LIQUID BIOPSY
WORKING GROUP

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].

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

Target	NCCN [5]	CAP/IASLC/AMP [6]	Recommended Therapies [5]	Response Rate
EGFR	X	X	Erlotinib, afatinib, gefitinib, osimertinib, dacomitinib	60% to 80% [7-14]
ALK	X	X	Alectinib, brigatinib, ceritinib, crizotinib, lorlatinib	40% to 90% [15-21]
ROS1	X	X	Ceritinib, crizotinib, lorlatinib	70%[b] [19]
BRAF	X	X	Dabrafenib + trametinib	65% [22]
NTRK	X	X	Larotrectinib	75% to 80% [23]
RET	X	X	Cabozantinib,[c] vandetanib	20% to 50% [24-26]
MET	X	X	Crizotinib[c]	40% to 50% [27, 28]
ERBB2	X	X	Ado-trastuzumab emtansine[c]	45% [29]
KRAS		X	---	---

[a]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.

[b]Response rate is for crizotinib.

[c]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.

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 occassionally 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.

APPROPRIATE PATIENTS AND TIMING OF USE

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

Study	Timing of ctDNA Collection	Method of ctDNA Analysis	Tissue Unavailable/ Insufficient for Genomic Profiling	ctDNA Results and Actions
NEXT-2 [15] Single-center, prospective study of metastatic cancer (n=194), with an aNSCLC cohort (n=73)	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] Single-center, prospective study of stage IIIB/IV NSCLC (n=180)	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])[a]

Study	Timing of ctDNA Collection	Method of ctDNA Analysis	Tissue Unavailable/ Insufficient for Genomic Profiling	ctDNA Results and Actions
Carpenter [32] Single-center, observational study of stage IV NSCLC (n=102)	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	NGS (tissue testing by NGS [n=50])	51% had unavailable or insufficient tissue for genomic profile 12% of tissue samples were insufficient for comprehensive genomic profiling	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
AURA3 [9] International, multi-center study of aNSCLC (n=419) with confirmed T790M mutation, and disease progression 1L EGFR-TKI	ctDNA collected at disease progression after 1L therapy with EGFR-TKI	PCR (tissue testing by PCR)	Not applicable	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

Study	Timing of ctDNA Collection	Method of ctDNA Analysis	Tissue Unavailable/ Insufficient for Genomic Profiling	ctDNA Results and Actions
EURTAC subgroup [48] 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)	ctDNA collected at baseline in the EURTAC trial (patients were treatment- naïve at time of blood draw)	PCR (tissue testing by PCR)	Not applicable	Of the 97 patients in this subgroup, EGFR mutations detected in 78% • 62% had exon 19 deletion • 38% had L858R PFS and overall RR in this subgroup compared well with that found in the EURTAC study 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)
AURA subgroup [43] Multi-center, retrospective study of aNSCLC with acquired resistance to EGFR-TKI (n=308)	ctDNA collected among patients on TKI therapy, but before osimertinib treatment	PCR (tissue testing by PCR)	Not applicable	• Sensitivity of ctDNA analysis for detection of T790M was 70% • Of 58 patients with T790M-negative tumors, T790M was detected by ctDNA in 31% of these cases • Objective RR and median PFS in T790M-positive ctDNA compared well with that found in T790M-positive tumor tissue • False-negative rate of 30% in ctDNA

Study	Timing of ctDNA Collection	Method of ctDNA Analysis	Tissue Unavailable/ Insufficient for Genomic Profiling	ctDNA Results and Actions
Janku [47] Single-center study of advanced cancers (n=55), with a NSCLC cohort (n=11)	Entire cohort: Median time from tissue to plasma sampling was 19.5 months	NGS (tissue testing by NGS or PCR)	Not applicable	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
PREDICT-UCSD [46] Single-center, retrospective study of solid cancers (n=168), with an NSCLC cohort (n=47)	• 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	NGS (tissue testing by NGS [n=101])	Not applicable	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
McCoach [54] Retrospective study of aNSCLC (n=85) with 2 primary cohorts; cohort 1 (n=42) consisted of newly diagnosed ALK-positive patients naïve to ALK inhibitors; cohort 2 (n=31) consisted of patients who progressed while on an ALK inhibitor	Cohort 1: blood draw at initial diagnosis Cohort 2: blood draw at disease progression	NGS (tissue testing by NGS or IHC)	26% of patients in cohort 1 had insufficient tissue for ALK testing	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

Study	Timing of ctDNA Collection	Method of ctDNA Analysis	Tissue Unavailable/ Insufficient for Genomic Profiling	ctDNA Results and Actions
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[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)

[a]Based on tissue genotyping results, although high positive predictive value was demonstrated against ctDNA results. [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.

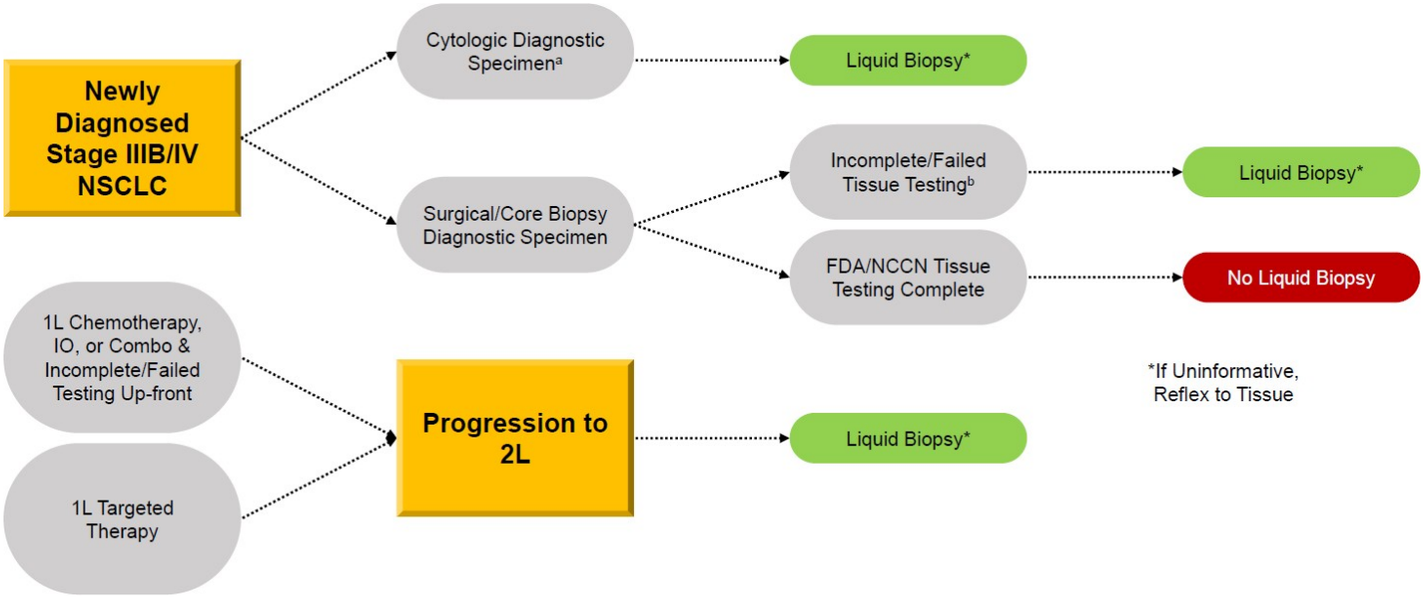


Figure 1. Algorithm for the use of liquid biopsy in patients with aNSCLC based on consensus from the Liquid Biopsy Working Group.

[a]Prioritize scant tissue for PD-L1 testing. [b]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 IIIB/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 IIIB/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.

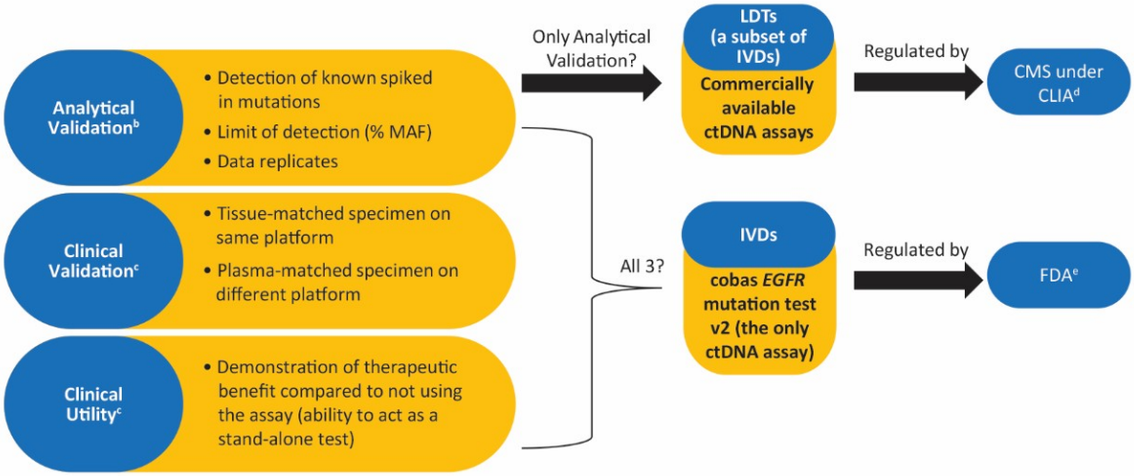
CONSIDERATIONS WHEN SELECTING A LIQUID BIOPSY PLATFORM

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

Hotspot or Comprehensive	Liquid Biopsy Platform	Coverage of NCCN Mutations							
		EGFR	*ALK*	*ROS1*	*BRAF*	*RET*	*MET*	*ERBB2*	*NTRK*[a]
Hotspot	ARUP® Laboratories	■							
	Roche Molecular Systems	■							
	Biodesix®	■	■	■	■	■			
	Biocept	■	■	■	■	■	■	■	
Comprehensive	Inivata™	■	■	■	■		■	■	
	NantHealth™	■	■	■	■		■	■	
	Foundation Medicine®	■	■	■	■	■	■	■	
	Guardant Health	■	■	■	■	■	■	■	■
	Personal Genome Diagnostics	■	■	■	■	■	■	■	■

B



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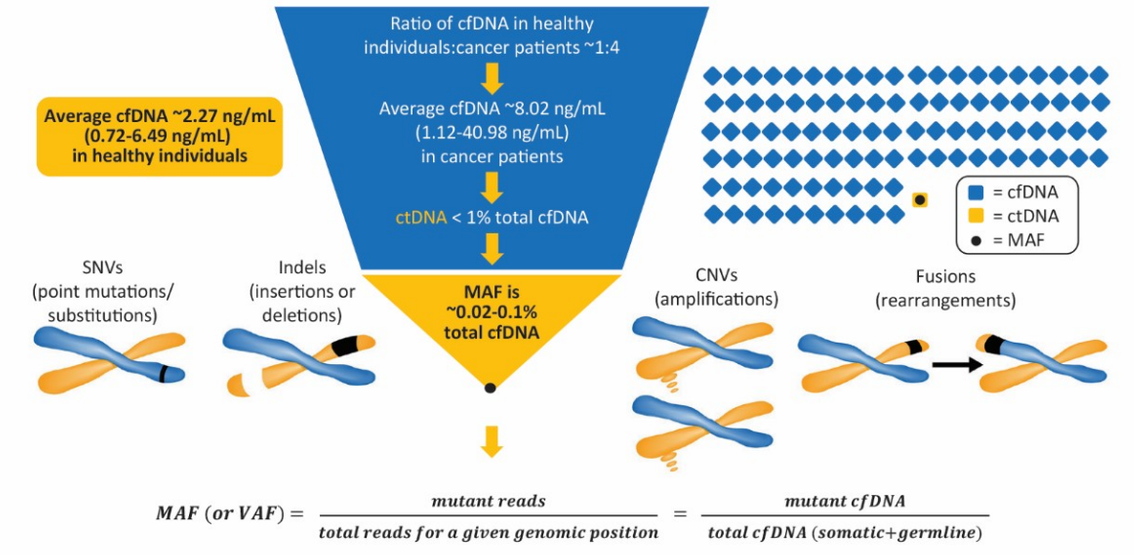

$$MAF\ (or\ VAF) = \frac{mutant\ reads}{total\ reads\ for\ a\ given\ genomic\ position} = \frac{mutant\ cfDNA}{total\ cfDNA\ (somatic+germline)}$$

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

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2018. CA Cancer J Clin 2018;68:7–30.

2. National Cancer Institute. SEER Cancer Statistics Review, 1975-2015. Available at https://seer.cancer.gov/csr/1975_2015/. Accessed March 19, 2019.

3. Cancer Genome Atlas Research Network. Comprehensive molecular profiling of lung adenocarcinoma. Nature 2014;511:543–550.

4. Kris MG, Johnson BE, Berry LD et al. Using multiplexed assays of oncogenic drivers in lung cancers to select targeted drugs. JAMA 2014;311:1998–2006.

5. NCCN Guidelines Version 3.2019 Non-small Cell Lung Cancer. 2019. Available at https://www.nccn.org/professionals/physician_gls/f_guidelines.asp. Accessed February 8, 2019.

6. Lindeman NI, Cagle PT, Aisner DL et al. Updated molecular testing guideline for the selection of lung cancer patients for treatment with targeted tyrosine kinase inhibitors: Guideline from the College of American Pathologists, the International Association for the Study of Lung Cancer, and the Association for Molecular Pathology. J Mol Diagn 2018;20:129–159.

7. Maemondo M, Inoue A, Kobayashi K et al. Gefitinib or chemotherapy for non-small-cell lung cancer with mutated EGFR. N Engl J Med 2010;362:2380–2388.

8. Mitsudomi T, Morita S, Yatabe Y et al. Gefitinib versus cisplatin plus docetaxel in patients with non-small-cell lung cancer harbouring mutations of the epidermal growth factor receptor (WJTOG3405): An open label, randomised phase 3 trial. Lancet Oncol 2010;11:121–128.

9. Mok TS, Wu YL, Ahn MJ et al. Osimertinib or platinum-pemetrexed in EGFR T790M-positive lung cancer. N Engl J Med 2017;376:629–640.

10. Rosell R, Carcereny E, Gervais R et al. Erlotinib versus standard chemotherapy as first-line treatment for European patients with advanced EGFR mutation-positive non-small-cell lung cancer (EURTAC): a multicentre, open-label, randomised phase 3 trial. Lancet Oncol 2012;13:239–246.

11. Sequist LV, Yang JC, Yamamoto N et al. Phase III study of afatinib or cisplatin plus pemetrexed in patients with metastatic lung adenocarcinoma with EGFR mutations. J Clin Oncol 2013;31:3327–3334.

12. Soria JC, Ohe Y, Vansteenkiste J et al. Osimertinib in untreated EGFR-mutated advanced non-small-cell lung cancer. N Engl J Med 2018;378:113–125.

13. Wu YL, Zhou C, Hu CP et al. Afatinib versus cisplatin plus gemcitabine for first-line treatment of Asian patients with advanced non-small-cell lung cancer harbouring EGFR mutations (LUX-lung 6): an open-label, randomised phase 3 trial. Lancet Oncol 2014;15:213–222.

14. Zhou C, Wu YL, Chen G et al. Erlotinib versus chemotherapy as first-line treatment for patients with advanced EGFR mutation-positive non-small-cell lung cancer (OPTIMAL, CTONG-0802): A multicentre, open-label, randomised, phase 3 study. Lancet Oncol 2011;12:735–742.

15. Kim ST, Banks KC, Lee S et al. Prospective feasibility study for using cell-free circulating tumor DNA-guided therapy in refractory metastatic solid cancers: an interim analysis. JCO Precis Oncol 2017;1:1–15.

16. Larkins E, Blumenthal GM, Chen H et al. FDA approval: alectinib for the treatment of metastatic, ALK-positive non-small cell lung cancer following crizotinib. Clin Cancer Res 2016;22:5171–5176.

17. Peters S, Camidge DR, Shaw AT et al. Alectinib versus crizotinib in untreated ALK-positive non-small-cell lung cancer. N Engl J Med 2017;377:829–838.

18. Shaw AT, Kim DW, Nakagawa K et al. Crizotinib versus chemotherapy in advanced ALK-positive lung cancer. N Engl J Med 2013;368:2385–2394.

19. Shaw AT, Ou SH, Bang YJ et al. Crizotinib in ROS1-rearranged non-small-cell lung cancer. N Engl J Med 2014;371:1963–1971.

20. Soria JC, Tan DSW, Chiari R et al. First-line ceritinib versus platinum-based chemotherapy in advanced ALK-rearranged non-small-cell lung cancer (ASCEND-4): a randomised, open-label, phase 3 study. Lancet 2017;389:917–929.

21. Solomon BJ, Besse B, Bauer TM, et al. Lorlatinib in patients with ALK-positive non-small-cell lung cancer: results from a global phase 2 study. Lancet Oncol 2018;19:1654–1667.

22. Planchard D, Smit EF, Groen HJM, et al. Dabrafenib plus trametinib in patients with previously untreated BRAF(V600E)-mutant metastatic non-small-cell lung cancer: an open-label, phase 2 trial. Lancet Oncol 2017;18:1307–1316.

23. Drilon A, Laetsch TW, Kummar S et al. Efficacy of larotrectinib in TRK fusion-positive cancers in adults and children. N Engl J Med 2018;378:731–739.

24. Drilon A, Rekhtman N, Arcila M et al. Cabozantinib in patients with advanced RET-rearranged non-small-cell lung cancer: an open-label, single-centre, phase 2, single-arm trial. Lancet Oncol 2016;17:1653–1660.

25. Gautschi O, Milia J, Filleron T et al. Targeting RET in patients with RET-rearranged lung cancers: results from the global, multicenter RET registry. J Clin Oncol 2017;35:1403–1410.

26. Yoh K, Seto T, Satouchi M et al. Vandetanib in patients with previously treated RET-rearranged advanced non-small-cell lung cancer (LURET): an open-label, multicentre phase 2 trial. Lancet Respir Med 2017;5:42–50.

27. Drilon AE, Camidge DR, Ou SI et al. Efficacy and safety of crizotinib in patients with advanced MET exon 14-altered non-small cell lung cancer (NSCLC). J Clin Oncol 2016:34(suppl 15): abstr 108.

28. Camidge DR, Ou SI, Shapiro G et al. Efficacy and safety of crizotinib in patients with advanced c-MET-amplified non-small cell lung cancer (NSCLC). J Clin Oncol 2014:32(suppl 15): abstr 8001.

29. Mazières J, Barlesi F, Filleron T et al. Lung cancer patients with HER2 mutations treated with chemotherapy and HER2-targeted drugs: results from the European EUHER2 cohort. Ann Oncol 2016;27:281–286.

30. Carbone DP, Reck M, Paz-Ares L et al. First-line nivolumab in stage IV or recurrent non-small-cell lung cancer. N Engl J Med 2017;376:2415–2426.

31. Hellmann MD, Ciuleanu TE, Pluzanski A et al. Nivolumab plus ipilimumab in lung cancer with a high tumor mutational burden. N Engl J Med 2018;378:2093–2104.

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

33. Villaflor V, Won B, Nagy R et al. Biopsy-free circulating tumor DNA assay identifies actionable mutations in lung cancer. Oncotarget 2016;7:66880–66891.

34. Hagemann IS, Devarakonda S, Lockwood CM et al. Clinical next-generation sequencing in patients with non-small cell lung cancer. Cancer 2015;121:631–639.

35. Aggarwal C, Thompson JC, Black TA et al. Clinical implications of plasma-based genotyping with the delivery of personalized therapy in metastatic non-small cell lung cancer. JAMA Oncol 2019;5(2):173-180.

36. Gutierrez ME, Choi K, Lanman RB et al. Genomic profiling of advanced non-small cell lung cancer in community settings: gaps and opportunities. Clin Lung Cancer 2017;18:651–659.

37. Abernethy AP, Arunachalam A, Burke T et al. Real-world first-line treatment and overall survival in non-small cell lung cancer without known EGFR mutations or ALK rearrangements in US community oncology setting. PLoS One 2017;12:e0178420.

38. Hussein M, Richards DA, Ulrich B et al. Biopsies in initial diagnosis of non-small cell lung cancer in US community oncology practices: implications for first-line immunotherapy: Topic: Medical oncology. J Thorac Oncol 2016;11:S249–S250 (abstr ORAL01.02).

39. Diaz LA, Jr., Bardelli A. Liquid biopsies: genotyping circulating tumor DNA. J Clin Oncol 2014;32:579–586.

40. Bettegowda C, Sausen M, Leary RJ et al. Detection of circulating tumor DNA in early- and late-stage human malignancies. Sci Transl Med 2014;6:224ra224.

41. Singh AP, Li S, Cheng H. Circulating DNA in EGFR-mutated lung cancer. Ann Transl Med 2017;5:379.

42. Burstein HJ, Krilov L, Aragon-Ching JB et al. Clinical Cancer Advances 2017: annual report on progress against cancer from the American Society of Clinical Oncology. J Clin Oncol 2017;35:1341–1367.

43. Oxnard GR, Thress KS, Alden RS et al. Association between plasma genotyping and outcomes of treatment with osimertinib (AZD9291) in advanced non-small-cell lung cancer. J Clin Oncol 2016;34:3375–3382.

44. Merker JD, Oxnard GR, Compton C et al. Circulating tumor DNA analysis in patients with cancer: American Society of Clinical Oncology and College of American Pathologists joint review. J Clin Oncol 2018;36:1631–1641.

45. Hu Y, Ulrich BC, Supplee J et al. False-positive plasma genotyping due to clonal hematopoiesis. Clin Cancer Res 2018;24:4437–4443.

46. Schwaederle M, Husain H, Fanta PT et al. Use of liquid biopsies in clinical oncology: pilot experience in 168 patients. Clin Cancer Res 2016;22:5497–5505.

47. Janku F, Zhang S, Waters J et al. Development and validation of an ultradeep next-generation sequencing assay for testing of plasma cell-free DNA from patients with advanced cancer. Clin Cancer Res 2017;23:5648–5656.

48. Karachaliou N, Mayo-de las Casas C, Queralt C et al. Association of EGFR L858R mutation in circulating free DNA with survival in the EURTAC trial. JAMA Oncol 2015;1:149–157.

49. Leighl NB, Page RD, Raymond VM et al. Clinical utility of comprehensive cell-free DNA analysis to identify genomic biomarkers in patients with newly diagnosed metastatic non-small cell lung cancer. Clin Cancer Res 2019 (in press). Available at https://doi.Org/10.1158/1078-0432.Ccr-19-0624. Accessed April 18, 2019.

50. Lanman RB, Mortimer SA, Zill OA et al. Analytical and clinical validation of a digital sequencing panel for quantitative, highly accurate evaluation of cell-free circulating tumor DNA. PLoS One 2015;10:e0140712.

51. Lebofsky R, Decraene C, Bernard V et al. Circulating tumor DNA as a non-invasive substitute to metastasis biopsy for tumor genotyping and personalized medicine in a prospective trial across all tumor types. Mol Oncol 2015;9:783–790.

52. Sacher AG, Paweletz C, Dahlberg SE et al. Prospective validation of rapid plasma genotyping for the detection of EGFR and KRAS mutations in advanced lung cancer. JAMA Oncol 2016;2:1014–1022.

53. Rozenblum AB, Ilouze M, Dudnik E et al. Clinical impact of hybrid capture-based next-generation sequencing on changes in treatment decisions in lung cancer. J Thorac Oncol 2017;12:258–268.

54. McCoach CE, Blakely CM, Banks KC et al. Clinical utility of cell-free DNA for the detection of ALK fusions and genomic mechanisms of ALK inhibitor resistance in non-small cell lung cancer. Clin Cancer Res 2018;24:2758–2770.

55. Nakamura Y, Yoshino T. Clinical utility of analyzing circulating tumor DNA in patients with metastatic colorectal cancer. The Oncologist 2018;23:1–8.

56. Sacher AG, Komatsubara KM, Oxnard GR. Application of plasma genotyping technologies in non-small cell lung cancer: a practical review. J Thorac Oncol 2017;12:1344–1356.

57. Rolfo C, Mack PC, Scagliotti GV et al. Liquid biopsy for advanced non-small cell lung cancer (NSCLC): a statement paper from the IASLC. J Thorac Oncol 2018;13:1248–1268.

58. Gyawali B, West H. Plasma vs tissue next-generation sequencing in non-small cell lung cancer-either, both, or neither? JAMA Oncol 2019;5(2):148-149.

59. Travis WD, Brambilla E, Noguchi M et al. Diagnosis of lung cancer in small biopsies and cytology: implications of the 2011 International Association for the Study of Lung Cancer/American Thoracic Society/ European Respiratory Society Classification. Arch Pathol Lab Med 2013;137:668–684.

60. Lozano MD, Echeveste JI, Abengozar M et al. Cytology smears in the era of molecular biomarkers in non-small cell lung cancer: doing more with less. Arch Pathol Lab Med 2018;142:291–298.

61. Roy-Chowdhuri S, Goswami RS, Chen H et al. Factors affecting the success of next-generation sequencing in cytology specimens. Cancer Cytopathol 2015;123:659-668.

62. Roy-Chowdhuri S, Stewart J. Preanalytic variables in cytology: lessons learned from next-generation sequencing-the MD Anderson experience. Arch Pathol Lab Med 2016;140:1191-1199.

63. Redig AJ, Costa DB, Taibi M et al. Prospective study of repeated biopsy feasibility and acquired resistance at disease progression in patients with advanced EGFR mutant lung cancer treated with erlotinib in a phase 2 trial. JAMA Oncol 2016;2:1240–1242.

64. Lokhandwala T, Bittoni MA, Dann RA et al. Costs of diagnostic assessment for lung cancer: a Medicare claims analysis. Clin Lung Cancer 2017;18:e27–e34.

65. Personal Genome Diagnostics, Inc. PlasmaSELECT™-R 64. Available at http://www.personalgenome.com/pharma-biotech-companies/liquid-biopsy/. Accessed March 14, 2019.

66. Guardant Health, Inc. Guardant360. Available at https://guardanthealth.com/. Accessed March 14, 2019.

67. Foundation Medicine, Inc. FOUNDATIONONE®LIQUID. Available at https://www.foundationmedicine.com/genomic-testing/foundation-one-liquid Accessed March 14, 2019.

68. NantHealth. Liquid GPS. Available at http://nanthealth.orbitdesignworks.com/liquid-gps/. Accessed March 14, 2019.

69. Inivata Ltd. InVisionFirst™-Lung. Available at https://www.inivata.com/our-products/invisionfirst/. Accessed March 14, 2019.

70. Biocept, Inc. Target Selector™ Biomarkers. Available at https://biocept.com/biomarkers-test/. Accessed March 14, 2019.

71. Biodesix, Inc. GeneStrat® Genomic Blood Test. Available at https://www.biodesix.com/products/biodesix-lung-reflex/genestrat-2/. Accessed March 14, 2019.

72. F. Hoffmann-La Roche Ltd. cobas® EGFR Mutation Test v2. Available at https://diagnostics.roche.com/global/en/products/params/cobas-egfr-mutation-test-v2.html. Accessed March 14, 2019.

73. ARUP Laboratories. EGFR T790M Mutation in Circulating Tumor DNA by Digital Droplet PCR. Available at https://www.aruplab.com/T790M. Accessed March 14, 2019.

74. Stetson D, Dougherty B, Ahmed A et al. Examination of analytical factors impacting concordance of 602 plasma-tumor testing by next-generation sequencing (NGS). Cancer Res 2017;77(13 suppl):abstract nr 603 LB-249.

75. Khodakov D, Wang C, Zhang DY. Diagnostics based on nucleic acid sequence variant profiling: PCR, hybridization, and NGS approaches. Adv Drug Deliv Rev 2016;105:3–19.

76. Engstrom PF, Bloom MG, Demetri GD, et al. NCCN molecular testing white paper: effectiveness, efficiency, and reimbursement. J Natl Compr Canc Netw 2011;9(suppl 6):S1–16.

77. Kurzrock R, Colevas AD, Olszanski A et al. NCCN Oncology Research Program's Investigator 609 Steering Committee and NCCN Best Practices Committee molecular profiling surveys. J Natl Compr Canc Netw 2015;13:1337–1346.

78. Mellert H, Foreman T, Jackson L et al. Development and clinical utility of a blood-based test service for the rapid identification of actionable mutations in non-small cell lung carcinoma. J Mol Diagn 2017;19:404–416.

79. Kuderer NM, Burton KA, Blau S et al. Comparison of 2 commercially available next-generation sequencing platforms in oncology. JAMA Oncol 2017;3:996–998.

80. Centers for Medicare & Medicaid Services. Decision memo for next-generation sequencing (NGS) for Medicare beneficiaries with advanced cancer (CAG-00450N). www.cms.gov/medicare-coverage-database/details/nca-decision-memo.Aspx?NCAID=290&timeframe=7&doctype=all&bc=aaaaiaaaqaaa&. Accessed February 13, 2019.

81. U.S. Food and Drug Administration. Discussion paper on laboratory developed tests (LDTs). January 13, 2017. Available at https://www.fda.gov/downloads/medicaldevices/productsandmedicalprocedures/invitrodiagnostics/laboratorydevelopedtests/ucm536965.pdf. Accessed April 3, 2018.

82. U.S. Food and Drug Administration. cobas® EGFR Mutation Test v2. Available at https://www.fda.gov/drugs/informationondrugs/approveddrugs/ucm504540.htm. Accessed March 27, 2018.

83. Levy KD, Pratt VM, Skaar TC et al. FDA's draft guidance on laboratory-developed tests increases clinical and economic risk to adoption of pharmacogenetic testing. J Clin Pharmacol 2015;55:725–727.

84. Rosenman MB, Decker B, Levy KD et al. Lessons learned when introducing pharmacogenomic panel testing into clinical practice. Value Health 2017;20:54–59.

85. U.S. Food and Drug Administration. Center for Biologics Evaluation and Research. Draft Guidance for Industry, Food and Drug Administration Staff, and Clinical Laboratories. Framework for regulatory oversight of laboratory developed tests (LDTs). Available at https://www.fda.gov/downloads/medicaldevices/deviceregulationandguidance/guidancedocuments/ucm416685.pdf. Accessed April 3, 2018.

86. Tempero M. Molecular diagnostics: another battle brewing. J Natl Compr Canc Netw 2018;16:225.

87. Palmetto finalizes LCD for Guardant360 assay in NSCLC. Available at https://www.Genomeweb.Com/reimbursement/palmetto-finalizes-lcd-guardant360-assay-nsclc#.Xkttipzfyv8. Accessed April 3, 2019.

88. Inivata Medicare Local Coverage Decision finalized for InvisionFirst-Lung Liquid Biopsy. Available at https://www.Genomeweb.Com/liquid-biopsy/inivata-medicare-local-coverage-decision-finalized-invisionfirst-lung-liquid-biopsy#.Xktu7pzfyr9. Accessed April 3, 2019.

89. Dienstmann R, Elez E, Argiles G et al. Analysis of mutant allele fractions in driver genes in colorectal cancer - biological and clinical insights. Mol Oncol 2017;11:1263–1272.

90. Phallen J, Sausen M, Adleff V et al. Direct detection of early-stage cancers using circulating tumor DNA. Sci Transl Med 2017;9:eaan2415.

91. Chen KZ, Lou F, Yang F et al. Circulating tumor DNA detection in early-stage non-small cell lung cancer patients by targeted sequencing. Sci Rep 2016;6:31985.

92. Szpechcinski A, Chorostowska-Wynimko J, Struniawski R et al. Cell-free DNA levels in plasma of patients with non-small-cell lung cancer and inflammatory lung disease. Br J Cancer 2015;113:476–483.

93. Vendrell JA, Mau-Them FT, Béganton B et al. Circulating cell free tumor DNA detection as a routine tool for lung cancer patient management. Int J Mol Sci 2017;18:264.

94. Singh AP, Cheng H, Guo X et al. Circulating tumor DNA in non-small-cell lung cancer: a primer for the clinician. JCO Precis Oncol 2017:1–13.

95. Hu Y, Alden RS, Odegaard JI et al. Discrimination of germline EGFR T790M mutations in plasma cell-free DNA allows study of prevalence across 31,414 cancer patients. Clin Cancer Res 2017;23:7351–7359.

96. Langabeer SE. The Jak2 V617F mutation in lung cancer: Caveat emptor. Exp Oncol 2018;40:343–344.

97. Severson EA, Riedlinger GM, Connelly CF et al. Detection of clonal hematopoiesis of indeterminate potential in clinical sequencing of solid tumor specimens. Blood 2018;131:2501-2505.

98. Ptashkin RN, Mandelker DL, Coombs CC et al. Prevalence of clonal hematopoiesis mutations in tumor-only clinical genomic profiling of solid tumors. JAMA Oncol 2018;4:1589-1593.

99. Jaiswal S, Fontanillas P, Flannick J et al. Age-related clonal hematopoiesis associated with adverse outcomes. N Engl J Med 2014;371:2488-2498.

100. Yang M, Topaloglu U, Petty WJ et al. Circulating mutational portrait of cancer: manifestation of aggressive clonal events in both early and late stages. J Hematol Oncol 2017;10:100.

101. Krug AK, Enderle D, Karlovich C et al. Improved EGFR mutation detection using combined exosomal RNA and circulating tumor DNA in NSCLC patient plasma. Ann Oncol 2018;29:700–706.

102. Dietz S, Schirmer U, Mercé C et al. Low input whole-exome sequencing to determine the representation of the tumor exome in circulating DNA of non-small cell lung cancer patients. PLoS One 2016;11:e0161012.

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

104. Davis AA, Chae YK, Agte S et al. Comparison of tumor mutational burden (TMB) across tumor tissue and circulating tumor DNA (ctDNA). J Clin Oncol 2017:35(suppl 15): abstr e23028.

105. Corcoran RB, Chabner BA. Application of cell-free DNA analysis to cancer treatment. N Engl J Med 2018;379:1754–1765.