Clinical Cancer Research

Clinical Utility of Comprehensive Cell-free DNA Analysis to Identify Genomic Biomarkers in Patients with Newly Diagnosed Metastatic Non–small Cell Lung Cancer

Natasha B. Leighl[1], Ray D. Page[2], Victoria M. Raymond[3], Davey B. Daniel[4], Stephen G. Divers[5], Karen L. Reckamp[6], Miguel A. Villalona-Calero[7], Daniel Dix[3], Justin I. Odegaard[3], Richard B. Lanman[3], and Vassiliki A. Papadimitrakopoulou[8]

Abstract

Purpose: Complete and timely tissue genotyping is challenging, leading to significant numbers of patients with newly diagnosed metastatic non–small cell lung cancer (mNSCLC) being undergenotyped for all eight genomic biomarkers recommended by professional guidelines. We aimed to demonstrate noninferiority of comprehensive cell-free DNA (cfDNA) relative to physician discretion standard-of-care (SOC) tissue genotyping to identify guideline-recommended biomarkers in patients with mNSCLC.

Patients and Methods: Prospectively enrolled patients with previously untreated mNSCLC undergoing physician discretion SOC tissue genotyping submitted a pretreatment blood sample for comprehensive cfDNA analysis (Guardant360).

Results: Among 282 patients, physician discretion SOC tissue genotyping identified a guideline-recommended biomarker in 60 patients versus 77 cfDNA identified patients (21.3% vs. 27.3%; $P < 0.0001$ for noninferiority). In tissue-positive patients, the biomarker was identified alone (12/60) or concordant with cfDNA (48/60), an 80% cfDNA clinical sensitivity for any guideline-recommended biomarker. For FDA-approved targets (*EGFR*, *ALK*, *ROS1*, *BRAF*) concordance was >98.2% with 100% positive predictive value for cfDNA versus tissue (34/34 *EGFR*-, *ALK*-, or *BRAF*-positive patients). Utilizing cfDNA, in addition to tissue, increased detection by 48%, from 60 to 89 patients, including those with negative, not assessed, or insufficient tissue results. cfDNA median turnaround time was significantly faster than tissue (9 vs. 15 days; $P <$ 0.0001). Guideline-complete genotyping was significantly more likely (268 vs. 51; $P < 0.0001$).

Conclusions: In the largest cfDNA study in previously untreated mNSCLC, a validated comprehensive cfDNA test identifies guideline-recommended biomarkers at a rate at least as high as SOC tissue genotyping, with high tissue concordance, more rapidly and completely than tissue-based genotyping.

See related commentary by Meador and Oxnard, p. 4583

Introduction

Clinical practice guidelines from numerous professional societies, including the National Comprehensive Cancer Network (NCCN), the American Society of Clinical Oncology (ASCO), the International Association for the Study of Lung Cancer (IASLC), the Association of Molecular Pathologists (AMP), and the College of American Pathologists (CAP) advocate for somatic genomic testing in all patients with newly diagnosed metastatic non–small cell lung cancer (mNSCLC; refs. 1–3). While the recommendations vary slightly, there is general consensus that alterations in up to seven genes should be assessed to identify patients who are likely to benefit from treatment with FDA-approved targeted therapies or promising targeted therapies available through late-stage clinical trials or off-label prescribing. These eight guideline-recommended biomarkers include *EGFR* mutations, *ALK* fusions, *ROS1* fusions, *BRAF* V600E mutation, *RET* fusions, *MET* amplification and *MET* exon 14 skipping variants, and *ERBB2* (HER2) mutations. Clinical practice guidelines continue to expand with the most recent version of the NCCN guidelines (v03.2019) advocating for assessment of a ninth biomarker, *NTRK* fusions. In addition, given the rarity of co-occurring oncogenic drivers in newly diagnosed mNSCLC, identifying a patient with an activating *KRAS* mutation is informative in not only ending the biomarker diagnostic odyssey (4), but also to identify patients for whom chemotherapy and/or immune checkpoint inhibitor (ICPi) therapy may be the best therapeutic course (1, 2).

In addition to targetable genomic biomarkers, the approval of ICPi has necessitated the use of other biomarkers to identify patients who may benefit from first-line ICPi monotherapy.

[1]Princess Margaret Cancer Centre, Toronto, Ontario, Canada. [2]Center for Cancer and Blood Disorders, Fort Worth, Texas. [3]Guardant Health, In, Redwood City, California. [4]Tennessee Oncology, Chattanooga, Tennessee. [5]Genesis Cancer Center, Hot Springs, Arkansas. [6]City of Hope Comprehensive Cancer Center, Duarte, California. [7]Miami Cancer Institute, Miami, Florida. [8]MD Anderson Comprehensive Cancer Center, Houston, Texas.

Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

N.B. Leighl and R.D. Page contributed equally to this article.

Corresponding Author: Natasha B. Leighl, Princess Margaret Cancer Centre, University of Toronto, 610 University Ave, Toronto, Ontario M5G 2C1, Canada. Phone: 416-946-4645; Fax: 416-946-6546; E-mail: Natasha.Leighl@uhn.ca

Clin Cancer Res 2019;25:4691–700

doi: 10.1158/1078-0432.CCR-19-0624

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Translational Relevance

In newly diagnosed advanced nonsquamous non–small cell lung cancer (NSCLC), undergenotyping, incomplete genotyping for genomic biomarkers that are guideline-recommended by numerous professional medical societies, poses a significant challenge to informative and timely clinical decision-making. In this prospective trial, we demonstrate that a validated, highly sensitive and highly specific, clinically utilized comprehensive cell-free DNA (cfDNA) test detects guideline-recommended biomarker-positive patients at a rate similar to physician discretion standard-of-care tissue genomic testing, with high tissue concordance and significantly faster return of results leading to more complete genotyping of the guideline-recommended biomarkers in more patients. These results prove cfDNA to be a clinically viable alternative to obtaining guideline complete genotyping for first-line therapy selection in patients with newly diagnosed advanced NSCLC.

Guidelines recommend that patients with newly diagnosed mNSCLC undergo IHC analysis for PD-L1 expression (1) with an estimated 25% of patients having "high" PD-L1 expression (>50% tumor proportion score) making them eligible for first-line ICPi monotherapy (5). Recently, the NCCN guidelines added tumor mutational burden (TMB) as an emerging biomarker for ICPi use (1). An important caveat to first-line treatment with ICPi monotherapy is that patients must have negative genomic testing for *EGFR* and *ALK* alterations as patients harboring somatic alterations in these targetable genes have higher response rates to first-line tyrosine kinase inhibitor (TKI) therapy as compared with ICPi therapy (6). Thus, genomic testing remains important even in the setting of high PD-L1 expression or high TMB.

The increasing number of therapeutic biomarkers to be assessed in patients with newly diagnosed mNSCLC adds time to the clinical evaluation and places strain on tumor tissue availability, especially when biomarkers are assessed in a sequential manner adding additional expense (7). Real-world studies of clinical practice have demonstrated that significant numbers of patients with mNSCLC are not tested for the four guideline-recommended biomarkers with FDA-approved targeted therapies, *EGFR* exon 19 deletions and L858R mutation, *BRAF* V600E mutation, *ALK* fusions, and *ROS1* fusions, and the majority are not tested for all eight guideline-recommended biomarkers (8, 9). Utilizing comprehensive tissue next-generation sequencing (NGS) has shown promise in the ability to fully assess patients for the recommended biomarkers but remains challenged by tissue availability and the time required for guideline-complete testing. In one large North American study, 19% of patients with nonsquamous mNSCLC initiated chemotherapy before *EGFR* or *ALK* results became available (10). In other studies, deterioration in performance status related to delays in obtaining tumor biopsy–based genotyping results disqualified 17% to over 50% of patients with mNSCLC from eligibility for clinical trials (11, 12).

Comprehensive cell-free DNA (cfDNA) analysis has consistently shown viability as an alternative to tissue genotyping, especially in tissue-limited or time-limited clinical scenarios (13, 14). In three large prospective clinical validation studies, a comprehensive and highly sensitive cfDNA NGS test showed high positive concordance with tissue-based genotyping (14–16). Despite such results, there continues to be a perception that cfDNA-based analysis finds relevant biomarkers at a markedly lower rate than current tissue-based standard-of-care (SOC) tissue genotyping and thus cfDNA testing should be reserved for reflex testing in cases of tissue insufficiency. Accordingly, we aimed to build on previous findings and help directly address this remaining perception by evaluating, in the clinical practice setting, whether a validated (17, 18) and highly sensitive comprehensive cfDNA test utilized at diagnosis of mNSCLC is noninferior to physician discretion SOC tissue genotyping to identify guideline-recommended genomic biomarkers and to evaluate potential advantages of cfDNA testing over physician discretion SOC given the known challenges with tissue-based molecular testing.

Patients and Methods

Patients

The NILE study (Non-invasive versus Invasive Lung Evaluation; ClinicalTrials.gov; NCT03615443) enrolled 307 patients with biopsy proven, previously untreated, nonsquamous mNSCLC (stage IIIB/IV) undergoing physician discretion SOC tissue genotyping at one of 28 North American centers. Eligible patients were prospectively consented to this institutional review board–approved study and enrolled between July 2016 and April 2018. Patients with previously treated localized NSCLC (stage I–IIIA) were eligible if primary surgical resection and/or radiation treatment was completed at least 6 months prior to the development of metastatic disease and adjuvant systemic therapy was completed at least 6 weeks prior to study enrollment. Patients with concurrent malignancy were ineligible with the exception of nonmelanoma skin cancer or noninvasive cervical cancer. Patients with a history of a prior cancer other than NSCLC were included if the previous diagnosis occurred more than 2 years prior to enrollment and the patient had no evidence of active disease.

This study was conducted in accordance with the U.S. Common Rule. Written informed consent was obtained from each patient or their guardian.

Study procedures

SOC tissue genotyping included genomic testing and PD-L1 expression analysis. In accordance with NCCN guidelines, SOC tissue genotyping may include NGS, PCR "hotspot" testing, FISH and/or IHC, or Sanger sequencing. The tissue genotyping methodology and spectrum of biomarkers assessed was allowable per physician discretion based on the genotyping they would pursue in a normal and customary SOC setting. Patients submitted a pretreatment blood sample for cfDNA analysis utilizing a CLIA-certified, CAP-accredited, New York State Department of Health–approved comprehensive NGS test (Guardant360; Guardant Health). The cfDNA test assesses for single-nucleotide variants (SNV) in 73 genes, insertion–deletion (indel) and fusion alterations, and copy-number amplifications in select genes including all eight guideline-recommended biomarkers and *KRAS* (17). The cfDNA test has demonstrated extensive analytical and clinical validity and clinical utility (19–21). A clinical report was issued to the ordering provider. Over the study period, the clinical cfDNA assay bioinformatics pipeline (BIP) underwent several modifications, including expanded probe coverage for fusion calling and discrimination of focal copy-number amplification (17). The primary analysis for this study was based on results reported to the ordering provider according to study procedures. To

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standardize results across BIP modifications, a *post hoc* analysis was completed utilizing the most current BIP on all samples. Results were compared with what was originally reported.

Statistical analyses

The NILE study aimed to enroll 300 patients with the primary objective to demonstrate the noninferiority of cfDNA-based versus SOC tumor tissue–based genotyping as it pertains to the detection of guideline-recommended biomarkers in first-line, treatment-naïve nonsquamous mNSCLC. Two preplanned interim analyses were conducted. The first interim analysis was conducted after enrollment of approximately 100 patients with pretreatment data. This second interim analysis was performed when 300 patients were enrolled with pretreatment data with the goal of reporting on the primary objective. Final study analysis will report on the secondary objective of objective response rate (ORR) in patients treated with a targeted therapy for the FDA on label genomic biomarkers—*EGFR*-activating alteration, *ALK* fusion, *ROS1* fusion, *BRAF* V600E mutation.

The original determined sample size was 190 patients, based upon the primary endpoint and the ability to show noninferiority in the identification rate of guideline-recommended biomarkers by cfDNA-based genotyping in the patient populations. Based upon preliminary data, it was predicted that 13% of patients who receive genotyping results from tumor tissue will be identified as having at least one of the eight guideline-recommended biomarkers (22). At the time of original sample size determination, it was calculated that 190 patients provide approximately 80% power with an alpha of 0.05 to reject a 1.3% (10% of the 13% identification based on tissue sequencing) or worse inferiority margin for cfDNA-based genotyping versus SOC tissue-based genotyping biomarker detection, assuming: (i) an actual difference of 4%, (ii) 85% sensitivity of the cfDNA test, (iii) a tumor not detected (TND) rate for the cfDNA test not exceeding 15%, and (iv) a quantity not sufficient (QNS) rate for tissue genotyping of approximately 20%. A sample size of 300 subjects provides power to better meet the secondary objective (final analysis) of ORR among subjects whose tumors have actionable activating mutations according to cfDNA results and who are treated with TKIs.

This second interim analysis aimed to assess the primary objective, identification rate of the eight guideline-recommended biomarkers in cfDNA versus tissue genotyping. Patients were either positive or not positive for a guideline recommended biomarker. The primary analysis is a noninferiority analysis of the null hypothesis

$$H_0 : \delta_B \leq \frac{9}{10}\delta_T$$

where δ_B and δ_T are the number of patients determined by cfDNA analysis and by SOC tissue genotyping (respectively) to have one of the eight guideline-recommended biomarkers. The primary analysis is conducted using a paired *t* test conducted at the 5% significance level.

For this analysis, QNS is a per biomarker tissue genotyping result that indicates insufficient tumor specimen for the lab genotyping tests for analysis of any of the eight guideline-recommended biomarkers, *KRAS*, or PD-L1 to be performed, which can be known prior to a test order being placed (e.g., limited or no residual tumor tissue available) or recognized at the testing lab; or tumor cellularity below lab-dictated minimal requirements; or no genotyping results available within 45 days of patient enrollment. Guideline-complete genotyping indicates that all eight guideline-recommended biomarkers were assessed in tissue genotyping or cfDNA and a positive or negative result was returned. Difference in number of patients achieving guideline-complete genotyping by each modality is calculated by paired *t* test. Tissue-incomplete or "undergenotyped" is a tissue genotyping result that denotes patients who were not completely assessed for all eight guideline-recommended biomarkers, including those samples identified as QNS for any of the guideline-recommended biomarkers or tissue samples that were not assessed for all eight guideline-recommended biomarkers. Because mutations among the guideline-recommended biomarkers are considered largely mutually exclusive, patients with tumors found by tissue testing to have one of the eight guideline-recommended biomarkers were not considered to be undergenotyped, even if all guideline-recommended biomarkers were not tested. Otherwise, the proportion of patients who are undergenotyped was calculated as the number of patients with undergenotyped results divided by the total number of patients enrolled in the study. TND is a cfDNA genotyping result, which indicates somatic mutations were not reported in the sequenced cfDNA.

The eight guideline-recommended biomarkers, *EGFR* mutations, *ALK* fusions, *ROS1* fusions, *BRAF* V600E mutation, *RET* fusions, *MET* amplification and *MET* exon 14 skipping variants, and *ERBB2* (HER2) mutations are reported as detected, not detected (but tumor DNA detected), TND (applicable only to cfDNA), QNS, or not assessed for each patient and for each sample type. *KRAS* mutations are reported in the same manner. The rate of detection for each sample type is equal to the number of patients with at least one biomarker detected divided by the total number of patients enrolled into the study. Tissue PD-L1 results are reported as positive [$\geq$1% tumor proportion score (TPS)], negative (<1% TPS), QNS, or not assessed. Turnaround time (TAT) is defined as the days between test order date and report date. In cases where serial reflex testing is used for tissue genotyping, the report date is calculated as the first of either (i) the date of return of the first positive guideline-recommended biomarker or (ii) date of return of all negative/QNS guideline-recommended biomarkers.

To understand the proportion of biomarker-positive patients who would have been detected by initiating molecular testing with tissue versus cfDNA genotyping, we compared the percentage of all patients who were biomarker positive by each modality and the percentage of biomarker-positive patients who would have been identified by reflex genotyping to the alternative modality. Paired *t* test calculation was applied to determine statistical difference.

Results

During the study period, 307 patients were consented. Four patients were excluded because they did not have a pretreatment cfDNA sample collected. Twenty-one patients were ineligible because they did not meet the inclusion criteria due to no tissue genotyping ordered ($N = 4$), metastatic disease not confirmed at enrollment ($N = 4$), received a prohibited treatment prior to enrollment ($N = 8$), or a diagnosis of squamous cell carcinoma ($N = 5$). Two hundred eighty-two patients met all inclusion criteria and were included in this analysis (Fig. 1). Median age at mNSCLC diagnosis was 69 years (range, 26–100). The majority

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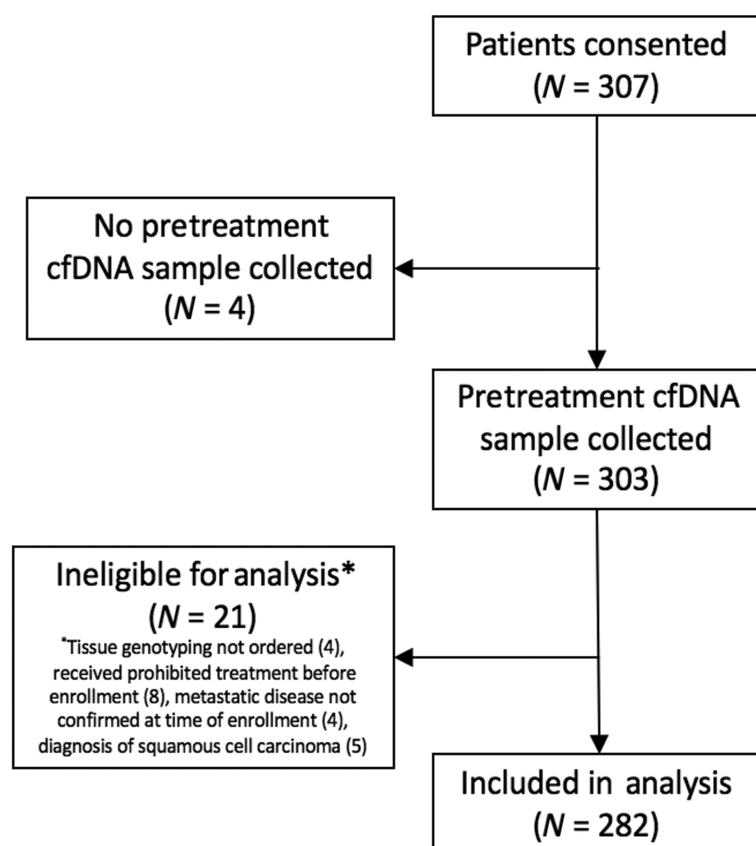

Figure 1.
Patient consort. This second interim analysis was preplanned at 300 patients enrolled with pretreatment samples collected. Twenty-one patients were ultimately deemed ineligible leaving 282 patients for the analysis.

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of patients were white (81.9%). About half (54.4%) of patients had a prior smoking history. Females and males were equally represented (Table 1).

Guideline complete genotyping rates

Tissue genotyping for all eight guideline-recommended biomarkers was completed in 18.1% ($N = 51$) of patients with an additional 13 patients attempting assessment of all biomarkers but were QNS for at least one ($N = 5$) or all ($N = 8$) of the biomarkers. Of the 51 patients who had complete genotyping, 35 (68.6%) had comprehensive tissue NGS genotyping while the remaining patients, 31.3% (16/51), had sequential individual biomarker testing of all eight biomarkers. The majority of patients underwent sequential individual biomarker tissue testing (84.8%; $N = 239$) with most patients undergoing testing for *EGFR* mutations, *ALK* fusions, and *ROS1* fusions (83%, 80%, 58%, Fig. 2). Tissue testing for the remaining guideline-recommended biomarkers occurred in one-quarter to one-third of patients; *BRAF* V600E mutation (35%), *RET* fusions (22%), *MET* amplifications (23%) and *MET* exon 14 skipping alterations (22%), and *ERBB2* mutations (20%). One hundred ninety-two patients (68.1%) were undergenotyped meaning they did not have a guideline-recommended biomarker identified and were not assessed for all guideline-recommended biomarkers. Two hundred eighty-one of 282 patients had a cfDNA result returned (99.6%) with 13 patients (4.6%) having a TND cfDNA result. Overall, 95% of patients were fully assessed for all eight guideline-recommended biomarkers in cfDNA (268 cfDNA vs. 51 tissue for guideline-complete genotyping; $P < 0.0001$; Supplementary Table S1).

Guideline-recommended biomarker detection

One of the eight guideline-recommended biomarkers was identified in tissue in 60 patients and in cfDNA in 77 patients (21.3% versus 27.3%) with a P value of <0.0001, concluding noninferiority of cfDNA versus SOC tissue genotyping (Table 2A). In the 60 tissue-positive patients, the guideline-recommended biomarker was identified in tissue alone ($N = 12$) or concordant with cfDNA ($N = 48$). This represented an overall clinical sensitivity of cfDNA relative to tissue of 80% for detection of any guideline-recommended biomarker. Positive predictive value (PPV) for cfDNA versus tissue genotyping for FDA-approved targets, *EGFR* exon 19 deletions and L858R mutations, *ALK* fusions, and *BRAF* V600E, was 100% with a greater than 98.2% concordance (Table 3). Tissue and cfDNA concordance for *ROS1* fusions was 98.7% (PPV not applicable). Concordance and PPV for the other guideline-recommended biomarkers was similarly high (Supplementary Table S2). Utilizing cfDNA, in addition to tissue, increased the number of patients with an identified guideline-recommended biomarker by 48%, from 60 patients to 89, including those with negative ($N = 7$) or QNS ($N = 6$) results, or those not assessed for the biomarker identified in cfDNA ($N = 16$; Table 2A; Supplementary Table S1). When analysis was restricted to those patients for whom assessment for all eight guideline-recommended biomarkers was attempted or completed in both cfDNA and tissue ($N = 64$), 22 patients had a guideline-recommended biomarker identified in tissue compared with 22 in cfDNA concluding noninferiority in this subcohort (Table 2B). The distribution of patients with guideline-recommended biomarkers identified in cfDNA and tissue

Table 1. Demographics of the 282 patients included in the analysis

		Number	Percentage (%)
Sex	Female	153	54.3
	Male	129	45.7
Median age at diagnosis (range) in years		69 (26–100)	
Race	White	231	81.9
	Black or African American	18	6.4
	Asian	17	6.0
	Native Hawaiian or other Pacific Islander	1	0.4
	Other	8	2.8
	Unknown	7	2.5
Ethnicity	Hispanic	23	8.2
	Non-Hispanic	259	91.8
ECOG status at enrollment	0	71	25.2
	1	151	53.5
	2	36	12.8
	3	12	4.3
	Unknown/missing	12	4.3
History of prior chemotherapy for early-stage NSCLC	Yes	45	16.0
	No	237	84.0
Stage of NSCLC at enrollment	IIIb	7	2.5
	IV	275	97.5
Type of NSCLC at enrollment	Adenocarcinoma	271	96.1
	Large cell carcinoma	5	1.8
	Other[a]	6	2.2
Smoking history	Never-smoker	61	21.4
	Previous smoker	153	54.4
	Current smoker	61	21.7
	Unknown	7	2.5

[a]Other types of NSCLC at time of enrollment include four cases of adenocarcinoma with mixed squamous histology, one case of sarcomatoid carcinoma, and one case where the specific type of NSCLC was not provided.

(Table 2C) was as expected from previous studies describing the prevalence of targetable genomic drivers in patients with newly diagnosed mNSCLC (23).

We compared the incremental add of each molecular testing modality. If the primary modality for molecular testing was tissue-based genotyping, 67% of the 89 patients with one of the eight guideline-recommended biomarkers would have been identified by tissue, with an additional 33% of patients identified on reflex cfDNA testing. Using cfDNA genotyping as the primary genotyping test, 87% of the 89 patients with a guideline-recommended biomarker would be identified in initial cfDNA testing, with the remaining 13% of patients identified with reflex tissue genotyping (Fig. 3A; $P < 0.0001$).

Median TAT

Median TAT was significantly lower for cfDNA as compared with tissue genotyping (9 vs. 15 days; $P < 0.0001$). Improvements in testing logistics enabled significant decrease in cfDNA TAT over the course of the study, a key quality metric for care delivery. The first 10 patients had a median cfDNA TAT of 14 days (range, 11–30 days) versus the last 10 patients who had median TAT of 7 days (range, 5–9 days; Fig. 3B).

EGFR T790M

Three patients (1.1%) had the *EGFR* T790M resistance alteration identified in their pretreatment sample. In two patients, both *EGFR* exon 19 deletion and T790M alterations were identified (patients 31 and 160). Neither patient had a reported prior exposure to an *EGFR* TKI. In the third patient (patient 243), the T790M mutation was detected in cfDNA in the absence of an *EGFR*-activating alteration and at a variant allele fraction (VAF) of 49.9%, while the next highest VAF was a *TP53* mutation at 0.2%. Utilizing a highly specific betabinomial algorithm that evaluates the VAF of the mutation in question versus a scaffold of common single-nucleotide polymorphisms (24), this sample was categorized by the BIP as a germline mutation and reported as an incidentally found germline mutation of potential clinical interest (Supplementary Table S3).

KRAS mutations

A total of 89 (31.6%) patients had an activating *KRAS* mutation identified, 21 detected in both tissue and cfDNA, 65 detected in cfDNA alone, and three detected in tissue alone (Supplementary Table S2).

Tissue PD-L1 expression analysis

Overall 199 patients underwent tumor tissue testing for PD-L1 expression and 127 patients (63.8%) had a positive result (TPS $\geq$1%). In 16 patients (5.7%), PD-L1 was the only tissue biomarker assessed either by physician ordering choice or because tissue was QNS for genomic biomarker testing. Eleven of 16 patients were positive, six of whom had a TPS $\geq$ 50%. Two of 16 patients had a guideline-recommended biomarker identified in cfDNA, one was negative for PD-L1 expression, and one had a TPS of $\geq$1% (patient 97). In a total of 34 patients (12.1%), PD-L1 tumor expression cooccurred with a guideline-recommended biomarker, *EGFR* mutation, 15; *ALK* fusion, 4; *ROS1* fusion, 1; *BRAF* V600E mutation, 1; *ERBB2* mutation, 2; *MET* amplification, 7; MET exon 14 skipping variant, 6. Over half of patients (18/34; 52.9%) with PD-L1 expression co-occurring with a guideline-recommended biomarker had a TPS $\geq$ 50% (Supplementary Table S4).

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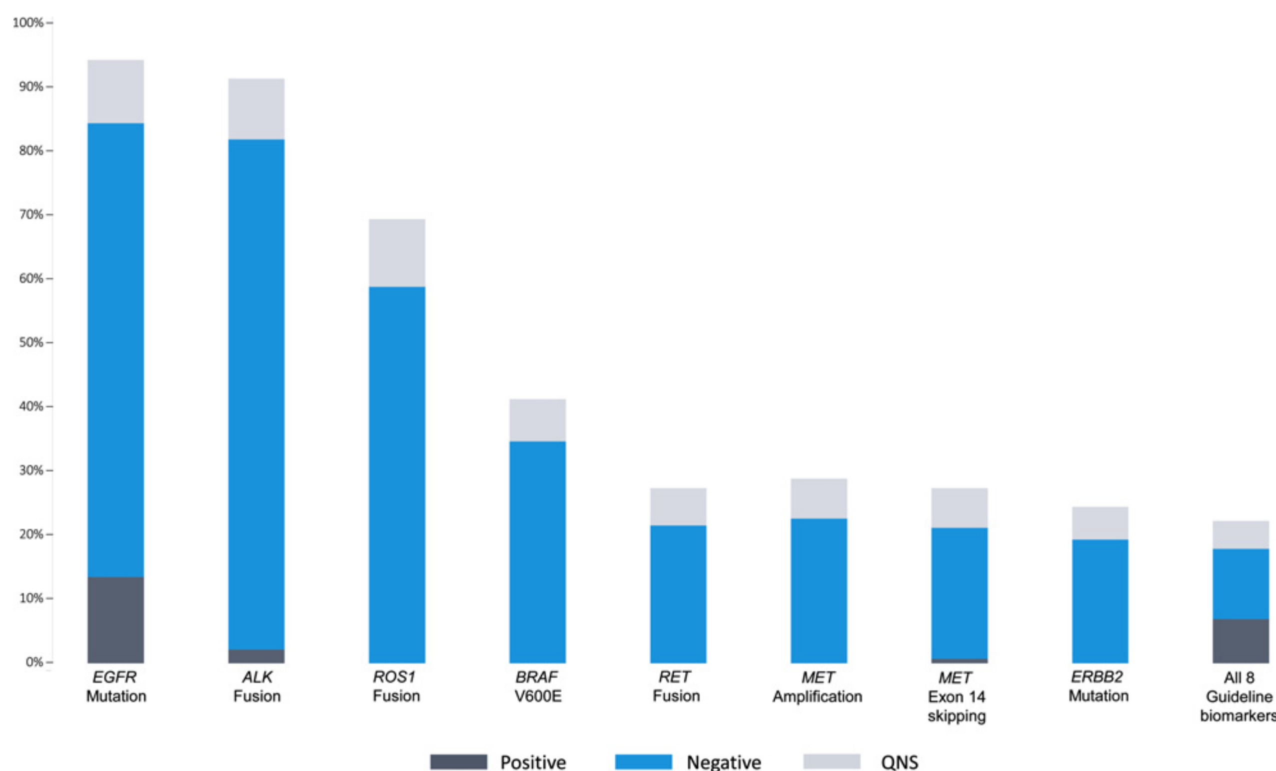

Figure 2.

Percentage of patients with completed tissue assessment for the guideline-recommended biomarkers. Only 18% of patients had complete tissue genotyping for all eight guideline-recommended genomic biomarkers. Most patients completed testing for *EGFR* mutations and *ALK* fusions (83%, 80%), followed by *ROS1* fusions (58%). Complete testing for *BRAF* V600E mutation (35%), *RET* fusions (22%), *MET* amplifications (23%) and exon 14 skipping alterations (22%), and *ERBB2* (HER2) mutations (20%) was rare. The QNS rates demonstrate where testing was attempted but was QNS for the biomarker of interest. For the eight guideline-recommended biomarker category, QNS denotes where tissue was QNS for all eight recommended biomarkers.

Continuous improvements in cfDNA assay performance

Because of the longitudinal nature of this study, we were able to assess the clinical impact of assay modifications over time. Modifications to the gene fusion detection algorithms enabled the current version of the clinical BIP to detect one of the three cfDNA *ALK* gene fusion false negatives in this study. Other assay feature modifications delivered over the course of the study included long indel detection, a rare but important category of *MET* exon 14 skipping alterations, and amplification aneuploidy discrimination. Of the 15 *MET* amplifications identified in cfDNA, the updated cfDNA BIP was able to differentiate 10 aneuploid samples from five samples with focal *MET* amplification (Supplementary Table S2), which is potentially important as clinical benefit of *MET* amplification–directed therapy for patients with aneuploidies are unproven.

Discussion

In the largest prospective, multicenter cfDNA study in previously untreated mNSCLC, we demonstrate that cfDNA genotyping utilizing a single, well-validated (17, 18), and highly sensitive comprehensive cfDNA test detects guideline-recommended biomarkers at a rate similar to tissue genotyping ($P < 0.0001$), meeting the primary study objective. This held true even when restricting the analysis to the patients who had attempted or completed tissue cfDNA genotyping for all eight guideline-recommended biomarkers. In this study, where SOC tissue genotyping was required for patient enrollment, the addition of cfDNA testing identified a guideline-recommended biomarker in 32% of patients (90/282) who otherwise would not have had guideline-complete genotyping, including those who were tissue QNS, incompletely genotyped, or negative for the guideline-recommended biomarker or *KRAS* alteration in tissue. Aggarwal and colleagues reported a similar rate of findings in their single-center study of patients with newly diagnosed and progressing mNSCLC. In those 323 patients, cfDNA testing identified a therapeutically targetable alteration in 35 patients (20.4%) including those who were negative, not assessed, or QNS for the biomarker of interest in tissue (20).

Undergenotyping, incomplete testing for all guideline-recommended biomarkers, continues to challenge the treatment of patients with mNSCLC. In a study of patients with newly diagnosed mNSCLC recruited from 15 community clinics, only 8% of 814 patients had complete tissue genotyping for all guideline-recommended biomarkers, with almost one-third not tested for *EGFR* mutations or *ALK* fusions, 75% untested for *ROS1* fusions, and more than 80% untested for the *BRAF* V600E mutation, *MET* amplifications or exon 14 skipping alterations, *RET* fusions, or *ERBB2* mutations (8). In a larger study conducted in 166 clinics, 25% of the almost 7,000 patients were not tested for *EGFR* mutations or *ALK* fusions (9). In this study, which represents an enriched

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Table 2A. Guideline-recommended genomic biomarker positivity by sample type

Guideline-recommended biomarker positivity by sample type		Tissue		
		Positive	Negative	Total
cfDNA	Positive	48	29	77
	Negative	12	193	205
	Total	60	222	282

NOTE: The NILE study met the primary endpoint of cfDNA noninferiority. cfDNA analysis identified one of the eight guideline-recommended biomarkers in 77 patients, while tissue analysis identified a guideline-recommended biomarker in 60 patients ($P = 0.0001$). For cfDNA, negative includes samples that were negative for the biomarkers of interest or those samples that were TND. For tissue, negative includes samples that were negative for all biomarkers of interest, QNS for all biomarkers, and/or biomarkers were not assessed.

Table 2B. Guideline-recommended genomic biomarker positivity by sample type in patients with all eight biomarkers attempted/completed in tissue and cfDNA

Guideline-recommended biomarker positivity by sample type in patients with attempted/completed genotyping for all eight biomarkers		Tissue		
		Positive	Negative	Total
cfDNA	Positive	19	3	22
	Negative	3	39	42
	Total	22	42	64

NOTE: Sixty-four patients attempted or completed assessment of all eight guideline-recommended biomarkers. Tissue identified a guideline-recommended biomarker in 22 patients. cfDNA identified a guideline-recommended biomarker in 22 patients.

population due to the requirement for SOC tissue genotyping to have been ordered, only 18% of patients had tissue genotyping for all eight guideline-recommended biomarkers, with 83% tested for *EGFR* mutations and 80% tested for *ALK* fusions. While practice patterns in this study are improved over previous years, the majority of patients remain undergenotyped. In contrast, cfDNA testing resulted in guideline-complete genotyping in 95% of patients.

Undergenotyping not only results in missed treatment opportunities but also in inappropriate use of therapies unlikely to be effective. This is particularly true with regard to immune checkpoint inhibitor use. In this cohort, PD-L1 expression analysis was the only tissue biomarker assessed in 5.7% of patients (16/282). In two of these 16 patients, a guideline-recommended biomarker was identified on cfDNA testing that was associated not only with superior clinical efficacy of targeted therapy (20–23) but also with decreased responsiveness to immune checkpoint inhibitors (6, 29–31). In 10 additional patients with PD-L1 expression, SOC tissue genotyping was negative, QNS, or not assessed, but cfDNA identified a guideline-recommended biomarker, seven of whom had a PD-L1 TPS $\geq$50%. The literature consistently reports superior response rates from first-line treatment with targeted therapy versus immune checkpoint inhibitors in patients with cooccurring high PD-L1 expression and a therapeutically targetable driver. In these 12 patients, the lack of full genomic assessment obtained by comprehensive cfDNA genomic profiling may have led to the patient being treated with a less efficacious therapy. The results from this study demonstrating that 95% of patients were able to be assessed for all guideline-recommended biomarkers, significantly faster, utilizing cfDNA, raises an interesting clinical algorithm of cfDNA for genomic biomarker assessment, preserving tissue for assessment of PD-L1 overexpression. This study illustrates that while the detection rate for cfDNA genotyping was 80% (perhaps related to low shedding of tumor DNA in some patients), in the real-world setting, the detection rate for SOC tissue genotyping was lower due, primarily, to incomplete testing, tissue insufficiency, and several cases of false-negative tissue tests.

One key limitation to this study is that, while cfDNA testing utilized a single platform, tissue genomic assessment was not standardized but was instead left to physician's discretion SOC, which included a variety of methodologies, including PCR, FISH, IHC, and/or NGS. As only 18% of patients successfully underwent comprehensive tissue genomic profiling, many alterations that were identified in cfDNA alone were, in fact, a result of incomplete tissue genotyping due to methodology choice and/or tissue testing failure as opposed to analytic discordance between the tests. As part of the study design, providers were specifically instructed to not make any changes to their SOC tissue genotyping practices; however, we cannot rule out the possibility that the receipt of a cfDNA clinical result may have influenced the decision to pursue further tissue genotyping in instances of sequential testing. Moreover, these findings may not apply to other cfDNA tests that are less

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Table 2C. Comparison of prevalence of biomarkers identified in cfDNA and tissue as compared with The Cancer Genome Atlas

		cfDNA		Tissue	
Guideline-recommended biomarkers	TCGA	Percent of total cohort	Frequency of alteration (%) in those with completed testing for biomarker of interest	Percent of total cohort	Frequency of alteration (%) in those with completed testing for biomarker of interest
EGFR mutation	11.3%	15.2%	16.0%	14.2%	17.3%
ALK fusion	1.3%	2.1%	2.2%	3.2%	4.0%
ROS1 fusion	1.7%	0.0%	0.0%	0.7%	1.2%
BRAF mutation (V600E)	7.0%	0.7%	0.7%	0.7%	2.1%
RET fusion	0.9%	1.1%	1.1%	0.0%	0.0%
ERBB2 mutation	1.7%	1.1%	1.1%	0.4%	1.6%
MET exon 14 skipping variant	4.3%	3.5%	3.7%	1.8%	7.5%
MET amplification	2.2%	5.3%	5.6%	0.4%	1.6%
MET focal amplification		1.8%	1.9%		
MET aneuploidy		3.5%	3.7%		
KRAS mutation	32.2%	31.6%	33.2%	8.5%	32.9%

NOTE: Biomarker frequency was calculated across the entire cohort ($N = 282$) and for those that had complete testing (positive or negative) for the biomarker of interest, 268 for cfDNA and for tissue see Table 3 and Supplementary Table S2.

Table 3. Comparison of tissue versus cfDNA results for the guideline-recommended biomarkers in newly diagnosed metastatic NSCLC with FDA-approved therapies, *EGFR* exon 19 deletion and L858R, *ALK* fusion, *ROS1* fusion, and *BRAF* V600E

		Tissue+	Tissue−	Tissue not assessed	Tissue QNS	Total		
EGFR exon 19 del	cfDNA+	18	0	0	1	19	Sensitivity	81.8%
	cfDNA−	4	201	19	25	249	PPV	100.0%
	cfDNA TND	0	11	1	1	13	Specificity	100.0%
	cfDNA cancelled	0	0	1	0	1	NPV	98.0%
	Total	22	212	21	27	282	Concordance	98.2%
EGFR L858R	cfDNA+	9	0	0	2	11	Sensitivity	90.0%
	cfDNA−	1	213	19	24	257	PPV	100.0%
	cfDNA TND	0	11	1	1	13	Specificity	100.0%
	cfDNA cancelled	0	0	1	0	1	NPV	99.5%
	Total	10	224	21	27	282	Concordance	99.6%
ALK fusion (original)	cfDNA+	5	0	0	1	6	Sensitivity	62.5%
	cfDNA−	3	207	27	25	262	PPV	100.0%
	cfDNA TND	1	10	2	0	13	Specificity	100.0%
	cfDNA cancelled	0	1	0	0	0	NPV	98.6%
	Total	9	218	29	26	282	Concordance	98.6%
ALK fusion (reanalysis)	cfDNA+	6	0	0	1	7	Sensitivity	75.0%
	cfDNA−	2	207	27	25	261	PPV	100.0%
	cfDNA TND	1	10	2	0	13	Specificity	100.0%
	cfDNA cancelled	0	1	0	0	1	NPV	99.0%
	Total	9	218	29	26	282	Concordance	99.1%
ROS1 fusion	cfDNA+	0	0	0	0	0	Sensitivity	-
	cfDNA−	2	151	85	30	268	PPV	-
	cfDNA TND	0	7	5	1	13	Specificity	100.0%
	cfDNA cancelled	0	1	0	0	1	NPV	98.7%
	Total	2	159	90	31	282	Concordance	98.7%
BRAF V600E mutation	cfDNA+	2	0	0	0	2	Sensitivity	100.0%
	cfDNA−	0	90	158	18	266	PPV	100.0%
	cfDNA TND	0	5	8	0	13	Specificity	100.0%
	cfDNA cancelled	0	0	1	0	1	NPV	100.0%
	Total	2	95	167	18	282	Concordance	100.0%

NOTE: Overall concordance across all four genes was greater than 98.2%, with a PPV of 100%. With continuous assay improvements, one cfDNA result originally reported as a false-negative for *ALK* fusion was identified as positive.

sensitive or less comprehensive. While this does limit certain comparisons, this design was critical to the fundamental question addressed by this study, whether a well-validated cfDNA test can match or even improve upon SOC tissue methods.

In conclusion, this prospective, multicenter study demonstrates that a comprehensive, sensitive, and specific cfDNA test used in patients with newly diagnosed mNSCLC successfully identifies guideline-recommended biomarkers at a rate, at least, as high as SOC tissue testing and returns these results significantly faster and for a significantly higher proportion of the population. Moreover, cfDNA-detected guideline-recommended biomarkers were invariably present in tissue, when tissue was successfully tested, reinforcing that cfDNA genotyping results may be used in clinical management in the same way tissue genotyping results are currently used. Finally, when modeled together, these results suggest that initial biomarker assessment using cfDNA rather than tissue ("blood first"), reserving tissue for PD-L1 IHC and reflex testing when cfDNA is negative for any known oncogenic driver muta-

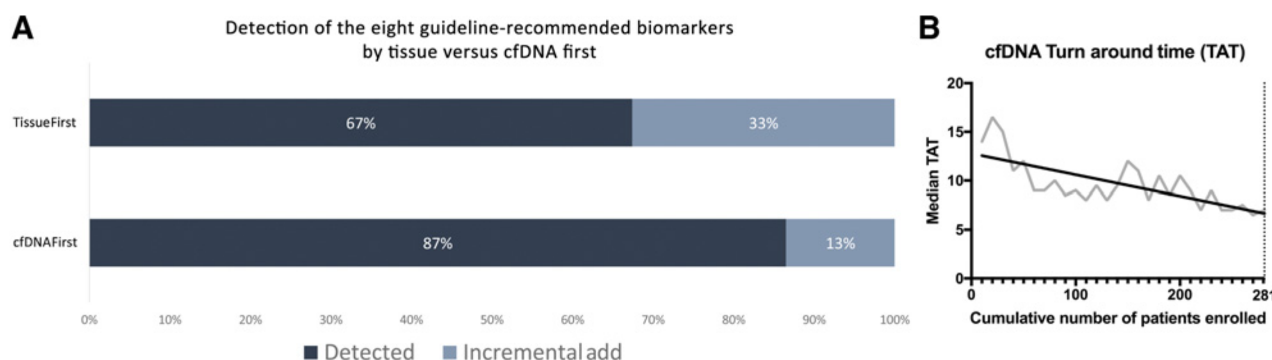

Figure 3.

A, Percentage of guideline-recommended biomarker detected by testing modality. All patients underwent both cfDNA testing and standard-of-care genotyping. In this cohort, leading with tissue testing, 67% of patients with a guideline-recommended biomarker would have been detected with 33% of patients identified on reflex cfDNA testing. If cfDNA was the first genomic testing modality, significantly more patients would be identified. **B,** TAT for complete cfDNA testing. The median TAT for the first 10 patients enrolled was 14 days (range, 11–30 days) versus the last 10 patients who had a median turnaround time (TAT) of 7 days (range, 5–9 days). One cfDNA test with a TAT of 2 days (test canceled) was an outlier and excluded.

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tions, improves biomarker discovery rate, TAT, and increases the number of patients with newly diagnosed mNSCLC who receive guideline-complete biomarker testing.

Disclosure of Potential Conflicts of Interest

N.B. Leighl reports receiving commercial research grants from Guardant Health. D.B. Daniel reports receiving commercial research grants from Guardant Health and other commercial research support from G1 Therapeutics, E R Squibb and Sons, Astra Zeneca, Boehinger Inglehemier, Genetech, Novartis Pharmaceuticals, Pfizer, Celgene Corporation, and Roche. K.L. Reckamp reports receiving commercial research grants from and is a consultant/advisory board member for Guardant Health. M.A. Villalona-Calero reports receiving other commercial research support from Guardant Health and Merck. D. Dix, V.M. Raymond, and J.I. Odegaard hold ownership interest (including patents) in Guardant Health. R.B. Lanman is an employee of Biolase, Inc., holds ownership interest (including patents) in Guardant Health, Biolase, Inc., and Forward Medical, Inc., and is a consultant/advisory board member for Forward Medical, Inc. V.A. Papadimitrakopoulou reports receiving other commercial research support from Eli Lilly, Novartis, Merck, Astra Zeneca, F Hoffman LaRoche, Nektar Therapeutics, Janssen, Bristol Myers Squibb, Checkmate, Incyte, and Guardant Health, and is a consultant/advisory board member for Guardant Health, Nektar Therapeutics, Astra Zeneca, Arrys, Merck, LOXO, Araxes, F Hoffman LaRoche, Janssen, Bristol Myers Squibb, Eli Lilly, Novartis, Takeda, Abbvie, Tesaro, Exelixis, Gritstone, Leads Biolabs, and Bolt Therapeutics. No potential conflicts of interest were disclosed by the other authors.

Authors' Contributions

Conception and design: V.M. Raymond, K.L. Reckamp, J.I. Odegaard, R.B. Lanman, V.A. Papadimitrakopoulou
Development of methodology: V.M. Raymond, D. Dix, R.B. Lanman
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): N.B. Leighl, V.M. Raymond, D.B. Daniel, S.G. Divers, K.L. Reckamp, M.A. Villalona-Calero, J.I. Odegaard, R.B. Lanman, V.A. Papadimitrakopoulou
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): N.B. Leighl, R.D. Page, V.M. Raymond, K.L. Reckamp, M.A. Villalona-Calero, D. Dix, J.I. Odegaard, R.B. Lanman, V.A. Papadimitrakopoulou
Writing, review, and/or revision of the manuscript: N.B. Leighl, R.D. Page, V.M. Raymond, , D.B. Daniel, S.G. Divers, K.L. Reckamp, M.A. Villalona-Calero, J.I. Odegaard, R.B. Lanman, V.A. Papadimitrakopoulou
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): V.M. Raymond, D. Dix, J.I. Odegaard, R.B. Lanman
Study supervision: V.M. Raymond, D. Dix, J.I. Odegaard, R.B. Lanman

Acknowledgments

This study was sponsored by Guardant Health.

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Received February 20, 2019; revised March 31, 2019; accepted April 11, 2019; published first April 15, 2019.

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