Incorporating Liquid Biopsy into Routine Practice for Patients with aNSCLC: A Practical Guideline for Community Oncologists from the Liquid Biopsy Working Group

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ABSTRACT

Genomic testing is recommended in patients with advanced non-small cell lung cancer (aNSCLC) to inform treatment. Logistical challenges arise when biopsy specimens are insufficient for adequate molecular profiling, potentially challenging appropriate patient care. Liquid biopsy may overcome these limitations and offers another avenue for identifying driver mutations. A multidisciplinary group convened with the goal of creating a practical guideline for the use of liquid biopsy in aNSCLC among community oncologists. The meeting objectives were to discuss the genomic testing challenges in aNSCLC and review evidence regarding use of liquid biopsy before first-line therapy and at progression. The purpose of this review is to disseminate the Working Group consensus to help community oncologists make informed decisions regarding use of liquid biopsy in practice. We discuss an algorithm drafted based on the Group’s consensus that there is sufficient evidence to support routine use of liquid biopsy in clearly defined subgroups of patients.
INTRODUCTION

In 2018, cancer of the lung and bronchus was anticipated to account for nearly a quarter of a million new diagnoses and over 150,000 cancer-related deaths [1]. According to data collected by the National Cancer Institute, 84% of all lung and bronchus cancers manifest as non-small cell lung cancer (NSCLC), and over half of these have a histologic subtype of adenocarcinoma [2]. Moreover, 55% of NSCLC presents as stage IV disease [2].

In this setting of lung adenocarcinoma, the presence of driver mutations is a well-documented phenomenon [3, 4]. Importantly, the detection of certain driver mutations has significant implications for treatment selection, as therapies can and should be chosen on the basis of a patient’s molecular signature [5].

There are currently 16 agents recommended by the National Comprehensive Cancer Network (NCCN) for the treatment of patients with advanced non-small cell lung cancer (aNSCLC) whose activity is tied to the mutational status of 1 of 8 genes (EGFR, ALK, ROS1, BRAF, NTRK, RET, MET, and ERBB2; Table 1) [5]. Furthermore, the NCCN also recommends 2 additional agents, based on a patient’s tumor mutational burden (TMB) [5]. A clear survival benefit has been documented in an assessment of 578 patients with advanced lung adenocarcinoma and an oncogenic driver mutation; here, those who received targeted therapy experienced a median survival of 3.5 years compared to 2.4 years in patients not on a targeted therapy [4].
According to NCCN guidelines (v3.2019), TMB is an evolving biomarker that is potentially useful for selecting patients for immunotherapy. Nivolumab and ipilimumab are available immunotherapy agents recommended by the NCCN, with a response rate range of 47% to 68% for those with a high TMB [30, 31]; however, neither of these agents have an approved use based on TMB.

Response rate is for crizotinib.

FDA-approved in another indication.

**Abbreviations:** CAP/IASLC/AMP, College of American Pathologists, the International Association for the Study of Lung Cancer, and the Association for Molecular Pathology; FDA, U.S. Food and Drug Administration; NCCN, National Comprehensive Cancer Network; TMB, tumor mutational burden.

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**Table 1. Genomic Testing Recommendations by NCCN and CAP/IASLC/AMP and Recommended Targeted Therapies by NCCN**

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<tr>
<td>EGFR</td>
<td>X</td>
<td>X</td>
<td>Erlotinib, afatinib, gefitinib, osimertinib, dacomitinib</td>
<td>60% to 80% [7-14]</td>
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<tr>
<td>ALK</td>
<td>X</td>
<td>X</td>
<td>Alectinib, brigatinib, ceritinib, crizotinib, lorlatinib</td>
<td>40% to 90% [15-21]</td>
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<tr>
<td>ROS1</td>
<td>X</td>
<td>X</td>
<td>Ceritinib, crizotinib, lorlatinib</td>
<td>70%(^b) [19]</td>
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<tr>
<td>BRAF</td>
<td>X</td>
<td>X</td>
<td>Dabrafenib + trametinib</td>
<td>65% [22]</td>
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<tr>
<td>NTRK</td>
<td>X</td>
<td>X</td>
<td>Larotrectinib</td>
<td>75% to 80% [23]</td>
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<tr>
<td>RET</td>
<td>X</td>
<td>X</td>
<td>Cabozantinib,(^c) vandetanib</td>
<td>20% to 50% [24-26]</td>
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<tr>
<td>MET</td>
<td>X</td>
<td>X</td>
<td>Crizotinib(^c)</td>
<td>40% to 50% [27, 28]</td>
</tr>
<tr>
<td>ERBB2</td>
<td>X</td>
<td>X</td>
<td>Ado-trastuzumab emtansine(^c)</td>
<td>45% [29]</td>
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<tr>
<td>KRAS</td>
<td>X</td>
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Guidance produced by the NCCN, and jointly by the College of American Pathologists, the International Association for the Study of Lung Cancer, and the Association for Molecular Pathology (CAP/IASLC/AMP), recommends broad molecular profiling in aNSCLC, including testing of EGFR, ALK, ROS1, BRAF, NTRK, KRAS, RET, MET, ERBB2, and TMB (Table 1) [5, 6]. Molecular profiling in aNSCLC, be it routine or broad, typically occurs via testing of tissue biopsy specimens; however, three associated challenges warrant attention. First, tumor heterogeneity could lead to misrepresentation of the overall mutational landscape when examining only a small fraction of the disease [32]. Second, prospective and retrospective studies have confirmed that tissue quality and/or quantity are often insufficient for molecular profiling, at rates ranging from 25% to 50% [4, 32-35]. Third, these analyses have shown that even with sufficient tissue, the extent of molecular profiling often falls short of recommended standards, with only 60% to 70% and 50% to 65% undergoing EGFR and ALK testing, respectively, and just 8% undergoing the full array of NCCN and CAP/IASLC/AMP testing [36-38]. To address these and other tissue-based concerns, liquid biopsy has emerged as an alternative means of analyte acquisition and genomic testing.

Under normal conditions, fragments of germline cell-free DNA (cfDNA) are present in circulation; however, among patients with cancer, more cfDNA is present via the additional contribution of tumor cfDNA [39]. Fragments of cell-free circulating tumor DNA (ctDNA) are produced largely as a result of apoptosis and are present in circulation at concentrations roughly dependent on cancer stage [39, 40]. Liquid biopsy tests are typically performed via blood samples with analysis of the cfDNA to identify tumor mutations present in the circulating tumor DNA. Relative to tissue biopsy, liquid biopsy enjoys several advantages: it is less invasive, allows for convenient serial testing, and has the potential for shorter turnaround time [41]. We should note, however, that although liquid biopsy benefits from the ability to capture tumor heterogeneity [42], this may occasionally confound interpretation of results if subclonal variants are detected or if an individual has clonal hematopoiesis [5, 43-45].

Although some professional societies have adopted liquid biopsy recommendations under limited circumstances, others have suggested that the lack of clinical validity and utility precludes widespread liquid biopsy use [5, 6, 44]. We believe this collective existing framework does not adequately consider the published literature on liquid biopsy nor align with the growing clinical adoption of liquid biopsy. Given the increasing clinical use of liquid biopsy in the absence of robust guidance on how to use and interpret results outside of limited circumstances, we convened as a group of community oncologists with vast liquid biopsy experience to review the literature, discuss the use-cases and limitations, and propose consensus on the clinically appropriate use of liquid biopsy among community oncologists in a manner both in keeping with known obstacles and sensitive to the needs of patients with aNSCLC. In this regard, we will focus on 3 key aspects of liquid biopsy: appropriate patients and timing of use, considerations when selecting a liquid biopsy platform, and interpretation of results. The next section describes a patient management algorithm specifically geared to meet the needs of the community oncologist.
The Working Group reviewed published data from retrospective and prospective liquid biopsy cohorts to establish boundaries regarding appropriate patient selection and time points for the use of liquid biopsy (Table 2). These studies assessed ctDNA (via polymerase chain reaction [PCR] or next-generation sequencing [NGS]) in stage IIIB/IV NSCLC patients who were either naïve or previously exposed to treatment. Based on the ctDNA results, 25% to 78% of patients could be matched to a targeted therapy or had a relevant clinical trial or off-label targeted therapy available [9, 15, 32, 35, 43, 46-49]. Importantly, these studies demonstrated high concordance or positive predictive value with tissue-matched samples and/or comparative response rates when targeted therapy was applied based upon liquid biopsy results, all of which provide high confidence that positive liquid biopsy results can be trusted (Table 2) [9, 15, 32, 35, 43, 46-54].
Table 2. Liquid Biopsy Cohort Data: Summary of Evidence From Retrospective and Prospective Studies Assessing the Use of ctDNA in Patients With aNSCLC

<table>
<thead>
<tr>
<th>Study</th>
<th>Timing of ctDNA Collection</th>
<th>Method of ctDNA Analysis</th>
<th>TissueUnavailable/Insufficient for Genomic Profiling</th>
<th>ctDNA Results and Actions</th>
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</table>
| NEXT-2 [15]  | aNSCLC cohort: ctDNA collected at diagnosis for 29%, at 2L therapy for 37%, and at ≥3L for 34% | NGS (tissue testing by PCR and IHC) | By trial design, patients had sufficient tumor tissue for hotspot analysis but not for comprehensive genomic profiling | aNSCLC cohort: 85% of patients (n=62) had detectable somatic alterations  
  aNSCLC cohort: 47% of patients (n=34) had targetable alterations (EGFR [n=29], ALK [n=2], RET [n=1] and ERBB2 [n=2])  
  • 50% (n=17) were matched to a targeted therapy  
  • Of the 15 patients who received targeted therapy, 13 achieved PR and 2 achieved SD |
| Sacher [52]  | ctDNA collected among patients naïve to treatment (67%) and resistant to EGFR-TKI (33%) | PCR (tissue testing by PCR or NGS) | To obtain sufficient tissue for genotyping:  
  • 19% of treatment-naïve patients required a repeat biopsy  
  • 21% of EGFR-resistant patients required a repeat biopsy  
  An additional 16% had insufficient tissue for KRAS testing (after EGFR testing) | 64% of patients (n=116) had detectable EGFR/KRAS mutations (EGFR exon 19 deletion [n=50], EGFR L858R [n=32], EGFR T790M [n=35] and KRAS G12X [n=26]) |
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<tbody>
<tr>
<td>Carpenter [32]</td>
<td>ctDNA collected from patients on TKI therapy (38%) and not on TKI therapy (62%) ctDNA collected between 0 days and 2 years after the tissue sample was collected</td>
<td>NGS (tissue testing by NGS [n=50])</td>
<td>51% had unavailable or insufficient tissue for genomic profile 12% of tissue samples were insufficient for comprehensive genomic profiling</td>
<td>84% of patients (n=86) had detectable somatic alterations (EGFR exon 19 deletion [n=16], EGFR L858R [n=10], EGFR T790M [n=10], ALK [n=2]) • 31% had an approved targeted therapy available • 55% had an off-label targeted therapy available • 70% had a relevant clinical trial available</td>
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<td>AURA3 [9]</td>
<td>ctDNA collected at disease progression after 1L therapy with EGFR-TKI</td>
<td>PCR (tissue testing by PCR)</td>
<td>Not applicable</td>
<td>Of the patients with tumor T790M-positive status (n=359), 172 had plasma T790M-positive status and were randomized to treatment, while 168 had plasma-negative status and were randomized to treatment • In a subgroup analysis of the 172 patients with plasma T790M-positive status, RR in both treatment arms (either osimertinib or platinum-pemetrexed) compared well with the RR found in the overall study population</td>
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<tr>
<td>Study</td>
<td>Timing of ctDNA Collection</td>
<td>Method of ctDNA Analysis</td>
<td>Tissue Unavailable/ Insufficient for Genomic Profiling</td>
<td>ctDNA Results and Actions</td>
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<td>EURTAC subgroup [48]</td>
<td>ctDNA collected at baseline in the EURTAC trial (patients were treatment-naïve at time of blood draw)</td>
<td>PCR (tissue testing by PCR)</td>
<td>Not applicable</td>
<td>Of the 97 patients in this subgroup, EGFR mutations detected in 78%</td>
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<td>• 62% had exon 19 deletion</td>
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<td>• 38% had L858R PFS and overall RR in this subgroup compared well with that found in the EURTAC study</td>
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<td>Of the 41 patients with L858R in tissue, those positive for L858R in ctDNA had shorter median survival than those in whom the L858R mutation was not detected in ctDNA (13.7 vs 27.7 months; HR, 2.22; P=.03)</td>
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<td>• Sensitivity of ctDNA analysis for detection of T790M was 70%</td>
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<td>• Of 58 patients with T790M-negative tumors, T790M was detected by ctDNA in 31% of these cases</td>
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<td>• Objective RR and median PFS in T790M-positive ctDNA compared well with that found in T790M-positive tumor tissue</td>
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<td>• False-negative rate of 30% in ctDNA</td>
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<tr>
<td>AURA subgroup [43]</td>
<td>ctDNA collected among patients on TKI therapy, but before osimertinib treatment</td>
<td>PCR (tissue testing by PCR)</td>
<td>Not applicable</td>
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EURTAC subgroup: Subgroup (n=97) of EURTAC study of aNSCLC with confirmed EGFR mutation (exon 19 deletion or L858R in exon 21) in tumor tissue (n=173)

AURA subgroup: Multi-center, retrospective study of aNSCLC with acquired resistance to EGFR-TKI (n=308)
<table>
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<tbody>
<tr>
<td>Janku [47]</td>
<td>Entire cohort: Median time from tissue to plasma sampling was 19.5 months</td>
<td>NGS (tissue testing by NGS or PCR)</td>
<td>Not applicable</td>
<td>Entire cohort: 89% of patients (n=49) had detectable somatic alterations; Entire cohort: 25% of patients (n=14) had potentially actionable mutations that had not been characterized in tumor tissue, including PIK3CA, IDH1, KRAS, and BRAF</td>
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<tr>
<td>PREDICT-UCSD [46]</td>
<td>• Entire cohort: Median time from tissue to plasma sampling was 10.5 months • Entire cohort: Patients had a median of 1 prior line of therapy before ctDNA testing</td>
<td>NGS (tissue testing by NGS [n=101])</td>
<td>Not applicable</td>
<td>NSCLC cohort: 72% of patients (n=34) had detectable somatic alterations (TP53 [45%], EGFR [23%], MET [15%], KRAS [11%], ALK [6%] and NOTCH1 [6%]) • 73.5% could be matched to an approved therapy • 2.9% could be matched to a clinical trial</td>
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<tr>
<td>McCoach [54]</td>
<td>Cohort 1: blood draw at initial diagnosis Cohort 2: blood draw at disease progression</td>
<td>NGS (tissue testing by NGS or IHC)</td>
<td>26% of patients in cohort 1 had insufficient tissue for ALK testing</td>
<td>Cohort 1: • ALK fusion identified in 16 patients who had been previously reported as tissue negative or QNS Cohort 2: • Mechanism of resistance identified in 77% of patients • 53% of patients were identified with at least 1 mutation in the tyrosine kinase domain</td>
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### Study

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| **UPenn [35]**  
Single-center, prospective study of stage IV NSCLC (n=323) | Blood draw at initial diagnosis or at disease progression; tissue sampling obtained within 24 weeks of plasma testing | NGS (tissue testing by NGS) | 44% of patients had unavailable or insufficient tissue for NGS | • Targetable mutations detected in 35% of patients overall (n=113) with 11% detected only in plasma  
• Adding ctDNA analysis to tissue analysis increased detection of targetable mutations from 20.5% to 35.8%  
• 86% of patients with targetable mutations detected by ctDNA responded to therapy or maintained SD |
| **NILE [49]**  
Multi-center, prospective study of untreated aNSCLC (n=282) | Blood draw and tissue sampling prospectively obtained pre-treatment | NGS (tissue testing by SOC\textsuperscript{b}) | 18.1% (n=51) of patients had complete tissue testing by SOC for all 8 guideline-recommended biomarkers with an additional 13 patients having QNS for at least 1 (n=5) or all (n=8) of the 8 biomarkers | • Guideline-recommended biomarkers detected in 27.3% of patients overall (n=77)  
• Adding ctDNA analysis to tissue analysis increased detection of targetable mutations by 48% (from 60 to 89 patients) |

\textsuperscript{a}Based on tissue genotyping results, although high positive predictive value was demonstrated against ctDNA results.  
\textsuperscript{b}SOC tissue genotyping was at physician discretion and may have included NGS, PCR, FISH, IHC, and/or Sanger sequencing.

**Abbreviations:** 1L, first-line; 2L, second-line; 3L, third-line; aNSCLC, advanced non-small cell lung cancer; ctDNA, circulating tumor DNA; FISH, fluorescence in-situ hybridization; HR, hazard ratio; IHC, immunohistochemistry; NSCLC, non-small cell lung cancer; NGS, next-generation sequencing; PCR, polymerase chain reaction; PFS, progression-free survival; PR, partial response; QNS, quantity not sufficient; RR, response rate; SD, stable disease; SOC, standard of care; TKI, tyrosine kinase inhibitor.
We and others acknowledge the challenges involved in evaluating the clinical impact of ctDNA in a retrospective setting [44, 55-57]. Namely, these studies: 1) report substantial variability in timing from initial tissue biopsy to time of blood draw for ctDNA profiling; 2) may use different platforms (NGS, also referred to as deep sequencing or comprehensive profiling, but may be used for hotspot sequencing; or PCR, the traditional “hotspot” sequencing method) for analysis of tissue and plasma-based samples; and 3) may report incomplete plasma-to-tissue matched samples in their data sets due to insufficient tissue [32, 46, 47]. All of these factors may influence concordance.

Our Working Group agrees with the joint American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) panel that evidence on the use of ctDNA assays for selecting treatment in advanced cancers is limited without data from prospective trials to demonstrate utility of ctDNA as a stand-alone test [44]. However, it is worth noting that since the literature cutoff date in March of 2017, established by the joint ASCO/CAP panel, three prospective feasibility studies (NEXT-2; UPenn; NILE) have been published [15, 35, 49]. NEXT-2 is a first-of-its-kind prospective study in which by design, patients had sufficient tumor tissue for hotspot analysis but not for comprehensive genomic profiling. This trial design highlights a new wave in genome-guided therapy, as it informs treatment on the basis of results obtained from comprehensive genomic profiling of ctDNA. In the study, the therapeutic response rate of 87% among the aNSCLC cohort, guided by comprehensive genomic profiling of ctDNA, compared well with response rates in studies using tissue-based samples, providing prospective trial demonstration of ctDNA clinical utility [15]. It is important to note the limitations of this study, namely, that patients were all from Korea, and only 29% of the overall cohort had aNSCLC.

In the UPenn study, a recently published prospective study including 323 patients with stage IV NSCLC, the clinical utility of liquid biopsy was evaluated in a real-world US clinical setting [35]. In this cohort, adding ctDNA analysis to tissue analysis increased detection of targetable mutations from 20.5% (47 of 229) to 35.8% (82 of 229). Among patients with targetable mutations detected by ctDNA, 86% responded to therapy or maintained stable disease [35]. Of note, tissue NGS was infeasible for 44% of patients and of all targetable alterations detected in this cohort, 58% were detected only by plasma. In the editorial accompanying the publication of this prospective study, the authors concluded “these results, combined with the patient satisfaction with the relative ease of providing blood rather than a solid tissue sample, suggest a clinical strategy of pursuing plasma NGS first, then tissue NGS if plasma NGS cannot detect relevant mutations [58].”

The planned interim analysis of the NILE study, a multi-center, prospective trial, demonstrated the study’s primary objective: non-inferiority of comprehensive cfDNA compared to standard of care (SOC) tissue genotyping in identifying guideline-recommended biomarkers in untreated patients with aNSCLC [49]. Specifically, among the 282 patients assessed, SOC tissue genotyping identified 60 patients with a guideline-recommended biomarker relative to the 77 patients identified by cfDNA. Considering only those patients with an identified guideline-recommended biomarker (n=89), 67% of these patients would have been identified by tissue alone and 87% of these patients would be
identified by plasma alone, demonstrating the utility of cfDNA testing and suggesting the potential for a “blood first” approach. Adding ctDNA analysis to tissue analysis increased the detection of targetable mutations by 48% (from 60 to 89 patients).

Additionally, it is important to note that the turnaround time, defined as time from test order to final results, was significantly shorter for the cfDNA results compared to the tissue genotyping results (9 vs. 15 days) [49]. One limitation to consider in the trial design of the NILE study is that the cfDNA was assessed using a single platform, while the SOC tissue genotyping was at the discretion of the physician and could have entailed comprehensive and/or sequential testing using any combination of NGS, PCR, FISH, IHC and/or Sanger sequencing approaches.

Figure 1. Algorithm for the use of liquid biopsy in patients with aNSCLC based on consensus from the Liquid Biopsy Working Group.

Prioritize scant tissue for PD-L1 testing.

Prioritize scant tissue for PD-L1 testing.

Tissue insufficient for testing or testing conducted, but not all FDA/NCCN targets assessed. Abbreviations: 1L, first-line; 2L, second-line; FDA, U.S. Food and Drug Administration; IO, immuno-oncology therapy; NCCN, National Comprehensive Cancer Network; aNSCLC, advanced non-small cell lung cancer; PD-L1, programmed death-ligand 1.

With these cohort data and recommendations from various professional societies in mind, we developed an algorithm to help guide the use of liquid biopsy (Fig. 1), the basis of which is to ensure that all patients with newly diagnosed stage IIB/IV NSCLC or whose disease has progressed on first-line therapy have sufficient genomic information to guide initial and/or subsequent therapy.

Specifically, patients with newly diagnosed stage IIB/IV NSCLC, confirmed on the basis of a cytologic specimen, should have their tissue prioritized for PD-L1
testing and undergo genomic profiling initially via liquid biopsy. For most patients presenting with advanced-stage lung cancer, minimally invasive techniques including small biopsy and cytology are the primary means of sample acquisition to facilitate a diagnosis, and pathologists (and increasingly, cytopathologists) are tasked with prioritizing scant tissue for molecular testing [5, 59, 60]. Although tremendous improvements have been made to the management of cytologic specimens for molecular testing, including rapid on-site evaluation of cytologic specimens to determine adequacy of the sample for comprehensive profiling, the overall success of tissue-based comprehensive molecular profiling on cytologic specimens still varies widely [34, 60] with tissue insufficiency rates ranging from 33% to 79% [34, 61, 62]. Recently, the NCCN has included liquid biopsy as a consideration at initial diagnosis for molecular testing when tissue quantity is not sufficient, provided there is a plan to reflex back to tissue if a test does not identify an oncogenic driver mutation. However, pursuing tissue testing of scant cytologic specimens for both PD-L1 and genomic biomarkers followed by reflex to liquid when insufficient significantly delays treatment planning for the 33% to 79% of patients whose specimen is deemed insufficient. Therefore, the rationale for this recommendation is that parallel work-up streamlines completion of all biomarker testing relevant to first-line care within the recommended 1-2 week window [57]. The Working Group concurs with NCCN on reflexing back to tissue if liquid biopsy is performed and does not result in the identification of an oncogenic driver mutation.

Those with aNSCLC confirmed on the basis of a surgical specimen fall into 1 of 2 categories: those with incomplete or failed genomic testing in tissue, who should reflex to liquid biopsy, and those whose tissue allows for complete genomic testing of all guideline-recommended genes, who have no immediate need for a liquid biopsy. Given the greater abundance of tissue from surgical specimens, the insufficiency rate is lower and thus we recommend a reflex to liquid approach in this clinical scenario.

Professional society guidelines recommend repeat biopsy in patients who have progressed on first-line therapy [6]. However, in a prospective feasibility study evaluating repeat biopsies in patients with acquired resistance to EGFR tyrosine kinase inhibitors, approximately 20% either could not undergo a repeat biopsy or did not comply with a repeat biopsy, and 11% did undergo a repeat biopsy but tissue was still insufficient for genomic analysis [63]. Given the incidence of biopsy-related adverse events, associated cost to the healthcare system, and the aforementioned challenges, repeat biopsy is not always feasible [63, 64].

To limit the number of patients needing a repeat biopsy, those who have progressed on first-line targeted therapy might rationally undergo contemporary genomic profiling via liquid biopsy; however, if the liquid biopsy is uninformative for treatment-relevant markers, we recommend reflexing to tissue biopsy, as has been recommended previously [43]. The Working Group acknowledges there are caveats to this recommendation: another review would be warranted to describe what progression looks like on a gene-by-gene basis for all 8 genes tied to a mutational status with an approved targeted therapy available. Liquid biopsy should not be undertaken in all instances of progression on first-line therapy, and it should be left to the discretion of the practicing oncologist to determine if a patient should undergo a liquid biopsy in clinical settings where
it is unlikely that an approved targeted therapy would be available at progression. However, the Working Group agrees that there is value in undergoing a liquid biopsy to inform of potential enrollment in clinical trials.

During the preparation of this manuscript, NCCN updated its algorithm for patients with advanced or metastatic disease, reflecting a change in the approach toward utilizing liquid biopsy upon progression on first-line EGFR therapy. Specifically, the algorithm now includes liquid biopsy for consideration upon progression on first-line EGFR therapy for evaluation of the T790M mutation [5]. Previously, this had been conditional upon obtaining tissue. After this recent change, the NCCN guidelines are now much more reflective of our liquid biopsy algorithm for patients progressing on first-line EGFR therapy. With osimertinib now the preferred first-line agent for patients with an EGFR driver mutation, the recommendation for liquid biopsy in patients who have progressed on first-line EGFR therapy may become less relevant, however remains important for those patients who progress on first-line erlotinib, afatinib, gefitinib, or dacomitinib. Lastly, for patients who have progressed on first-line chemotherapy, immunotherapy, or a combination of these agents, and for whom up-front genomic testing was incomplete for all guideline-recommended genes, we recommend liquid biopsy at progression to complete the genotyping.

Recently, a multidisciplinary panel convened by IASLC drafted 2 patient management algorithms, stratified by treatment status (treatment naïve patients and those on 2L and beyond) that align with ours [57], and we feel this further substantiates the need for consensus in the ever-expanding field of molecular profiling to provide the patient with the best care.

There are key differences to point out. First, our algorithm further differentiates patients by the methods used in obtaining biologic specimens (type of biopsy performed). Second, our algorithm recommends that all patients regardless of treatment status undergo genomic profiling for all 9 genes recommended by NCCN and CAP/IASLC/AMP.

**CONSIDERATIONS WHEN SELECTING A LIQUID BIOPSY PLATFORM**

When selecting a liquid biopsy platform, one should consider: the method of genetic analysis (hotspot vs comprehensive), coverage of important mutations, the nature of assay validation, and regulatory factors. The recent IASLC statement paper compares various methods of ctDNA analysis in great detail which we will not replicate here [57]. Briefly, hotspot sequencing tests can be either PCR- or NGS-based, and these tests look for a prespecified, often limited set of mutations with a matched therapy. It is worth noting that a hotspot test generally refers to an assay powered to detect a specific biological molecule or fragment, and detection can be through sequencing or via other technologies, including immunohistochemistry or fluorescence in situ hybridization (FISH). In contrast, comprehensive testing, which utilizes NGS-based sequencing methods, can be used to assess multiple actionable mutations in a single test. Figure 2A highlights liquid biopsy tests that are commercially available, whether these tests are hotspot or comprehensive, and their respective coverage of NCCN mutations [65-73]. When the decision is made by a clinician to move forward with a liquid biopsy test, it is paramount that he/she understands each test is different, in terms of the coverage of alterations and assay validation [74].
A

<table>
<thead>
<tr>
<th>Hotspot or Comprehensive</th>
<th>Liquid Biopsy Platform</th>
<th>Coverage of NCCN Mutations</th>
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</thead>
<tbody>
<tr>
<td>Hotspot</td>
<td>ARUP® Laboratories</td>
<td>EGFR</td>
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<tr>
<td></td>
<td>Roche Molecular Systems</td>
<td>ROS1</td>
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<td>Biodiesix®</td>
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<td>Personal Genome Diagnostics</td>
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</table>

B

- **Analytical Validation**
  - Detection of known spiked in mutations
  - Limit of detection (% MAF)
  - Data replicates

- **Clinical Validation**
  - Tissue-matched specimen on same platform
  - Plasma-matched specimen on different platform

- **Clinical Utility**
  - Demonstration of therapeutic benefit compared to not using the assay (ability to act as a stand-alone test)

Only Analytical Validation?

- **LDTs (a subset of IVDs)**
  - Regulated by CMS under CLIA®
  - Commercially available ctDNA assays

- **IVDs**
  - Regulated by FDA®
  - cobas EGFR mutation test v2 (the only ctDNA assay)

C

Ratio of cfDNA in healthy individuals: cancer patients ~1:4

Average cfDNA ~2.27 ng/mL (0.72-6.49 ng/mL) in healthy individuals

Average cfDNA ~8.02 ng/mL (1.12-40.98 ng/mL) in cancer patients

cfDNA <1% total cfDNA

MAF is ~0.02-0.1% total cfDNA

SNVs (point mutations/substitutions)

Indels (insertions or deletions)

CNVs (amplifications)

Fusions (rearrangements)

\[
MAF \text{ (or VAF)} = \frac{\text{mutant reads}}{\text{total reads for a given genomic position}} = \frac{\text{mutant cfDNA}}{\text{total cfDNA (somatic+germline)}}
\]
Figure 2. Considerations when deciding among genomic testing assays and when interpreting a report.

(A) Commercially available liquid biopsy platforms and their respective coverage of NCCN mutations. Blue boxes indicate hotspot assays and coverage of the specified NCCN mutations. Yellow boxes indicate comprehensive assays and coverage of the specified NCCN mutations. (B) Nature of assay validation and how molecular testing is regulated are practical factors to consider when choosing a genomic test. (C) Mutant allele fraction (MAF) is an important concept to understand when interpreting a genomic testing report.

\(^a\)Assays detecting fusions in at least 1 NTRK gene are indicated below. \(^b\)Analytical validation based on assay performance. \(^c\)May include prospective or retrospective assessments. \(^d\)Commercial assays must meet or exceed CLIA standards; however, CLIA does not regulate clinical validation or clinical utility. Therefore, although many ctDNA assays have demonstrated clinical validation, this is not a prerequisite for commercialization. \(^e\)CMS evaluates clinical utility of a specific test, and this evaluation typically follows from FDA review of analytical and clinical validation. Abbreviations: cfDNA, cell-free DNA; CLIA, Clinical Laboratory Improvement Amendment; CMS, Centers for Medicare and Medicaid Services; CNV, copy number variant; ctDNA, circulating tumor DNA; FDA, U.S. Food and Drug Administration; indel, insertion or deletion; IVD, in vitro diagnostic; LDT, lab-developed test; MAF, mutant allele fraction; NCCN, National Comprehensive Cancer Network; SNV, single nucleotide variant; VAF, variant allele fraction.

Both assay validation and regulatory factors are key considerations (Fig. 2B) [44, 50, 56, 75-80]. Molecular testing used to inform patient care must be carried out in a Clinical Laboratory Improvement Amendment (CLIA)-certified laboratory, the standards for which are regulated by the Centers for Medicare and Medicaid Services (CMS), and include, at a minimum, demonstration of analytical validation. Commercial assays must meet or exceed CLIA standards, but CLIA does not regulate clinical validation or clinical utility; therefore, although many ctDNA assays have demonstrated clinical validation, this is not a prerequisite for commercialization and thus an important datapoint for clinicians to consider when selecting a test [44, 75, 76, 81].

Establishing analytical and clinical validation of ctDNA assays requires an evaluation of concordance among variants detected in ctDNA against a reference standard within a specified tumor type, stage, and point in treatment [56]. To establish clinical utility of a ctDNA assay, it must be demonstrated that the use of the assay shows statistically significant therapeutic benefit compared to not using the assay. While the Working Group agrees with the joint ASCO/CAP panel that demonstrating clinical utility in a prospective, observational setting has the potential to shift the treatment paradigm, prospectively establishing clinical utility of ctDNA for use as a stand-alone diagnostic test is not a prerequisite for introducing liquid biopsy into routine care for patients with aNSCLC [44]. To date, the cobas® EGFR Mutation Test v2 is the only U.S. Food and Drug Administration (FDA)-approved plasma genotyping test, demonstrating clinical utility for patients with NSCLC in the absence of sufficient tumor tissue [82]. However, other liquid biopsy tests have demonstrated clinical utility in both
retrospective and prospective settings [15, 32, 46, 52, 54].

Many genomic profiling tests are considered laboratory-developed tests (LDTs), a subset of in vitro diagnostics (IVDs), which are regulated by CMS under CLIA (Fig. 2B) [76]. Although the FDA has the authority to regulate LDTs, no framework is in place, and in January 2017, the FDA announced that it would not regulate LDTs at this time [81] after issuing draft guidance in 2014 that would have imposed additional restrictions on CLIA-certified labs, potentially causing delays in patient care and increasing developmental costs [75, 83-85]. As such, FDA-approval is not required to offer a genomic profiling test for clinical care.

Another key consideration is insurance coverage. While variability of coverage for molecular testing in private and public payer policies does currently confound routine use of molecular testing assays, broader insurance coverage will likely be adopted as greater evidence of clinical validation and utility comes to light [76, 77, 86]. Indeed, in 2018, CMS implemented a national coverage determination policy that includes FDA-authorized NGS tests for patients with advanced-stage cancers. In addition, Medicare Administrative Contractors may determine coverage for other NGS tests that are not FDA-approved or - cleared as a companion diagnostic, provided the patient meets specific requirements [80]. In regards to liquid biopsy specifically, there are at least two Medical Local Coverage Determination policies providing coverage for specific aNSCLC patients and the Working Group encourages clinicians to directly ask liquid biopsy providers regarding coverage by other insurance parties [87, 88].

**INTERPRETATION OF RESULTS**

Proper interpretation of a liquid biopsy report will help inform patient care but will require an understanding of the components and terminology associated with an output report. The following are answers to questions frequently asked in the community oncologist setting.

**What components comprise a liquid biopsy output report?**

Reports generally include patient information and a list of the genomic alterations detected by the assay. Based on the alterations detected, the report may also include relevant FDA-approved therapies, clinical trials, and off-label therapeutic options associated with the patient’s molecular profile.

**What is the mutant allele fraction, and how does this information help to inform targeted therapy?**

In the context of a liquid biopsy report, the mutant allele fraction (MAF) is the amount of cfDNA with mutant reads divided by the total number of reads (mutant plus germline) for the given allele position (Fig. 2C) [39, 89-92]. Because ctDNA assays differ in their limits of detection for a specified genomic alteration, the importance of analytical validation is appreciated when an MAF approaches the lower limit of detection for a ctDNA assay: a variant detected near the lower limit of detection may confound interpretation of a report, raising concern about applying targeted therapy to a low-level allele. Multiple reports have demonstrated response to ctDNA-detected alterations at low allele fractions, suggesting that response is independent of allele fraction [15].
What are the 4 classes of genomic alterations?

The 4 classes of genomic alterations include: single nucleotide variants (SNV), also known as point mutations or substitutions; insertions or deletions (indels); amplifications or copy number variants (CNVs); and fusions, also known as Rearrangements (Fig. 2C). MAFs may be reported for each of these alterations. ctDNA assays do not necessarily detect all 4 of these alterations, so it is imperative that a clinician review the full list of genes and genomic alterations detected in a given test. Indeed, ctDNA assays are not created equal [5], and even if they do cover the same genes, the 4 classes of alterations may be detected with varying sensitivity and specificity that is largely dependent on the technology used [39, 93, 94].

What is the difference between a somatic and a germline mutation?

Somatic mutations are not inherited, while germline mutations are inherited and may be heterozygous (~50% MAF) or homozygous (~100% MAF) in nature [95]. If a clinician is questioning whether a given variant is somatic- or germline-derived, an MAF of much less than 50% suggests it is somatic in nature. It should be noted that MAFs >50% do not rule out somatic alterations in cases with high ctDNA burden or where a mutant allele has high copy number amplification of the gene containing the variant. If there is still ambiguity, germline sequencing assays may be used to guide clinical decision making.

Are there other sources of cfDNA, and how may these confound interpretation of a report?

Clinicians must be aware that interpreting plasma genotyping results may be complicated by the detection of somatic mutations in genes that are not tumor-derived, but arise from individuals with clonal hematopoiesis [43-45, 96]. This phenomenon has also been reported in tissue NGS sequencing with the suggestion of pairing tumor testing, be it cfDNA or tissue, with peripheral blood testing to help distinguish tumor-derived mutations from other incidental somatic mutations [45, 97, 98]. Differentiating tumor-derived from other-derived mutations will be of paramount importance as liquid biopsies move into recurrence monitoring and primary detection where any positive call will be interpreted as “cancer present.” However, the current use of liquid biopsy is to identify targetable alterations that guide application of therapy. Mutations associated with clonal hematopoiesis typically occur in DMNT3A, TET2, JAK2, TP53, and other genes not associated with any FDA-approved therapies and as such are unlikely to lead to inappropriate oncologic care if detected and reported via tissue or ctDNA assays [99].

What constitutes an actionable mutation?

The term “actionable mutation” is used loosely in the literature to refer to a genomic alteration that informs therapeutic decision making. For instance, there are 16 FDA-approved targeted agents recommended by the NCCN for the treatment of aNSCLC, and these agents target actionable mutations in 8 genes. It must be cautioned that this term is context-dependent. An output report may include detectable alterations in genes beyond those with approved targeted therapies, and these alterations should also be considered actionable. Here, the Working Group has advised prioritizing the actionable mutations from an output report. Specifically, as
a first priority, clinician should address actionable mutations with an approved targeted therapy in aNSCLC. If no such therapies exist for a given mutation, the second priority should be consulting clinical trials, followed lastly by assessing off-label therapies. Off-label therapies are not actively recommended by the Working Group.

If a test comes back with “no alterations detected,” this result should be considered uninformative as opposed to negative, as the possibility of a false negative cannot be ruled out. Other explanations in a scenario like this may include minimal shedding of tumor DNA, the inability of an assay to detect a specified alteration, or the absence of genomic alterations in ctDNA. It is also possible that a test comes back as “alterations detected but nothing actionable found,” and this should be considered informative; the clinician should remember that for many genes sequenced in comprehensive panel testing, either the impact of identified mutations is not well understood or the mutation does not have an available targeted therapy.

**CONCLUSIONS**

In the view of the Working Group, professional society recommendations fall short in addressing the problem of under-genotyping in the community oncologist setting. This further highlights the need for consolidating and disseminating pertinent information on the practical use of liquid biopsy in clinical practice. Our Working Group supports molecular testing for the 9 genes recommended by NCCN and CAP/IASLC/AMP. Specifically, we support the routine use of liquid biopsy as a first resort in treatment-naïve patients with aNSCLC newly confirmed by cytological diagnosis, provided the cytologic tissue specimen is prioritized for PD-L1 testing and profiling of the remaining scant tissue is done in parallel to avoid delays in treatment planning. Liquid biopsy should be used as a second resort after traditional biopsy-based molecular testing in patients whose disease is confirmed by surgical diagnosis but in whom tissue genotyping is insufficient or incomplete. Patients who have progressed on first-line targeted therapy should undergo genomic profiling via liquid biopsy as a first resort with reflex to tissue testing in uninformative cases. Patients who have progressed while taking chemotherapy, immunotherapy, or a combination should undergo liquid biopsy if up-front profiling was incomplete.

Technologies informing molecular testing practices in precision medicine are rapidly evolving, and patients can benefit tremendously from these advances. Data to support the use of liquid biopsy in clinical settings other than for selecting treatment in advanced disease are emerging but are beyond the scope of this review. Namely, evidence to support liquid biopsy for screening of earlier stage cancers, to monitor or track therapy in advanced cancers by means of serial sampling, to evaluate tumor mutational burden as a prognostic biomarker, or for whole exome sequencing, extraction of ctDNA from urine, and isolation of tumor genomic fragments from sources other than ctDNA are topics that will be assessed as additional clinical evidence emerges [90, 93, 100-105].

**REFERENCES**


