Utility of Genomic Assessment of Blood-Derived Circulating Tumor DNA (ctDNA) in Patients with Advanced Lung Adenocarcinoma

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Abstract

Purpose: Genomic alterations in blood-derived circulating tumor DNA (ctDNA) from patients with non–small cell lung adenocarcinoma (NSCLC) were ascertained and correlated with clinical characteristics and therapeutic outcomes.

Experimental Design: Comprehensive plasma ctDNA testing was performed in 88 consecutive patients; 34 also had tissue next-generation sequencing; 29, other forms of genotyping; and 25 (28.4%) had no tissue molecular tests because of inadequate tissue or biopsy contraindications.

Results: Seventy-two patients (82%) had ≥1 ctDNA alteration(s); among these, 75% carried alteration(s) potentially actionable by FDA-approved (61.1%) or experimental drug(s) in clinical trials (additional 13.9%). The most frequent alterations were in the TP53 (44.3% of patients), EGFR (27.3%), MET (14.8%), KRAS (13.6%), and ALK (6.8%) genes. The concordance rate for EGFR alterations was 80.8% (100% vs. 61.5%; ≤1 vs. >1 month between ctDNA and tissue tests; \(P = 0.04\)) for patients with any detectable ctDNA alterations. Twenty-five patients (28.4%) received therapy matching ≥1 ctDNA alteration(s); 72.3% (\(N = 16/22\)) of the evaluable matched patients achieved stable disease ≥6 months (SD) or partial response (PR). Five patients with ctDNA-detected EGFR T790M were subsequently treated with a third generation EGFR inhibitor; all five achieved SD ≥6 months/PR. Patients with ≥1 alteration with ≥5% variant allele fraction (vs. <5%) had a significantly shorter median survival (\(P = 0.012\)).

Conclusions: ctDNA analysis detected alterations in the majority of patients, with potentially targetable aberrations found at expected frequencies. Therapy matched to ctDNA alterations demonstrated appreciable therapeutic efficacy, suggesting clinical utility that warrants future prospective studies.

Clin Cancer Res; 1-11. ©2017 AACR.

Introduction

Lung cancer is by far the leading cause of death in the United States (1). Non–small cell lung cancer (NSCLC), adenocarcinoma subtype, is the most common histologic variant. Despite advances in early detection and standard of care, NSCLCs are often identified at advanced stages and have a poor prognosis. Adenocarcinoma is the most frequent histology in lifetime never-smokers, women, and younger adults (<60 years; ref. 2).

The classification and treatment paradigm for NSCLC changed drastically in 2004 with the discovery of the epidermal growth factor receptor (EGFR) mutations as a driver in NSCLC, occurring in about 10% to 30% of patients (3, 4). The presence of EGFR-activating mutations impacts not only the response rate, but also progression-free survival (PFS) in patients with NSCLC treated with EGFR tyrosine kinase inhibitors (TKIs; refs. 5–7). Anaplastic lymphoma kinase (ALK) fusions were later found to also play a driver role in NSCLC, though these anomalies are less frequent (about 4%–5% of NSCLC). Patients bearing ALK abnormalities respond well to small molecule ALK inhibitors such as crizotinib (8). In addition to EGFR and ALK, The National Comprehensive Cancer Network (NCCN) NSCLC guidelines recommend testing for 5 additional markers with associated targeted therapies. As the list of targetable alterations grows, tissue biopsy specimens are often inadequate for comprehensive evaluation and a subset is insufficient for any genomic profiling (9). For patients successfully genotyped and treated with targeted therapy, acquired resistance (e.g., EGFR T790M or ALK L1196M resistance mutation; refs. 10–12) ultimately limits the long-term utility of targeted therapies despite impressive initial response rates. Second-line therapies targeting the resistance mutations (e.g., osimertinib or alectinib) are becoming available but require contemporary tumor profiling to identify the acquired mutations.

Genomic profiling of circulating tumor DNA (ctDNA) provides an attractive alternative to repeat biopsy in patients whose tissue is insufficient in the first line and at progression. These noninvasive liquid biopsies are increasingly entering the clinical setting for patients with NSCLC. Indeed, because ctDNA tests require only a small amount of blood, they are far easier to obtain and less...
Translational Relevance

Genomic profiling of circulating tumor DNA (ctDNA) provides an attractive alternative over repeat invasive biopsy in patients whose tissue is insufficient in the first line and at progression. Noninvasive liquid biopsies are increasingly entering the clinical setting for patients with non–small cell lung adenocarcinoma (NSCLC) and while there is a growing body of literature describing targeted ctDNA performance for specific alterations assayed in association with investigational drug clinical trials, there are little data evaluating the performance and clinical outcomes of multigene ctDNA assays in clinical use. In this clinical use study, ctDNA analysis detected alterations in the majority of NSCLC patients, with potentially targetable aberrations found at expected frequencies, and therapy matched to ctDNA alterations demonstrated appreciable therapeutic efficacy, suggesting clinical utility. Further prospective validation of an approach that uses ctDNA to guide management is warranted.

Materials and Methods

Patients

We retrospectively reviewed the clinicopathologic and outcome data of 88 consecutively tested patients with lung adenocarcinoma followed at UC San Diego Moores Cancer Center, for whom molecular testing (ctDNA test) had been performed on their plasma (August 2014 until October 2015). Data were abstracted from the electronic medical record and performed in accordance with the Declaration of Helsinki. For all patients, this study (PREDICT-UCSD [Profile Related Evidence Determining Individualized Cancer Therapy; NCI02478931]) was performed and consents obtained whenever necessary after approval by UCSD Institutional Review Board guidelines.

Sequencing

Digital Sequencing of ctDNA (DNA) in all patients was performed by Guardant Health, Inc. (Guardant360, Redwood City, CA; http://www.guardanthealth.com/guardant360/), a Clinical Laboratory Improvement Amendment (CLIA)-certified and College of American Pathologists (CAP)-accredited clinical laboratory. The analytical and clinical validation of Guardant360 was conducted in conformance with evidentiary standards established by the Standards for Reporting of Diagnostic Accuracy (STARD), Reporting of tumor MARKer Studies (REMARK), Evaluation of Genomic Applications in Practice and Prevention (EGAPP), and the recent Next-generation Sequencing: Standardization of Clinical Testing (Nex-StoCT) biomarker guidelines (17).

As described in Lanman and colleagues (17), 5 to 30 ng of ctDNA was isolated from plasma (two 10 ml Streck tubes drawn for each patient) and sequencing libraries were prepared with custom in-line barcode molecular tagging and complete sequencing at 15,000x read depth (~4,000 unique double-stranded cfDNA fragments, each represented by 3–5 sequencing reads). The current panel utilizes hybrid capture followed by NGS of the critical exons in a panel of 70 genes and reports all four major types of genomic alterations (point mutations, indels, fusions, and copy-number amplifications). Post-sequencing bioinformatics matches the complementary strands of each barcoded DNA fragment to remove false positive results (17). The variant allele fraction (VAF) is computed as the number of mutated DNA molecules divided by the total number (mutated plus wild-type) of DNA fragments at that allele; VAF is reported as a percentage. The majority of cell-free DNA is wild-type (germline); thus, the median VAF of somatic alterations is <0.5%.

For 40 patients, a 54-gene panel was used, which identified potential tumor-related alterations in 54 cancer-related genes (Supplementary Table S1), including copy-number amplifications in ERBB2, EGFR, and MET (indels and fusions were not detected as part of this panel). For 47 patients, a 68-gene version of the original panel (expanded to all four major alteration types) was used, and for one patient, the most recent 70-gene panel version (further expanded to amplifications in 18 genes and fusions in 6 genes) was applied (Supplementary Tables S2 and S3). Only nonsynonymous alterations were included in our analysis [variants of unknown significance (VUSs) did not include synonymous alterations; Supplementary Table S4].

In addition, 34 of 88 patients (42%) who had ctDNA results also had CLIA/CAP-accredited next-generation sequencing (NGS) performed on tumor tissue (FoundationOne; http://www.foundationone.com). An additional 29 patients had various other types of tissue molecular testing.

Concordance rate

For the 34 patients who had both types of tests (tissue NGS and plasma ctDNA testing that covered the same genes and alteration types revealed in the tissue NGS), we assessed the concordance for EGFR alterations and corresponding kappa statistics (Supplementary Table S5), which are a conservative measurement of relative agreement that takes into account agreement by chance. Kappa values range from $\kappa = 1$ (perfect agreement) to $\kappa = 0$ (no agreement other than would be expected by chance; ref. 18). We also performed this analysis excluding the patients that had “no alterations detected” as the lack of alterations could be because no ctDNA was detected in the plasma ($N = 26$ patients included). In these 26 patients, we also conducted a concordance analysis including all alterations that were potentially detectable by both tests (i.e., included in both gene panels).

Matched therapy and actionability

Treatment was considered “matched” if at least one agent in the treatment regimen targeted at least one abnormality or pathway component aberrant in a patient’s molecular profile either prospectively or retrospectively.
Actionability implies that the protein product of a genomic abnormality can be affected by a specific targeted drug (19). A potentially actionable alteration was defined as an alteration that was either the direct target (such as an EGFR inhibitor targeting an EGFR mutation), or a pathway component (such as an mTOR inhibitor for a PIK3CA mutation (because mTOR is downstream of PIK3CA)) that could be targeted by at least one FDA-approved or investigational drug in a clinical trial. Actionability was considered at the variant level; variants of unknown significance (functional consequences and clinical significance of these gene variants are not established, as opposed to characterized alterations) were considered nonactionable.

Statistical analysis
When appropriate, median and 95% confidence intervals (95% CI) or range were reported. The following clinical endpoints were considered: (i) rate of [stable disease (SD) ≥ 6 months/partial response (PR)/complete response (CR)]; (ii) progression-free survival (PFS); and (iii) overall survival (OS). SD, PR, or CR was determined per assessment of the treating physician. PFS and OS were defined as the time from the beginning of therapy to progression (for PFS)/death event (for OS) or the time to last follow-up. For the analysis examining the correlation between the percentage of ctDNA and survival, we defined OS as the time from the ctDNA test results to death or last follow-up date for patients who were alive (the latter were censored on that date). The cutoff date of the analysis was December 28, 2015; all patients who were progression-free (for PFS) or alive (for OS) as of the date of analysis were censored on that date unless their date of last follow-up was earlier, in which case that was the date of censoring.

Whenever appropriate, χ² tests were used to compare categorical variables and the nonparametric Mann–Whitney U test to compare two groups on one continuous variable. Binary logistic regressions were performed for categorical endpoints and multiple linear regressions for continuous variables. PFS and OS were analyzed by the Kaplan–Meier method (20), and the log-rank test was used to compare variables. Statistical analysis was performed by MS with IBM Statistics SPSS version 22.0.

**Results**

**Patient characteristics**

Our population was comprised of 88 patients with NSCLC (adenocarcinoma) who had an NGS ctDNA test performed on their plasma. Patient’s median age at diagnosis was 66.2 years old (range, 36.3–89.5 years). There was a predominance of women over men [n = 58 (66%): n = 30 (34%)]. The majority of patients were smokers (N = 50, 56.8%); 36 patients were never smokers (40.9%), and for 2 patients the smoking status was unknown (Table 1). Twelve patients had intrathoracic disease while the others had metastatic disease.

**ctDNA sequencing results**

The median time from diagnosis to the ctDNA test results was 11.7 months (95% CI, 6.2–15.8 months; range, 0–123 months), and the median time from the blood draw to results (turnaround time) was 15 days (95% CI, 14–15 days; range, 8–35 days). In our 88 tested patients, a total of 257 alterations were identified, with the majority being point mutations (n = 205, 79.8%), followed by amplifications (10.5% of alterations (27/257)). The most frequent alterations (all nonsynonymous alterations considered) discerned were in the following genes: TP53 (44.3% of patients), followed by EGFR (27.3%), MET (14.8%), KRAS (13.6%), and ALK (6.8%; Fig. 1A and B). The majority of patients had mutations; 17 demonstrated amplification and 3 showed rearrangements.

Of the total, 72 patients (82%) had at least one detectable ctDNA alteration. Among these 72 patients, 75% (N = 54 patients; 61.4% of the total 88 patients) had ≥1 anomaly that was potentially actionable (by FDA-approved drugs; 61.1%) or by an experimental drug(s) in a clinical trial (if an FDA-approved drug was not available; an additional 13.9%; Fig. 1C). When we examined the differences between the most frequent alterations detected in smokers versus nonsmokers, we observed that KRAS aberrations were only detected in smokers in our population (24% (12/50) versus 0% (0/36) in nonsmokers, P = 0.001); TP53 alterations were also more frequent in smokers, with a statistically significant difference when focusing on characterized alterations only (50% versus 25% in nonsmokers, P = 0.026; Fig. 2). In addition, characterized EGFR alterations were the most frequent alterations found in nonsmokers (36% (13/36) versus 16% (8/50) in smokers, P = 0.043), Fig. 2).

In the 88 patients, the median number of alterations was two (average 2.7; range, 0–21). Specifically, 19.3% of patients had one alteration, and 62.5% had at least two alterations. In an univariable analysis, smokers had a higher median number of alterations (2 vs. 1.5, P = 0.030), and it remained a trend in the multivariable analysis (P = 0.083). The presence of TP53 and MET alterations was an independent predictor of a higher number of alterations in the multivariable analysis, with P = 0.002 and P = 0.021, respectively (Table 2).

There was no difference in the rate of ctDNA detection between those patients with and without metastases. Two of the 12 (16%) patients with intrathoracic disease alone (no metastases) had no alterations; 14 of the 76 patients (18%) with

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<td><strong>Number of patients with ≥1 alteration</strong></td>
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*a Included Hispanic, N = 2; African American, N = 1, Middle Eastern, N = 1, and unknown, N = 8.

bPatients whose medical chart indicated that they smoked <100 cigarettes in their lifetime were considered never-smokers for the purpose of this study.

*Categories defined at the variant level; variants of unknown significance (functional consequences and clinical significance of these gene variants are not established, as opposed to characterized alterations) were considered nonactionable.*
Figure 1.
Frequent alterations and potential actionability. A, Most frequent alterations given as a percentage of all (\(N = 88\)) patients. Some patients had multiple alterations in the same gene. Only genes with alterations in \(\geq 3\) patients are displayed; all alterations were nonsynonymous. Alterations were also identified in the following genes (albeit in one or two patients): APC, BRCA1, CDKN2A, CTNNB1, FGFR1, KIT, MAP2K1, RAF1, RHOA, ATM, CCND1, CCNE1, CDK6, ERBB4, IDH2, JAK2, MAP2K2, MLH1, NFE2L2, NRAS, PTEN, RBL, RET, RIT1, SRC, and VHL. *Alterations of unknown significance (variant of unknown significance; VUSs) versus characterized mutations (indels, amplifications, fusions, and single nucleotide variant (SNV) point mutations) were considered at the variant level. (”–” indicates variant type not assessed for that gene.) B, Oncoprint of the most frequent alterations (\(N = 88\) patients). Only the most frequent alterations were represented (at least 10 patients with the alteration). We also included ALK for its driver role in adenocarcinoma, even though there were less than 10 patients. Dark blue are characterized alterations, and light blue represents variants of unknown significance. C, Potential actionability of the detected alterations. Pie chart representing the frequency of patients with actionable alterations in patients with alterations identified (\(N = 72\)). Fifty-four patients (75% of patients with alterations) had potentially actionable alterations (61.4% of all 88 patients). The 18 patients with nonactionable alterations carried only variant(s) of unknown significance (VUS) and were therefore considered nonactionable by definition.
Patients whose medical chart indicated that they smoked metastatic disease had no alterations. There was also no difference in alteration detection rate between those with ≤1 prior therapies versus those with >1 prior therapies, with no alterations detected in about 18% of each group. ctDNA levels were also not significantly different.

Concordance analysis

**EGFR alterations.** Thirty-four patients (38.6%) also had a multi-gene panel NGS tissue testing (see Materials and Methods; ref. 21). The median time interval between the blood draw and tissue biopsy was 0.8 months (and hence this was used as a cut point for discerning differences between tissue and blood based results). The overall concordance rate for **EGFR** alterations was 76.5%. (Concordance rate remained similar (75%) when **EGFR** tissue testing of non-NGS types were included in the calculations). The concordance rate was 88.2% compared with 64.7% when the time interval was ≤0.8 versus >0.8 months between the blood draw and the tissue biopsy for NGS (P = 0.22; Supplementary Table S5 and Fig. 3).

We also performed this analysis excluding the patients that had "no ctDNA alterations detected," as the absence of alterations could be because no tDNA was detected in the plasma. In those 26 patients, the median time interval between the blood draw and tissue biopsy was 1.0 months, and the overall concordance rate was 80.8% for **EGFR** alterations. The concordance rate was 100% in patients for whom the time interval between the blood draw and tissue biopsy was ≤1.0 months (corresponding to the median) compared with 61.5% when this time interval was >1.0 month (P = 0.04; Supplementary Table S5; Fig. 3A). Of the total, there were 7of 26 patients with positive concordance, 14 of 26 patients with negative concordance and 5 of 26 patients with discordance (2 patients were positive for **EGFR** alterations in ctDNA only and 3 were positive for EGFR alterations in tissue only).

**Overall concordance.** We also performed an analysis including all the alterations in these 26 patients (we included alterations in common in the gene panel of both the ctDNA and tissue testing, N = 62 alterations). Thirty-three alterations were found in both tests, for a positive concordance rate of 53.2%. In total, 52 alterations were found in the tissue and 43 in the plasma, leading to a sensitivity of 63.5% (33/52) when the tissue is used as a comparator and 76.7% (33/43) when the plasma is used as the comparator. Nineteen alterations were found in the tissue only and 10 in the plasma only. In 70% of patients (18/26), we found at least one alteration in common in their ctDNA and tissue testing. Of interest, when we classified the alterations in the plasma into a %ctDNA ≥ 1% or <1%, we found that 100% of the 21 alterations with a %ctDNA ≥ 1% were also identified in the tissue compared with 71.4% (10/14) for the 14 alterations with a %ctDNA < 1%, P = 0.0191 (Fisher exact test; N = 8 alterations were amplifications and were not included in this analysis). We also found in sub-analyses that the concordance rates were higher for the alterations tested in pretreated patients (64.7% vs. 48.9%; median of 1.3 months was used as cutoff), although these differences were not statistically significant.

Analysis of patient outcome

Patients had a median of one prior line of therapy (range, 0–13) before ctDNA testing. Of the 88 patients, 25 (28.4%) had an alteration in their ctDNA results that was targeted by a matched therapy, with 20 of 25 patients (80%) receiving a therapy directed to **EGFR** (n = 18; activating **EGFR** mutations [mostly exon 19, but also L858R in exon 21 or G719X in exon 18 for first/second-generation inhibitors; **EGFR** T790M for third-generation inhibitors]) or **ALK** alterations (n = 2; the other targeted alterations included **KIT**, **PDGFRα**, or **BRAF**).
Sixty-three patients (71.6%) did not receive a treatment that matched their ctDNA results (including 36 patients who were not evaluable for treatment) mainly because they died, were lost to follow-up before ctDNA results, or were not treated yet (Supplementary Fig. S1).

Of the 25 matched patients, all received FDA-approved drugs (15 (60%) on-label, 5 (20%) off-label) except for the patients (N = 5) matched to EGFR T790M. These patients received osimertinib, which was being used in a clinical trial at the time, but has since been FDA approved. Twenty-two patients were evaluable for response (3 patients were too early to assess); 16 (72.3%) achieved stable disease (SD) ≥ 6 months (N = 5) or partial response (PR; N = 11). In comparison, 12 of 20 (60%) of the never matched evaluable patients (N = 27 patients never matched; 7 too early to assess) achieved SD ≥ 6 months/PR (P = 0.515 for matched versus never matched).

The median PFS for the 25 matched patients was 14.7 months [95% CI, 3.7–25.7; 15.3 months for nonsmokers vs. 8.1 months for smokers (P = 0.202; not significant)] and was longer in patients carrying EGFR alterations versus not (medians: 17.6 vs. 5.1 months, P = 0.009). Comparing matched and never matched patients [the treatment with the longest PFS for both matched and never-matched was chosen (prospectively or retrospectively)], the median line of therapy in the recurrent or metastatic setting was 2 (range, 1–10) for the longest PFS in matched patients versus 1 (range, 1–3) for the longest PFS in never matched patients (P = 0.015). [For the matched patients, the longest PFS refers to longest PFS on matched therapy (in case the patient received more than one regimen of matched therapy); for the never matched patients, we chose the longest PFS on any therapy that the patient may have received (including chemotherapy)]. The median longest PFS was 14.7 months for the matched patients (n = 25) vs. 7.8 months for the never-matched patients (n = 27), with P = 0.280. The median OS was not reached for the matched patients (n = 25; median follow-up time of 18.6 months) versus 36.7 months for the never-matched patients (n = 27; P = 0.928; PFS and OS survival were calculated from the initiation date of treatment with the longest PFS for both the matched and the never-matched groups).

EGFR T790M and third-generation EGFR inhibitors. There were 5 patients who were given a third-generation EGFR inhibitor after their ctDNA results showing the EGFR T790M resistance mutation, and all five achieved SD ≥ 6 months (N = 3)/PR (N = 2). The PFS for these 5 patients were 3.2+, 7.2, 8.1+, 14+, and 15+ months. All 5 also had a tissue biopsy showing EGFR T790M at some point in their course.

Levels of ctDNA and survival. Among the 88 patients, the median overall survival (calculated from the ctDNA results until death or last follow-up date) was not reached at a median follow-up of
A, EGFR concordance between the ctDNA and tissue tests. We included patients with EGFR alterations (all EGFR alterations considered) that were tested for in both the ctDNA and tissue gene panels. Overall concordance included positive (+) and negative (−) concordant cases, i.e., when both the tissue and the ctDNA were positive or negative. Thirty-four patients had both ctDNA and a common tissue molecular test. For these 34 patients, the median time interval between tissue biopsy to blood draw was 0.8 months. Twenty-six patients had both ctDNA and a common tissue molecular test, and had alterations in their ctDNA. For these 26 patients, the median time interval between tissue biopsy to blood draw was 1.0 months (we performed this analysis excluding patients that had “no alterations detected” as it could be because no ctDNA was detected in the plasma). P values compare the percentage of overall concordance between patients with biopsy interval time ≤ or > median (two-sided χ² test). Alterations were examined at the gene level (i.e., no distinction in the location of alteration within gene).

B, Venn diagrams representing the positive concordance of EGFR alterations detected in the ctDNA and tissue tests. The upper part of the chart displays the results in the 34 patients who had a ctDNA test and tissue molecular testing. For these 34 patients, the median biopsy interval time (between blood draw and tissue biopsy) was 0.8 month. The bottom part of the chart displays the results in the 26 patients who had alterations detected in their ctDNA test and had tissue molecular testing. For these 26 patients, the median biopsy interval time (between blood draw and tissue biopsy) was 1.0 months. All EGFR alterations were considered.
In this study investigating the use of liquid biopsies in 88 patients with lung adenocarcinoma, we show that the most frequent alterations detected were in TP53 (44.3% of patients), *EGFR* (27.3%), *MET* (14.8%), *KRAS* (13.6%), and *ALK* (6.8%) genes. In comparison, The Cancer Genome Atlas (TCGA) showed similar rates of tissue alterations of 46% (TP53), 17% (*EGFR*), 12% (*MET*), 36% (*KRAS*), and 8% (*ALK*; ref. 22). For the *EGFR* and *KRAS* genes, the rates differed between the ctDNA and TCGA tissue. The reasons for the variance could be related to the fact that TCGA was performed on untreated patients, while our population was previously treated (22, 23). Further, resistance mutations such as *EGFR* (T790M) or *KRAS* emerge after matched therapy and would be less likely to be present in the TCGA cohort. Other reasons for these differences could include, but not be limited to, factors related to tumor heterogeneity and the propensity of tumor DNA to shed into the blood, as well as the fact that the local area has a relatively high Asian population and low smoking rates. We also observed that *KRAS* alterations were only found in smokers (24% vs. 0%; *P* = 0.001) and that characterized *EGFR* alterations were more common in nonsmokers (36% vs. 16%, *P* = 0.04), consistent with what was previously described in analyses performed on tissue (24, 25).

Overall, 82% of patients had at least one detectable alteration (s), and for 75% of them (61.4% of the total 88 patients), the alterations were potentially actionable by an FDA-approved drug or an experimental agent in a clinical trial. This actionability rate is within the same range of the results of our prior study that detected alterations in the plasma of patients with diverse cancer types (17).

We also examined the concordance rate of *EGFR* alterations for patients who had both ctDNA and multigene panel NGS tissue testing (21). The concordance rates were in the same range (61.5%–100%; Supplementary Table S5) of those previously reported (about 67% to 99%; refs. 22–24). Further, we observed that the concordance rate was increased when the time interval between the blood draw and the tissue biopsy was shorter, which can be explained by the dynamic changes occurring in the tumor, especially when the patient is receiving treatment (26–30). Of interest, a focused analysis on the positive cases (Fig. 3B) revealed that both tests could independently detect alterations not found in the other test, highlighting the clinical value and complementary nature of both techniques.

The overall analysis of the concordance, including all alterations potentially detectable by both tests, also showed that some alterations were found in tissue or ctDNA only and resulted in a specificity of 63.5% when the tissue was used as a reference and 76.7% when the plasma was used as a reference. In order to observe more accurate concordance rates, the comparison of tissue and plasma results should include samples temporally concurrent and pretreatment so that ctDNA is not suppressed to undetectable levels, and only the genetic footprint in common between the two tests can be compared. Several publications meet these study design criteria (two pan-cancer, one each in NSCLC, breast, colorectal, anaplastic thyroid and pancreatic cancer) and all show high diagnostic concordance for concurrent samples (99%, 86%, 100%, 100%, 100%, 72%, and 98%, respectively; refs. 17, 27, 29, 31–34). Reasons that results might be positive in tissue but not in ctDNA include the following: suppression of ctDNA shedding by treatment; and the fact that not all tumors shed DNA into the bloodstream even pretreatment or at progression. On the other hand, ctDNA may be

**Discussion**

Overall survival (OS) analysis. Kaplan–Meier curves comparing the OS (from ctDNA test results until death or last follow-up date) for patients with a percentage of ctDNA < 5% (n = 62; median not reached) versus ≥5% (n = 18; median 4.2 months), *P* = 0.012. Eight patients died before their ctDNA results were available and were not included in this analysis.
positive when tissue is not because ctDNA reflects shed DNA from multiple metastases whereas a biopsy typically samples a single lesion. Furthermore, gene mutations acquired under treatment pressure may not be found in archival tissue (temporal separation). There may also be technical differences between the tests. Considering the distinct advantages and disadvantages of each technique may therefore be pertinent in the clinical setting. Tissue biopsies are critical in the first line to define histopathologic cancer diagnosis and genotyping both, and at progression, especially when ctDNA is negative. Conversely, ctDNA may be useful at progression to reduce the need for repeat biopsies.

Some genomic alterations found in ctDNA may not be found in sequenced tissue samples because of tumor heterogeneity or because the plasma sequencing method is more sensitive and will pick up mutations at lower allele fractions than with tissue NGS. In Goyal and colleagues (35) five FGFR2 resistance mutations were found in a cholangiocarcinoma patient on targeted FGFR2 therapy, but only one was detected on liver biopsy. At warm autopsy, three of the five ctDNA-detected FGFR2 mutations were confirmed in tissue and all five were found to drive resistance on functional modeling (35). In Rozenblum and colleagues (36), ctDNA NGS changed treatment in 32% of patients negative for NSCLC genomic targets via local tissue-based EGFR qPCR and ALK FISH testing, including two EGFR driver mutations not detected in tissue (36). Of interest in this regard, it has previously been shown that matched therapy based on ctDNA findings of EGFR L858R, exon 19 deletion, and T790M produced responses similar to those based on tissue biopsy-based assays, even in cases when ctDNA was positive and tissue was wild-type for the EGFR mutation (37–39). In our study, we found that all ctDNA-detected EGFR T790M alterations were confirmed (100%) in corresponding tissue.

One goal of our study was to investigate the outcome of patients with lung adenocarcinoma who were treated (either prospectively or retrospectively) with a therapy matching alteration(s) detected in their ctDNA test. At the time of our analysis, 25 patients (28.4%) received a matched therapy consistent with their ctDNA results. When considering only patients carrying EGFR characterized mutations (N = 19/88), 95% (18/19) received matched therapy. Seventy-three percent (16/22 patients evaluable for response) of matched patients achieved SD ≥ 6 months/PR. Response correlated with PFS, as the 16 patients with SD ≥ 6 months/PR had a significantly prolonged median PFS as compared with nonresponders (17.6 months vs. only 1.8 months, \( P < 0.0001 \)). Other studies have described the benefit of matching patients using a biomarker. Indeed, three recent meta-analyses looking at trials leading to FDA approval (40), phase II studies (41) and phase I studies (42) concluded that the strategy of selecting patients with a biomarker led to improved response rates, longer PFS and OS. In addition, our previous retrospective analysis performed on more than 300 patients with various malignancies and using NGS tissue testing revealed that the strategy of matching patients led to increased rates of SD ≥ 6 months/PR/CR and longer PFS (43).

In our population, we found that 37.5% of patients with EGFR alterations (9/24) carried the EGFR T790M resistance mutation, consistent with previous reports (34, 35, 42). Not surprisingly, these 9 patients had previously been treated with EGFR tyrosine kinase inhibitors. The initial detection of EGFR alterations in patients with lung adenocarcinoma as well as the monitoring during treatment for the appearance of resistance mutations may be an important application of liquid biopsies. This is particularly true because obtaining serial tissue samples post EGFR tyrosine-kinase inhibitors is challenging in clinical practice due to the invasiveness of the procedure and the frailty of patients with metastatic disease.

In addition, tissue biopsies may not reflect tumor heterogeneity if multiple metastatic sites are involved. Indeed, in our study, 25 of 88 patients (28.4%) only had ctDNA results available (no other molecular tissue testing), with the most common reasons being inadequate tissue and/or the potential risk for repeat biopsy.

Several studies have monitored EGFR alterations in the plasma of patients with NSCLC during their disease course (12, 44–48). For instance, Zheng and colleagues (44) examined 318 patients with advanced or recurrent NSCLC who were receiving a tyrosine kinase inhibitor, and focused on the 117 patients who acquired resistance. EGFR T790M ctDNA was detected in the plasma of 55 of 117 patients (47%). In addition, they showed that almost half of the T790M ctDNA-positive patients were identified at a median time of 2.2 months prior to clinically progressive disease (PD). Similarly, Sueoka-Aragane and colleagues (45) looked at sequential examinations in 89 patients with NSCLC. They detected EGFR T790M in 40% of patients who developed progressive disease after treatment with a kinase inhibitor. These studies highlight the feasibility of monitoring EGFR mutation dynamics in serial plasma samples from NSCLC patients receiving TKI therapy.

Matched versus never-matched patients had higher rates of SD ≥ 6 months/PR (72.3% vs. 60%; \( N = 22 \) vs. 20 evaluable) and longer median PFS (14.7 vs. 7.8 months; \( N = 25 \) vs. 27 evaluable), although the differences were not statistically significant, perhaps due to the limited number of patients. Interestingly, there were five patients whose ctDNA demonstrated the EGFR T790M resistance mutation who were then given a third-generation EGFR inhibitor, and all five achieved SD ≥ 6 months (\( N = 3 \))/PR (\( N = 2 \)).

Lastly, our study demonstrated that patients with at least one alteration with a percentage of ctDNA ≥ 5% had a shorter overall survival (median 4.2 months versus not reached at a median follow-up of 7.5 months, \( P = 0.012 \); Fig. 4), consistent with a prior study performed on diverse cancer types (48) and various other studies describing the prognostic value of ctDNA, including in patients with lung cancer (33, 46–49).

Our study had several limitations. First, because of its retrospective and single center nature and small number of patients, there could be unknown biases that influenced our analysis. In addition, some of our patients did not have concurrent tissue and ctDNA biopsies, and the ctDNA test and tissue NGS are two different techniques each having its own advantages and disadvantages. On the other hand, the range of intervals between tissue and ctDNA tests allowed us to investigate the influence of time interval between the tissue biopsy and ctDNA test and to observe that a shorter time interval between these tests resulted in increased concordance rates. In future studies, it will be important to examine larger, well-timed cohorts for concordance in a wide range of genes. Another limitation was that a subset of patients (\( N = 16 \)) did not have detectable ctDNA. It is plausible that some tumors do not shed ctDNA. However, it is also conceivable that improvements in technology will eventually prove able to detect ctDNA in such patients. Alternative
techniques such as those that evaluate circulating tumor cells could also be assessed. Finally, response assessment in our study was performed by individual physicians rather than by centralized standardized review. Overall, larger prospective studies are needed to further define the value of ctDNA tests to inform treatment and their use as a tissue surrogate.

In summary, our study reveals the frequent detection of altered ctDNA in patients with NSCLC adenocarcinoma, including in difficult-to-biopsy patients. The concordance rate for EGFR alterations was 80.8% [100% vs. 61.5% (≤1 vs. >1 month between tests; P = 0.04)]. This confirms and strengthens the findings of Thompson and colleagues (29) who showed EGFR concordance rates of 79%, with a shorter time interval between tissue and blood collection associated with increased concordance (P = 0.038). Patients who received cognate therapies demonstrated a high rate of SD ≥ 6 months/PR. Indeed, all five patients with EGFR T790M resistance mutation detected in ctDNA and then treated with a third generation EGFR inhibitor achieved SD ≥ 6 months/PR. Importantly, high levels of ctDNA (≥5%) predicted for poor survival, suggesting clinical utility for ctDNA tests. It appears that several groups of patients could benefit from ctDNA evaluation, including those with hard-to-biopsy lesions, those whose biopsy tissue is exhausted for histopathology or immunohistochemistry testing, or those patients who had non-NGS tissue testing and no actionable alterations were detected (49). In addition, there are patients in whom serial monitoring for emergence of resistance mutations might be important (e.g., patients with EGFR mutations or ALK fusions who are treated with first-generation EGFR or ALK inhibitors, respectively). However, when ctDNA results in no alterations detected, a tissue biopsy would be recommended as some tumors may not shed sufficient DNA into circulation to be detectable. Further prospective validation of an approach that uses ctDNA to guide management and assesses the cost: benefit ratio of serial testing is warranted.

Disclosure of Potential Conflicts of Interest
S.P. Patel reports receiving speakers bureau honoraria from Boehringer Ingelheim and Merck, and is a consultant/advisory board member for Eli Lilly and Novartis. R. Lamman and A. Talasaz have ownership interests (including patents) in Guardant Health, Inc. R. Kurzrock is an employee of and has ownership interests (including patents) at CureMatch, Inc., is a consultant/advisory board member for Actuate Therapeutics and Xbiotech, and reports receiving commercial research grants from Foundation Medicine, Genentech, Guardant, Pfizer and Sequenom. No potential conflicts of interest were disclosed by the other authors.

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Grant Support
This study was funded in part by the Joan and Irwin Jacobs Fund philanthropic fund and by NCI grant P30 CA016672 (R. Kurzrock, rkurzrock@ucsd.edu).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received October 6, 2016; revised January 7, 2017; accepted May 18, 2017, published OnlineFirst May 24, 2017.

References
Use of ctDNA in Patients with Advanced Lung Adenocarcinoma


Clinical Cancer Research

Utility of Genomic Assessment of Blood-Derived Circulating Tumor DNA (ctDNA) in Patients with Advanced Lung Adenocarcinoma


Clin Cancer Res  Published OnlineFirst May 24, 2017.

Updated version  Access the most recent version of this article at: doi:10.1158/1078-0432.CCR-16-2497

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