Case Report

Brief Report: Discordance Between Liquid and Tissue Biopsy-Based Next-Generation Sequencing in Lung Adenocarcinoma at Disease Progression

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Clinical Practice Points

- Liquid biopsy and next-generation sequencing of circulating tumor DNA is increasingly used at the time of diagnosis of non-small cell lung cancer. Its role at the time of progression—and whether it will complement or even supplant tissue biopsy-based methods of identifying targetable resistance mutations in lung adenocarcinoma—remains unclear.
- In this brief report, we demonstrate using 138 paired tests that tissue-liquid concordance rates at diagnosis are similar to previously published findings (~80%), followed by steep decline in concordance at the time of progression (~40%).
- These findings suggest ctDNA or tissue biopsy alone is therefore likely to be insufficient to identify resistance mutations at progression in the majority of patients. We encourage practitioners to pursue both methods of testing to maximize the likelihood of identifying a targetable mutation.

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Introduction

Molecularly targeted therapies have dramatically altered the landscape of lung adenocarcinoma treatment.[1] Panel-based next-generation sequencing (NGS) to detect targetable somatic genetic events, including point mutations, indels, and fusion events, is guideline-recommended initial diagnostic testing in lung adenocarcinoma.[2]

Though tumor biopsy with tissue-based NGS is guideline-recommended, it has many limitations: expense and adverse events associated with tissue acquisition, turnaround time, and sampling error due to intratumoral heterogeneity or circulating tumor subclones.[1,3] Blood-based liquid biopsy utilizing circulating tumor DNA (ctDNA) as NGS substrate has emerged as an important tool in NSCLC management. Liquid biopsy appears useful and cost-effective as a component of initial lung adenocarcinoma evaluation.[4-6] It is also a useful adjunct in monitoring longitudinal response to therapy or as an early predictor of progression.[7] Intratumoral heterogeneity in terms of acquired resistance to targeted therapy, ctDNA shedding, reimbursement challenges, and uncertainty in concordance between liquid and tissue-based NGS at tumor progression through targeted therapy have all limited liquid biopsy's uptake.[1] In particular, for patients with oncogene-driven cancers who receive targeted therapy, whether tissue and liquid biopsy-based NGS are equally capable of identifying at disease progression the acquired resistance mechanisms resulting from branching tumoral evolution is unknown. Further, given intratumoral heterogeneity in response to targeted therapy, whether tissue and liquid biopsy-based NGS results are concordant in this setting is an unanswered, clinically relevant question.

In this Brief Report, we evaluated a large, single-center database of concurrent liquid and tissue biopsies in a patient population enriched for targetable mutations to assess real-world concordance at time of diagnosis and progression.

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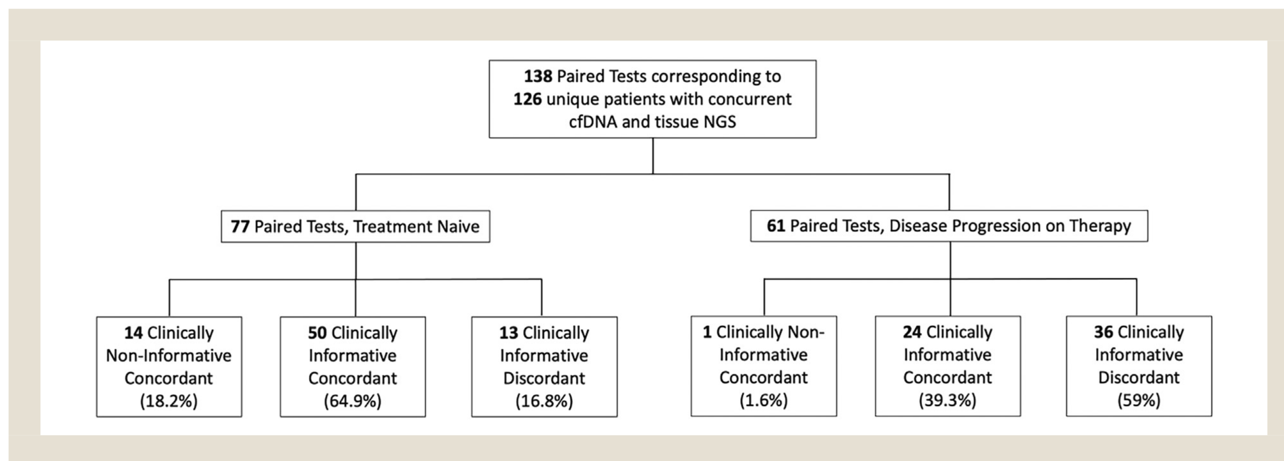
Figure 1 Flow chart summary of concurrent ctDNA- and tissue-based NGS test results. Clinical relevance and concurrence are as defined in the methods. Among paired tests with at least one clinically informative result (n = 123), concordance rate at diagnosis was 79.4% (n = 50/63), compared to 40.0% (n = 24/60) at disease progression ($P < 0.001$). ctDNA = circulating tumor DNA.



Materials and Methods

Patients and Test Identification

This single-center retrospective study was conducted at the University of Chicago Medicine (UCM), with approval by the local Institutional Review Board (IRB20-0162). We retrospectively identified patients with histologically confirmed lung adenocarcinoma who had both ctDNA- and tissue-based NGS performed within 24 weeks of one another without therapeutic change in between (hereafter referred to as *concurrent pairs*) between April 2016 and September 2020. Each individual test could only be a part of one concurrent pair. If a given test was performed within 24 weeks of multiple others, the nearest complementary test was used as the other member of the pair.

Tissue testing was performed using in-house UCM-OncoPlus (University of Chicago Medicine; Chicago, IL). UCM-OncoPlus reports 155 genes for clinical use and 1,057 additional genes for research purposes, and has > 99% sensitivity in identifying variants within the reported territory.[8] Tissue analysis from August 2020 forward also included assessment by the University of Chicago OncoPlus RNA Fusion panel, a large-scale RNA capture NGS assay validated for detection of fusions in *NTRK1, NTRK2* and *NTRK3*. Patients were excluded if other tissue-based NGS platforms had been used (Figure 1). Liquid biopsy was performed using Guardant360 CDx (Guardant Health; Redwood City, CA), which reported variants in 55 genes for the entirety of the study period. Genes included in the UCM-OncoPlus test are summarized in Supplemental Table 1.

Definitions of Clinical Relevance and Concordance

Variants of either ctDNA analysis or tumor tissue NGS were deemed *clinically actionable* if they guided systemic therapy decisions, or informed prognosis and risk of histologic transformation. These clinically actionable variants included: 1) targetable single-nucleotide variants, indels, or fusion events in known driver genes; 2) mutations that may have prognostic importance with respect to ongoing clinical trials; and 3) variants portending transformation to a different histology as a resistance mechanism to targeted therapy. Clinically actionable mutations included: any sensitizing *EGFR* point mutation, *ALK* fusion event, *RET* fusion event, *NTRK* fusion events, *MET* fusion event, *MET* exon 14 skipping mutation, *MET* amplification, *BRAF* mutation, *KRAS* G12C, *ROS1* fusion event, *ERBB2* amplification, *TP53* single nucleotide variant in the context of targetable *EGFR* mutation,[9] and *RB1* loss or inactivating mutation in the context of targetable *EGFR* mutation.[10] All listed anomalies, with the exception of *NTRK* fusion (covered by the RNA Fusion assay instead), are detected by the UCM-OncoPlus assay.

For each individual test, we assessed whether it contained a clinically actionable gene mutation. Tests with at least one clinically actionable result were deemed *clinically informative.* Those without were deemed *clinically uninformative*. If both tests in the pair generated clinically informative results leading to the same clinical action, this was defined as *clinically informative concordance*. If one test identified a clinically informative result and the other failed to do the same, leading to a difference in clinical decision, this was deemed *clinically informative discordance*. If neither test identified a clinically informative result, the pair was defined as *clinically uninformative concordance*.

Statistical Analysis

We generated descriptive statistics of the patient population and test characteristics. Concordance rates were calculated for the paired tests with at least one clinically informative result. Comparison of concordance rates between samples obtained at diagnosis and upon disease progression were performed using a generalized estimating equations approach and logit link to account for correlation among multiple tests within the same patient.[11] Among clinically informative discordant pairs, we assessed each methodology's failure to detect rate.

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Table 1 Patient and Next-Generation Sequencing Test Characteristics of the Study Population

Age at diagnosis	
Median (year)	66.2
Range (year)	30-83
Gender	*No. (%)*
Male	49 (38.9%)
Female	77 (61.1%)
Median ECOG performance status	
At initial diagnosis	1
At time of progression	1
Smoking status	*No. (%)*
Never smoker	51 (40.5%)
Former smoker	70 (55.6%)
Current smoker	4 (3.2%)
No answer	1 (0.8%)
Ethnicity	*No. (%)*
Caucasian	76 (60.3%)
African American	38 (30.1%)
Asian	7 (5.6%)
Latinx	3 (2.4%)
Multiracial	1 (0.8%)
No answer	1 (0.8%)
Most common treatment prior to progression	*No. (%)*
Any tyrosine kinase inhibitor	42 (31.8%)
Osimertinib	22 (16.7%)
Erlotinib	8 (6.1%)
Afatinib	5 (3.8%)
Alectinib	2 (1.5%)
Crizotinib	1 (0.8%)
Selpercatinib	1 (0.8%)
Tepotinib	1 (0.8%)
TKI combination	2 (1.5%)
Chemotherapy	5 (3.8%)
Immunotherapy	3 (2.8%)
Chemotherapy and TKI	2 (1.5%)
Chemotherapy and immunotherapy	1 (0.8%)
Untreated	77 (58%)
Unknown treatment history	2 (1.5%)

Characteristics were recorded at the time of sampling for each pair of tests; multiple samples were included multiple times.
Abbreviation: TKI = Tyrosine Kinase Inhibitor

Results

Patient Population and Test Characteristics

We identified 126 unique patients with 138 paired tests, 77 at initial diagnosis and 61 at the time of disease progression. Characteristics of the patient population are summarized in Table 1. Patients were predominantly Caucasian female, never- or former-smokers with good performance status (median ECOG 1). The most common therapies immediately preceding progression were osimertinib (n = 22, 15.9%), erlotinib (n = 8, 5.8%), afatinib (n = 5, 3.6%), and chemotherapy (n = 5, 3.6%) (Table 1).

Test Concordance at Diagnosis and Progression

Among all paired tests, the concordance rate among the treatment-naïve group was 83.1% (n = 64/77) and 41.0% (n = 25/61) at disease progression ($P < .001$). Among paired tests in treatment-naïve patients demonstrating at least one clinically informative finding, concordance rate was 79.4% (n = 50/63 pairs). Among the 60 paired tests collected at disease progression that demonstrated at least one clinically informative finding, the concordance rate was 40.0% (n = 24/60; $P < 0.001$, compared to concordance rate at diagnosis) (Figure 1). Of the combined set of 49 clinically informative discordant results, the relevant mutation was identified by liquid biopsy only in 13 cases (26.5%) and by tissue biopsy only in 34 cases (69.4%). The most common clinically informative genetic events identified on liquid biopsy but not on tissue included *EGFR* T790M mutations (n = 8). Tissue biopsy identified *EGFR* exon 19 deletion (n = 5) and *EGFR* missense mutations (n = 3) that were not identified by liquid biopsy (Table 2).

Test Concordance in the Osimertinib-Refractory Population

Sixteen paired tests were obtained at disease progression through osimertinib. Six (37.5%) provided clinically informative concordant results while 10 (62.5%) provided clinically informative discordant results. Among the clinically informative discordant results, liquid biopsy identified 7 actionable mutations while tissue biopsy identified 3. In 2 cases, tissue and liquid biopsies identified two different targetable mutations *(EGFR* T790M on liquid biopsy and *ROS1* fusion and *K1F5B-RET* fusion on tissue). Common clinically informative genetic events missed by tissue biopsy upon disease progression through osimertinib included *EGFR* T790M (n = 6) and *CCDC6-RET* fusion (n = 1). The clinically informative mutations missed on liquid biopsy were *GOPC-ROS1* fusion (n = 1), *RB1 loss* (n = 1), and *K1F5B-RET* fusion (n = 1).

Discussion

In this report we demonstrate a liquid-tissue biopsy-based NGS concordance rate of 79.4% at initial diagnosis in treatment-naïve patients with clinically actional findings that declined precipitously, to 40.0%, following disease progression through first-line therapy. In particular, tissue-liquid biopsy NGS concordance was 37.5% in the subpopulation of patients with lung adenocarcinoma who had recently progressed through osimertinib. Taken together, these findings suggest that in patients with lung adenocarcinoma whose disease has progressed through recent treatment, including targeted therapy, ctDNA or tissue biopsy alone is unlikely to be sufficient to identify all possible resistance mutations. These results are consistent with a model in which rising intratumoral heterogeneity in response to systemic therapy increase the potential for sampling error. Paired liquid and tissue-based biopsy at progression may represent a more comprehensive strategy to prolong time to cytotoxic chemotherapy, especially after consideration of turnaround time, procedure safety, and heterogeneity in tissue sampling.[1]

Concordance rates at the time of initial diagnosis in our cohort are similar to previously published prospective studies of tissue and liquid biopsy NGS in lung adenocarcinoma.[4] One advantage of our study compared to the previously published literature is the inclu-

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Table 2 Summary of Discordant Variants Between ctDNA and Tissue-Based NGS Tests

	Mutations Detected on Tissue Only	Mutations Detected on G360 Only
EGFR exon 19 deletion	5	
EGFR missense	3	
ALK Fusion	2	
RB1 loss	4	
EGFR T790M	4	8
BRAF V600E	3	
TP53	4	1
KRAS mutation	2	1
MET amplification	2	1
MET fusion	2	
KIF5B-RET fusion	1	
ROS1 fusion	2	1
CCDC6-RET fusion		1
Total	*34*	*13*

Abbreviations: ctDNA = circulating tumor DNA; NGS = next-generation sequencing.

sion of fusion events, *KRAS*^{G12C}, and prognostic variants among the set of clinically informative results. Despite the inclusion of this broader set of variants and the evolution of ctDNA-based NGS technologies over time, the above events are not common enough to meaningfully alter the conclusions of prior studies. Together, our data suggest ctDNA-based NGS to be a useful adjunct at both diagnosis and progression, independent of the results of initial tissue-based testing. One notable strength of our study is utilization of a patient population enriched for targetable mutations, offering a more realistic and perhaps sobering assessment of tissue-liquid concordance at progression.

There are limitations to this study. It is a retrospective study at a single, urban academic medical center. Prospective validation in a more heterogeneous patient population or meta-analysis with aggregate datasets may be useful. Paired tests occurred within 24 weeks of each other and without an intervening change in therapy. While this time window was based on prior literature, we acknowledge this is a wide window and ideally this testing would have occurred simultaneously. Only a single platform (Guardant360) was included, and it is unclear if these results are generalizable to all ctDNA testing. Additionally, liquid biopsy tests have historically performed poorly in detecting fusions, which may have limited liquid biopsy sensitivity. Additionally, our analysis centered on variants that are clinically actionable now, at the present time. Both the threshold variant allele frequencies and variants themselves can change over time, thus becoming more (or less) actionable. Therefore, an update to this study will be needed in the future.

Disclosures

Dr. Strohbehn is an employee of the US Federal Government; the contents of this article do not reflect the official position of the US Federal Government, serves in an uncompensated Board of Directors position for the Optimal Cancer Care Alliance (Ann Arbor, MI), reports a consulting role for VIVIO Health, and research funding from Merck. Dr. Rouhani reports grant support from the NIH T32 grant and travel from Turning Point Therapeutics. Dr. Shergill reports advisory board participation from regeneron/Sanofi and Catalyst Pharmaceuticals, as well as research support from Hutchison MediPharma, Merck, INHIBRx, VERASTEM, Turning Point Therapeutics, and Pfizer. Dr. Garassino reports receiving grants from AstraZeneca and Merck and personal fees from AstraZeneca, Merck, Roche, Bristol-Myers Squibb, Celgene, Boehringer Ingelheim, Novartis, GlaxoSmithKline, Pfizer, Eli Lilly, and Takeda. Dr. Vokes reports consulting fees from AstraZeneca, Beigene, BioNTech, Eli Lilly, EMD Serono, GeneCentric, Genentech, GlaxoSmithKline, Novartis, and stock/stock options from McKesson and Coordination Pharmaceuticals. Dr. Bestvina reports consultant/advisor/speaker fees from AstraZeneca, Bristol-Myers Squibb, CVS, Genentech, Jazz, Johnson & Johnson, Merck, Novartis, Pfizer, Seattle Genetics, and Takeda; and has received funds for research support from AstraZeneca and Bristol-Myers Squibb. All other authors report no disclosures.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.cllc.2023.01.003.

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