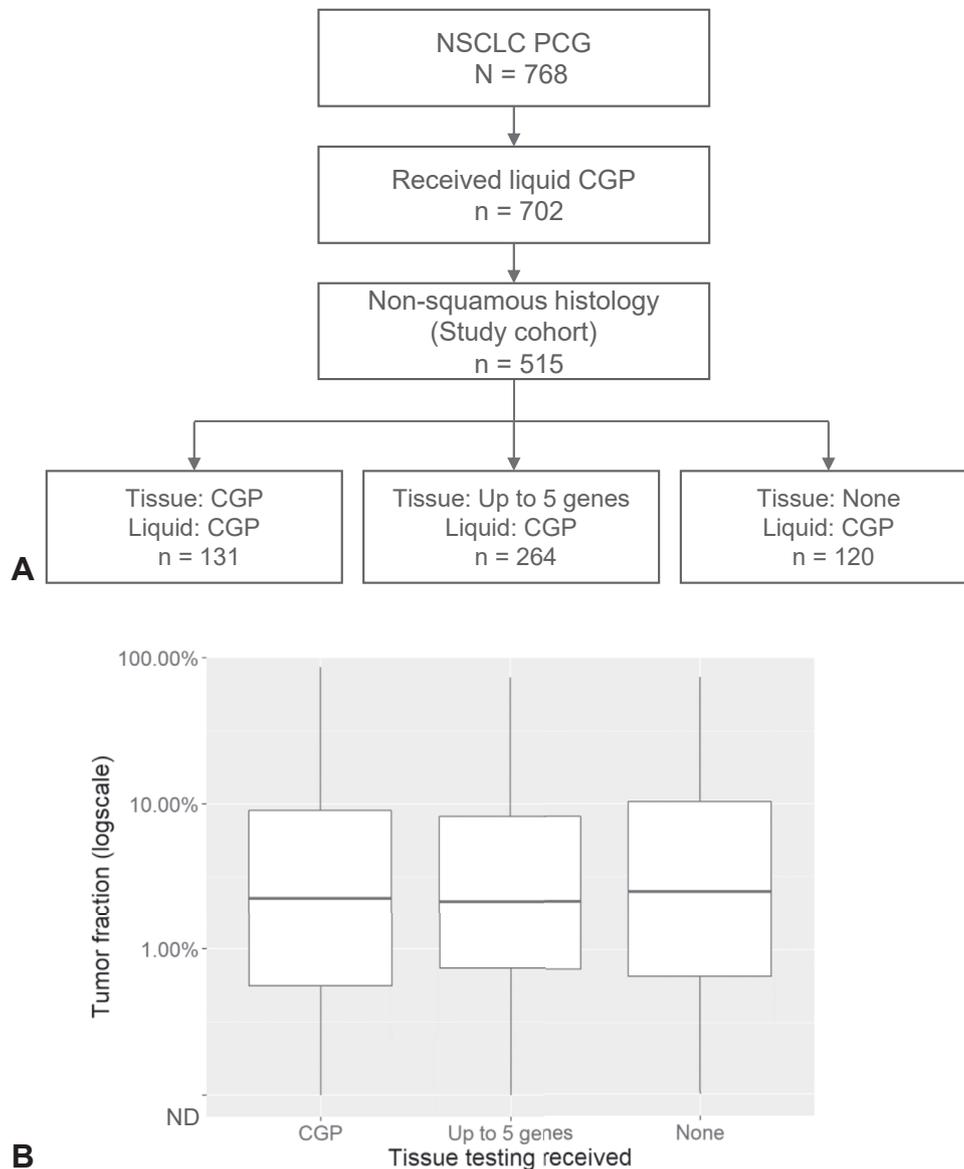


Figure 1. Study population. (A) Analysis subcohorts are on the basis of available tissue testing results. (B) Tumor fraction distributions were similar across the three subcohorts. Whiskers span the full range of observed values, whereas the box indicates the quartiles. CGP, comprehensive genomic profiling; ND, not detectable.

19.5–42, $n = 131$) for tissue CGP, which includes the time between order and specimen procurement and transit time between the site of care and the central laboratory where CGP was performed, noting this CGP testing was done for study research and may not be representative of routine clinical care.

Liquid biopsy demonstrated the expected PPA for the detection of driver alterations detected in tissue. The overall PPA was 69% (95% confidence interval [CI]: 62%–75%) (Supplementary Fig. 1 and Supplementary Table 4); the PPA was 73% (95% CI: 64%–81%) for first-line patients. Hypothesizing that the lack of driver detection was related to ctDNA shed, we studied the 35 patients with positive tissue biomarkers and elevated

ctDNA shed (>10%), and PPA was found to be 100% (95% CI: 88%–100%).

Focusing on 131 patients with both tissue and liquid CGP results (tissue specimens collected median of 31 days before liquid specimens), we found that liquid CGP detected fewer patients with actionable NCCN biomarkers than tissue CGP (43% [34%–52%] versus 66% [57%–74%], $p < 0.001$) (Fig. 2A). This was primarily driven by ctDNA shed, with similar rates of detection of NCCN biomarkers when limited to 31 cases in which liquid CGP showed elevated ctDNA shed (55% [36%–72%] versus 55% [36%–72%], $p = 1$) (Supplementary Fig. 2). Then focusing on patients with tissue testing for up to five genes, liquid biopsy trended toward

Table 1. Cohort Clinical and Pathologic Characteristics

Patient Characteristic	All (N = 515)	Tissue CGP (n = 131)	Up to Five Tissue Biomarkers (n = 264)	No Tissue (n 120)	p-Value
Practice type					0.678
Academic	4 (0.78)	0 (0.00)	3 (1.14)	1 (0.83)	
Community	511 (99.2)	131 (100)	261 (98.9)	119 (99.2)	
Age at enrollment	68.0 [63.0-76.0]	68.0 [62.0-74.0]	68.0 [63.0-77.0]	69.0 [63.8-78.0]	0.339
Sex					0.795
F	262 (50.9)	67 (51.1)	131 (49.6)	64 (53.3)	
M	253 (49.1)	64 (48.9)	133 (50.4)	56 (46.7)	
Race					0.107
Black or African American	59 (11.5)	7 (5.34)	38 (14.4)	14 (11.7)	
Other race	58 (11.3)	16 (12.2)	32 (12.1)	10 (8.33)	
Unknown	39 (7.57)	12 (9.16)	15 (5.68)	12 (10.0)	
White	359 (69.7)	96 (73.3)	179 (67.8)	84 (70.0)	
Smoking status					0.329
History of smoking	447 (86.8)	112 (85.5)	226 (85.6)	109 (90.8)	
No history of smoking	68 (13.2)	19 (14.5)	38 (14.4)	11 (9.17)	
ECOG Performance Score					0.843
0	154 (29.9)	38 (29.0)	78 (29.5)	38 (31.7)	
1	206 (40.0)	57 (43.5)	106 (40.2)	43 (35.8)	
2	89 (17.3)	23 (17.6)	47 (17.8)	19 (15.8)	
3+	16 (3.11)	3 (2.29)	7 (2.65)	6 (5.00)	
Not assessed	50 (9.71)	10 (7.63)	26 (9.85)	14 (11.7)	
AJCC stage at diagnosis:					0.639
I-II	63 (12.2)	19 (14.5)	34 (12.9)	10 (8.33)	
III-IV	449 (87.2)	111 (84.7)	229 (86.7)	109 (90.8)	
Unknown	3 (0.58)	1 (0.76)	1 (0.38)	1 (0.83)	
Study line number:					0.009
Previously treated at study line start	147 (30.4)	45 (37.5)	83 (31.7)	19 (18.8)	
Study line is first-line	336 (69.6)	75 (62.5)	179 (68.3)	82 (81.2)	

Note: Categorical variables are presented with counts and percentages, with chi-square tests used to compare among the subcohorts. Continuous variables are presented as medians and interquartile ranges, with Kruskal-Wallis tests to compare among the subcohorts.

AJCC, American Joint Committee on Cancer; CGP, comprehensive genomic profiling; F, female; M, male.

identifying more patients with actionable NCCN biomarkers than tissue testing (45% [39%–52%] versus 39% [33%–45%], $p = 0.05$). Dividing this subcohort by the number of biomarkers tested, we found that the liquid biopsy detection rate overall was comparable to the tissue detection rate for patients who received tissue testing of all five genes (48% [40%–57%] versus 52% [43%–60%], $p = 0.5$, $n = 153$), but was superior to tissue testing of fewer than five genes (41% [32%–51%] versus 21% [14%–30%], $p < 0.001$, $n = 111$) (Supplementary Fig. 3). Yet the improved detection with liquid CGP over limited tissue testing was quite apparent when focusing on the 61 cases in which liquid CGP showed elevated ctDNA shed (49% [36%–62%] versus 28% [18%–41%], $p = 0.002$). Finally, of the 120 patients without tissue testing, liquid biopsy detected an actionable NCCN biomarker in 56 (47%, 95% CI: 38%–56%).

We then studied whether the addition of liquid biopsy testing to tissue testing increased the overall identification of patients with actionable NCCN

biomarkers. Studying 131 patients who completed tissue CGP, actionable biomarkers were detected in the tissue of 86 patients (66%), and zero additional patients with actionable NCCN biomarkers were detected with the addition of liquid biopsy (Fig. 2B). In contrast, the liquid biopsy was additive in 264 patients receiving tissue testing of up to five genes, with 102 (39%) actionable NCCN biomarkers detected in tissue and 48 (18% of the subcohort) additionally detected with liquid biopsy, for 150 in total (57%). Of the 48 patients detected to have an actionable NCCN biomarker in liquid biopsy but not tissue testing of up to five genes, 18 (38%) of these patients had drivers in *RET*, *MET*, or *ERBB2* that were not included in the 5-gene testing paradigm (Fig. 3A).

Conversely, we looked at whether tissue testing adds meaningfully to liquid biopsy. Studying the 131 patients who completed tissue CGP, actionable biomarkers were detected in 56 patients with the liquid CGP, with an additional 30 patients (23% of the subcohort) who were liquid-negative detected to harbor an NCCN biomarker

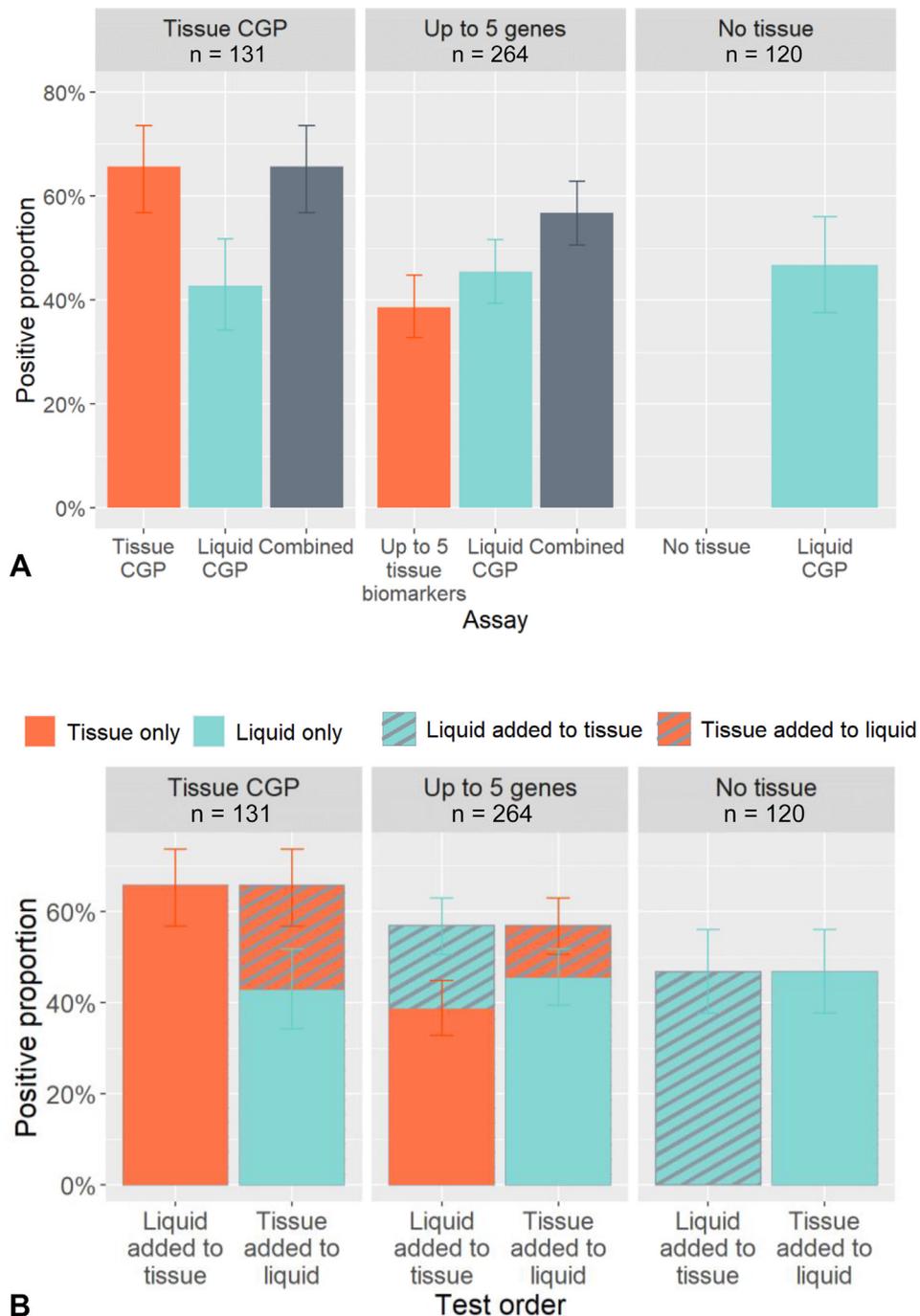


Figure 2. Detection of NCCN biomarkers on the basis of available tissue results. (A) Liquid CGP consistently detects biomarkers across subcohorts, whereas tissue CGP detects biomarkers in the highest proportion of patients. (B) In patients with tissue CGP results, liquid CGP detects no additional patients with NCCN biomarkers, whereas liquid CGP adds meaningfully in patients getting more limited tissue testing. In patients with liquid CGP results, the reflex to tissue CGP increases the overall biomarker detection rate. CGP, comprehensive genomic profiling; NCCN, National Comprehensive Cancer Network.

with tissue CGP (Figs. 2B and 3B). Similarly, in the 264 patients receiving tissue testing of up to five genes, 120 had an actionable biomarker detected with liquid CGP, with an additional 30 (11% of the subcohort) detected with up to five gene tissue testing. We further explored whether TF on liquid CGP could inform which patients

might benefit from reflex to tissue CGP after liquid biopsy. Among 14 patients with negative liquid CGP but elevated ctDNA shed, none had an NCCN biomarker detected on tissue CGP. But among 61 patients with a negative liquid biopsy without elevated ctDNA shed, 30 (49%) had an NCCN biomarker on tissue CGP.

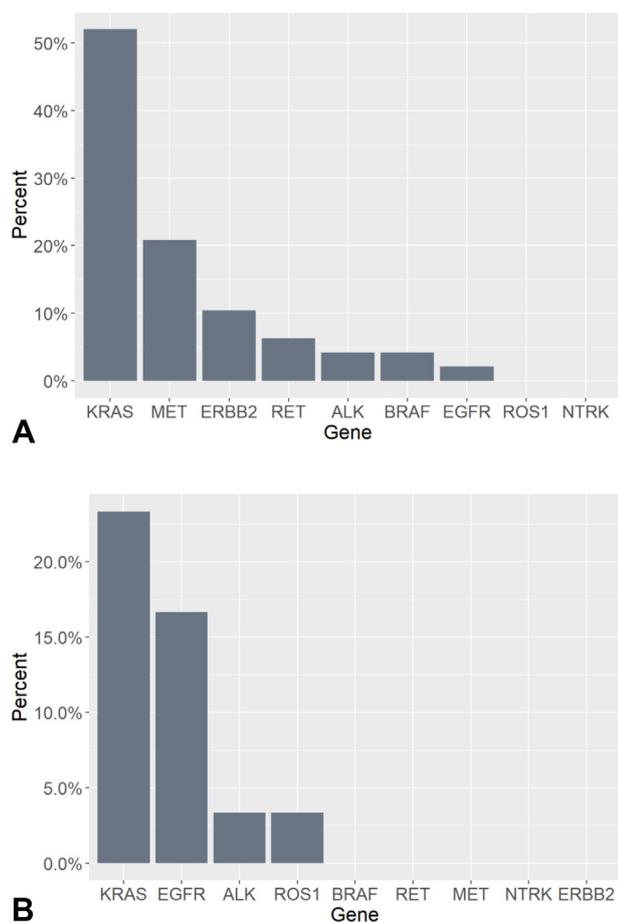


Figure 3. Additional NCCN biomarkers detected through testing a second specimen. (A) Patients (n = 48) whose liquid CGP detected an NCCN biomarker though limited tissue testing were negative. (B) Patients (n = 30) whose tissue CGP detected an NCCN biomarker though liquid CGP were negative, highlighting the value of reflex-to-tissue testing after a negative liquid biopsy. CGP, comprehensive genomic profiling; NCCN, National Comprehensive Cancer Network.

Discussion

In this analysis of nine NSCLC oncogenes identified in the NCCN guidelines,⁴ studying a prospective cohort of 515 patients with advanced nonsquamous NSCLC, we find that tissue CGP and liquid CGP are complementary tools providing different degrees of value for different patients. In patients with no tissue results or more limited tissue testing results, liquid CGP meaningfully increases the number of patients found to be positive for an NCCN biomarker. In contrast, in those patients who receive a tissue CGP result, liquid CGP does not increase the overall detection of patients with NCCN biomarkers, suggesting the limited value of liquid CGP in those patients who can receive tissue CGP. This positions tissue CGP and liquid CGP as complementary tools that may be suited for different patients with NSCLC depending on

biologic factors (ctDNA shed) and practical factors (tissue availability).

Our analysis did find that liquid biopsy increases the detection of actionable biomarkers in patients with more limited tissue testing, confirming its value in patients with tissue unavailable for genomic analysis. In addition, despite guidelines recommending broad molecular profiling for patients with NSCLC, some with tissue available still receive more limited tissue testing.²³ Evaluating a subset of patients who had more limited testing results, we found that adding liquid biopsy resulted in 11% more patients being detected with actionable NCCN biomarkers (Fig. 2), suggesting a possible role of concurrent liquid CGP in patients receiving limited tissue testing. Liquid biopsy may, thus, represent a pragmatic tool for accessing broad-panel CGP for those centers that offer more limited tissue testing in-house, with tissue CGP used as needed for negative cases.

In contrast, with our focus on NCCN biomarkers, we find limited added value of concurrent liquid biopsy in patients receiving tissue CGP results. In those with tissue CGP results, 66% were found to harbor an actionable biomarker in one of these NCCN genes, whereas a subset of these was detected with liquid CGP. This supports the recent Consensus Statement by the International Association for the Study of Lung Cancer (IASLC) working group—in patients with advanced NSCLC able to undergo tissue testing, tissue next-generation sequencing is recommended.^{17,24} Because liquid biopsy did not increase the number of patients with an actionable NCCN biomarker, the role of ctDNA testing in patients who receive tissue CGP results is unclear in this analysis, even among patients with high-shed tumors. However, we acknowledge that, in some patients, tissue is not immediately available when testing is needed such that liquid biopsy is ordered initially, another point acknowledged in the IASLC Consensus Statement. In these patients in which the liquid is ordered first, tissue CGP can detect an additional 23% of patients with actionable NCCN biomarkers missed in ctDNA, pointing out the need for a sequential reflex to tissue CGP after liquid biopsy when there are no actionable alterations detected. One pragmatic strategy when tissue availability is uncertain is to order liquid CGP while requesting the tissue specimen in parallel, which can enable earlier decisions for symptomatic patients with positive liquid results while waiting for the subsequent tissue results when the liquid is negative. This highlights that the value of liquid biopsy may not be fully captured by focusing on the detection of NCCN biomarkers alone. For example, for some patients, the rapidity of results returning from a

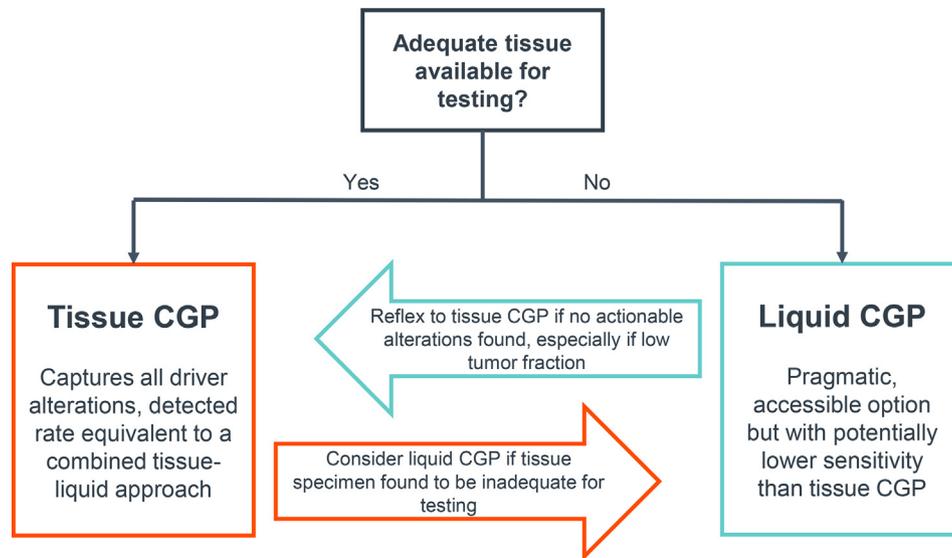


Figure 4. For advanced NSCLC, tissue CGP and liquid CGP are complementary approaches that may be preferred in different clinical scenarios. Tissue CGP is preferred when a sufficient, high-quality tissue specimen is available as it offers the highest sensitivity to detect clinically actionable driver biomarkers. When tissue is not available, liquid CGP offers a pragmatic alternative but with lower sensitivity; if liquid CGP fails to detect actionable biomarkers, a reflex to tissue CGP is indicated, particularly if there is low circulating TF. Similarly, liquid CGP can be used as a backup if tissue CGP fails to generate a result. CGP, comprehensive genomic profiling; TF, tumor fraction.

liquid biopsy can enable timely first-line therapy that may not be feasible on the basis of how tissue testing is operationalized at their institution.²⁵

Finally, we found that in patients without any tissue biomarker testing, ctDNA-based CGP can detect a range of actionable alterations, confirming its value in patients with insufficient tissue for genomic analysis. Whereas overall PPA for liquid biopsy in this study was consistent with previous publications,²⁴ we find that the biomarker detection with liquid CGP depends on levels of ctDNA shed. When there is elevated TF (>10%), suggesting a liquid biopsy as rich in tumor DNA as a tissue biopsy, the sensitivity for actionable alterations is 100%. In other words, a negative liquid CGP in an elevated TF specimen can be taken to be a reliable negative result. However, when ctDNA shed is lower there is a risk of false negatives such that negative results should be confirmed with tissue testing (Fig. 4). This variable ctDNA shed has resulted in the FDA label recommending negative liquid biopsy results to be confirmed with tissue testing. In patients whose tissue is deemed to be inadequate for CGP, this means clinicians must be prepared to perform a repeat tumor biopsy in a patient with a negative liquid biopsy (when feasible) to rule out the possibility of an actionable alteration missed in ctDNA. Fortunately, it has been described that patients with lower levels of ctDNA shed have a more favorable prognosis,^{14,26,27} so perhaps may be clinically more suitable for repeat biopsy to complete their genomic testing. Fundamentally, this points to tissue CGP as the preferred option when tissue

is immediately available, with liquid CGP favored when tissue availability is uncertain, as illustrated in the recent IASLC Consensus Statement.¹⁷

The prospective design of this observational study is a strength, yet we must acknowledge some limitations. This study was designed at a time when only 5 NSCLC biomarkers were standard and more limited genomic testing was more common. The study abstracted only five genes from SOC tissue biomarker testing, allowing the study of a more limited testing paradigm but not fully capturing the diversity of alterations potentially detectable with SOC assays, including additional testing that these patients may have received. Nevertheless, this cohort within this study served as a pragmatic representation of the experience at oncology sites of care where limited tissue testing may still be standard practice. Furthermore, because the CGP testing in the study was performed as research, we were not able to capture an accurate measure of turnaround time, which we expect would be different for liquid versus tissue CGP. In addition, because follow-up continues on the study, we are not able to yet analyze the outcome of how these actionable NCCN biomarkers were used to guide clinical care. A complete report of the Prospective Clinico-genomic Study will be forthcoming to more fully describe the genomics of the pretreatment and on-treatment biomarker testing and its relationship to patient outcomes. Our data point to ctDNA shed as a useful tool for predicting the utility of liquid biopsy but estimating this carries limitations as well. For example,

higher ctDNA levels are needed to detect tumor aneuploidy, whereas a variant allelic frequency-based approach can struggle to distinguish between tumor DNA and clonal hematopoiesis—these may limit the dynamic range of ctDNA shed estimation and motivate further development efforts.

In conclusion, this analysis of liquid biopsy CGP in a diverse group of patients with advanced NSCLC highlights the complementary roles of tissue CGP and liquid CGP for enabling the identification of patients with actionable NCCN biomarkers (Fig. 4). Our data support the prioritization of tissue CGP in patients for whom adequate tissue is immediately available as it offers the most reliable detection of NCCN biomarkers. In our analysis, we did not find that liquid CGP increases the detection of NCCN biomarkers in such patients, though we acknowledge it may provide other value through supporting trial enrollment, improving prognostication, and enabling germline insights.^{14,28} However, when tissue is not immediately available for timely CGP, liquid biopsy can be used for expeditious multigene testing initially, with follow-on tissue testing when liquid biopsy does not detect an actionable alteration, particularly when there are lower levels of ctDNA shed. The high sensitivity of liquid biopsy in the setting of elevated TF offers optimism that, with adequate validation, a negative liquid biopsy in patients with elevated TF can be trusted to rule out the presence of an actionable alteration, reducing the burden to reflex to tissue testing, and preserving confirmatory tissue testing for those patients that truly need it.

CRedit Authorship Contribution Statement

Lee S. Schwartzberg: Conceptualization, Writing - review & editing.

Gerald Li: Formal analysis, Visualization, Data curation, Software, Writing - review & editing.

Khaled Tolba: Conceptualization, Writing - review & editing.

Ariel B. Bourla: Resources, Writing - review & editing.

Katja Schulze: Resources, Writing - review & editing.

Rujuta Gadgil: Project administration, Writing - review & editing.

Alexander Fine: Formal analysis, Visualization, Data curation, Writing - review & editing.

Katherine Lofgren, Ryon Graf: Writing - review & editing.

Geoffrey R. Oxnard: Conceptualization, Supervision, Writing - original draft, Writing - review & editing.

Davey Daniel: Conceptualization, Writing - review & editing.

Acknowledgments

The study was funded by Foundation Medicine and Genentech.

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.2022.100386>.

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