4-18-2021

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Moom R. Roosan

Isa Mambetsariev

Rebecca Pharaon

Jeremy Fricke

Hatim Husain

See next page for additional authors

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Authors

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CHEST

Usefulness of Circulating Tumor DNA in Identifying Somatic Mutations and Tracking Tumor Evolution in Patients With Non-small Cell Lung Cancer

Moom R. Roosan, PharmD, PhD; Isa Mambetsariev, BA; Rebecca Pharaon, BA; Jeremy Fricke, BS; Hatim Husain, MD; Karen L. Reckamp, MD; Marianna Koczywas, MD; Erminia Massarelli, MD, PhD; Andrea H. Bild, PhD; and Ravi Salgia, MD, PhD

BACKGROUND: The usefulness of circulating tumor DNA (ctDNA) in detecting mutations and monitoring treatment response has not been well studied beyond a few actionable biomarkers in non-small cell lung cancer (NSCLC).

RESEARCH QUESTION: How does the usefulness of ctDNA analysis compare with that of solid tumor biopsy analysis in patients with NSCLC?

METHODS: We retrospectively evaluated 370 adult patients with NSCLC treated at the City of Hope between November 2015 and August 2019 to assess the usefulness of ctDNA in mutation identification, survival, concordance with matched tissue samples in 32 genes, and tumor evolution.

RESULTS: A total of 1,688 somatic mutations were detected in 473 ctDNA samples from 370 patients with NSCLC. Of the 473 samples, 177 showed at least one actionable mutation with currently available Food and Drug Administration-approved NSCLC therapies. *MET* and *CDK6* amplifications co-occurred with *BRAF* amplifications (false discovery rate [FDR], < 0.01), and gene-level mutations were mutually exclusive in *KRAS* and *EGFR* (FDR, 0.0009). Low cumulative percent ctDNA levels were associated with longer progression-free survival (hazard ratio [HR], 0.56; 95% CI, 0.37-0.85; $P = .006$). Overall survival was shorter in patients harboring *BRAF* mutations (HR, 2.35; 95% CI, 1.24-4.6; $P = .009$), *PIK3CA* mutations (HR, 2.77; 95% CI, 1.56-4.9; $P < .001$) and *KRAS* mutations (HR, 2.32; 95% CI, 1.30-4.1; $P = .004$). Gene-level concordance was 93.8%, whereas the positive concordance rate was 41.6%. More mutations in targetable genes were found in ctDNA than in tissue biopsy samples. Treatment response and tumor evolution over time were detected in repeated ctDNA samples.

INTERPRETATION: Although ctDNA analysis exhibited similar usefulness to tissue biopsy analysis, more mutations in targetable genes were missed in tissue biopsy analyses. Therefore, the evaluation of ctDNA in conjunction with tissue biopsy samples may help to detect additional targetable mutations to improve clinical outcomes in advanced NSCLC.

CHEST 2021; 160(3):1095-1107

KEY WORDS: circulating tumor DNA; non-small cell lung cancer; overall survival; precision oncology; progression-free survival

ABBREVIATIONS: ctDNA = circulating tumor DNA; FDA = Food and Drug Administration; FDR = false discovery rate; HR = hazard ratio; IQR = interquartile range; NSCLC = non-small cell lung cancer; OS = overall survival; PFS = progression-free survival; SNV = single nucleotide variant

AFFILIATIONS: From the Chapman University School of Pharmacy (M. R. Roosan), Irvine, the City of Hope Comprehensive Cancer Center (I. Mambetsariev, R. Pharaon, J. Fricke, K. L. Reckamp, M. Koczywas, E. Massarelli, and R. Salgia), the Division of Molecular Pharmacology (A. H. Bild), Department of Medical Oncology

Take-home Points

Study Question: How does the usefulness of circulating tumor DNA (ctDNA) compare with that of solid tumor biopsy analysis in patients with non-small cell lung cancer (NSCLC)?

Results: Treatment response and tumor evolution over time were detected in repeated ctDNA samples, with more mutations detected in targetable genes through ctDNA than tissue biopsy samples.

Interpretation: ctDNA analysis offers similar usefulness to tissue biopsy analysis in detecting somatic mutations, assessing mutual exclusivity, analyzing co-occurrences, and determining prognosis along with additional mutations detected and can serve as a less invasive option for monitoring the temporal evolution of NSCLC.

Despite advances in cancer treatments over the last decade, lung cancer continues to be the leading cause of cancer-related deaths.[1] Non-small cell lung cancer (NSCLC) accounts for 85% of all lung cancer cases and is relatively resistant to chemotherapy. Although targeted therapies such as *EGFR* and *ALK* tyrosine kinase inhibitors often are used to treat NSCLC, intratumor heterogeneity limits the efficacy of targeted treatments owing to the nature of genetically distinct subpopulations (ie, subclones).[2,3] Tissue biopsies have been considered as the gold standard in guiding treatment, however, they are costly, painful, and risky and often are not feasible owing to the worsening conditions of patients with metastatic diseases. Moreover, tissue biopsies are inherently limited by spatial heterogeneity.[4] Because tumors are known to shed cell-free DNA into the blood, less invasive plasma samples may be an alternative to tissue biopsies for solid tumors.[5] These cell-free circulating tumor DNAs (ctDNA)—also known as liquid biopsy—are short-lived fragments of extracellular DNA (approximately 160-180 bp; half-life, approximately 2 h) and are thought to be the result of the enzymatic degradation of dead tumor cells.[6] Numerous studies have demonstrated promising results by using ctDNA to detect cancers, to assess response to treatment and resistance, and to monitor the evolution of cancers.[7-10] However, most of the retrospective studies that have used ctDNA in patients with NSCLC have focused on the relationship between somatic mutations in a single gene (often in the *EGFR* gene) and corresponding targeted treatments.[2,3,9-13] With the exception of *EGFR* mutations and clinical responses to *EGFR*-targeting tyrosine kinase inhibitors, the complementary roles of the tumor and liquid biopsies in metastatic lung cancer have yet to be explored.[3,7,8,11] In addition, concordance in paired tumor and ctDNA NSCLC samples to establish the clinical validity of few actionable mutations for routinely using ctDNA assays to monitor biomarkers is limited.[14-16] A recent large-scale ctDNA study reported identification of actionable driver and resistant mutations in ctDNA at comparable frequencies and distributions as in tissue biopsies and presented ctDNA potentially as a first-line biomarker in cases of insufficient or incomplete or lack of tissue biopsy samples.[17] Therefore, the present study sought to evaluate a retrospective repeated-measure NSCLC dataset from the City of Hope Cancer Center to investigate (1) somatic mutations and their role in assessing prognosis, (2) concordance between tissue and ctDNA samples, and (3) the evolution of cancer as captured by repeated ctDNA sampling.

Methods

Participants

A retrospective analysis was performed using 473 ctDNA reports from a cohort of 370 patients with NSCLC treated at the City of Hope Cancer Center. ctDNA assays were analyzed using the Guardant360 platform (Guardant Health) between November 2015 and August 2019 (e-Fig 1). The Guardant360 panel is a Clinical Laboratory Improvement Amendments-certified and College of American Pathologists-accredited assay that detects single nucleotide variants (SNVs) of 73 genes, copy number amplifications for 18 genes, fusions in six genes, and insertions or deletions in 23 genes.[18,19] Somatic mutations from 295 formalin-fixed paraffin-embedded tissue biopsy samples were evaluated.

and Therapeutics Research, City of Hope, Duarte, the UC San Diego Health Moores Cancer Center (H. Husain), La Jolla, and the Division of Medical Oncology (K. L. Reckamp), Department of Medicine, Cedars-Sinai Medical Center, Los Angeles, CA.

Dr Roosan and Mr Mambetsariev contributed equally to this manuscript.

FUNDING/SUPPORT: This study was funded by the National Cancer Institute, National Institutes of Health [Grants P30CA033572, U54CA209978, R01CA247471, and R01CA218545], and the Chapman University Kay Family Foundation Data Analytics Grant.

CORRESPONDENCE TO: Ravi Salgia, MD, PhD; email: rsalgia@coh.org

DOI: https://doi.org/10.1016/j.chest.2021.04.016

Tumor analyses were performed according to the guidelines of an institutional review board-approved protocol (City of Hope Identifier: 19085; Chapman University Identifier: IRB-20-8). De-identified patient data for treatment history, sex, ethnicity, smoking status, tumor stages, age at diagnosis, vital status, progression, and last follow-up were abstracted from electronic health records.

ctDNA Mutation Analyses

To assess the usefulness of mutations detected in ctDNA samples, we evaluated mutation frequencies, variant allele frequencies (VAFs), co-occurrences, mutual exclusivity, and prognostic values. All somatic mutations detected by the Guardant360 panel in patients were considered to be of NSCLC origin. *ALK*, *EGFR*, *ERBB2*, *ROS1*, *BRAF*, *AKT1*, *PIK3CA*, *MTOR*, *RET*, and *MET* genes were considered targetable genes. *ALK-EML4* fusions, *BRAF V600E*, *EGFR* exon 19 deletions, *EGFR L858R*, *EGFR L861Q*, *EGFR T790M*, *EGFR C797S*, *EGFR G719A*, *EGFR G719C*, *EGFR G719S*, *EGFR C797S*, *MET* exon 14 skipping mutations, *RET* fusions, *ROS1* fusions, and *NTRK1/2/3* fusions were considered actionable mutations for current availability of Food and Drug Administration (FDA)-approved NSCLC treatments targeting these mutations according to the National Comprehensive Cancer Network guidelines.[20] VAF was calculated as the fraction of ctDNA molecules harboring the variant of interest divided by the total number of unique ctDNA molecules mapped to the variant position. The cumulative percent ctDNA was defined as the sum of VAFs from all detected mutations in a ctDNA sample. The cumulative percent ctDNA level in a sample that was equal or more than the median cumulative ctDNA was considered high, with other percentages considered to be low. Overall survival (OS) was assessed for mutations in *EGFR*, *TP53*, *KRAS*, *PIK3CA*, *MET*, *BRAF*, and *ERBB2* genes. OS and progression-free survival (PFS) were assessed for cumulative percent ctDNA levels. Both OS and PFS analyses were performed from the first ctDNA sample for gene mutations in cases of $\geq$ 30 patients, and only the first ctDNA sample was included for patients with multiple ctDNA samples.

Concordance Analyses

Concordance analyses were performed in matched samples to evaluate the agreement in mutation detection in both tissue biopsies and ctDNA samples. Tissue and ctDNA samples were considered to be matched if both were collected within 7 days of each other regardless of the other clinical factors (ie, number of prior treatments received, stage of cancer, tissue biopsy sources), assuming that tumors remained unchanged during the 7 days. Overall concordance was defined as the absence or presence of somatic mutations at the gene level in both tissue and liquid biopsies. Positive concordance was defined as the concurrent detection of positive mutation at a target gene. Although ctDNA results were obtained using the Guardant360 platform, 12 different platforms were used to analyze tissue biopsy samples (e-Table 1). To assess concordance in common genes consistent across all platforms, tissue biopsy results from platforms that interrogated $\leq$ 10 genes were excluded (n = 6). Another patient had two tissue samples matched with the same ctDNA sample. Of these two, the tissue biopsy collected on the same day as the ctDNA sample was included. Thus, somatic mutations in 64 patients with NSCLC were analyzed for the concordance of SNVs and insertions or deletions (32 genes), amplification (10 genes), and fusion (one gene) across all molecular testing platforms (e-Table 2). The sources of tissue for the biopsy are listed in e-Table 3.

Tumor Evolution Analyses

To assess tumor evolution, intratumor heterogeneity, and treatment response, somatic mutations, corresponding VAFs, tumor biopsy findings, and treatment data were investigated in five patients with NSCLC. Treatment response or failure was assessed using progression status and vital status at the final follow-up as noted in the progress report in the City of Hope electronic health records.

Statistical Analyses

Patient demographics and somatic mutations were summarized using descriptive statistics. To assess the impact of ctDNA-derived biomarkers on survival, Cox regression models were used for univariate and multivariate analysis of PFS and OS end points with complete observations for variables considered. Cancer stages were not considered in the PFS and OS because all the patients with complete observation harbored stage IV disease. For survival analyses, $P < .05$ was considered to be significant. For multiple testing corrections, a false discovery rate (FDR) of < 0.05 was considered to be statistically significant for analyses of co-occurrence and mutual exclusivity. The R software packages used were: ComplexHeatmap, GenVisR for heatmap analyses, discover for mutation co-occurrence and mutual exclusivity, and survival and survminer for OS and PFS analyses.[10,21-25] All analyses were performed using R version software 3.6.2 (R Foundation for Statistical Computing).[26]

Results

Of the 370 patients included, 55.4% were women, 58.9% were White, and 95.9% received a diagnosis of stage IV NSCLC. The median age at diagnosis was 65 years (range, 32-91 years), and most patients had lung adenocarcinoma (93.2%) (Table 1).

ctDNA Mutation Analyses

Of 473 ctDNA samples from the included patients, no somatic mutations were detected in 64 samples. Although 290 (of 473 [61.3%]) ctDNA samples harbored at least one somatic mutation in targetable genes, 582 (of 1688 [34.5%]) somatic mutations were found in ten targetable genes. Of 1,688 mutations, 235 mutations (13.9%) were considered actionable and 177 ctDNA samples (of the 473 [37.4%]) had at least one of the 17 actionable mutations with currently available FDA-approved NSCLC therapies. Somatic mutation frequencies in the earliest ctDNA samples were highest in *TP53* (18.8%) and *EGFR* (15.6%), whereas other prominent mutations were observed in *KRAS* (5.2%), *PIK3CA* (4%), *MET* (3.9%), and *NF1* (3.6%) (Fig 1, e-Table 4). Mutations such as *BRAF* V600E sometimes may arise from nontumorous sources (benign nevi or polyps) and may be detected in the ctDNA samples.[27] However, none of the patients with NSCLC harbored nevi or polyps to our knowledge. Thus, the detected *BRAF* alterations were related to the lung cancer. *EGFR L858R* and *EGFR E746_A750 deletion* mutations were mutually exclusive (FDR, 0.009). *KRAS* and *EGFR*

TABLE 1] Patient Demographics and Tumor Characteristics of the 370 Patients With Lung Cancer in Whom ctDNA Was Analyzed for the Present Study

Variables	
Patient characteristics (n = 370)	
Sex	
Female	205 (55.4)
Male	165 (44.6)
Race	
Black	12 (3.2)
Asian	129 (34.9)
White	218 (58.9)
Other or unknown	11 (3)
Smoking status	
Smoker	180 (48.6)
Never smoker	185 (50)
Unknown	5 (1.4)
ctDNA samples per patient	1 (1-5)
Age at diagnosis, median (range)	65 (32-91)
Age at diagnosis of metastasis, median (range)	65 (32-91)
Tumor stage	
I	1 (0.3)
II	2 (0.5)
II	7 (1.9)
IV	355 (95.9)
Unknown	6 (1.4)
Tumor type (n = 473 samples)	
Lung adenocarcinoma	345 (93.2)
Squamous cell carcinoma	13 (3.5)
Adenosquamous carcinoma	2 (0.5)
Large cell carcinoma	2 (0.5)
Adenocarcinoma to SCLC	2 (0.5)
Other or unspecified NSCLC	6 (1.6)
Genes altered per sample, median (range)	3 (0-15)
Mutations per sample, median (range)	3 (0-18)
Patients with ctDNA samples (n = 473 samples), time point	
1	293 (79.2)
2	57 (15.4)
3	15 (4.1)
4	4 (1.1)
5	1 (0.3)
Type of mutation (n = 1,688 mutations)	
SNVs or indels	1,474 (87.3)
Amplification	196 (11.6)
Fusion	18 (1.1)
Interventions received before first ctDNA sample obtained	
None	8 (2.1)
First line	163 (44.1)
Second line	62 (16.8)
Third line	18 (4.9)
Unknown	60 (16.2)

Data are presented as No. (%) or median (range). ctDNA = circulating tumor DNA; indels = insertions or deletions; NSCLC = non-small cell lung cancer; SCLC = small cell lung cancer; SNV = single nucleotide variant.

gene-level mutations were mutually exclusive (FDR, 0.0001). The co-occurrences of amplifications in *MET* with *BRAF* and *CDK6* with *BRAF* were statistically significant (FDR, 0.006 and 0.007, respectively).

Survival analyses were used to assess the usefulness of ctDNA results in defining prognostic indices. The median follow-up time in 330 patients with follow-up data was 5.6 months (IQR, 2.3-14 months). A low cumulative percent ctDNA was an independent predictor of longer PFS (hazard ratio [HR], 0.56; 95% CI, 0.37-0.85; $P = .006$), after adjusting for patient age, sex, and smoking status (Fig 2). Univariate analyses of OS were performed to evaluate mutations in commonly mutated or targetable genes that had at least 30 or more cases in our dataset (eg, *EGFR*, *BRAF*, *KRAS*, *TP53*, *PIK3CA*, and *MET*). *BRAF*, *KRAS*, and *PIK3CA* mutations were associated with a shorter OS (Fig 2). The presence of *BRAF* (HR, 2.35; 95% CI, 1.24-4.5; $P = .009$), *PIK3CA* (HR, 2.77; 95% CI, 1.56-4.9; $P < .001$), and *KRAS* (HR, 2.32; 95% CI, 1.30-4.1; $P = .004$) remained significant predictors of the shorter OS when all significant single-mutation statuses—along with age, sex, and smoking statuses—were included in multivariate Cox regression models. The presence of *EGFR*, *MET*, and *TP53* was not associated significantly with OS.[28] Therefore, ctDNA results can be used for the assessment of PFS and OS, similar to tissue biopsy findings.[29]

Concordance Between Tissue and ctDNA Samples

A concordance analysis in 64 lung adenocarcinoma patients with matched tissue and ctDNA samples identified a total of 214 unique somatic mutations at the gene level. Only gene-level concordance in matched

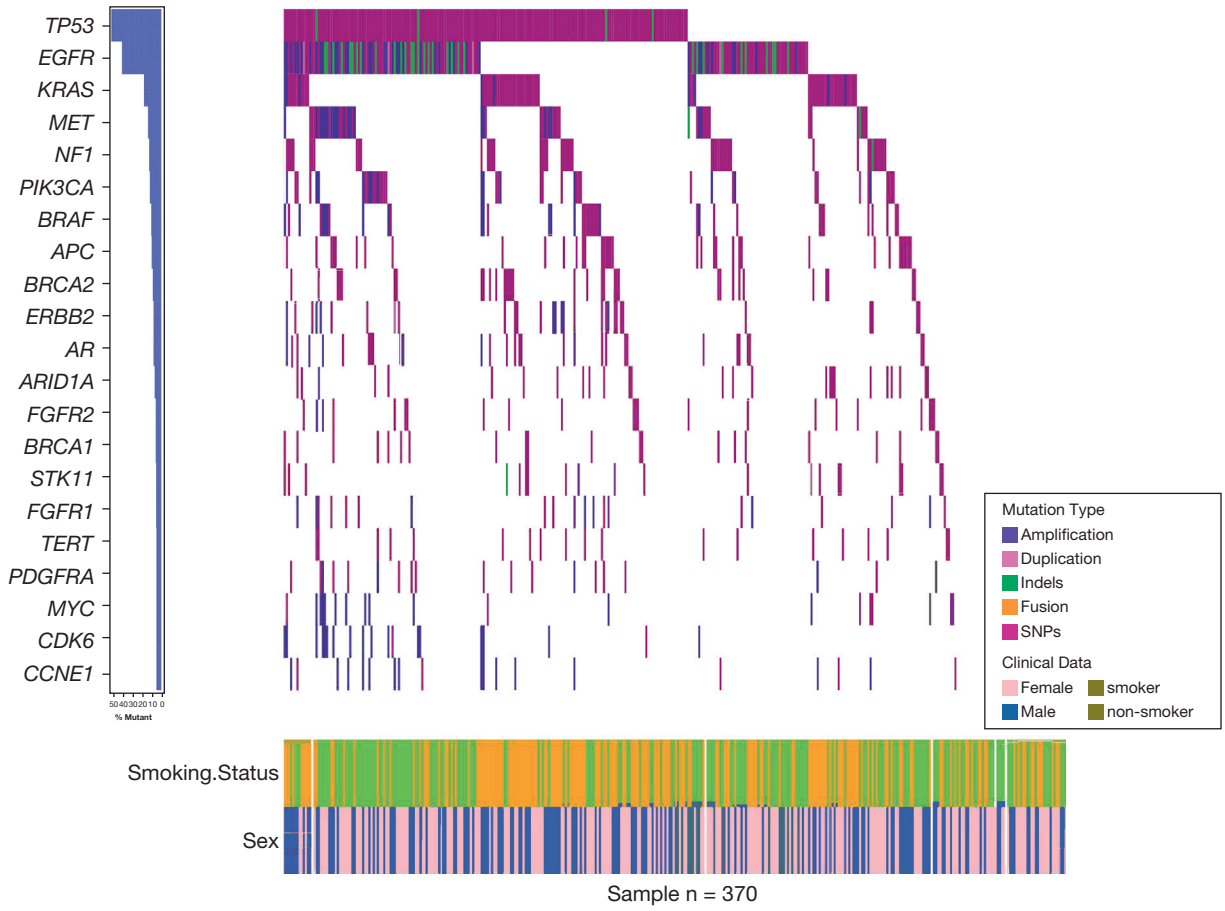

Figure 1 – *Circulating tumor DNA (ctDNA) analyses from 370 patients with non-small cell lung cancer. Co-mutation plot showing somatic mutations identified in the ctDNA samples for the most prevalent genes (ie, with a mutation recurrence cutoff of ≥ 0.05).*

samples from both tissue and ctDNA sources was analyzed without differentiating them on actionability. For example, the *BRAF* gene was considered to be a targetable gene and *BRAF* V600E mutations were considered actionable. However, all *BRAF* mutations (fusion, E24E, V600E, K601E, L537L, L597R, I617V, and amplification) were assessed for gene-level concordance. The positive concordance rate—the concordance within the detected mutations—was 41.6%. Sixty-six of the detected mutations (30.4%) and 62 of the detected mutations (28.6%) were found in ctDNA only and tissue only, respectively (Fig 3). The overall concordance rate between tissue and ctDNA samples was 93.8%. A subanalysis of concordance for four patients with non-stage IV NSCLC showed a high overall concordance of 90.6%. However, more discordant somatic mutations were detected in tissue, 7% compared with 2.3% in ctDNA, potentially because of low shedding of tumor DNA in earlier stages.

Although somatic mutations detected via tissue biopsy and ctDNA were highly concordant at the gene level, both sources exhibited additional unique mutations that were missed by the other (e-Fig 2). The concordance analysis for amplification in 10 genes showed 39 unique amplifications in 21 patients. Although the overall concordance rate of amplification was 97.1%, the positive concordance rate was 28.2%. Eighteen discordant amplifications (46.2%) and 10 discordant amplifications (25.6%) were found in ctDNA and tissue, respectively (Fig 4). Similarly, the overall concordance rate for *ALK-EML4* fusion was 95.3%, with a 40% positive concordance rate. Overall, more SNVs, insertions or deletions, and amplifications were detected via ctDNA than by tissue biopsy analysis. In the matched samples, ctDNA samples showed a higher number of mutations in 10 targetable genes than tissue biopsies.

Intratumor Heterogeneity and Evolution

Intratumor heterogeneity and tumor evolution were investigated over time in all patients with two or more ctDNA results (Fig 5, e-Figs 3-6, e-Table 5). All patients

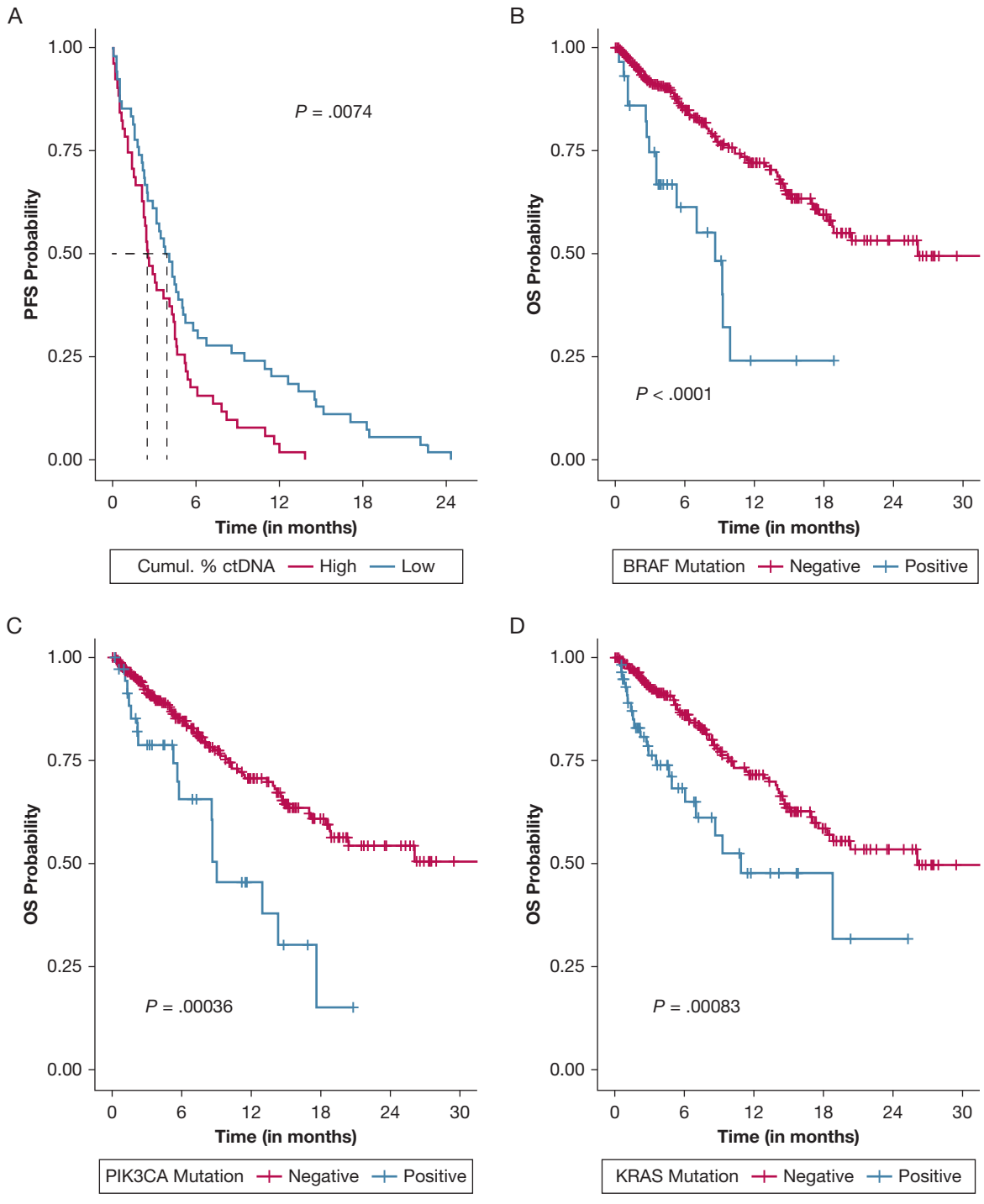

Figure 2 – *Survival analysis in ctDNA samples. A, Kaplan-Meier plot showing PFS for the cumulative percent ctDNA variant allele frequency (VAF). Patient samples with a cumulative ctDNA VAF of ≥ 1.8% (ie, median cumulative ctDNA VAF of all samples) were considered to be high. A low cumulative percent ctDNA VAF was associated significantly with a longer PFS after adjusting for age at diagnosis, sex, and smoking status. B-D, Kaplan-Meier overall survival (OS) curves for patients with known* BRAF *(B),* PIK3CA *(C), and* KRAS *(D) mutations detected in ctDNA. Shorter OS was associated significantly with the presence of* BRAF, PIK3CA, *and* KRAS *mutations after adjusting for age at diagnosis, sex, and smoking status. ctDNA = circulating tumor DNA; Cumul. % = cumulative percent; PFS = progression-free survival.*

exhibited variations in the number of mutations detected, and VAFs fluctuated during the course of disease and therapy. Specifically, four ctDNA samples were collected from patient G1 within 3 years of the first sample. Only an *ALK E1407E* mutation was detected at the first ctDNA time point at 0.1% VAF, whereas *EGFR* sensitizing mutations

Figure 3 – *Gene level overall concordance analysis. Concordance landscape of somatic mutations (single nucleotide variants, insertions or deletions indels, amplifications, and fusions) at the gene level for 32 genes in 64 patients with non-small cell lung cancer with both tissue and circulating tumor DNA (ctDNA) samples available. Each row represents a gene, and each column represents a patient. Gray indicates that no somatic mutation is detected in either tissue or ctDNA. Cadet blue indicates that a somatic mutation is detected in the tissue sample, and brown indicates that a somatic mutation is detected in the ctDNA sample. Percentages on the left show the detection rate for each gene across all patients, whereas the stacked bars on the top represent the number of somatic mutations detected in each patient. The stacked bars on the right represent the number of somatic mutations detected for each gene. Female and male patients are shown as pink and blue, respectively. Nonsmokers and smokers are indicated in light green and orange, respectively.*

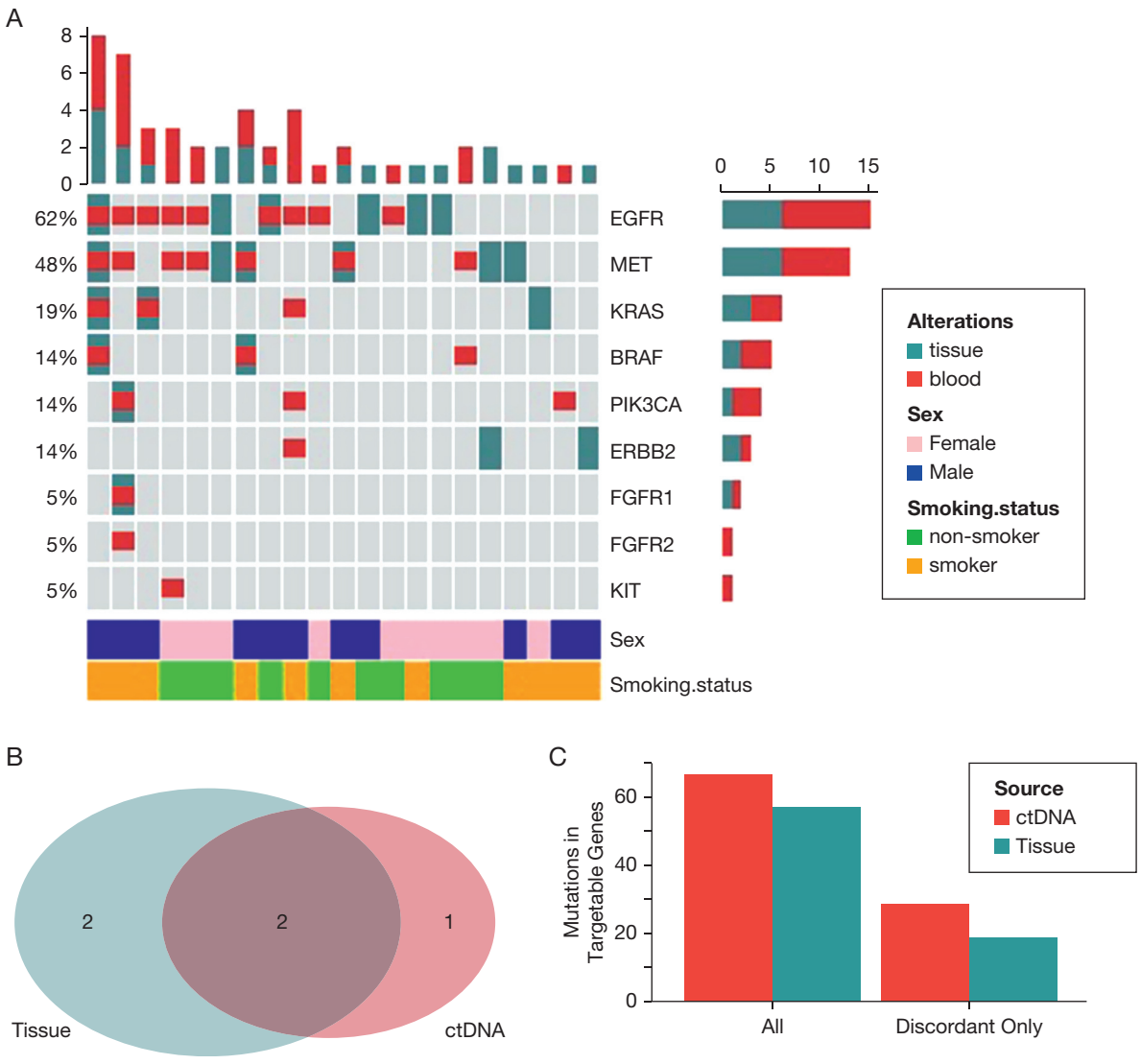

Figure 4 – *Concordance analysis of amplifications, fusion, and actionable mutations. A, Concordance landscape of somatic amplifications at the gene level for nine genes in 23 patients with non-small cell lung cancer with both tissue and ctDNA samples available. Each row represents a gene, and each column represents a patient. Cadet blue indicates that an amplification is detected in the tissue sample, and brown indicates that an amplification is detected in the ctDNA sample. Percentages on the left show the detection rate for that gene across all patients, whereas the stacked bar on the top represents the number of amplifications detected in each patient. The stacked bars on the right represent the number of amplifications detected in each gene. Missing clinical information is shown in white. B, Venn diagram showing the number of patients with the* ALK-EML4 *fusion detected in tissue and ctDNA samples. C, Bar graph showing percentage of mutations in targetable genes detected.* ALK, AKT1, BRAF, EGFR, ERBB2, MET, MTOR, PIK3CA, RET, *and* ROS1 *genes were considered to be targetable. Of all the detected mutations, the number of mutations in targetable genes were higher in ctDNA samples across all genes and discordant genes. Female and male patients are shown as pink and blue, respectively. Nonsmokers and smokers are indicated by light green and orange, respectively.*

were detected in a tissue biopsy sample at that time. Patient G1 received afatinib as a first-line targeted treatment. At the second time point, three novel somatic mutations (*EGFR*, *TP53*, and *TERT*) emerged in ctDNA, of which *EGFR* and *TP53* persisted into the third and fourth time points. Additionally, *TSC1*, *ATM* SNVs, and *MYC* amplification were detected at the fourth ctDNA time point. Patient G1 participated in a blinded clinical trial after the second time point, and further treatment information, other than restarting afatinib, was unavailable. Patient G9 showed one of the highest numbers of gene mutations detected in four ctDNA samples within 3 years and showed multiple mutations in *EGFR* in the first ctDNA sample (including *EGFR* T790M), which was undetected in the tissue biopsy examinations performed at that time. Patient G9 received carboplatin plus docetaxel as a first-line treatment and erlotinib as a second-line treatment after the first ctDNA sample. Significant decreases in the percentage of detected VAFs were observed from the first to the second ctDNA time point after initiating osimertinib as a third-line

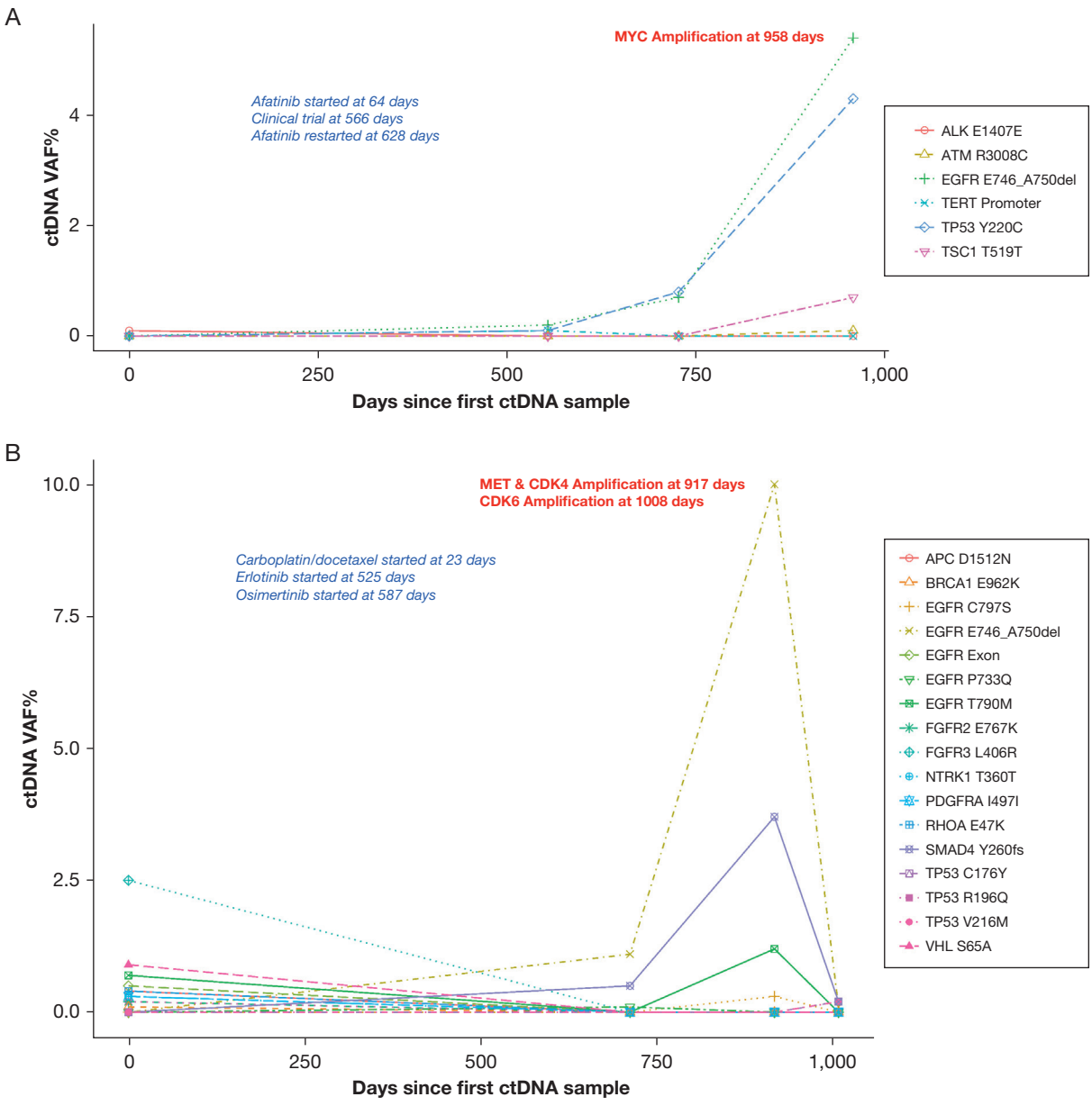

Figure 5 – *Tumor evolution analysis. Graphs showing tumor heterogeneity evolution as detected in ctDNA samples over time for patient G1 (A) and patient G9 (B). The y-axis represents the VAF%, whereas the x-axis shows time in days since the first ctDNA sample was collected. Patient G1 began afatinib therapy after the first ctDNA time point. Although the clinical trial drug was unknown, EGFR* E746_A750 deletion *continued to increase despite the treatments. ctDNA from patient G9 showed lower* EGFR *T790M mutation after osimertinib treatment. However, EGFR* E746_A750 deletion *increased in ctDNA. ctDNA = circulating tumor DNA; VAF% = percent variant allele frequency.*

treatment, indicating a response. However, *EGFR* T790M persisted in the two subsequent ctDNA samples. Similar to patient G9, *EGFR* T790M mutations were detected in ctDNA samples in patient G220, which remained undetected in tissue biopsy samples, highlighting the usefulness of ctDNA in detecting mutations missed in tissue biopsy analyses. In contrast, patient G59 showed *EGFR* T790M mutation detected both in ctDNA and tissue biopsy samples. However, after osimertinib treatment, the *EGFR* T790M mutation was undetected in tissue biopsy samples, but was detected in ctDNA. Patient G8 harbored *EGFR* and *KRAS* mutations that were undetected in all four ctDNA samples compared with tissue biopsy samples. Therefore, although ctDNA assays often identified actionable mutations in patients G1, G9, G59, and G220, it was limited in detecting mutations in patient G8.

Discussion

NSCLC is a molecularly heterogeneous disease with wide variations in presentation, response to treatment, and overall prognosis. Although repeated tissue biopsy examinations have been the gold standard to guide therapy and study tumor evolution, the collection of multiple tissue biopsy samples is costly, painful, and often risky. Recently, ctDNA has proven to be promising in detecting and monitoring multiple cancers by offering a less invasive option for biopsy.[13,30,31] ctDNA also addresses the inherent limitation of solid tumor biopsies for spatial heterogeneity. Currently, ctDNA tests such as Guardant360 have been approved by FDA as an alternative to tissue-based biopsies only when a tissue biopsy sample is unattainable. Although *EGFR* is the most studied biomarker in the treatment of NSCLC, the role of other known biomarkers such as *KRAS*, *ERBB2*, *PIK3CA*, *MET*, *MEK1*, *ALK*, *BRAF*, *ROS1*, and *RET*, and other commonly mutated genes such as *TP53*, have yet to be explored using ctDNA. Furthermore, concordance between tissue and liquid biopsy analyses has not been assessed beyond actionable biomarkers in patients with NSCLC.[12,15,32] Therefore, our study evaluated a retrospective single-center cohort of 370 patients to assess clinical outcomes, concordance with tissue biopsy findings in 32 genes, and tumor evolution in five patients. To our knowledge, this collection of ctDNA and clinical data from patients with NSCLC is the largest compiled to date. Furthermore, our concordance analysis was evaluated in a broader panel of genes from patients with diverse clinical statuses than previously published in patients with NSCLC.

In agreement with biopsy-based findings, we found that *KRAS* and *EGFR* gene mutations were significantly mutually exclusive,[33-35] whereas *MET* and *CDK6* amplifications were significantly mutually co-occurring with *BRAF* amplifications. A low cumulative percent VAF in ctDNA was an independent predictor of longer PFS after adjusting for patient age, sex, and smoking status. Low levels of cumulative percent ctDNA potentially indicate a low tumor burden as detected in the blood, and thus less severe disease. Given the fleeting nature of ctDNA, it is not surprising to find no significant association of ctDNA with OS. Other studies also have demonstrated the prognostic value of pretreatment or presurgery cell-free DNA levels as a surrogate marker for tumor burden and treatment response in many cancers.[29,36-39]

Studies have demonstrated improved clinical outcomes in patients harboring *EGFR*, *ALK*, *ROS1*, and *BRAF* mutations when targeted agents were directed against these driver and resistant mutations.[40,41] In our study, patients with *BRAF*, *PIK3CA*, or *KRAS* mutations, as detected by ctDNA, showed significantly worse survival rates. Although FDA-approved *BRAF*-targeted therapy is available for NSCLC, patients with positive *BRAF* findings received *EGFR*-targeted therapies or chemotherapies because of the presence of actionable *EGFR*-sensitizing or *KRAS* mutations. Despite the *PIK3CA* mutations, *PIK3CA*-targeted therapies were rare because *PIK3CA* mutations often went undetected in tissue biopsy samples and are not considered actionable in NSCLC. Currently, no *KRAS*-targeted therapy is available, and biomarker detection is driven by tissue biopsy findings. Hence, the worse prognosis associated with *BRAF*, *KRAS*, and *PIK3CA* mutations could be associated with a lack of targeted therapy use in these patients. Similar to data derived from tissue biopsy samples, *EGFR*, *MET*, and *TP53* mutations were not associated significantly with OS.[28]

Overall concordance, including presence and absence in somatic mutations at the gene level, was 93.8%. Herein, we assessed concordance in each patient regardless of clinical status (eg, age, stage, sex, or smoking status) or prior therapy received. Other studies that have investigated gene-level concordance reported values of 52% to 94.2% for various cancers in demonstrating potential clinical use of ctDNA for NSCLC treatment.[12,42-45] Concordance at the mutation level has been shown to be 62.2% to 88.8% in patients with NSCLC for *EGFR* mutations.[46-49] Concordance rates varied from 50% to 55% in early-stage cancers and 64% to 83% in late-stage and metastatic cancers.[49-54] The lower concordance in early-stage NSCLC may be contributed by a lack of sensitively to DNA shedding of early tumors and overall low tumor mutation burden.[16,55] Furthermore, more gene mutations and actionable gene mutations were found in ctDNA than in tissue biopsy samples in the matched samples, in agreement with the findings in other studies.[12,42] A prospective trial observed 98.2% concordance with 100% positive predictive value for cell-free DNA vs tissue genotyping of *EGFR*, *ALK*, *ROS1*, and *BRAF* genes in previously untreated metastatic NSCLC.[56] Some previous studies have suggested that the discordant mutations found in ctDNA are potential false-negative results from tissue biopsy samples and can be related to temporal and spatial heterogeneity.[18,32,57] The

discordant mutations found only in tissue could be indicative of the low disease burden or nonporous nature of the tumor, and thus are undetected in plasma.

To assess tumor evolution, we selected five lung adenocarcinoma patients with NSCLC with ≥ 4 ctDNA samples. Not all mutations were detected in both tissue and ctDNA samples when they were collected at approximately the same time, similar to other studies.[17] However, the trends in ctDNA data were more consistent (ie, although the same mutations often were detected in subsequent samples, they would go undetected in the tissue biopsy samples obtained between ctDNA sample collections). Incidences of mutations that were undetected in ctDNA but were detectable in tissue biopsy samples also have been reported, and the use of ctDNA in addition to tissue biopsy examination has been suggested for detecting more targetable mutations.

It is worth noting that our study has several limitations. Most of the patients in the cohort had advanced-stage NSCLC. Therefore, our findings may not be generalizable to patients with early-stage NSCLC. However, a prospective trial showed similar concordance and usefulness in early-stage cancer.[58] Additionally, because this was a single-center retrospective study, the patient population at City of Hope may not be representative of other patient populations with NSCLC. Many observations had to be excluded owing to missing data for the PFS and OS analyses. Also, the multivariate models were not exhaustive in developing the comprehensive OS and PFS prognostic models. Instead, oversimplified multivariate models were assessed to evaluate the risk of ctDNA-derived biomarkers on PFS and OS, controlling for common clinical factors. Some patients were part of masked clinical trials, and thus treatment information was not specified in their electronic health records. Because the first ctDNA sample was obtained at different time points of the disease course, OS and PFS analyses were biased. The prognostic value of using ctDNA biomarkers may be biased owing to having targeted therapies available to some, but not all, patients. Concordance analyses were performed for only 32 genes that were common across the ctDNA and tissue-based next generation sequencing platforms, and concordance may vary depending on the gene mutations analyzed. Furthermore, samples were matched if the patient's ctDNA and tissue biopsy samples were collected within 7 days of each other with the underlying assumption that the tumor likely would not change significantly within 7 days. However, concordance rates would be underreported if tumors indeed evolved at a detectable rate during the 7-day period. In addition, the tissue sources for biopsy were heterogeneous, which may show variation in intratumor mutational burden. Because the mutational frequencies predominately were less than 20 counts, the City of Hope dataset may not capture the true diversity of somatic mutational co-occurrences or mutual exclusivity, at least at the mutation level. Since most patients had advanced NSCLC, the median follow-up time was short for both OS and PFS analysis. In the future, larger clinical trials are necessary to explore the prospective role of ctDNA in the treatment of patients with NSCLC at all stages to address these limitations.

Interpretation

ctDNA captured clinically useful, actionable, and dynamic information by identifying targetable mutations regardless of patients' clinical status. Thus, ctDNA analysis can provide complementary information to tissue biopsy examination in cancer management and surveillance, and may offer additional targetable opportunities beyond when a tissue biopsy samples are not attainable.

Acknowledgments

Author contributions: R. S. had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. M. R. R, I. M., A. H. B, and R. S. contributed substantially to the study concept and design. M. R. R, I. M., R. P., J. F., K. L. R., M. K., E. M., H. H., A. H. B, and R. S. made substantial contributions to the acquisition, analysis, or interpretation of data for the manuscript. M. R. R. and I. M. contributed substantially to the drafting of the manuscript. R. P., J. F., K. L. R., M. K., E. M., H. H., A. H. B, and R. S. contributed to the critical revision of the manuscript for important intellectual content, final approval of the version to be published, and agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Financial/nonfinancial disclosures: None declared.

Role of sponsors: The study sponsors had no role in the design and conduct of the study; collection, management, analysis, or interpretation of the data; preparation, review, or approval of the manuscript; or decision to submit the manuscript for publication.

Additional information: The e-Figures and e-Tables can be found in the Supplemental Materials section of the online article. Study data and codes can be found at https://github.com/mumtahena/ctDNA_lung.

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