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# Interventional pulmonology use of cell-free DNA assay for metastatic non–small cell lung cancer: the UC Davis experience

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***Abstract***

**Background:** Interventional pulmonologists (IPs) are often the first specialist to see patients with suspected metastatic non–small cell lung cancer (mNSCLC). Consequently, they are potentially ideally positioned to expedite the identification of actionable molecular mutations by ordering blood-based cell-free DNA (cfDNA), prior to or upon tissue diagnosis of mNSCLC.

**Methods:** Retrospective review of cfDNA ordered by IP as part of a routine clinical practice. Patients were categorized into two groups based on when cfDNA was ordered by IP: (1) IP suspected mNSCLC prior to histologic confirmation or (2) IP diagnosed mNSCLC based on histologic confirmation of NSCLC.

**Results:** Twenty patients were identified. Twelve of 13 in group 1 were confirmed to have mNSCLC by oncology and 1 had stage IIIA. Seven of 7 in group 2 were confirmed to have mNSCLC by oncology. Fifteen of 20 also had next-generation tissue molecular testing. Thirteen of 20 (65%) had targetable alterations. Seven of 13 (54%) were identified on cfDNA and tissue, 5/13 (38%) on cfDNA only, and 1/13 (8%) on tissue alone. Tissue results were available a medium of 24 days after, and cfDNA results a medium of 4 days prior to, the patients' first oncology visit.

**Conclusions:** IP appears to be able identify patients who have mNSCLC and for whom testing for molecular mutations is appropriate even prior to tissue confirmation of NSCLC. A strategy whereby IP employ blood-based cfDNA testing in suspected and tissue confirmed mNSCLC could potentially provide medical oncologists with more timely information on actionable mutations than tissue-based testing first, potentially expediting patient treatment.

***Keywords:*** actionable molecular mutations, cell-free DNA, interventional pulmonology, metastatic non–small cell lung cancer

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## Introduction

Lung cancer remains the leading cause of cancer-related deaths in the United States, although in recent years with continued tobacco avoidance and cessation programs, as well as advances in the development of therapeutics, including immune checkpoint inhibitors (ICIs) and targeted therapies, the prognosis of advanced-stage non–small cell lung cancer (NSCLC) has dramatically improved.[1] In patients with metastatic non–small cell lung cancer (mNSCLC) with a targetable genomic alteration (TGA), such as *EGFR* L858R, targeted therapy has a decided progression-free survival advantage over chemotherapy[2–5] and is the recommended first-line treatment. At present, the National Comprehensive Cancer Network (NCCN) NSCLC guidelines recommend molecular testing for the following: *EGFR* (sensitizing alterations), *ALK* (fusions), *ROS1* (fusions), *BRAF* (V600E alterations), KRAS (G12C), *NTRK1-3* (fusions), *MET* (exon 14 skipping alterations), *RET* (fusions), and PD-L1 expression levels.[6] Unfortunately, only 61–87% of patients with mNSCLC undergo at least single



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gene mutation analysis, and far fewer (8–59%) undergo multi-gene mutational analysis[7,8] as recommended in the NCCN guidelines. Moreover, studies have shown that less than half of patients with molecular testing showing a targetable alteration receive the corresponding targeted therapy[9,10] and 30% of *EGFR* mutations are found during first-line chemotherapy.[7] While many factors contribute to these problems, three major barriers have been identified: (1) available tissue is not suitable for molecular profiling, (2) rebiopsy is problematic, and (3) the urgency to do something drives the patient and provider to start chemotherapy and or immunotherapy-based treatment rather than waiting days or weeks for the results of molecular tissue testing that might ultimately be insufficient and or not alter the treatment plan.[11]

At present, tissue analysis remains the standard of care for making an initial histologic diagnosis and has historically been used to identify a growing number of TGAs associated with Food and Drug Administration (FDA)-approved or emerging targeted therapies. However, there has been increasing use of 'liquid biopsies' of peripheral blood. Studies by Aggarwal,[12] Schrock,[13] and Leighl[14] suggest that liquid and tissue molecular testing is complementary, that liquid testing added to tissue testing increases yield and that there is high concordance between the two.

ICIs are widely used in the first-line treatment of mNSCLC, but have no added efficacy or may be inferior in patients with EGFR mutations[15,16] and patients with an actionable mutation should have disease progression on an FDA-approved targeted therapy prior to initiation of ICI therapy[17,18] given concerns for immune-mediated toxicities.[19,20] Unfortunately, the short turn-around time (TAT) of PD-L1 expression increasingly presents the disconcerting dilemma of delaying treatment of a patient with a 'high' PD-L1 with ICIs while awaiting results of molecular testing.

Taken together, these data suggest that a strategy employing early cfDNA testing at the time of, or prior to a definitive tissue diagnosis of mNSCLC, could potentially provide medical oncologists with more timely molecular profiling, allowing patients to start appropriate treatment sooner. To better understand the value and feasibility of molecular profiling of mNSCLC driven by pulmonologists, we performed a retrospective review of our interventional pulmonology (IP) practice in which we used cfDNA for molecular profiling of known or suspected mNSCLC where there was high suspicion for an oncogenic driver.

## Methods

We retrospectively identified all patients seen by the University of California, Davis IP practice from 1 February 2015 to 1 November 2020 for whom cfDNA analysis was ordered by the treating IP as part of routine clinical practice. Only when the IP ordered cfDNA analysis for mNSCLC were patients included for further analysis. These patients were categorized into two groups: (1) IP suspected diagnosis of mNSCLC based on clinical and imaging results without histologic confirmation of lung cancer or (2) IP diagnosis of mNSCLC based on histologic confirmation of NSCLC in addition to clinical and imaging results. Patients with previous early-stage NSCLC (stages I–III) treated with curative intent were included if surgical resection and/or radiation therapy with or without adjuvant chemotherapy had been completed at least 2 years prior without prior suspected progression. Given the retrospective nature of this study, there were no formal inclusion/exclusion criteria in terms of selecting which patients would receive cfDNA testing, although it seems highly plausible that IP selected patients with a high potential for an actionable mutation, namely, no or remote smoking history with known or suspected adenocarcinoma histology. Patients with previous biomarker testing for NSCLC were excluded. Current smokers were defined as having quit less than 6 months prior to the first IP visit, ex-smokers as having quit less than 15 years prior and remote smoking defined as having quit more than 15 years prior and never smokers defined as having smoked less than 100 cigarettes daily

cfDNA analysis was performed using a commercially available, targeted next-generation sequencing (NGS) assay (Guardant360®, Guardant Health). Guardant360 is Clinical Laboratory Improvement Amendments (CLIA)-certified, College of American Pathologist-accredited, and New York State Department of Health-approved, with analytic and clinical validation previously reported.[21,22] Over the course of the study period, the assay expanded from a 68- to a 74-gene panel and includes assessment of single-nucleotide variants (SNVs), insertion–deletions (indels), fusions,

and copy number gain in select genes (Supplemental Table 1). Tissue molecular testing was ordered by the IP or at the discretion of the medical oncologist, and in all cases involved send-out testing.

Following identification of all eligible patients, a retrospective chart review was done to collect information regarding patient demographics, timing of, and indication for pulmonology visits, tissue biopsy, final staging/pathological diagnosis, cfDNA molecular testing, tissue molecular testing, first visit to medical oncology, date of treatment recommendation, date of treatment initiation, and outcome of treatment. This information was maintained in a de-identified database. For a subset of patients who returned to outside health care professionals for follow-up oncology care best effort was made to gather information on treatment recommendations and outcomes, though in some cases, this information was limited. First pulmonology visit was defined as the patient's first visit with an IP for evaluation of possible lung cancer (e.g. pulmonary nodule, pleural effusion, mediastinal adenopathy), staging for NSCLC, management of mNSCLC, or suspicion of recurrence of lung cancer. First oncology visit was defined as the patient's first visit with their treating medical oncologist following the patient's first IP visit as previously defined.

Results of cfDNA and tissue molecular testing were compared in terms of identification of TGAs. Consistent with NCCN guidelines, TGAs in *EGFR*, *ALK*, *ROS1*, *BRAF*, *ERBB2*, *MET*, *NTRK*, and *RET* were considered actionable. In addition, given emerging targeted therapy for *KRAS* G12C and the general mutual exclusivity between activating *KRAS* alterations and other oncogenic drivers, we assessed the number of patients with activating *KRAS* alterations as these alterations may also inform patient treatment. The TAT for cfDNA and tissue molecular testing was defined as the time from the date the sample was received by the testing laboratory to the return of results. We also assessed the 'real-world' TAT, defined as the time from the date the order for molecular testing was placed to the date of the receipt of the results.

## Results

During the study period, a total of 20 patients met the inclusion criteria detailed above and were included for further analysis. Seven patients had an IP diagnosis of mNSCLC, and all were confirmed to have mNSCLC by the patient's medical oncologist. Thirteen patients had a suspected diagnosis of mNSCLC determined by an IP's clinical assessment, and cfDNA was ordered prior to histologic confirmation of malignancy. Twelve of 13 patients were later confirmed to have mNSCLC by histology and by the patient's medical oncologist, and one patient had stage IIIA NSCLC with a large pleural effusion that was negative on cytology.

The median age at the time of cfDNA blood draw was 70.5 years (range: 53–87 years) (Table 1). The majority of patients were female (13/20, 65%) and Caucasian (15/20, 75%). The most common indication for referral to IP was a lung mass ± adenopathy (11/20, 55%), followed by hilar and/or mediastinal adenopathy (4/20, 20%), pleural effusion (2/20, 10%), or other (3/20, 15%). At final diagnosis, the majority of patients had adenocarcinoma (15/20, 75%) and either stage IVA (9/20, 45%) or stage IVB (10/20, 50%). All patients had (1) adenocarcinoma (15/20, 75%), (2) mixed adenocarcinoma and small cell carcinoma (1/20, 5%), or (3) other NSCLC (4/20, 20%) with a never or remote smoking history (15/20, 75%).

At least, one cfDNA alteration was identified in 100% of samples analyzed. Fifteen of 20 patients had tissue molecular testing ordered in addition to cfDNA analysis, and DNA quantity was sufficient for tissue molecular analysis in 80% (12/15) of patients. In all cases, tissue testing consisted of NGS testing via a multi-gene panel. Fourteen of 15 analyses were performed by FoundationOne®CDx (Foundation Medicine, Cambridge, MA) and one test performed by NeoGenomics NGS (NeoGenomics, Fort Myers, FL). A targetable alteration associated with an FDA-approved or emerging targeted therapy in NSCLC was identified in 65% (13/20) of patients (Table 2), including four patients with *EGFR* drivers (exon 19 deletions or L858R), two *BRAF* V600E alterations, one *EGFR* exon 20 insertion, two *ERBB2* exon 20 insertions, one *MET* exon 14 skipping alteration, and three patients with *KRAS* G12C. Of these, 54% (7/13) were identified on both the cfDNA and tissue platforms, 38% (5/13) were identified on cfDNA only, and 8% (1/13) were identified on tissue alone.

**Table 1.** Patient characteristics.

| Patient characteristic | All patients *n* = 20 (%) |
|---|---|
| Age | |
| Median | 70.4 |
| Range | 53–87 |
| Sex | |
| Female | 13 (65) |
| Male | 7 (35) |
| Race | |
| White | 15 (75) |
| Asian | 2 (10) |
| Hispanic | 2 (10) |
| Black or African American | 1 (5) |
| Smoking status | |
| Current | 5 (25) |
| Former | 9 (45) |
| Never | 6 (30) |
| Indication for interventional pulmonology visit | |
| Lung mass ± adenopathy | 11 (55) |
| Hilar and/or mediastinal adenopathy | 4 (20) |
| Pleural effusion | 2 (10) |
| Other | 3 (15) |
| Histology | |
| Adenocarcinoma | 15 (75) |
| Squamous | 2 (10) |
| Mixed adenocarcinoma and small cell | 1 (5) |
| NSCLC not otherwise specified | 1 (5) |
| Inconclusive[a] | 1 (5) |
| Final staging | |
| IIIA | 1 (5) |
| IVA | 9 (45) |
| IVB | 10 (50) |

NSCLC, non–small cell lung cancer.
[a]Patient declined biopsy.

Next, we assessed the median times (calendar days) from patients' first IP visit and the patients' first medical oncology visit to specified endpoints (Figure 1). The time from the first IP visit: (1) to tissue biopsy was 6 days (range: 21 days prior to 64 days after), (2) to the first medical oncology visit was 21 days (range: 5–83 days), and (3) to cfDNA results was 12 days (range: 6–92 days). The median time from the first medical oncology visit to cfDNA results was negative 4 days (range: 62 days prior to 18 days after). Of the seven patients with tissue molecular testing ordered by IP with tissue sufficient for analysis, the time from the first IP visit to receipt of tissue results was 30 days (range: 21–126 days), and tissue results were received 15 days after the patient's first visit with medical oncology (range: 14 days prior to first visit to 43 days after). When including patients with tissue testing ordered by either IP or the medical oncologist, the median time from the patient's first medical oncology visit to the tissue result was 24 days (range: 14 days prior to first visit to 71 days after). A single patient had significant delays for both first medical oncology visit and molecular testing, and accounted for several of the maximum values above, including the 83 days to first medical oncology visit, 92 days to cfDNA results, and 126 days to tissue molecular testing results. This patient was an outlier who was thought to have early-stage disease and only at the time of the surgical resection were pleural metastases noted. Eight days later, IP was notified and ordered both cfDNA and tissue molecular testing simultaneously upon notification.

The median time (calendar days) between when cfDNA was ordered and when tissue molecular testing was ordered was 7 days (range: 8 days prior to 124 days after). There was a median of 2 days (range: 1 day prior to 29 days after) between when tissue was determined to be sufficient for molecular testing and imaging supported mNSCLC to when tissue molecular testing was ordered. In only two cases was there more than a 3-day delay, one of which was due to the fact that the medical oncologist had treated the patient based on cfDNA and ordered tissue molecular testing only at progression, and the other being the patient described above was only found to have metastatic disease at the time of surgery. In all other cases, delays greater than 3 days between when cfDNA was ordered and when tissue molecular testing was ordered were due to delays in obtaining sufficient tissue.

**Table 2.** Summary of targetable alterations detected via liquid and tissue assays, as well as timing of treatment initiation and treatment type initiated.

| Patient | cfDNA-detected TGA | Tissue-detected TGA | Treatment initiated? | Timing of treatment initiation | Time from first medical oncology visit to treatment initiation | First-line treatment category |
|---|---|---|---|---|---|---|
| Patient 1 | *BRAF* V600E | Not assessed | No[a] | N/A | N/A | N/A |
| Patient 2 | *EGFR* exon 19 deletion | *EGFR* exon 19 deletion | Yes | After cfDNA result, prior to tissue result | 0 | Targeted therapy |
| Patient 3 | None | None | Yes | After cfDNA and tissue result | 33 | Chemotherapy + immunotherapy |
| Patient 4 | None | *KRAS* G12C | Yes | After cfDNA and tissue result | 98 | Chemotherapy + immunotherapy |
| Patient 5 | None | *KRAS* G12V | Yes | After cfDNA and tissue result | 56 | Radiation |
| Patient 6 | *ERBB2* exon 20 insertions | *ERBB2* exon 20 insertions | Yes | After cfDNA and tissue result | 63 | Clinical trial |
| Patient 7 | *KRAS* G12C | *KRAS* G12C | Yes | After cfDNA and tissue result | 44 | Chemotherapy + immunotherapy |
| Patient 8 | *EGFR* L858R | Not assessed | No[b] | N/A | N/A | N/A |
| Patient 9 | *ERBB2* exon 20 insertion | *ERBB2* exon 20 insertion | Yes | After cfDNA, result, prior to tissue result | 21 | Chemotherapy + immunotherapy |
| Patient 10 | *MET* exon 14 skipping | Not assessed | Yes | After cfDNA result | 1 | Targeted therapy |
| Patient 11 | None | *KRAS* Q61P | Yes | After cfDNA and tissue result | 49 | Chemotherapy + immunotherapy |
| Patient 12 | *BRAF* V600E | Not assessed | Yes | After cfDNA result | 21 | Targeted therapy |
| Patient 13 | None | Tissue QNS | Yes | After cfDNA and tissue QNS result | 11 | Chemotherapy |
| Patient 14 | *EGFR* exon 20 insertions | *EGFR* exon 20 insertions | Yes | After cfDNA result, prior to tissue result | 3 | Targeted therapy |
| Patient 15 | None | None | No[c] | N/A | N/A | N/A |
| Patient 16 | None | Tissue QNS | Yes | After cfDNA and tissue result | 13 | Chemotherapy + immunotherapy |
| Patient 17 | *EGFR* L858R | *EGFR* L858R | Yes | After cfDNA result, prior to tissue result | 1 | Targeted therapy |
| Patient 18 | *KRAS* G12C | Not assessed | Yes | After cfDNA result | 16 | Chemotherapy + immunotherapy |
| Patient 19 | None | Tissue QNS | Yes | After cfDNA and tissue QNS result | 45 | Immunotherapy |
| Patient 20 | *EGFR* exon 19 deletion | *EGFR* exon 19 deletion | Yes | After cfDNA result, prior to tissue result | 4 | Targeted therapy |

cfDNA, cell-free DNA; QNS, quantity not sufficient; TGA, targetable genomic alteration.
[a]Chemotherapy was recommended before cfDNA resulted, but the patient opted for hospice.
[b]Patient declined diagnostic biopsy on multiple occasions, diagnosis not confirmed, and no treatment started.
[c]Patient referred to hospice.

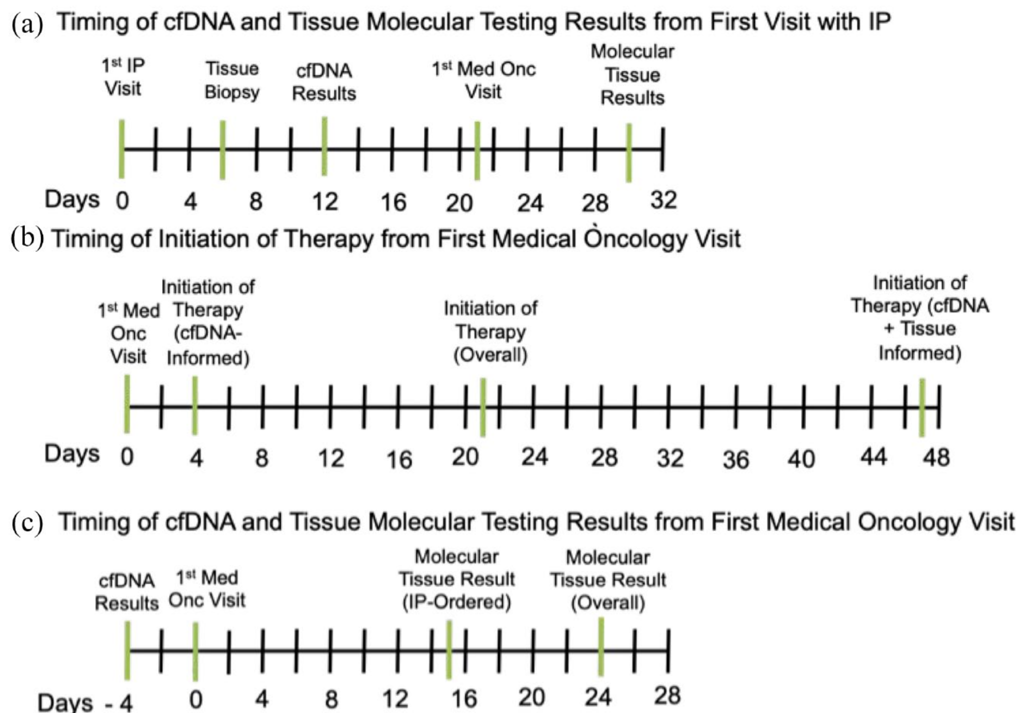


**Figure 1.** Timeline of molecular mutational profiling: cfDNA vs Tissue. (a) Illustration of median time from first interventional pulmonology (IP) visit to tissue biopsy, receipt of cfDNA results, first medical oncology (med onc) visit, and receipt of molecular tissue testing result. (b) Median time from first med onc visit to initiation of therapy for patients treated based on cfDNA result only, the overall cohort ($n=17$), and patients treated after both cfDNA and tissue molecular testing results were available. (c) Median time from first med onc visit to receipt of cfDNA results and tissue testing results (including tissue testing ordered by IP only or tissue testing ordered by either IP or med onc). Timing from cfDNA result and tissue result to first med onc visit differs in (a) and (c) because (a) uses the first IP visit as 'Day 0' from which median time is measured, while (c) uses first med onc visit as 'Day 0'.

The TAT (defined by when blood or tissue was sent for analysis and when results were received) for cfDNA testing was 7 calendar days and the TAT for tissue molecular testing was 14 calendar days. However, the 'real-world' TAT (defined by when blood or tissue testing was ordered and when results were received) for cfDNA testing was 8 calendar days *versus* 20 calendar days for tissue molecular testing. The differences in laboratory TAT and 'real-world' turnaround for blood were due to delays related to phlebotomy arrangements, while the delays for tissue related to the logistics of identifying and arranging for shipments of appropriate tissue and the occasional need for re-biopsy.

Eighty-five percent (17/20) of patients went on to receive therapy (Table 2). Of these, the median time from the patient's first visit to medical oncology to the initiation of therapy was 21 days (range: 0–98 days). In 53% (9/17) of cases, the treating oncologist began treatment based on cfDNA results and before tissue molecular testing was available, either because cfDNA revealed a targetable mutation (6/9, 67%) or because chemotherapy ± immunotherapy was deemed appropriate pending tissue molecular analysis (3/9, 33%). In these cases, treatment was initiated a median of 4 days after the patient's first oncology visit, and in no cases, did subsequent tissue molecular testing alter therapy. For the eight patients who were not treated based on cfDNA alone, five received chemotherapy and immunotherapy after tissue molecular profiling results were received, one patient received radiation therapy alone, one was treated for the small cell component of their mixed small cell and adenocarcinoma, and one was enrolled in a clinical trial for treatment of an *ERBB2*(HER2) mutation found on both tissue molecular profiling and cfDNA. In these cases, treatment was initiated a median of 47 days after the patient's first oncology visit. Of these eight patients, two had

matching targetable alterations on both assays, three had no targetable alterations on either assay, and three had an activating *KRAS* alteration identified on tissue alone (one being *KRAS* G12C). Notably, two out of three samples with an activating *KRAS* alteration identified on tissue but not on cfDNA had a maximum variant allele fraction (equivalent to the tumor-derived alteration with the highest level of cfDNA in the sample) below the assay's median level of cfDNA detection, suggesting that these tumors were low-shedding tumors. As patients had to be treatment free for 2 years prior to be included in this study, slow tumor growth or more limited disease burden may explain these low-shedding tumors. Since cfDNA is naturally limited by the degree of tumor shed, it is possible that in these cases the *KRAS* alterations detected via tissue may have been occurring below the assay's limit of detection.

## Discussion

Liquid biopsy techniques encompass a variety of potential analyses and can include evaluation of circulating cfDNA, circulating tumor cells (CTCs), circulating proteins, cytokines, and exosomes. At present, there are three FDA-approved liquid biopsy tests, Guardant360® CDx, cobas® EGFR MutationTest v2, and FoundationOne Liquid CDx®, all of which test for plasma cfDNA. In 2019, Aggarwal *et al.* compared a plasma-based cfDNA assay with a tissue-based test, both of which used NGS 11. They found a concordance of 88.9% for targetable alterations in *EGFR*, *ALK*, *MET*, *RET*, *BRCA1*, *ERBB2*, and *BRAF* between tissue and plasma in newly diagnosed patients. In addition, cfDNA increased the proportion of patients with a targetable alteration from 20.5% to 35.8% via identification of alterations in patients who were unable to get tissue molecular testing or identification of alterations in patients who had negative tissue testing.[12] Concordance between tissue and liquid tests in NSCLC has been extensively studied and suggests high levels of agreement, ranging from 85% to 98% for targetable alterations in concurrently collected samples.[13] A study from Leighl *et al.* compared cfDNA with tissue testing (physician's choice) in 282 newly diagnosed NSCLC patients and found concordance for *EGFR*, *ALK*, *ROS1*, and *BRAF* to be $>98.2\%$, with 100% positive predictive value for cfDNA compared with tissue. In addition, cfDNA results were returned significantly faster than tissue results (9 *versus* 15 days, $p < 0.0001$).[14] These studies support the utility of using liquid biopsy as a 'first pass' molecular testing platform, with follow-up tissue testing in patients with uninformative liquid biopsy results.

ICIs have been approved and widely adopted as monotherapy or in combination with chemotherapy in the first-line setting for mNSCLC. While ICI therapy can be an effective treatment for mNSCLC, it has no added efficacy or is inferior in patients with *EGFR* mutations.[15,16] Notably, drug labels for all three ICI with FDA-approval in mNSCLC state that patients with *EGFR* or *ALK* alterations should have disease progression on the corresponding FDA-approved targeted therapy prior to initiation of ICI therapy.[17,18,23] Importantly, recent studies have suggested that the order in which patients receive therapy may be important, as an increase in immune-mediated toxicities has been seen in patients receiving sequential (ICI prior to targeted therapy) and concurrent immunotherapy and EGFR tyrosine kinase inhibitors.[19,20] PD-L1 expression (as measured by immunohistochemical assays) is currently the most widespread biomarker used to identify patients who may benefit from ICI therapy, and generally has a relatively short TAT. Longer TAT for tissue molecular testing means that oncologists are now increasingly faced with the dilemma of either delaying treatment for a mNSCLC patient with a high PD-L1 score, or potentially inappropriately treating with an ICI while awaiting results of molecular profiling.

At present, the relative value and timing of cfDNA from blood and tissue molecular testing is in a state of flux. Certainly, reducing the wait time before definitive treatment is a priority for patients and oncologists and a strategy employing early cfDNA testing at the time of, or prior to a, definitive tissue diagnosis of mNSCLC could potentially provide medical oncologists with more timely molecular profiling, allowing patients to start appropriate treatment sooner. Pulmonologists are often at the front line of the diagnosis and staging of mNSCLC patients and are potentially ideally situated to utilize this technology, but only if they understand the appropriate indications and timing. To better understand the value and feasibility of molecular profiling of mNSCLC driven by pulmonologists, we performed a retrospective review of our IP practice in which we used cfDNA for molecular

profiling of known or suspected mNSCLC where there was high suspicion for an oncogenic driver.

In this small retrospective study, we assessed the potential real-world value of cfDNA analysis in the setting of known or suspected mNSCLC as utilized in the routine of an academic IP practice. Our main questions were: (1) can IP-directed use of cfDNA potentially reduce time to treatment, (2) how accurate were IPs at predicting mNSCLC as well as the need for molecular profiling based on clinical and radiologic features, and (3) how did cfDNA testing ordered by IP inform treatment in these patients?

Notably, cfDNA results were available a median of 4 days prior to the patient's first oncology visit while tissue molecular results were not available until a median of 15 days after the patient's first oncology visit. This difference was not due to an inappropriate delay in ordering tissue molecular tests, regardless of who ordered tissue testing. This, in combination with the finding that a potentially targetable mutation was identified by cfDNA in 12/20 (60%) patients, suggest that IP-directed cfDNA testing can potentially allow oncologists to start treatment sooner than a strategy based on tissue molecular testing first. The finding that cfDNA testing had a shorter TAT than tissue-based testing is consistent with previous studies comparing cfDNA testing with tissue molecular testing.[14] Furthermore, in half of our cases with oncogene driver mutations, the treating oncologist commenced their initial treatment based on cfDNA results, not waiting for tissue molecular testing results. This suggests a high degree of confidence in the results and this strategy, which is consistent with recommendations from international guidelines on the use of well-validated liquid biopsy assays in mNSCLC as long as a validated assay is used. Tissue testing only detected one additional patient with a TGA in our study, and no targeted therapy initiated based on cfDNA was altered by the tissue test results.

How accurate and appropriate was pre-emptive ordering of cfDNA before a histologic and clinical diagnosis of mNSCLC was established? There are three parts to this question: (1) how often was a suspected diagnosis of NSCLC confirmed, (2) how often was the final stage confirmed metastatic by an oncologist, and (3) how often was broad molecular testing consistent with NCCN guidelines? Of the 13 patients with suspected mNSCLC, all but one were confirmed metastatic by standard radiographic evaluation; one patient had non-operable stage IIIA adenocarcinoma. Recommendations to test for *EGFR* mutation status in earlier stage NSCLC will likely become more widespread given the recent FDA-approval of osimertinib in the adjuvant setting based on the results of the ADAURA trial[24] (though it should be noted that the cfDNA assay used in this study is only intended for use in patients with advanced NSCLC). As this was a retrospective study of clinical practice, explicit inclusion/exclusion criteria were not initially used to select patients for cfDNA analysis. However, given the high rate of targetable alterations (60%) identified in this cohort, it seems likely that empiric data suggesting that young, non-smokers with adenocarcinoma are more likely to harbor targetable alterations impacted IP decision-making when selecting which patients warranted cfDNA analysis. Given the fact that current updated NCCN guidelines recommend comprehensive molecular profiling for patients with mNSCLC regardless of patient age, sex, or smoking status, and that an FDA-approved therapy now exists for NSCLC patients with *KRAS* G12C, expansion of cfDNA testing to other demographic groups (e.g. smokers, males) is clinically relevant.

Currently, the NCCN NSCLC guidelines state that cfDNA should not be used in lieu of histologic tissue diagnosis, but that cfDNA testing can be considered for newly diagnosed patients who are either unfit for invasive biopsy or who have insufficient tissue to allow for comprehensive molecular analysis. Notably, 40% of patients in our study did not undergo tissue molecular testing either because the patient did not wish to pursue an invasive biopsy, the re-biopsy was considered unsafe by the clinicians or the medical oncologist chose to treat the patient based on cfDNA results. Several guidelines have suggested 14 days as a clinically acceptable TAT for molecular testing, though Kim *et al.*[25] suggest this should be the 'upper limit' given that both patients and providers typically want to initiate therapy as quickly as possible.[26] In our study, the TAT for cfDNA results was 7 days, compared with 14 days for tissue. Perhaps more importantly, the 'real-world' TAT (the time from the date the order for molecular testing was placed to the date of the receipt of the results) was 8 days for cfDNA and 20 days for tissue. Admittedly, our testing strategy is novel and is not currently supported by guidelines. However, we believe that these data clearly

illustrate that the implementation of cfDNA testing earlier into a mNSCLC patient's journey provides medical oncologists with molecular data more quickly, as shown by the fact that cfDNA results were available a median of 4 days prior to the patient's first medical oncology visit while tissue molecular testing was available a median of 24 days after the first medical oncology visit, despite the fact that tissue molecular testing was ordered on a timely basis once sufficient tissue was confirmed. Previous studies have demonstrated that the combination of cfDNA testing and tissue molecular testing increases the yield of discovering potentially targetable mutations in advanced mNSCLC.[12,14] We feel our data further support a 'liquid first' paradigm in the newly diagnosed mNSCLC setting; this would allow patients with targetable alterations detected via cfDNA to begin treatment without delay, while patients with 'uninformative' cfDNA results could go on to receive tissue molecular testing at the discretion of their medical oncologist.

Our study is limited by the fact that it is small, retrospective and conducted within a single practice and there is no comparator control group. Therefore, results may vary depending on the institution and in particular according to country. In addition, as a real-world study, we could not prevent variations in practice between IPs or oncologists, we could not intuit the reasoning behind the timing of an oncologist's appointment, nor could we control or fully understand the practice behavior or thought process behind an IP's rationale that a patient warranted cfDNA testing even before a diagnosis of mNSCLC was made or before tissue molecular profiling had been performed. Time to testing results for both cfDNA and tissue molecular testing were influenced by socio-economic factors and patient's belief systems (such as biopsy of a cancer will cause it to rapidly spread and become metastatic) that affected the patient's ability to make or keep appointments and comply with testing recommendations, and delays in testing were in some cases significantly affected, more so for tissue testing than for cfDNA testing. As a real-world study though, these delays represent challenges that are faced in clinical practice. Whether this strategy is cost-effective or generalizable to other pulmonologists, IPs, and institutions remains unproven. Finally, unseen consequences of uninformative cfDNA results, conflicting tissue and cfDNA results, conflicts between IP and oncology practices regarding ordering of molecular tests, and patient burden of cost cannot be assessed.

## Conclusion

IP-directed cfDNA testing early in the diagnostic workup for suspected or confirmed mNSCLC prior to or concurrent with tissue molecular profiling appears to provide oncologists with more timely molecular profiling information and to expedite treatment of mNSCLC compared with tissue-based testing first. Such a testing paradigm may eliminate the treatment delay medical oncologist face and the anxiety patients' experience, when molecular profiling is not available in the setting of mNSCLC. While promising, this testing strategy deserves further study. We recommend that all stakeholders work closely and continually regarding strategies to expedite molecular testing for NSCLC in this rapidly evolving field and to integrate IP into a multidisciplinary workflow strategy to expedite molecular testing in mNSCLC.

## Declarations

### *Ethics approval and consent to participate*

This study was reviewed and approved by the UC Davis Institutional Review Board Administration, Davis, CA (1502378-1). Due to the retrospective nature of the study and the fact that only patient data was accessed and downloaded in a deidentified and coded fashion, consent from participants was not deemed necessary.

### *Consent for publication*

Not applicable.

### *Author contributions*

**Chinh Phan:** Conceptualization; Data curation; Writing – review & editing

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**Tianhong Li:** Conceptualization; Data curation; Formal analysis; Methodology; Validation; Writing – review & editing.

**Ken Y Yoneda:** Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Project administration; Resources; Supervision; Validation; Writing – original draft; Writing – review & editing.

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**Supplemental material**
Supplemental material for this article is available online.

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