Original Study

Liquid Biopsy Versus Tissue Biopsy to Determine Front Line Therapy in Metastatic Non-Small Cell Lung Cancer (NSCLC)

Luis E. Raez,[1,#] Kayla Brice,[2,#] Katerine Dumais,[2] Alejandro Lopez-Cohen,[3] Delia Wietecha,[3] Paola A. Izquierdo,[2] Edgardo S. Santos,[4] Hermán W. Powery[2]

Abstract

Next generation sequencing (NGS) of tumors to find actionable genes has been the standard of care in metastatic non–small cell lung cancer. We demonstrated that liquid biopsy NGS can replace tissue NGS in front line therapy decisions in most of the patients with shorter turnaround time than tissue NGS and similar clinical outcomes.

In the last decade, non–small-cell lung cancer (NSCLC) treatment has improved with the approval of multiple therapies to target specific genetic alterations. Though, next generation sequencing (NGS) has traditionally been conducted from tissue biopsy samples, developing data supports the use of plasma-based circulating tumor DNA (ctDNA), also known as "liquid biopsy," to complement tissue biopsy approaches in guiding front-line therapy. This study is a retrospective analysis of 170 new NSCLC patients treated at 2 cancer centers within a 5-year period who received both tissue and liquid biopsy NGS as standard of care. Based on a treatment schema defined by testing sufficiency, biomarker detection, and turnaround time (TAT), physicians based the majority of their treatments on liquid biopsy results (73.5%) versus tissue biopsy (25.9%). Liquid biopsy NGS returned results on average 26.8 days faster than tissue and reported higher testing success. For guideline-recommended biomarkers, liquid biopsy was 94.8% to 100% concordant with tissue. In comparing testing modalities, a liquid-first approach identified guideline-recommended biomarkers in 76.5% of patients versus 54.9% in a tissue-first approach. There was no significant difference in time-to-treatment, or survival outcomes (overall survival and progression free survival) based on liquid versus tissue biopsy findings. This research demonstrates that liquid biopsy NGS is an effective tool to capture actionable genetic alterations in NSCLC. Due to its high concordance to tissue, faster TAT, and similarity in outcomes and time-to-treatment, liquid biopsy can be used either as a first-line test or concordantly with tissue biopsy to guide treatment decisions in NSCLC.

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Introduction

Non–small-cell lung cancer (NSCLC) is one of the leading causes of death worldwide with a 5-year survival rate of 26% in patients with metastatic disease.[1] In recent years, the development of targeted agents has improved patient outcomes, as targeted therapy provides superior survival advantage to chemotherapy and/or immunotherapy.[2,3] There are multiple tools available to assess for actionable genetic alterations in NSCLC. Typically, clinicians opt for molecular analysis via next-generation sequencing (NGS), which allows for ultra-high throughput sequencing of the genome to detect targetable alterations in solid tumors.[4] NGS provides more comprehensive sequencing than hotspot testing, PCR (polymerase chain reaction) or IHC/FISH (Immunohistochemistry/fluorescence in situ hybridization).[5] Indeed, comprehensive testing via broad molecular profiling panels is currently supported by several best practice guidelines for NSCLC, including the National Comprehensive Cancer Network (NCCN), the American Society of Clinical Oncology, the International Association for the Study of Lung Cancer, and the European Society for Medical Oncology.[5-8] Beyond first-line therapy, the guidelines also support molecular profiling at certain events of progression to evaluate for mechanisms of resistance.

[1]Thoracic Oncology Program, Memorial Cancer Institute/Florida Atlantic University, Pembroke Pines, FL
[2]Memorial Cancer Institute, Pembroke Pines, FL
[3]Department of Medicine/Memorial Healthcare System, Pembroke Pines, FL
[4]Florida Precision Oncology/Genesis Care/Florida Atlantic University

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Address for correspondence: Luis E. Raez, MD, FACP, FCCP, Thoracic Oncology Program, Memorial Cancer Institute/Florida Atlantic University, 801 N Flamingo Rd # 11, Pembroke Pines, FL 33028.
E-mail contact: LRaez@mhs.net

[#] Both authors contribute equally for this manuscript.

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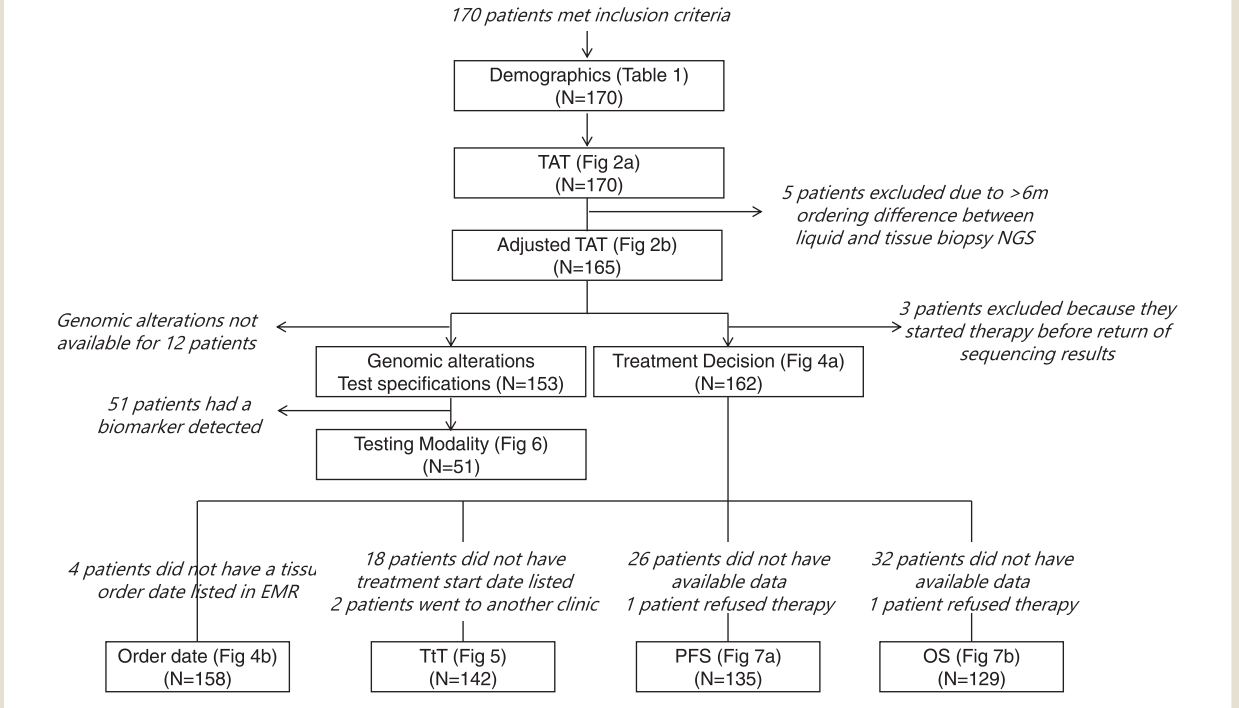
Figure 1 Patient exclusion schema by analysis



Historically, NGS has been conducted on tumor tissue biopsy samples to identify potentially actionable biomarkers in patients with NSCLC. Though effective, NGS via tissue has numerous disadvantages, including requiring an invasive procedure, potential limitations in performing biopsies due to anatomic site, limited tissue quantities, potential insufficient tissue sample available after pathology, and limitations in capturing tumor heterogeneity.[7,9,10] In recent years, NGS via liquid biopsy has emerged originally as a complementary testing option to tissue. Unlike tissue biopsy, liquid biopsy captures circulating tumor DNA (ctDNA) shed from the tumor, typically as a response to cell death.[11] Plasma-based liquid biopsy NGS offers multiple advantages to tissue. First, liquid biopsy uses a minimally invasive biopsy procedure that provides opportunities for sequencing beyond front-line therapy selection and amongst all stages of lung cancer, including screening, detection of minimum residual disease, longitudinal surveillance, assessment of mechanisms of resistance at progression, and real-time monitoring.[5,11-13] Second, liquid biopsies require less sample preparation than tissue; thus, allowing for a rapid turnaround time (TAT) to reporting sequencing results.[11] NGS tissue biopsies take about 3 to 4 weeks to provide results compared to NGS liquid biopsies that take about 8 business days.[12,13] One consequence of slower NGS TAT is that patients may be disqualified for clinical trial enrollment or may become ineligible for treatments due to decompensation during delayed genomic test results.[14,15] Third, liquid biopsy captures cell-free DNA shed into the plasma, and thus can capture tumor heterogeneity more broadly as compared to tissue biopsies.[7,11] As a complementary test, liquid biopsy also has some disadvantages compared to tissue. First, liquid biopsy requires active tumor shed to detect ctDNA, and tumor shed can be limited by slow growing or indolent tumors, as well as brain tumors, which ultimately can report false negatives.[11] Second, liquid biopsies can be obfuscated by nontumor findings arising from clonal hematopoiesis of indeterminate potential, which must be considered when interpreting data.[7,11]

The use of liquid biopsy to identify actionable alterations in mNSCLC has increased dramatically. Multiple studies have demonstrated noninferiority of liquid biopsies to identify actionable genetic alterations in patients with mNSCLC relative to physician discretion standard-of-care tissue genotyping.[12,16] Additional evidence supports similar patient outcomes to targeted therapies prescribed based on NGS from tissue versus liquid biopsy.[17,18] In this study, we aimed to illustrate that liquid biopsy can be the new standard tool for decision making for patients with stage IV NSCLC, particularly, for patients treated in the first line setting. This protocol can help close the gap for patients who do not have a sufficient tissue sample, but additionally, will also provide opportunities for patients with mNSCLC to initiate targeted treatment options faster.

Table 1 Demographics of 170 Patients Included in the Analysis

Gender	N	%
Male	82	48.2%
Female	88	51.8%
Age (26-94)		
Average	66	
Race		
White Caucasian	93	54.7%
Hispanics	51	30.0%
African American	15	8.8%
Asian	10	5.9%
Pacific Islander/Native Hawaiian	1	0.6%
Smoking history		
Former smoker	107	62.9%
Never smoked	52	30.6%
Current smoker	11	6.5%
Histology		
Adenocarcinoma	159	93.5%
Poorly differentiated	5	2.9%
Adenosquamous carcinoma	2	1.2%
Squamous cell carcinoma	2	1.2%
Not listed	2	1.2%
Treatment line		
First line	124	72.9%
Second line	11	6.5%
Third line or greater	10	5.9%
Not listed	25	14.7%

Materials and Methods

Patients and Electronic Medical Record Data

This was a retrospective study of adult patients (≥ 18 years) treated at 2 centers, Memorial Cancer Institute and Florida Precision Oncology between July 1, 2015 and June 30, 2020. Patients included in the analysis were diagnosed with stage IV NSCLC and received both tissue and liquid biopsy as part of routine clinical care. Liquid biopsy was conducted from a blood test to analyze plasma-derived cell-free DNA using a CLIA (Clinical Laboratory Improvement Amendments) certified, CAP (College of American Pathologists)-accredited, New York State Department of Health approved comprehensive NGS test consisting of 68 to 83 genes (Guardant360; Guardant Health, Redwood City, CA). The liquid biopsy test has previously demonstrated analytical and clinical validity and clinical utility in solid tumors.[18-20] In contrast, tissue genotyping was conducted according to physician preference and included mainly 2 NGS panels from CLIA certified and CAP-accredited laboratories. The electronic medical record did not capture all tissue biopsy panels used in this study, but of those defined, all included broad genomic profiling of NCCN guideline supported biomarkers. The Memorial Healthcare System IRB approved this study.

Data for this study was collected from the EMR (Electronic medical records) via REDCap, and included patient demographics, liquid and tissue biopsy sequencing ordering and reporting dates, reported genomic alterations, clinician decision of biopsy used to make the treatment decision (ie, liquid, tissue, or both biopsies), prescribed treatment, date of treatment initiation, and dates of disease progression and death. The patient cohort began with 170 eligible patients. Certain patients were excluded by analysis for variable reasons as described in the results section. For clarity, these exclusions are outlined in Figure 1.

TAT

The TAT was defined as the time between NGS date ordered in the EMR and the date results were reported by the laboratory and entered into the EMR. In this study, a subset of patients had large periods of time between the ordering date of liquid and tissue biopsy. Previous literature has demonstrated that concordance between paired liquid and tissue biopsy samples decreases over time, considered as a consequence of temporal heterogeneity.[11] In a prior publication, paired Guardant360® and tissue biopsy samples from diagnosed or suspected NSCLC patients maintained >90% concordance in identifying *EGFR* alterations when the biopsy samples were collected within 6 months of 1 another.[21] The current study aimed to conserve the clinical impact of testing efficacy on treatment decisions made using liquid versus tissue biopsy NGS. To best evaluate the clinical impact of genetic testing efficiency on treatment decisions, patients whose test order dates were greater than 6 months apart (180 days) were excluded from the TAT sub analysis and additional analyses (N = 5). These were cases where 1. the sample was not easily found or 2. the supply of the tissue sample was delayed because the biopsy was done in an outside referral hospital or a new tissue sample had to be cut from the block and submitted for analysis because the original was not enough. Descriptive statistics for TAT were conducted with an unpaired student t test calculation.

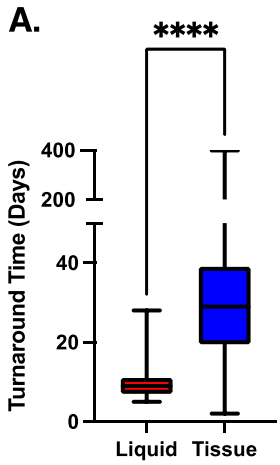
Schema for Treatment Decision Based on Biopsy Result

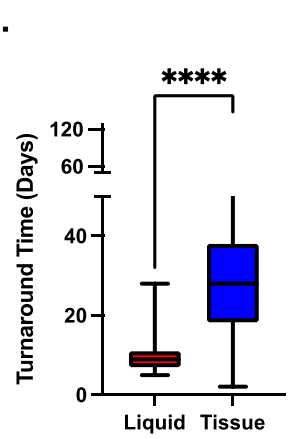
The clinicians in this study were asked to identify the NGS biopsy sample used to determine treatment. This decision was based on multiple factors. To begin, the treating clinician determined if NGS results were effectively reported by 1 or both tests. Next, the clinician assessed if an actionable genomic alteration was detected by neither test, 1 test, or both tests. For this study, an actionable genomic alteration is defined as an alteration in the NCCN guideline recommended biomarkers associated with a targeted agent (ie, targeted therapy). If an actionable alteration was detected, the clinician chose the test which returned results earlier. If neither test reported an actionable genomic alteration, then the clinician chose the NGS assay that returned results earliest because that allowed them to make a decision for the use of chemotherapy and/or immunotherapy when genomic alterations were not present.

Time to Treatment, Performance Specifications, Testing Modality, and Survival Analyses

The time to treatment (TtT) was defined as the time between NGS date reported by laboratory and entered into the EMR and treatment initiation. Descriptive statistics for TtT were conducted with an unpaired student t test calculation. The sensitivity, speci-

Figure 2 Turnaround time (TAT) of liquid versus tissue biopsy NGS. (A) TAT of all samples (N = 170). Liquid biopsy NGS had a significantly faster TAT than tissue biopsy ($P < .0001$, 2-tailed unpaired student *t*-test). (B) Adjusted TAT for samples excluding patients with order dates >6 m between liquid and tissue (N = 165). Liquid biopsy NGS had a significantly faster TAT than tissue biopsy ($P < .0001$, 2-tailed unpaired student *t*-test)





N=170	Liquid	Tissue
TAT Mean	9.6	36.4
TAT Median	9	29
Samples QNS	0	18 (10.6%)

N=165	Liquid	Tissue
TAT Mean	9.6	30.5
TAT Median	9	28
Samples QNS	0	18 (10.6%)

ficity, positive predictive value, negative predictive value, and concordance were calculated.

For biomarker-positive patients, a testing modality assay was conducted to determine the proportion of patients who would have been detected by initiating genomic profiling with tissue versus liquid biopsy sequencing. Methods are similar to those from prior publications.[12,17] "Liquid-first detected" denotes the proportion of patients who had a genomic alteration identified with liquid biopsy – either alone or in addition to tissue biopsy NGS. "Liquid first incremental add" refers to the proportion of biomarker-positive patients who would have not been identified without complementary tissue NGS. In contrast, "Tissue-first detected" refers to biomarker-positive patients identified with tissue NGS and "Tissue-first incremental add" refers to biomarker-positive patients rescued via liquid NGS. Outcomes for progression free survival (PFS) and overall survival (OS) were conducted via Kaplan-Meier survival curves. PFS was defined as the time between patient treatment initiation and date of progression-if patient had progressed, or date of last analysis (12/9/21). OS was defined as the time between patient treatment initiation and date of death-if patient had died, or date of last analysis (12/9/21). To address bias in the therapy initiation date, an additional sensitivity analysis was performed for PFS and OS using cohorts of patients with >2 years and >3 years of follow-up. Descriptive statistics for outcomes were conducted using a Log-rank (Mantel-Cox) test.

Results

Patient Characteristics

Based on the inclusion criteria, 170 patients were eligible for this study. The demographics are presented in Table 1. Of the patients tested, 82 were male (48.2%) and 88 were female (51.8%). The average age was 66 years old (range 26-94). The majority of patients were Non-Hispanic White (54.7%) followed by Hispanics (30.0%), African American (8.8%), Asian (5.9%), and Pacific Islander/Native Hawaiian (0.6%). The majority of patients were former smokers (62.9%) followed by never smokers (30.6%) and current smokers (6.5%). The majority of patients had the histology of adenocarcinoma (93.5%). Most patients, 72.9%, were treated in the first line setting, 12.4% were treated in second line and beyond, and 14.7% of patients did not have treatment line listed. In the following analyses, some patients were excluded based on unavailable data in the EMR. For visibility, these exclusions are outlined in Figure 1.

Liquid Biopsy Demonstrated a Higher Rate of Test Reporting and a Significantly Faster TAT Than Tissue Biopsy NGS

Each patient had concurrent NGS performed from a tissue and a liquid biopsy sample. Between the 2 biopsies, liquid biopsy had a higher frequency of successfully reported results (assay success rate) as compared to tissue biopsy. All liquid biopsy NGS samples were sufficient and successfully reported with an assay success rate

of 100%. In contrast, 18 (10.8%) of the tissue samples were not sufficient for NGS, that is, quality not sufficient (QNS), with a tissue assay success rate of 89.2%. For these 18 patients, diagnostic decisions were based exclusively on liquid biopsy (Figure 2A, "sample QNS").

Next, the TAT was calculated for both liquid and tissue NGS (Figure 2A). Liquid biopsy had a significantly faster TAT than tissue, reporting results on average 26.8 days earlier than tissue ($P < .0001$; 2-tailed, unpaired, student *t*-test). The average liquid biopsy TAT was 9.6 days with a range of 5 to 28 days versus a tissue average of 36.4 days with a range of 2 to 399 days. To best evaluate the clinical impact of genetic testing efficiency on treatment decisions, a TAT sub analysis was conducted on samples from patients who received tissue and liquid sequencing results within a 6-month period to reduce bias from noncontemporaneous samples as described in the methods (N = 165). In this sub analysis, patient exclusion did not affect the mean or median TAT for liquid biopsy NGS, however, tissue TAT was reduced to a mean of 30.5 days, a median of 28 days, and a range of 2 to 111 days (Figure 2B). Even by preserving these contemporaneous samples, liquid TAT was on average faster than tissue by 20.9 days ($P < .0001$; 2-tailed, unpaired, student *t*-test).

Clinicians Based Treatment Decisions on Liquid Biopsy Results More Often Than Tissue, Primarily Due to Faster TATs

To assess the clinical utility of liquid versus tissue biopsy, treatment decisions were assessed. The schema for treatment decision was described above in the methods for the 165 patients who were included with contemporaneous sequencing tests (Figure 3). To begin, it was determined if NGS results were effectively reported by 1 or both tests. There were 3 patients who began treatment prior to the return of their respective NGS results because they had rapidly progressing disease. For the remaining 162 patients, if an actionable alteration was detected, the clinician chose the test which returned results earlier. For 1 sample, the tissue and liquid biopsy results reported on the same day. If an actionable alteration was not detected, the clinician chose the test which returned results sooner.

Based on these factors, clinicians defined their treatment decision on liquid biopsy NGS in 73.5% of patients, while treatments were based on tissue biopsy in 25.9% of patients (N = 162) (Figure 4A). One patient had liquid and tissue results reported on the same day and was treated based on both tests (0.6%). As described above, the 3 patients who began treatment prior to biopsy results returned were excluded from this analysis. The majority of treatments chosen based on liquid biopsy were due to faster reporting of liquid versus tissue NGS. For most patients, liquid and tissue biopsies were not ordered on the same day. Thus, to evaluate for a potential bias resulting from order sequence (ie, a bias that liquid was consistently ordered before tissue), the dates between orders were compared between liquid and tissue biopsies (Figure 4B). Four patients did not have a tissue order date defined and were excluded from this analysis. In the remaining cohort (N = 158), tissue was consistently ordered earlier than liquid biopsy NGS in 70.3% of patients and ordered on the same day as liquid biopsy NGS in 9.9% of patients.

Table 2 Comparison of Liquid Versus Tissue Biopsy NGS Results for Guideline-Recommended Biomarkers in mNSCLC With FDA-Approved Therapies That Were Identified in Patients in This Study

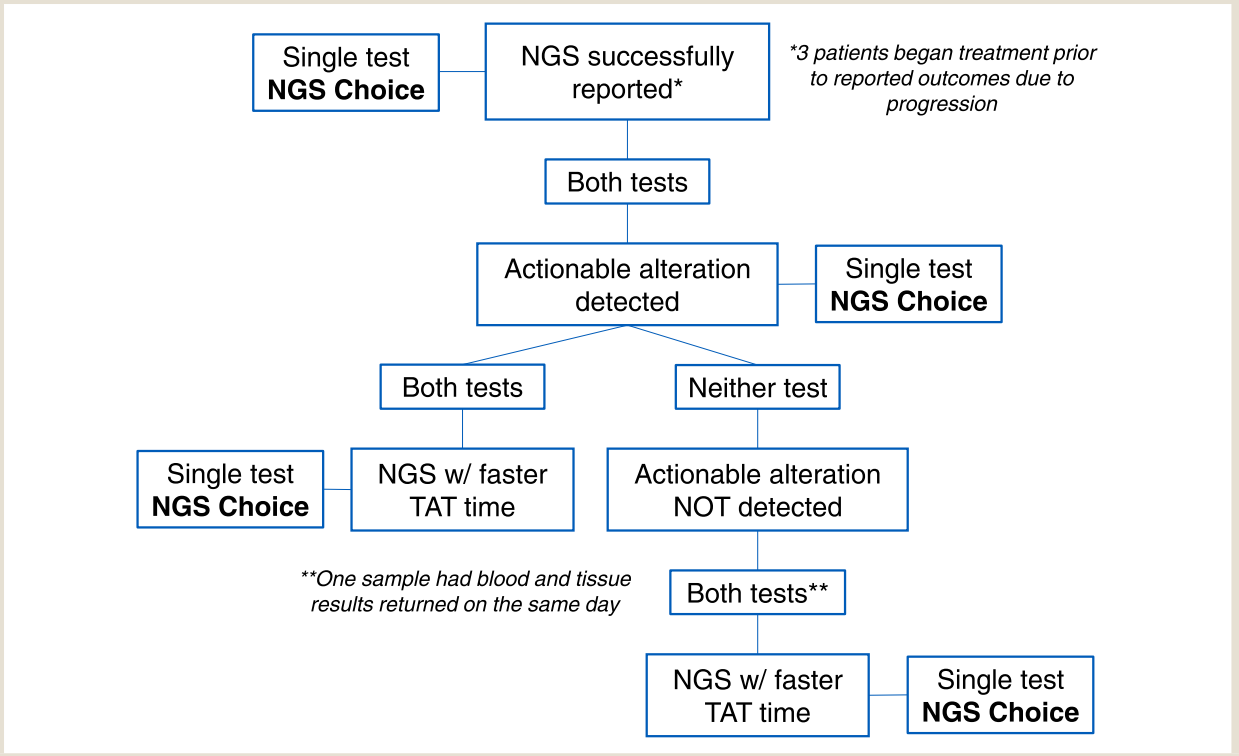
EGFR		Tissue+	Tissue-	Sensitivity	66.7%
	Liquid +	14	18	Specificity	86.4%
	Liquid -	7	114	PPV	43.8%
	Total	21	132	NPV	94.2%
				Concordance	94.8%
BRAF		Tissue+	Tissue-	Sensitivity	0.0%
	Liquid +	0	2	Specificity	98.7%
	Liquid -	2	149	PPV	0.0%
	Total	2	151	NPV	98.7%
				Concordance	98.7%
ALK		Tissue+	Tissue-	Sensitivity	NA
	Liquid +	0	2	Specificity	98.7%
	Liquid -	1	150	PPV	0.0%
	Total	1	152	NPV	99.3%
				Concordance	99.3%
MET		Tissue+	Tissue-	Sensitivity	50.0%
	Liquid +	1	1	Specificity	99.3%
	Liquid -	1	150	PPV	50.0%
	Total	2	151	NPV	99.3%
				Concordance	99.3%
NTRK		Tissue+	Tissue-	Sensitivity	0.0%
	Liquid +	0	0	Specificity	100.0%
	Liquid -	1	152	PPV	NA
	Total	1	152	NPV	99.3%
				Concordance	99.3%
ROS1		Tissue+	Tissue-	Sensitivity	100.0%
	Liquid +	1	0	Specificity	100.0%
	Liquid -	0	152	PPV	100.0%
	Total	1	152	NPV	100.0%
				Concordance	100.0%

Thus, a trend toward earlier ordering of liquid biopsy did not bias the result of clinicians choosing liquid biopsy to define treatment. Likely, the faster TAT, despite ordering sequence, led to utility of liquid biopsy in guiding treatment decisions.

As described, treatment decisions were influenced by the identification of an actionable alteration defined from sequencing. In this cohort, 57 (35.2%) patients were prescribed a targeted therapy, 103 (63.6%) patients were prescribed a nontargeted therapy (chemotherapy or immune checkpoint inhibitor therapy), and 2 patients were prescribed no treatment. The genomic alterations were available in the EMR for 153 patients in the adjusted cohort (N = 165). Of these patients, 51 had an actionable alteration detected with either liquid biopsy, tissue biopsy, or both biopsy sequencing assays. The actionable alterations identified involved *EGFR* (76%), *BRAF* (8%) *ALK* (6%), *MET* (6%) *NTRK* (2%), and *ROS1* (2%). Concordance between liquid and tissue NGS ranged between 94.8% and 100% (Table 2).

The TtT was compared between patients prescribed therapies based on tissue versus liquid biopsy NGS (Figure 5). The TtT was

Figure 3 Schema describing clinician choice in using liquid biopsy or tissue biopsy NGS to make treatment decision. This is based on patients receiving both liquid and tissue biopsy NGS as SOC prior to therapy selection



calculated by subtracting the biopsy report date from the treatment start date in the adjusted cohort. As described, 3 patients started therapy prior to results returned and were excluded from this analysis. Additionally, 18 patients did not have the treatment start date listed and were excluded from this analysis. Two other patients had clinical notes detailing that the patient had switched to a secondary clinic prior to treatment start date and were also excluded from the analysis (N = 142). There was no significant difference in TtT between patients prescribed therapy based on tissue versus liquid biopsy NGS (23.7 days vs. 17.8 days) ($P = .4410$; 2-tailed, unpaired student *t*-test). Of note, TtT is dependent on multiple patient variables that were not controlled for, for example, if the patient wanted a second opinion, the patient got sick and was admitted to the hospital, or the patient was lost to follow up. Nonetheless, the data from our study align with previous literature.[12,16]

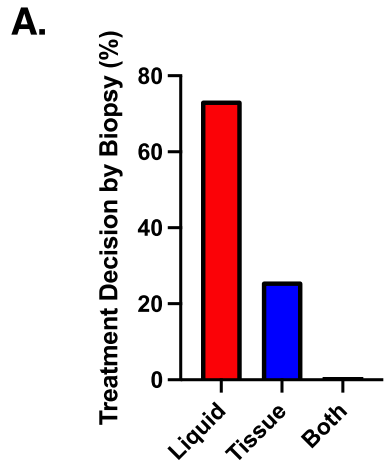
Liquid Reflexed to Tissue Biopsy Captures a Greater Number of Actionable Alterations Than Tissue Reflexed to Liquid Biopsy

While tissue and liquid biopsies are highly concordant and complementary, we aimed to understand how reflexing from 1 modality to the next would improve actionable alteration identification. For this, testing modalities were similar to a previous study for patients with identified actionable alterations (N = 51) (Figure 6).[12] To clarify, the modality of a liquid-first paradigm (ie, proportion of patients identified by either liquid biopsy only or liquid and tissue biopsy and then rescued by tissue biopsy), was compared to a tissue-first paradigm (ie, proportion of patients identified by either tissue biopsy only or tissue and liquid biopsy and then rescued by liquid biopsy). In this study, the use of a liquid-first paradigm would have identified actionable genomic alterations in 76.5% of patients with reflex to tissue biopsy identifying an additional 23.5%. In contrast, a tissue-first paradigm would have identified actionable genomic alterations in 54.9% of patients with liquid biopsy identifying an additional 45.1%. Thus, in this analysis, liquid and tissue biopsies were shown to complement one another in capturing a larger proportion of actionable genomic alterations than either test alone. However, a liquid-first paradigm captured more actionable alterations as a first-line testing modality than tissue-first.

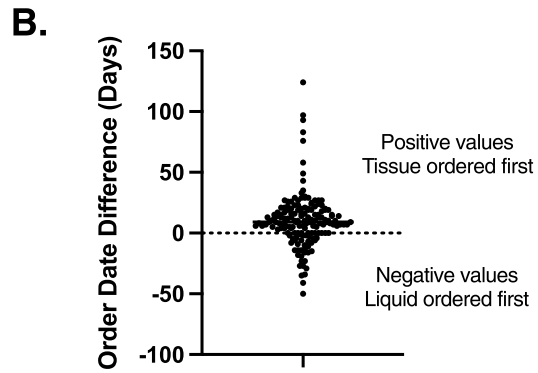
Treatment Decisions Based on Liquid Versus Tissue Biopsies Have Similar Outcomes

Finally, to demonstrate the noninferiority of liquid biopsy NGS to tissue, PFS and OS were compared between liquid and tissue biopsies (Figure 7). For patients with available information, PFS was calculated as start date of treatment subtracted from the date of progression or the last date of analysis, depending on if the patient had progressed (Figure 7A) (N = 135). OS was calculated as the start date of treatment subtracted from the date of death or the last date of analysis, depending on if patient was still alive (Figure 7D) (N = 129). In this cohort, there were no significant differences in

Figure 4 Frequency of treatment decision based on liquid versus tissue biopsy NGS. (A) Clinicians in this study based treatment decisions on liquid biopsy results more frequently than tissue. One patient had liquid and tissue results returned on the same day and was treated using both tests ("Both"). (B) The time between liquid and tissue ordering was plotted to assess for bias between test ordering dates. Data points inform the following: >0 that tissue was ordered prior to liquid biopsy, =0 tests were ordered on the same day, <0 liquid was ordered prior to tissue. Most patients had tissue ordered prior to liquid biopsy NGS



	Liquid Guided	Tissue Guided	Both
Patients	119	42	1



PFS or OS in patients treated based on liquid or tissue biopsy NGS results (Mantel-Cox PFS $P = .9653$, OS $P = .8322$).

Though most patients received at least 2 years of follow up, there were a subset of patients with less than 2 years of follow up time (N = 27 for PFS and N = 31 for OS). To address any bias for patients with reduced follow up time (<2 years), a sensitivity analysis was performed for PFS and OS by focusing the sample population to patients with either a >2 year (Figure 7B PFS, 7e OS) or >3 year (Figure 7C PFS, 7f OS) follow up time. Each of these cohorts demonstrated no difference in survival outcome for patients treated based on liquid versus tissue biopsy (>2 year cohort: Mantel-Cox PFS $P = .4662$, OS $P = .3562$; >3 year cohort: Mantel-Cox PFS $P = .5368$, OS $P = .3360$). Overall, this supports the conclusion that patients experience similar outcomes when their treatment decision is based on either a liquid or tissue biopsy.

Discussion

In patients with stage IV NSCLC, NGS performed on liquid biopsies has the potential to be incorporated as standard of care to inform front-line therapeutic decisions. The median TAT in our study, was significantly faster with liquid NGS compared to tissue NGS (9.6 days vs. 36.4 days ($P < .0001$). These findings are similar to the NILE trial that demonstrated noninferiority of liquid biopsies

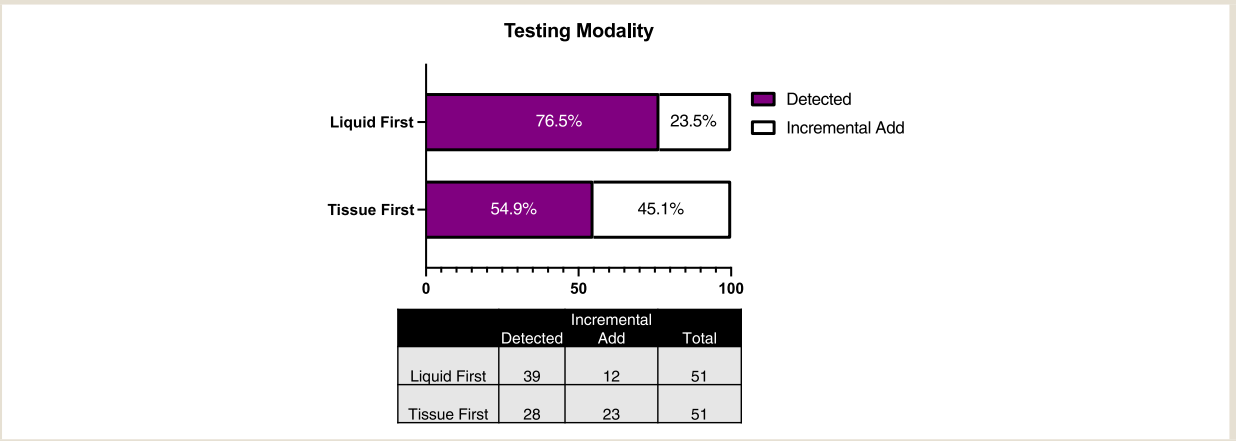
compared to tissue, and concluded that liquid NGS median TAT was significantly faster than tissue (9 vs. 15 days; $P < .0001$).[12] Our study is a "real world study" that validates the results from the NILE trial which concluded that liquid biopsy identifies guideline-recommended biomarkers at a similar rate as standard of care tissue genotyping with a faster TAT and higher frequency of complete assessment of all guideline-recommended biomarkers.[12] In addition, liquid biopsy NGS reported an assay success rate of 100% versus 10.8% of tissue biopsy NGS who were not reported because the tissue quality was not sufficient for testing. These data are similar to previously reported literature estimating that 10% to 20% of tissue biopsy samples are not evaluable due to QNS.[16,22-24]

Due to TAT and effective reporting, liquid biopsies were used in this study to make approximately 73% of the treatment decisions in patients with mNSCLC, the majority of whom were newly diagnosed and treated in the first line setting. Though TtT was not statistically different between liquid and tissue, there was a trend favoring TtT, similar to previous publications.[12,16] The faster TAT contributed to the success of liquid biopsies because oncologists were able to get meaningful results in less than 10 days rather than waiting more than 3 weeks for tissue NGS results.[12,13] Indeed, one major limitation to tissue NGS that our center has experienced is the length of time it takes to obtain tissue blocks from outside hospitals, as most of our patients come to our center from community referrals. Clinically, faster and more consistent TAT are helpful in reducing patient anxiety and easing the logistics of follow-up scheduling.

Though liquid biopsy is sometimes considered as a complement assay to identify actionable biomarkers, research from the NILE demonstrated concordance rates between 98.2% and 100% in targetable alterations.[12] Similarly, our research demonstrated high concordance between liquid and tissue in identifying actionable alterations. Analysis of testing modalities demonstrated that a liquid-first approach identified actionable alterations in 21.6% more patients than a tissue-first approach. Additionally, liquid biopsy rescued tissue biopsy detection in 45.1% of patients. Our results as well as the literature demonstrate that combined modalities capture the greatest proportion of actionable alterations. Altogether, these data support a tiered approach with liquid first as a preferred method to detect higher proportions of actionable alterations with a fast TAT.[12,16,25,26] This is especially pertinent, as many US payers are unwilling to extend coverage for 2 NGS tests at time of evaluation. To consider a reflex to tissue-based testing modality when liquid biopsy is negative is not only evidence supported, but also a potentially useful strategy to preserve tissue for clinical trial enrollments and alternative biomarker studies such as PD-L1. Best practice guidelines are including liquid biopsy NGS into more opportunities within testing in NSCLC. For example, the recent nternational Association for the Study of Lung Cancer consensus states that in patients with oncogene-addicted NSCLC, liquid biopsy is emerging as not only complementary to tissue-based analysis but also acceptable as the initial approach ("plasma first") for biomarker evaluation at the time of diagnosis and for monitoring the efficacy of targeted therapies.[7]

Limitations of this study included those expected in a retrospective study (however, this is also a strength because this a community-based "real world" study), such as obtaining records of patients who had a tissue biopsy performed at an outside facility and some patients having expired prior to the completion of the diagnostic workup. Also, if we were to correct for tumor burden, the success of liquid biopsy would most likely increase because we did not consider this to evaluate the accuracy of the liquid biopsy. A prospective study with a larger sample population is needed to assess the clinical outcomes between patients whose treatment decisions were based on liquid versus tissue biopsy as well as to validate these findings. Nevertheless, this study presents compelling evidence that liquid biopsy should be considered as an initial approach to therapy decision making in NSCLC because of its fast TAT, high concordance to tissue, and noninferiority in TtT and treatment outcomes.

Figure 6 **Frequency of guideline-recommended biomarkers detected by testing modality. In this cohort, leading with liquid testing, 76.5% of patients with a guideline-recommended biomarker would have been detected with 23.5% of patients identified on reflex tissue testing. If tissue biopsy was the first genomic testing modality, substantially less patients would have been identified**



	Detected	Incremental Add	Total
Liquid First	39	12	51
Tissue First	28	23	51

Clinical Practice Points

- DNA NGS done with liquid biopsies have shown already to be as effective as tissue NGS in diagnosing genetic aberrations; however, traditionally because they arrived later liquid biopsies are being used as a complement to tissue biopsies when there is not enough tissue.
- This publication is not only corroborating that fact but showing that there might be a utility in using liquid biopsies first based on a shorter time of reporting.
- We have also shown here that treatment decisions based on liquid biopsies do not affect clinical outcomes.

Disclosure

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Figure 7 **Kaplan-Meier survival outcomes between patients treated based on liquid versus tissue biopsy NGS results. (A) Median PFS for liquid biopsy was 43 months and 46 months for tissue biopsy (N = 135) (n.s., *P* = .9847; HR, 0.9886; 95% 0.5679-1.597; median follow-up time 90 months. (B and C) Sensitivity analyses for PFS of patients with at least 2 years (B) and at least 3 years (C) of follow up data. (D) Median OS for liquid biopsy was not reached and 82 months for tissue biopsy (N = 129) (n.s., *P* = .8322; HR, 0.9350; 95% CI, 0.5020-1.741; median follow-up time 87 months). (E and F) Sensitivity analyses for OS of patients with at least 2 years (E) and at least 3 years (F) of follow up data**

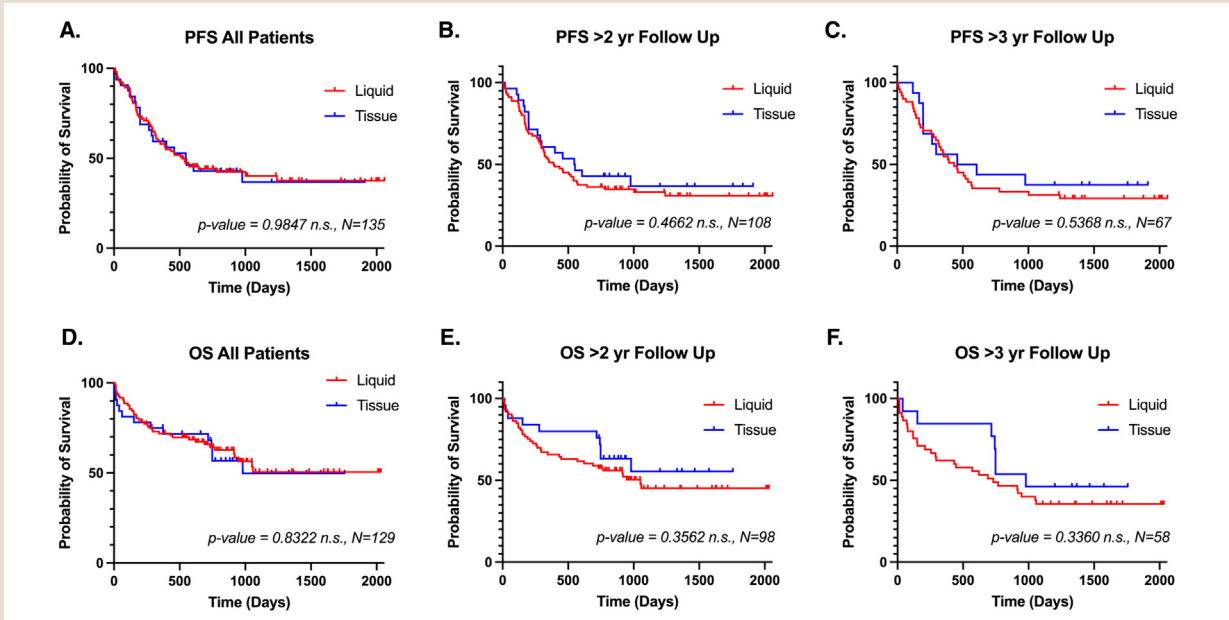
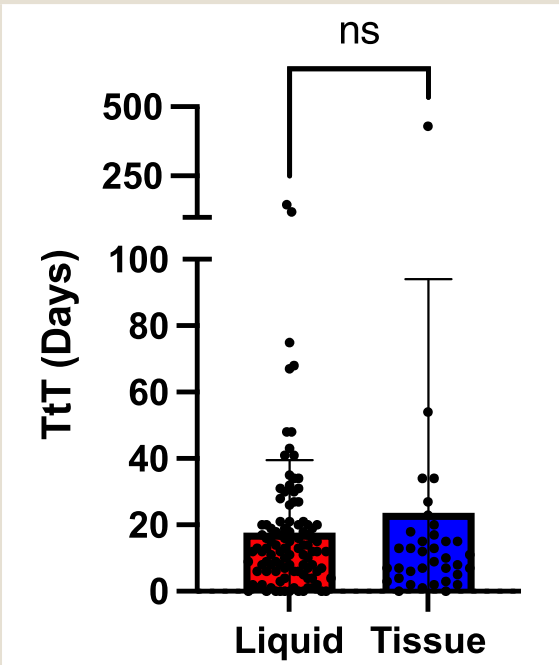


Figure 5 Time-to-treatment (TtT) for patients treated with results from liquid biopsy versus tissue biopsy (N = 142). No significant differences between TtT in patients treated based on liquid versus tissue biopsy ($P = .4410$, 2-tailed unpaired student *t*-test)



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