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Overall survival with circulating tumor DNA-guided therapy in advanced non-small-cell lung cancer

A full list of authors and affiliations appears at the end of the article.

Abstract

Circulating tumor DNA (ctDNA) sequencing guides therapy decisions but has been studied mostly in small cohorts without sufficient follow-up to determine its influence on overall survival. We prospectively followed an international cohort of 1,127 patients with non-small-cell lung cancer and ctDNA-guided therapy. ctDNA detection was associated with shorter survival (hazard ratio (HR), 2.05; 95% confidence interval (CI), 1.74–2.42; $P < 0.001$) independently of clinicopathologic features and metabolic tumor volume. Among the 722 (64%) patients with detectable ctDNA, 255 (23%) matched to targeted therapy by ctDNA sequencing had longer survival than those not treated with targeted therapy (HR, 0.63; 95% CI, 0.52–0.76; $P < 0.001$). Genomic alterations in ctDNA not detected by time-matched tissue sequencing were found in 25% of the patients. These ctDNA-only alterations disproportionately featured subclonal drivers of resistance, including *RICTOR* and *PIK3CA* alterations, and were associated with short survival. Minimally invasive ctDNA profiling can identify heterogeneous drivers not captured in tissue sequencing and expand community access to life-prolonging therapy.

Multiplex tissue sequencing can match patients with non-small-cell lung cancer (NSCLC) to a growing number of life-prolonging targeted therapies^{1–3}. In many real-world settings, however, patients do not receive multiplex tumor sequencing due to an inability to acquire tissue, sequencing failure or lack of access^{4–6}. Sequencing of circulating tumor DNA

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Correspondence and requests for materials should be addressed to Bob T. Li. lib1@mskcc.org.

Author contributions

Conception: J.J. and B.T.L. Radiomic analysis: J.J., E.S.L., R.Y. and J.P.D. Patient accrual: J.J., A.N., P.K.P., J.E.C., Y.R.M.-G., B.D., H.A.Y., M.O., M.D.H., K.C.A., M.G.Z., M.G.K., K.K.N., J.E., I.P., W.V.L., J.J.F., A.I., D.M., G.R., B.J.P., D.L.C., C.I.D., M.I., S.C., N.P., A.L., N.R., J.C., W.D.T., G.J.R., V.W.R., A.R., D.G., A.D., D.R.J., C.M.R., J.M.I. and B.T.L. Genomic data collection and analysis: J.J., G.J., A.R.B., R.B., A.Z., M.D., N.S., D.C., R.K., R.M., S.P.S., M.F.B., M.E.A., M.L., R.L., L.P.L. and M.L. Clinical data collection and analysis: J.J., E.S.L., N.S., Y.R.M.-G., H.-Y.T., C.-R.X., C.T.-L. and M.D.S. Administration: A.M., J.G., D.B.S., A.D., H.I.S., P.L., L.P.L., M.F.B., M.E.A., M.L., P.R., J.S.R.-F., D.R.J., C.M.R., J.M.I. and B.T.L. Statistical plan: J.J., M.G., R.S. and S.P.S. Writing: J.J., C.W., P.R., J.S.R.-F. and B.T.L. All co-authors reviewed and approved the final draft of the manuscript.

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Reporting summary

Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Code availability

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(ctDNA) from plasma ('liquid biopsies') may similarly identify targetable alterations. Community adoption of such methods is rising^{7,8}, although the effectiveness of ctDNA for targeted therapy matching has been studied mostly in small cohorts with limited follow-up^{9–16}.

Short-term studies have suggested that ctDNA-matched targeted therapy produces equivalent rates of radiologic response to tissue-matched therapy^{12,17}, but whether it translates to equal overall survival (OS) benefit is unknown. ctDNA detection is also thought to reflect aggressive tumor biology and disease burden^{18–23}, but whether its prognostic value is independent of tumor volume or clinical factors is not yet defined. Some ctDNA alterations may be observed in plasma but not in tissue²⁴. This genomic diversity between plasma and tissue may represent a mixture of artifacts²⁵, clonal hematopoiesis (CH)²⁶ and spatial tumor heterogeneity²⁷. Whether alterations corresponding to tumor heterogeneity have prognostic relevance or common genomic features is unclear. Despite increasing commercial use of ctDNA detection, clinical interpretation is challenging without adequately powered studies to address such biological questions.

Recent reviews have highlighted the need for large prospective studies confirming the clinical utility of liquid biopsies^{28,29}. To define the usefulness of ctDNA as a genomic biomarker to guide treatments and its association with and impact on survival, we conducted a prospective international cohort study of patients with metastatic NSCLC undergoing plasma ctDNA sequencing.

Results

Patient characteristics

Patients were enrolled at Memorial Sloan Kettering Cancer Center (MSK, New York, NY, USA), an academic cancer center, and GenesisCare (Sydney, Australia), a community-based oncology practice including physicians affiliated with the University of Sydney. The study included adults with stage IV or recurrent NSCLC and either no known driver mutation before enrollment or progression of disease following targeted therapy. Of the 1,357 patients initially enrolled in the study, 1,127 were included in the analysis (Fig. 1 and Table 1).

Sequencing overview

All patients received ctDNA sequencing via the Resolution Bioscience ctDx Lung platform, a targeted next-generation sequencing panel including known molecular drivers of NSCLC (Supplementary Table 1)^{10,30–32}. Plasma was drawn on the day of study enrollment and could be redrawn subsequently at the provider's discretion. Most patients ($n = 1,003$, 89%) provided a single sample. Only mutations passing a filter for germline variants were included in the analysis (Methods and Supplementary Fig. 1). A summary of the pathogenic alterations detected on first ctDx Lung sequencing is shown in Supplementary Table 2. All patients enrolled at MSK consented to tissue next-generation sequencing with Integrated Mutation Profiling of Actionable Cancer Targets (MSK-IMPACT), a targeted capture sequencing assay cleared by the Food and Drug Administration and approved by the New York State Department^{33,34}. From 10 June 2019, patients were eligible for additional

plasma sequencing with Analysis of Circulating cfDNA to Evaluate Somatic Status (MSK-ACCESS)³⁵ at the provider's discretion. Both MSK assays use matched white blood cell (WBC) sequencing to remove CH and germline variants.

We confirmed lower failure rates and faster turnaround time with ctDNA compared to tissue; 164 of 1,219 (13%) tissue sequencing attempts failed after sample receipt due to insufficient material or sequencing failure. By contrast, 37 of 1,919 (2%) ctDx Lung or MSK-ACCESS samples failed. Only two patients did not have successful molecular profiling of either plasma or tissue. Time from blood draw (that is, ctDNA sample collection or matched WBC collection for MSK-IMPACT) to sequencing report was shorter for plasma than for tissue sequencing: 33 days (interquartile range (IQR), 25–41) for MSK-IMPACT versus 11 days (IQR, 9–14) for MSK-ACCESS or ctDx Lung (Extended Data Fig. 1). Mutations detected in time-matched MSK-ACCESS and ctDx Lung assays were highly concordant (Methods (“ctDx Lung versus MSK-ACCESS” and Supplementary Fig. 2).

Independent prognostic value of ctDNA alterations

To test whether ctDNA alterations are prognostic, we compared OS among patients with versus without detectable ctDNA on the earliest ctDx Lung sequencing, correcting for the timing of sample collection (Methods). Because the majority of plasma DNA is derived from non-tumor tissue^{14,26,36}, we define ctDNA detection here as the identification of at least one mutation or copy number change, in keeping with other studies using DNA sequencing-based liquid biopsies^{25,37}.

Of the 1,127 patients, 722 (64%) had at least one detectable ctDNA alteration by ctDx Lung. Patients with ctDNA alterations had shorter survival (hazard ratio (HR), 2.05; 95% CI, 1.74–2.42; $P < 0.001$; Fig. 2a). This observation could be generalized to the MSK and Sydney cohorts and in treatment-naïve and post-treatment settings (Fig. 2b).

Previous studies conflict as to whether ctDNA burden does³⁸ or does not¹⁷ associate with survival. In our large NSCLC cohort, higher numbers of ctDNA alterations and higher maximum variant allele frequency (VAF) were associated with shorter survival (Fig. 2b and Supplementary Table 3). We sought to assess whether circulating tumor fraction (cTF), that is, an estimate of tumor burden in plasma based on clonal mutations that is corrected for copy number changes (Methods), might provide additional prognostic stratification. Higher cTF itself was not associated with shorter survival, possibly because the requirement that clonal mutations be present in plasma was itself a strong prognostic sign. cTF could not be calculated for patients without clonal alterations in ctDNA; patients in this group had better survival than those with calculable cTF (Fig. 2b).

Some patients had only variants of unknown significance (VUS; that is, pathogenic status unknown³²) in ctDNA. These patients also had worse survival than those without ctDNA alterations, suggesting that ctDNA portends worse outcomes even in the absence of pathogenic alterations. Among patients with known pathogenic alterations (in *TP53*, *EGFR* or *KRAS*), we tested whether it is the presence of those alterations themselves, rather than their detection in ctDNA, that is associated with worse prognosis. In these subgroups,

detection of the pathogenic alteration in ctDNA was associated with worse prognosis than detection in tissue only (Fig. 2b).

We tested whether ctDNA is more likely to be detected in patients with higher tumor mutational burden (TMB) and whether this might affect our conclusions regarding survival. TMB on MSK-IMPACT sequencing was not associated with greater ctDNA burden, possibly due to the small footprint of the ctDx Lung assay (Supplementary Fig. 3a). Among patients with TMB of <10 mutations per Mb³⁹, those with ctDNA detection had shorter survival; among those with TMB of ≥ 10 mutations per Mb, ctDNA detection was associated with a trend toward worse survival (Supplementary Fig. 3b). Finally, it is possible that ctDNA's prognostic value may vary based on treatment patterns. In subgroup analyses limited to patients treated with targeted therapy, immunotherapy or chemotherapy only, ctDNA detection remained an independent predictor of worse outcome. Among patients treated with immunotherapy, our findings of ctDNA detection as a poor prognostic marker held true regardless of TMB stratification (Supplementary Fig. 3c). In summary, we found that ctDNA is prognostic in a manner that scales with ctDNA burden but is independent of treatment patterns and tumor genomics.

Multivariate and radiologic analysis

ctDNA detection is influenced by histology²⁷, disease extent and clinical factors³⁸, findings that were confirmed in this cohort (Extended Data Fig. 2). Of 335 patients with extrapulmonary disease on time-matched positron emission tomography (PET) imaging (Supplementary Table 4), 248 (74%) had detectable ctDNA; by contrast, 49 of 122 (40%) patients with intrapulmonary disease had detectable ctDNA (chi-squared test, $P < 0.001$). In a multivariate model including extrapulmonary disease and clinical features as variables, ctDNA detection independently predicted worse survival (Fig. 2c). In addition, advanced age was associated with worse survival, whereas receipt of targeted therapy was associated with better survival.

Previous studies differ in whether ctDNA levels correlate with metabolic tumor volume (MTV)^{40,41}. It is also unknown whether the prognostic value of ctDNA reflects tumor burden or another aspect of tumor biology not captured by metabolic imaging. To examine the relationships among ctDNA levels, MTV and survival, we computed MTV for treatment-naïve patients with adenocarcinoma who had time-matched PET imaging and MSK-IMPACT sequencing (Methods).

MTV was correlated with ctDNA VAF, although there were cases in which high ctDNA levels were observed with low MTV and vice versa (Fig. 2d). Among patients with high or low MTV (relative to the median), ctDNA remained independently prognostic (Fig. 2e). It is possible that ctDNA may appear independently prognostic among patients with high or low MTV because it reflects relative tumor burden within those discrete cohorts. In multivariate analyses treating MTV as a continuous variable, higher MTV was associated with a trend toward worse survival, while ctDNA detection was significantly associated with worse survival (Fig. 2f). We repeated these analyses with other continuous radiologic features, namely, maximum standardized uptake value (SUV), SUV_{mean} , the product of MTV and SUV_{mean} (total lesion glycolysis) and the number of lesions. In all cases, ctDNA levels were

correlated with radiologic features but remained independently prognostic (Supplementary Fig. 4).

Patients treated at MSK had higher ctDNA burden than those treated at Sydney (Supplementary Fig. 5b). In multivariate analysis including treatment site as a variable, ctDNA detection remained prognostic, but treatment site was not (Supplementary Fig. 5c). In summary, all multivariate analyses confirmed the independent prognostic value of ctDNA detection.

ctDNA matching to targeted therapy

We examined the clinical utility of ctDNA-matched targeted therapy and its impact on survival, a subject of equivocal results in previous smaller short-term studies^{12,17,23}. A total of 418 (37%) patients were treated with targeted therapy (Supplementary Table 5) after study entry; 255 (23%) patients had molecular targets identified on ctDNA (Supplementary Table 6), and 163 (14%) patients were matched based on tissue analysis only (Fig. 1). The odds of matching to targeted therapy by ctDNA were lower in smokers, patients with non-adenocarcinoma histology and patients with intrapulmonary disease only (Supplementary Table 7).

Among patients treated with targeted therapy, those with detectable ctDNA had worse survival (Supplementary Fig. 3c and Fig. 3a). Among patients with detectable ctDNA, those matched to targeted therapy by liquid biopsy had longer OS than those not receiving targeted therapy (HR, 0.63; 95% CI, 0.52–0.76; $P < 0.001$; Fig. 3a). Among patients without detectable ctDNA, those treated with targeted therapy did not have longer survival than those not treated with targeted therapy to a statistically significant extent (HR, 0.92; 95% CI, 0.7–1.2; $P > 0.1$), suggesting greater relative benefit to targeted therapy among those patients with detectable ctDNA. Genes targeted in the ctDNA-matched and tissue-only-matched groups are shown in Fig. 3b. OS varied by gene target; trends toward shorter OS among patients with ctDNA were observed in all genes (Supplementary Fig. 6).

To test whether our findings regarding ctDNA-matched targeted therapy might be confounded by the histopathologic and treatment diversity of our cohort, we repeated these analyses restricted to treatment-naïve patients with adenocarcinoma (Fig. 3) and in multivariate analyses including clinical and radiologic variables (Supplementary Fig. 7). The relationships for patients treated or not treated with targeted therapy, stratified by ctDNA presence, were similar.

In summary, despite the lower baseline survival of patients with detectable ctDNA, among those patients, ctDNA-matched targeted therapy was associated with survival benefit, to an even greater extent than with tissue-matched therapy among those without detectable ctDNA. Previous studies have suggested that ctDNA presence does not affect radiologic response to targeted therapy^{12,17}; however, we found that ctDNA-matched targeted therapy was associated with shorter OS than tissue-only-matched therapy, emphasizing the importance of long-term follow-up in evaluating the full benefits of any biomarker-driven therapy.

ctDNA may match patients to targeted therapy when tissue sequencing is unavailable⁶. Among the patients without tissue sequencing, ctDNA-matched targeted therapy was associated with better survival than no targeted therapy (Extended Data Fig. 3). ctDNA may also be useful for repeat sampling that is impractical with tissue. Here, ctDNA matched 11 patients to a second targeted therapy based on detection of an additional oncogenic driver at progression (Supplementary Fig. 8), although it should be noted that the evidence supporting targeted therapy directed against a second oncogene ranges considerably^{42–44}.

Plasma–tissue divergent genomics

It is established that tumor mutations may be absent in ctDNA due to lower rates of DNA shedding or limits of plasma assay sensitivity^{8,10,12}; these tumor-only mutations are associated with better survival (Fig. 2b). By contrast, the clinical relevance of alterations found on plasma but not clinical tissue sequencing is less clear. Of 429 patients with tissue sequencing (MSK-IMPACT) within 30 days of ctDx Lung sequencing, 109 (25%) patients had mutations or copy number alterations (CNAs) detected in ctDNA that were absent on tumor sequencing ('ctDNA-only' alterations). Three of these patients had *EGFR* T790M mutations, leading to changes in treatment. Additional potentially actionable ctDNA-only mutations included one *KRAS* G12C mutation, one *BRAF* V600E mutation and one *RET* fusion; none of these led to changes in therapy due to their absence in tissue.

We sought to evaluate whether ctDNA-only alterations associate with survival. The subgroup of patients with ctDNA-only alterations had worse survival than those with tissue-matched ctDNA alterations (Fig. 4a). These findings were consistent when the ctDNA-only alteration was either a mutation or CNA (Fig. 4b and Supplementary Table 8).

Using MSK-IMPACT, we quantified the proportion of ctDNA-only mutations attributable to CH or germline variation and tumor mutations missed because of stringent base-calling thresholds. Of the 109 patients with ctDNA-only alterations, 66 patients had ctDNA-only mutations. Among these 66 patients, 92 ctDNA-only mutations were detected. Of these 92 mutations, 11 (12%) were filtered out in MSK-IMPACT based on WBC sequencing suggesting a CH or germline mutation (Fig. 4c). Of the remaining 81 mutations, 44 (54%) were detectable in tissue sequenced by MSK-IMPACT at subthreshold levels (Fig. 4c). Similarly, of the 110 ctDNA-only CNAs, 56 (51%) were detectable at subclinical reporting levels in tissue MSK-IMPACT (Fig. 4d). Both called and subthreshold MSK-IMPACT mutations could be detected in WBC controls at low frequencies (Supplementary Fig. 9); it is unclear whether these mutations represent artifacts, low-level CH or tumor DNA in buffy coat. We investigated whether subthreshold detectability in MSK-IMPACT had any bearing on OS. Patients with ctDNA-only alterations had shorter survival than those with tissue-only-matched ctDNA alterations whether or not the alterations were at a detectable subthreshold level (Fig. 4b).

In summary, we found that ctDNA-only alterations are common and have significant prognostic value. A subgroup of 26 patients had no common alterations observed between ctDNA and tissue. These patients also had worse survival than those with tissue-matched ctDNA alterations (Fig. 4b).

Correlates of ctDNA-only alterations

We sought to assess whether ctDNA-only alterations might reflect higher cTF, tumor burden or other clinical variables. Patients with ctDNA-only alterations did not have higher average cTF (Fig. 5a). Patients without ctDNA alterations had smaller MTV (median, 122 mL; IQR, 58–284 mL) than those with tissue-matched ctDNA alterations (median, 252 mL; IQR, 109–484 mL; $P < 0.001$; Fig. 5b). The latter, in turn, had smaller MTV than patients with ctDNA-only alterations (median, 737 mL; IQR, 125–1,598 mL; $P < 0.001$; Fig. 5b). Patients with extrapulmonary disease were more likely to have ctDNA-only alterations, but patients with advanced age, male sex, smoking history, previous treatment and non-adenocarcinoma histologies were not more likely to have these alterations (Supplementary Fig. 10a). ctDNA-only alterations were not associated with TMB, tumor purity, sequencing depth (Supplementary Fig. 10b) or sequencing site (Supplementary Fig. 10c). Together, these findings suggest that genomic divergence between liquid and tumor biopsies is most related to tumor burden.

We sought to study the genomic composition of ctDNA-only alterations. The gene-level distribution of pathogenic alterations³² in ctDNA-only versus tissue alterations is shown in Fig. 5c. The altered genes more commonly seen in tissue were also more commonly seen in only ctDNA (Pearson's $R = 0.86$, $P < 0.001$; Fig. 5c). The relative proportions of ctDNA-only alterations to tissue alterations, however, showed over-representation of commonly subclonal alterations in secondary resistance genes^{45,46}, in particular, *RICTOR*, *NTRK1*, *MET* and *ERBB2* amplifications and *PIK3CA* mutations (Fig. 5c). These genes remained over-represented in alterations absent or at subthreshold levels in MSK-IMPACT and in treatment-naïve patients (Supplementary Fig. 11). In patients without overlap in genomic alterations between ctDNA and tissue, no significant over-representation was observed, leaving open the possibility that these cases may indicate a second primary rather than a single heterogeneous disease.

The presence of the aforementioned alterations in secondary resistance genes, even in tissue, might confer worse prognosis. To test whether the worse survival seen in patients with ctDNA-only alterations is a result of underlying tumor genetics, we performed multivariate survival analysis including the presence of any ctDNA alteration, the presence of a ctDNA-only alteration and the presence of specific pathogenic gene alterations in either ctDNA or tissue. As expected based on previous tissue-based analyses⁴⁷, patients with *ALK*, *RET* and *EGFR* alterations had better survival, as expected given frequent treatment with targeted therapy among this group, while the presence of *STK11* alterations was associated with worse survival. The presence of *RICTOR*, *NTRK*, *MET*, *ERBB2* and *PIK3CA* alterations was not associated with worse survival. Conversely, the presence of ctDNA alterations and the presence of ctDNA-only alterations were both independently associated with worse survival (Fig. 5d). These findings suggest that ctDNA–tissue divergence, and not the genomic changes themselves, is prognostic and highlight the utility of liquid biopsies for detecting meaningful spatial genomic heterogeneity when paired with tissue sequencing.

We studied whether CH database filtering or serial sampling might differentiate whether ctDNA-only alterations are related to the tumor or CH. Mutations commonly seen in NSCLC (that is, ‘hotspots’) were more likely to be recovered as ctDNA mutations; however,

mutations could not be discriminated from CH on this basis because these mutations were also likely to be CH ‘hotspots’ (Supplementary Fig. 12)⁴⁸. CH mutations were not associated with statistically significantly worse survival in this cohort (Supplementary Fig. 13). Although CH mutations are associated with subtle differences in survival^{48–50}, the worse survival seen in patients with ctDNA-only alterations here was evidently not attributable to CH. Among the seven patients with ctDNA-only mutations and multiple ctDNA samples, ctDNA-only mutations qualitatively appeared in multiple samples or followed VAF trends of analogous tissue-matched ctDNA mutations (Supplementary Fig. 14).

Discussion

Despite the increased attention received in the technology industry⁷ and community adoption of liquid biopsy as a promising means of monitoring treatment response and selecting targeted therapy⁵¹, robust studies on its survival impact are lacking. We present the results of the largest prospective international cohort ($n = 1,127$) of patients with advanced NSCLC who underwent ctDNA-guided therapy with longitudinal follow-up for survival.

We find that ctDNA levels are correlated with radiologic features but have independent prognostic power in the general metastatic setting. The robust, independent prognostic utility of ctDNA suggests that liquid biopsies are a marker for increased micrometastatic or especially aggressive tumor biology and should supplement radiologic reports when assessing disease burden and chance of relapse following systemic therapy.

Overall, 23% of the patients were matched to targeted therapy by ctDNA testing. Targeted therapy was associated with greater relative survival benefit in patients with detectable ctDNA than in those without, highlighting the potentially greater survival gains with liquid biopsy-matched therapy. Future studies that use ctDNA for patient selection⁵¹ should account for the lower baseline OS of patients with detectable ctDNA to avoid underestimating the effects of therapy. At the same time, even though patients without detectable ctDNA have better prognosis overall, those patients may still further benefit from tissue-matched targeted therapy. Patients treated with targeted therapy, such as those with *EGFR*-mutant lung cancer, may not derive the same benefit from nontargeted treatments such as immunotherapy as those without driver mutations⁵², who comprise the control arm here. Randomized trials of novel therapies should be adequately stratified and powered to determine the relative survival benefits of particular targeted agents in patients with and without detectable ctDNA.

Plasma sequencing without paired WBC sequencing is common among commercial liquid biopsy assays⁷. We found that appropriate germline filters and a focus on NSCLC drivers may greatly reduce but not eliminate inclusion of CH and germline mutations. Even with WBC pairing, methods such as fragment-length analysis⁵³ may be helpful in differentiating tumor DNA from CH at especially low VAF.

Of the patients with time-matched clinical tissue sequencing, 25% had alterations in ctDNA not detected in tissue. These ctDNA-only alterations disproportionately featured commonly

subclonal drivers of resistance such as *RICTOR* amplification and *PIK3CA* mutation^{45,46} even in the treatment-naïve setting. Patients with ctDNA-only alterations had worse survival and greater MTV than even those with tumor-matched ctDNA alterations, which suggests that ctDNA-only alterations are related to tumor heterogeneity missed by spatially restricted tissue sequencing⁵⁴. Genomic heterogeneity in lung cancer is widespread and may be associated with worse prognosis⁵⁵. The presence of ctDNA-only alterations did not increase with age, unlike CH, and was not commonly found in matched WBCs. Plasma may have higher specificity for tumor mutations than previously reported¹⁰ and may be a valuable means of detecting subclonal genomic diversity when paired with tissue sequencing.

This study has a number of limitations. The nonrandom, real-world nature of this study makes it challenging to assess the survival benefit of any single targeted therapy with ctDNA matching. We find that ctDNA-matched therapy, like tissue-matched therapy¹, is associated with longer survival; however, the baseline of patients not treated with targeted therapy is heterogeneous and consists of driver-negative and nontargetable driver groups treated with a variety of therapies, as expected given the evolution of NSCLC treatments over the past decade^{2,56}. Patients with previous treatment reflect a particularly heterogeneous genomic landscape, and further studies on the interaction between specific resistance alterations and ctDNA detection are warranted. Despite our attempts to correct for immortality bias using variable entry times, randomized trials are necessary to confirm the benefits of particular targeted therapies. Reassuringly, our findings align with historic randomized trials and propensity-matched analyses, suggesting that targeted therapy matched by either ctDNA or tissue is associated with survival benefit^{1,57}. Our cohort was predominantly recruited from an academic cancer center in New York; although findings were validated in a community oncology practice in Sydney, further exploration of ctDNA use in community settings is warranted. Indeed, our findings suggest that treatment patterns differ between primary and referral settings, as do levels of ctDNA, likely due to differences in disease burden at presentation. Both cohorts also had lower smoking rates than expected from a typical population of patients with lung cancer. Generalization of our results to cohorts should be performed with caution. Both tumor and ctDNA sequencing were performed using targeted panels; estimates of tumor fraction, clonality and copy number changes will likely improve with broader sequencing approaches^{22,40}. Of the 230 patients with plasma sequenced by the 129-gene MSK-ACCESS panel, 188 (82%) had at least one detectable ctDNA alteration. The higher proportion of patients with detectable alterations using a broader panel aligns with other studies, suggesting that the rate of ctDNA detection is higher with such panels^{35,58}. The focused ctDx Lung panel was centered on therapeutic actionability. The calculation of cTF itself is challenging, particularly with sparse sequencing panels, and methods for cTF calculation vary considerably; our findings in this respect should be interpreted with caution.

Nonetheless, given the growing number of targeted therapies for NSCLC², next-generation sequencing of tumor DNA is crucial. Liquid biopsy-matched therapy provided survival benefit in patients with failed or missing tissue sequencing for practical reasons. Even in an academic setting in which consent to tissue sequencing was routine, tissue sequencing was absent in 20% of the patients. In real-world settings, the prevalence of incomplete tissue sequencing may exceed 50% and is particularly low in disadvantaged groups^{4,5,59}.

Although tumor sequencing remains valuable, our study suggests that in both academic and community settings, ctDNA-guided therapy may increase survival. ctDNA sequencing may increase clinical trial enrollment⁶⁰, which is also lower in disadvantaged patients^{4,61}, owing to fast turnaround of results³⁷. Lower failure rates and faster turnaround time with liquid versus tissue biopsies here corroborate this notion, especially after accounting for the time required to obtain tissue from external institutions, schedule biopsies or process samples. The minimally invasive nature of ctDNA collection may also increase clinical trial participation among older patients also underrepresented due to comorbidities and time demands on both the patient and provider^{61–63}. Owing to their low failure rate coupled with a fast turnaround time, liquid biopsies are well suited to expansion into retail clinics within pharmacies and home settings, further lowering barriers to precision cancer medicine for communities at large^{64,65}. Greater availability of these assays will in turn provide an invaluable tool for monitoring disease relapse^{19,20,66}, emergence of secondary actionable drivers^{67–69} and tumor evolution⁷⁰. As technologies continue to improve, liquid biopsies may ultimately offer similar benefits in the early detection of cancer^{40,71}. By helping to overcome barriers to sequencing, liquid biopsies provide an opportunity to match patients to life-prolonging therapies on a previously unseen scale.

Methods

Statistics and reproducibility

The study included adults with stage IV or recurrent NSCLC and either no known driver mutation before enrollment or progression of disease following targeted therapy. The enrollment began on 21 October 2016; this analysis included patients enrolled until 1 November 2020 with follow-up until 31 December 2021. Patients were followed for a median of 445 (IQR, 171–826) days following study enrollment. Patients provided written informed consent and were enrolled in a continuous, nonrandom fashion. Patients were not compensated for participation in the study. The study was independently approved by the institutional review board of each site. Patients in MSK were enrolled as part of a prospective sequencing protocol (NCT01775072). No statistical method was used to predetermine sample size. Only patients not meeting the criteria for study inclusion (Fig. 1) were excluded from the analysis; no data were otherwise excluded unless they did not meet the criteria for particular analyses. The experiments were not randomized. The investigators were not blinded to allocation during experiments and outcome assessment.

Germline variant filtering and benchmarking

To reduce inclusion of incidental germline mutations, we applied two filters to variant calls from the plasma-only ctDx Lung assay. Filter 1 removed variant calls present in gnomAD⁷² at greater than 0.5% population frequency, a threshold with high specificity for germline mutations⁷³ that is standard for ctDx Lung clinical reporting. Filter 2 removed mutations present in gnomAD at any population frequency with a VAF of 35–65%, a range in which germline mutations are frequently recovered in plasma³⁵, but retained known oncogenic driver mutations³². There were 10,410 ctDx Lung unfiltered ctDNA mutations identified across all 1,631 samples; of these, 7,165 (69%) mutations were removed by filter 1 and an

additional 156 (1%) were removed by filter 2. The remaining 3,089 mutations were analyzed (Supplementary Fig. 1).

To examine the accuracy of this filtering method, we examined filtered mutations in samples with time-matched WBC filtering (MSK-IMPACT or MSK-ACCESS), which served as a gold-standard control for the detection of germline mutations. A total of 555 ctDx Lung samples had time-matched MSK-IMPACT or MSK-ACCESS sequencing. Among these samples, 1,616 plasma mutations with a VAF of 35–65% were filtered out and had WBC concentrations with a VAF of 35–65% (true negatives), 31 were unfiltered and had WBC VAF < 35% (true positives), 4 were unfiltered and had WBC VAF > 35% (false positives) and one was excluded but had WBC VAF < 35% (false negative). The positive predictive value for tumor mutations with a VAF of 35–65% was 89%, and the negative predictive value was >99%.

Survival analyses

All OS analyses measured time from diagnosis of stage IV or recurrent NSCLC to the time of death, right-censored at the date of the last follow-up. To mitigate immortality bias, left truncation at the time of cohort entry was applied, and targeted therapy treatment was encoded as a time-dependent variable. HRs, CIs and *P* values were calculated using Cox proportional hazards models. *P* values < 0.05 were considered significant, although in the setting of multiple hypotheses, this should be interpreted as exploratory. Analyses were conducted in Python 3.7.4 using lifeline package 0.25.7. In the analyses of the prognostic value of ctDNA, those with failed ctDx Lung testing (*n* = 24) were excluded. In multivariate analyses including radiologic features as variables, those without time-matched PET scans were excluded.

Prognostic value and correlates of ctDNA

We compared the survival of patients with any mutation or CNA on their first ctDx Lung test (that is, those with ‘detectable’ ctDNA) to those without any alteration. Patients with ctDNA testing failure (*n* = 24) were excluded from this analysis.

We assessed the relationships between age (greater or less than median), smoking status (current/former versus never smoker), sex, histologic subtype (adenocarcinoma, squamous cell carcinoma or other), previous NSCLC systemic therapy and radiologic extrapulmonary disease (at least one SUV avid extrapulmonary lesion on [18F]fluorodeoxyglucose ([18F]FDG) PET within 30 days of ctDNA draw) with ctDNA alteration number and VAF using histograms and Mann–Whitney *U* or Kruskal–Wallis tests. We evaluated the prognostic value of ctDNA alterations in a multivariate model, including the aforementioned clinical features and receipt of targeted therapy as variables.

Targeted therapy

We compared OS for patients matched to targeted therapy following study entry to that for patients treated with other therapies; patients were further grouped based on matching (ctDNA-only versus tissue-only). Only targeted therapies initiated after study enrollment were considered. Patients were matched to targeted therapy based on OncoKB level 1 or

2 alterations, with the majority being matched based on level 1 alterations (Supplementary Table 6). Patients with ctDNA testing failure were treated as lacking ctDNA alterations (either mutations or CNAs) for this analysis.

ctDNA–tissue discordance

We measured the incidence of ctDNA alterations absent on time-matched (from a specimen obtained within 30 days of plasma draw) tissue sequencing with MSK-IMPACT. Only loci overlapping between panels from the earliest sample pair were considered. Mutations present in ctDNA that were absent in MSK-IMPACT were genotyped for subthreshold evidence of mutation from MSK-IMPACT Binary Alignment Map files³⁴; CNAs were similarly reevaluated using a previously validated algorithm⁷⁴. Among the 429 patients with time-matched ctDx Lung and MSK-IMPACT sequencing, 131 CNAs were detected across the 11 genes in both panels by the MSK-IMPACT clinical pipeline. Of these, 31 (24%) were detected by ctDx Lung. An additional 110 CNAs were detected by ctDx Lung but not MSK-IMPACT. Among the 290 MSK-IMPACT mutations in regions covered by ctDx Lung, 178 (61%) mutations were detected by ctDx Lung. An additional 96 mutations were detected by ctDx Lung but not MSK-IMPACT. We compared the OS of patients who had ctDNA-only alterations to those who did not.

cTF calculation

The relationship between VAF and cTF was assumed as

$$\text{VAF} = \frac{\text{Mutant DNA from tumor}}{\text{Healthy tissue DNA} + \text{tumor DNA}} = \frac{\text{cTF} \times \text{TCN} \times \text{BAF}}{2 \times (1 - \text{cTF}) + \text{TCN} \times \text{cTF}}$$

where TCN is the tumor copy number at a given allele and BAF is the B allele frequency or proportion of TCN assumed to be mutant. cTF in ctDNA was thus calculated as follows.

$$\text{cTF} = \frac{2 \times \text{VAF}}{\text{TCN} \times \text{BAF} - \text{VAF} \times (\text{TCN} - 2)}$$

The cTF of a sample was taken as the maximum cTF of all mutations. Given the small panel size used for ctDNA sequencing, TCN and BAF were calculated using Fraction and Allele-Specific Copy Number Estimates from Tumor Sequencing (FACETS) applied to time-matched tumor IMPACT sequences. Only clonal mutations (that is, those with either cancer cell fraction >0.8 or cancer cell fraction >0.7 and upper 95% CI of cancer cell fraction >0.9 according to FACETS) were considered. Patients in whom at least one clonal tissue mutation did not appear in ctDNA or for whom FACETS did not converge were labeled as having incalculable cTF.

PET–CT imaging analysis

Time-matched FDG PET–computed tomography (CT) scan images were retrieved from the picture archiving and communication system. The MTV of all tumor lesions on PET–CT was semiautomatically contoured using the Beth Israel PET–CT viewer plugin for FIJI (ImageJ version 1.53q)⁷⁵. MTV was calculated using the recommended 41% SUV_{max}

threshold⁷⁶. Based on the MTVs, additional PET metrics of SUVs (SUV_{max} and SUV_{mean}) and total lesion glycolysis ($SUV_{mean} \times MTV$) were calculated. For MTV and total lesion glycolysis, the sum of all lesions was used for analysis.

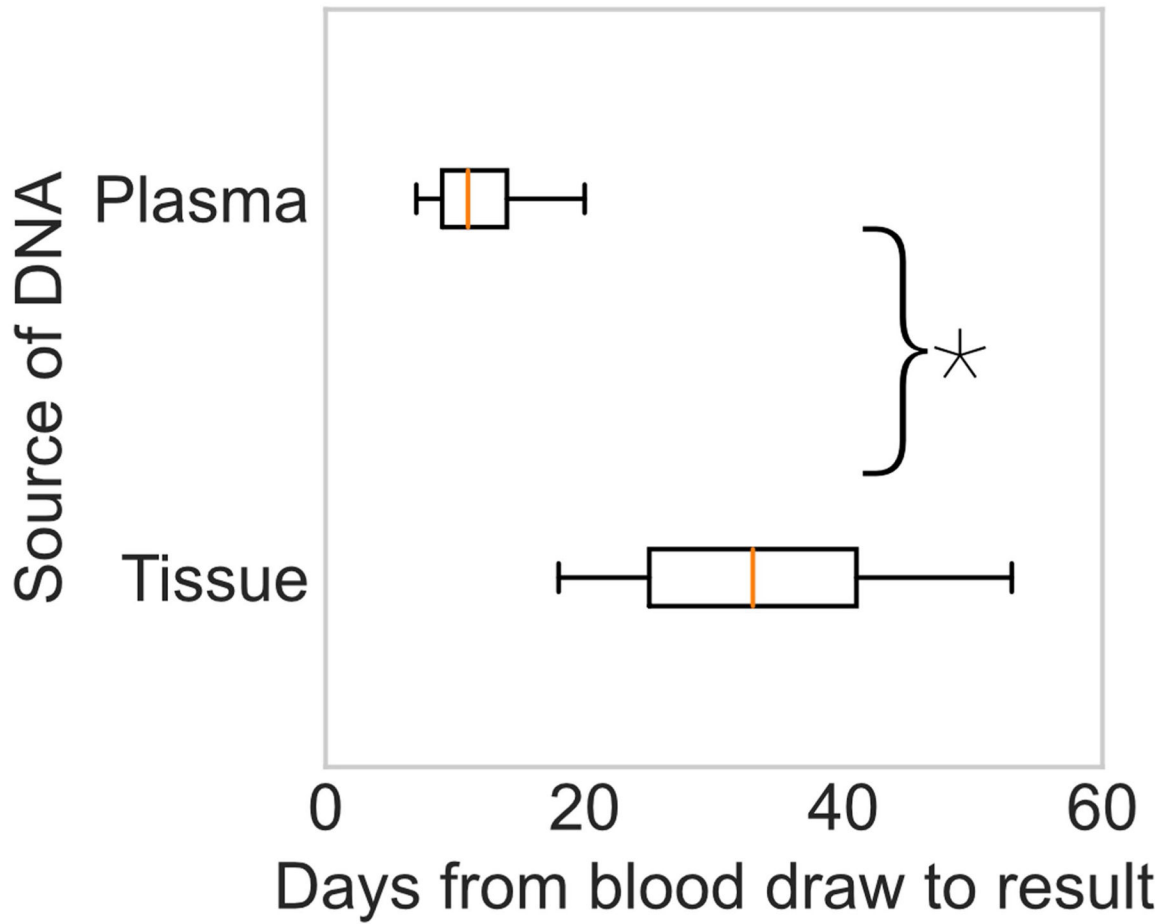
MSK-ACCESS pipeline

MSK-ACCESS is a hybrid-capture, duplex barcoded sequencing panel that allows for two bioinformatic methods of calling mutations: (1) de novo base calling, requiring a given mutation to be present in at least three separate duplex consensus reads for recurrently observed ‘hotspot’ mutations and at least five separate duplex consensus reads for non-hotspot mutations, and (2) ‘genotyping’, in which a variant previously observed in a given patient (in either MSK-IMPACT or MSK-ACCESS samples with de novo base calling) is called as a mutation with a lower threshold (at least one duplex consensus read or two simplex (single-stranded) consensus reads)³⁵. In this study, a total of four actionable mutations resulting in therapy matching were identified in MSK-ACCESS ctDNA samples using genotyping alone (that is, these would not have been discovered were they not previously discovered in a given patient’s tissue sample): one *EGFR* L858R mutation, one *EGFR* exon 19 deletion and two *KRAS* G12C mutations. Because this is the default base calling algorithm for MSK-ACCESS and because it is possible for driver mutation profiles to change across time independently of base calling methods (that is, for biological reasons), these patients were treated as being matched to targeted therapy by ctDNA for the purposes of analysis.

ctDx Lung versus MSK-ACCESS

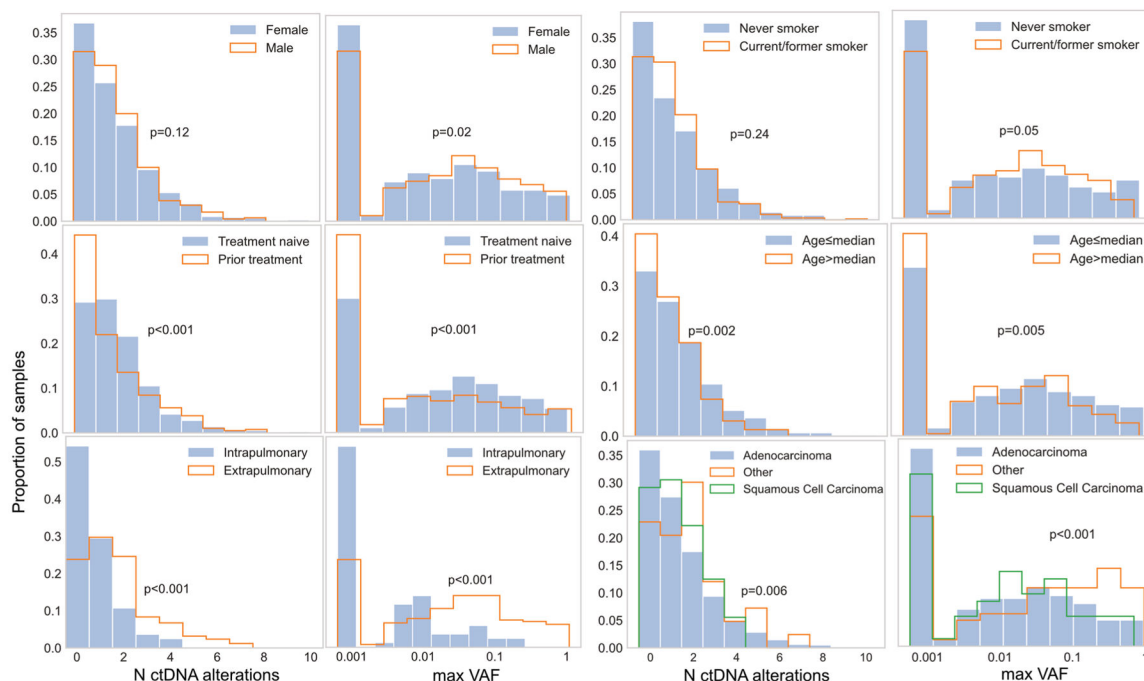
Among the 163 patients with ctDx Lung and MSK-ACCESS tests within 30 days of each other, 54 mutations were present on ctDx Lung testing only, while 55 mutations were present on MSK-ACCESS only. Of the 54 unique ctDx Lung mutations, 30 (56%) of them were filtered out based on WBC sequencing (Extended Data Fig. 3). The 24 (44%) remaining mutations were below the subthreshold or not detected at any frequency in MSK-ACCESS. A total of 216 mutations were detected on both assays. The concordance correlation coefficient of all mutations together was 0.98; the Pearson’s *R* for these same mutations was also 0.98, $P < 0.001$. Of the 230 patients with both ctDx Lung and MSK-ACCESS testing, 13 were matched to targeted therapy based on a ctDNA alteration not detected on ctDx Lung testing, while 3 patients were matched based on a ctDNA alteration not detected on MSK-ACCESS.

Extended Data



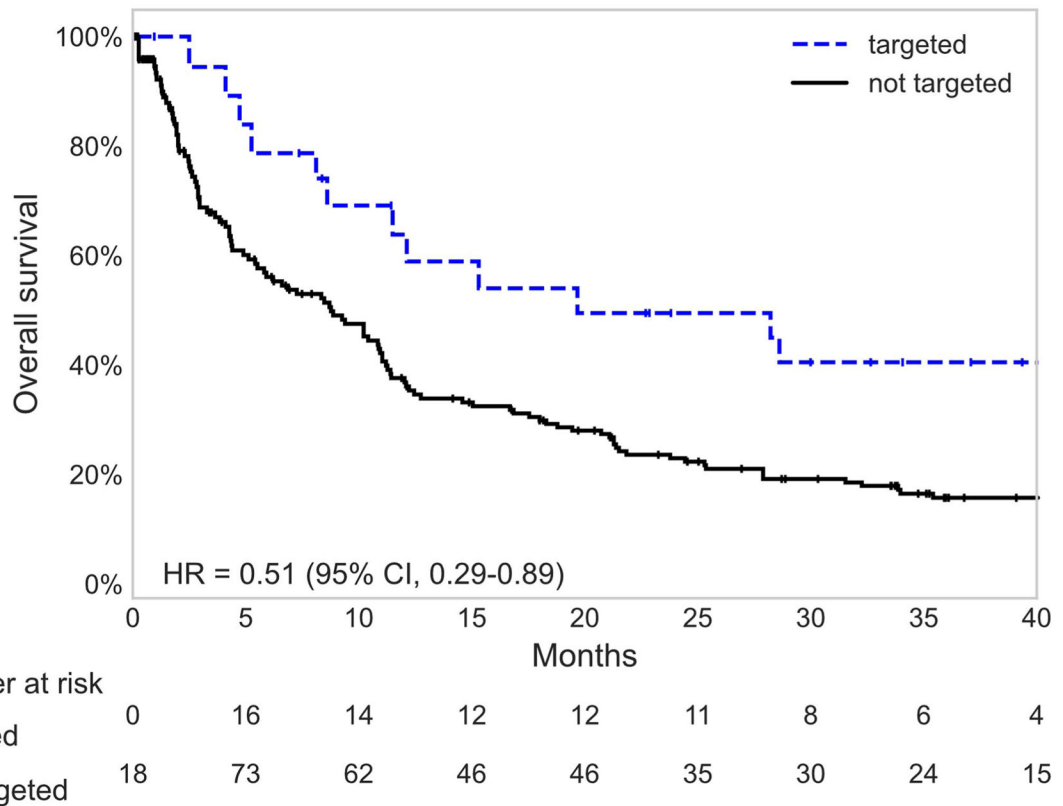
Extended Data Fig. 1 | Turnaround time of plasma and tissue sequencing.

Boxplots showing median (center) \pm 25%ile (boxes) and 95%ile (whiskers) of turnaround time for liquid biopsy (MSK-ACCESS and ctDx Lung, N independent samples = 2,162) and tissue (MSK-IMPACT, N independent samples = 612) sequencing from date of blood collection. Tissue start time is the date of white blood cell control collection. The turnaround time for plasma ctDNA sequencing was significantly faster than for tissue sequencing (*two-sided Mann-Whitney U, $p < 0.001$).



Extended Data Fig. 2 | Correlates of ctDNA alteration levels.

Histograms showing the proportion of patients with either number of ctDNA alterations or maximum mutation variant allele frequency (VAF) per sample. P-values are from Mann-Whitney U tests or Kruskal-Wallis tests for histologic subgroups. Raw VAFs of zero are set to the minimum value of the log axis.



Extended Data Fig. 3 |. Survival of patients without tissue sequencing matched to targeted therapy by ctDNA.

Kaplan-Meier survival curves for patients without tissue sequencing matched or not matched to targeted therapy. Number at risk in each category is adjusted for left truncation and time-dependent nature of targeted therapy variables.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Authors

Justin Jee¹, Emily S. Lebow¹, Randy Yeh¹, Jeeban P. Das¹, Azadeh Namakydoust¹, Paul K. Paik^{1,2}, Jamie E. Chaff^{1,2}, Gowtham Jayakumar¹, A. Rose Brannon¹, Ryma Benayed¹, Ahmet Zehir¹, Mark Donoghue¹, Nikolaus Schultz¹, Debyani Chakravarty¹, Ritika Kundra¹, Ramyasree Madupuri¹, Yonina R. Murciano-Goroff¹, Hai-Yan Tu^{1,3}, Chong-Rui Xu^{1,3}, Andrés Martínez¹, Clare Wilhelm¹, Jesse Galle¹, Bobby Daly^{1,2}, Helena A. Yu^{1,2}, Michael Offin^{1,2}, Matthew D. Hellmann^{1,2}, Piro Lito^{1,2}, Kathryn C. Arbour^{1,2}, Marjorie G. Zauderer^{1,2}, Mark G. Kris^{1,2}, Kenneth K. Ng^{1,2}, Juliana Eng^{1,2}, Isabel Preeshagul^{1,2}, W. Victoria Lai^{1,2}, John J. Fiore^{1,2}, Afsheen Iqbal^{1,2}, Daniela Molena^{1,2}, Gaetano Rocco^{1,2}, Bernard J. Park^{1,2}, Lee P. Lim⁴, Mark Li⁴, Candace Tong-Li^{5,6}, Madhawa De Silva⁵, David L. Chan⁵, Connie I. Diakos⁵, Malinda Itchins⁵, Stephen Clarke⁵, Nick Pavlakis⁵, Adrian Lee⁵, Natasha Rekhtman^{1,2}, Jason Chang^{1,2}, William D. Travis^{1,2}, Gregory J. Riely^{1,2}, David B.

Solit^{1,2}, Mithat Gonen¹, Valerie W. Rusch^{1,2}, Andreas Rimner^{1,2}, Daniel Gomez^{1,2}, Alexander Drilon^{1,2}, Howard I. Scher^{1,2}, Sohrab P. Shah¹, Michael F. Berger¹, Maria E. Arcila^{1,2}, Marc Ladanyi^{1,2}, Ross L. Levine^{1,2}, Ronglai Shen¹, Pedram Razavi^{1,2}, Jorge S. Reis-Filho^{1,2}, David R. Jones^{1,2}, Charles M. Rudin^{1,2}, James M. Isbell^{1,2}, Bob T. Li^{1,2,✉}

Affiliations

¹Memorial Sloan Kettering Cancer Center, New York, NY, USA.

²Weill Cornell Medicine, Cornell University, New York, NY, USA.

³Guangdong Lung Cancer Institute, Guangdong Provincial People's Hospital and Guangdong Academy of Medical Sciences, Guangzhou, China.

⁴Resolution Bioscience, Agilent Technologies, Kirkland, WA, USA.

⁵GenesisCare, University of Sydney, Sydney, Australia.

⁶Massachusetts Institute of Technology, Cambridge, MA, USA.

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Data availability

Genomic and clinical data are available on cBioPortal (https://www.cbioportal.org/study/summary?id=nsclc_ctdx_msk_2022). The raw sequencing data for MSK-IMPACT and MSK-ACCESS are protected and are not broadly available due to privacy laws. Researchers at MSK with appropriate IRB permission may request the data from the Center for Molecular Oncology (skicmopm@mskcc.org).

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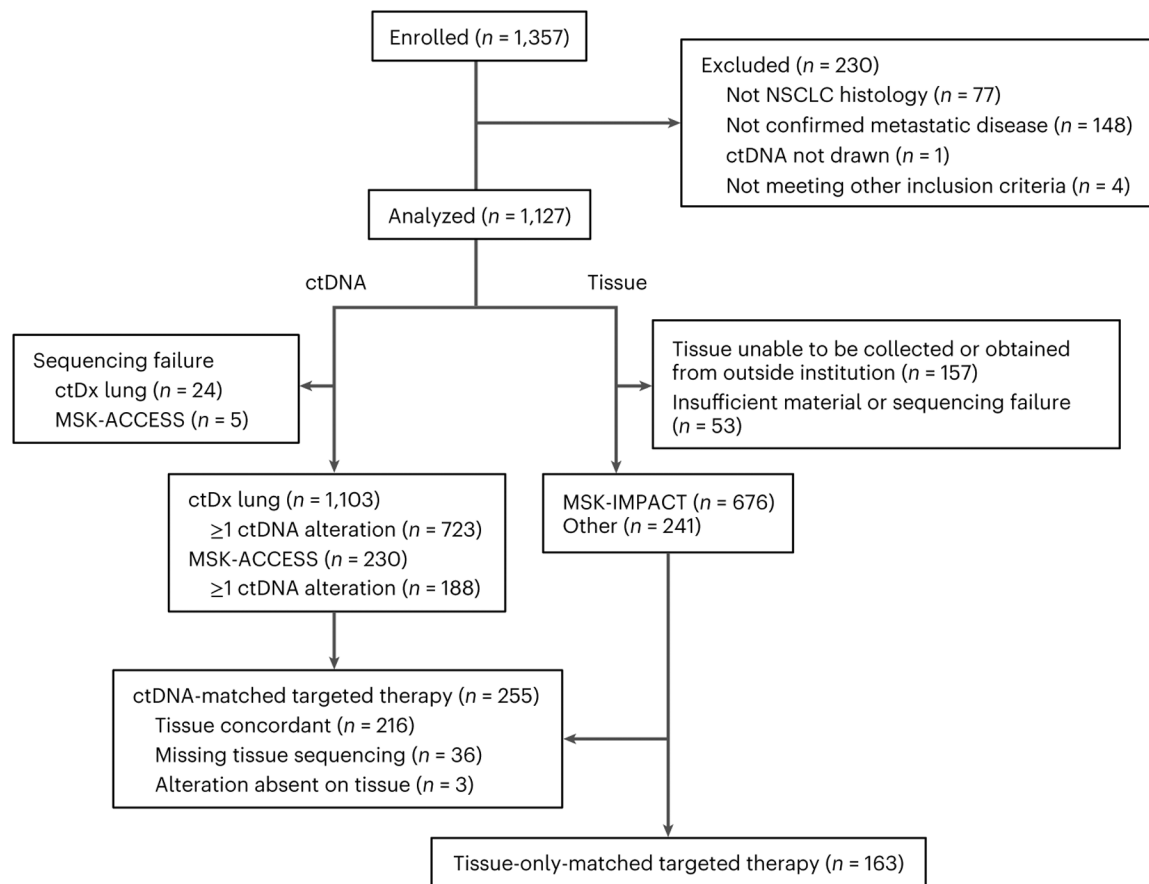


Fig. 1 |. Status of enrolled patients. CONSORT diagram showing excluded patients as well as status of ctDNA/tissue sequencing and targeted therapy during the study for included patients.

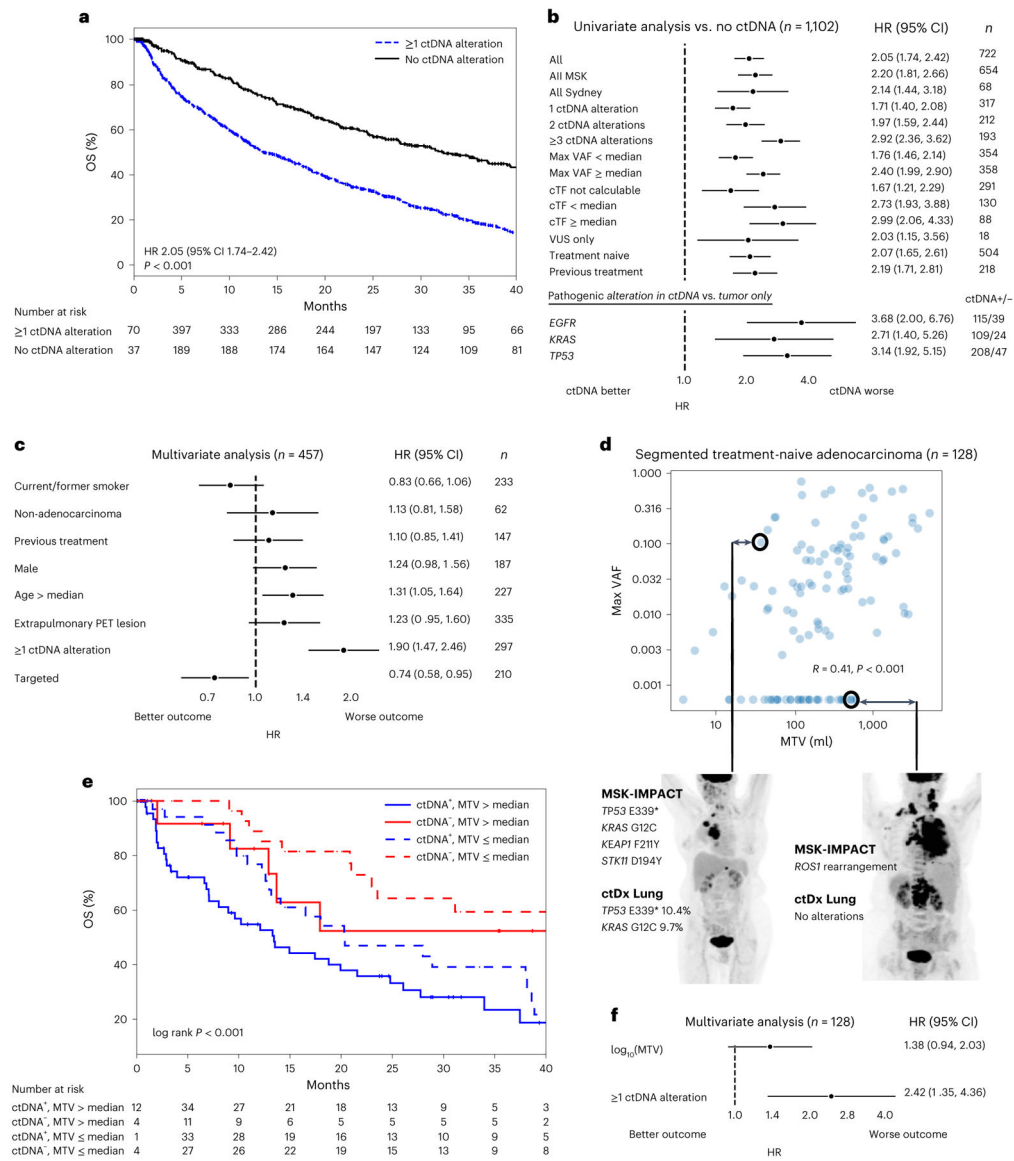


Fig. 2 | ctDNA alterations and OS.

a, Kaplan–Meier survival curves for patients with versus without ctDNA detected (defined by alteration presence). Number at risk adjusted for study/variable entry time. **b**, Forest plots comparing patients with versus without ctDNA alterations in the listed subgroups (median OS and the number of independent patients in both the ctDNA⁺ and ctDNA⁻ arms are given in Supplementary Table 3). Error bars represent 95% CI. *EGFR*, *KRAS* and *TP53* subgroups compare patients with alterations in these genes in ctDNA to those with alterations in these genes in tumor tissue only. **c**, Multivariate Cox proportional hazards model with the listed variables in patients with time-matched PET imaging. ‘Targeted’, treated with targeted therapy. Error bars represent 95% CI. Total cohort size and number of independent patients in each variable arm are listed in the figure (*n*). **d**, Scatterplot showing the relationship between ctDNA maximum VAF and MTV among segmented patients with treatment-naïve adenocarcinoma. Values of zero are set to the minimum of the respective log axes. *R* and

P values from two-sided Spearman's correlation coefficient ($P = 2 \times 10^{-6}$). Representative examples of discordance between MTV and VAF are shown. **e.** Kaplan–Meier survival curves for patients stratified by MTV and ctDNA detection. Number at risk adjusted for study/variable entry time. **f.** Multivariate Cox proportional hazards model with $\log_{10}(\text{MTV})$ as a continuous variable and ctDNA detection. Error bars represent 95% CI. *n*, number of independent patients in the total analysis.

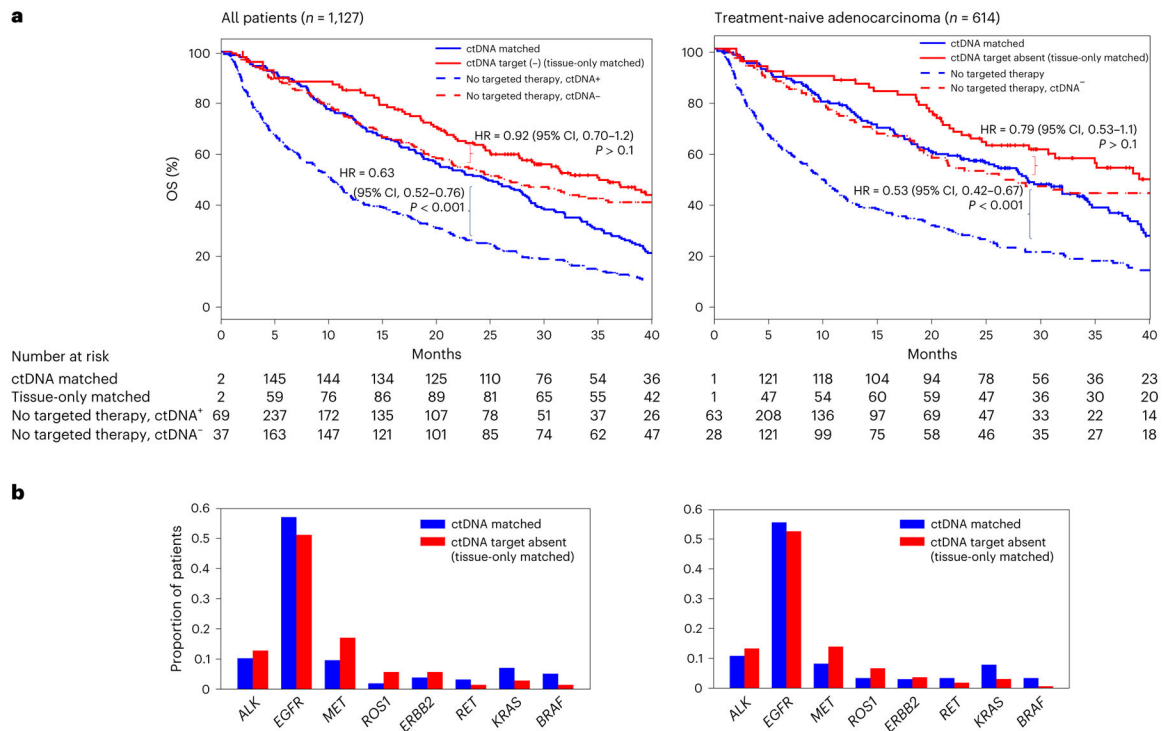


Fig. 3 |. Clinical utility of ctDNA and tissue sequencing.

a, Kaplan–Meier survival curves for patients matched to targeted therapy by ctDNA, not treated with targeted therapy but ctDNA⁺, matched to targeted therapy by tissue only and not treated with targeted therapy and ctDNA⁻. Number at risk adjusted for study/variable entry time. *P* values are from a two-sided Cox proportional hazards model and were not adjusted for multiple hypotheses. **b**, Number of patients matched to a given molecular target by ctDNA and tissue sequencing or tissue sequencing only.

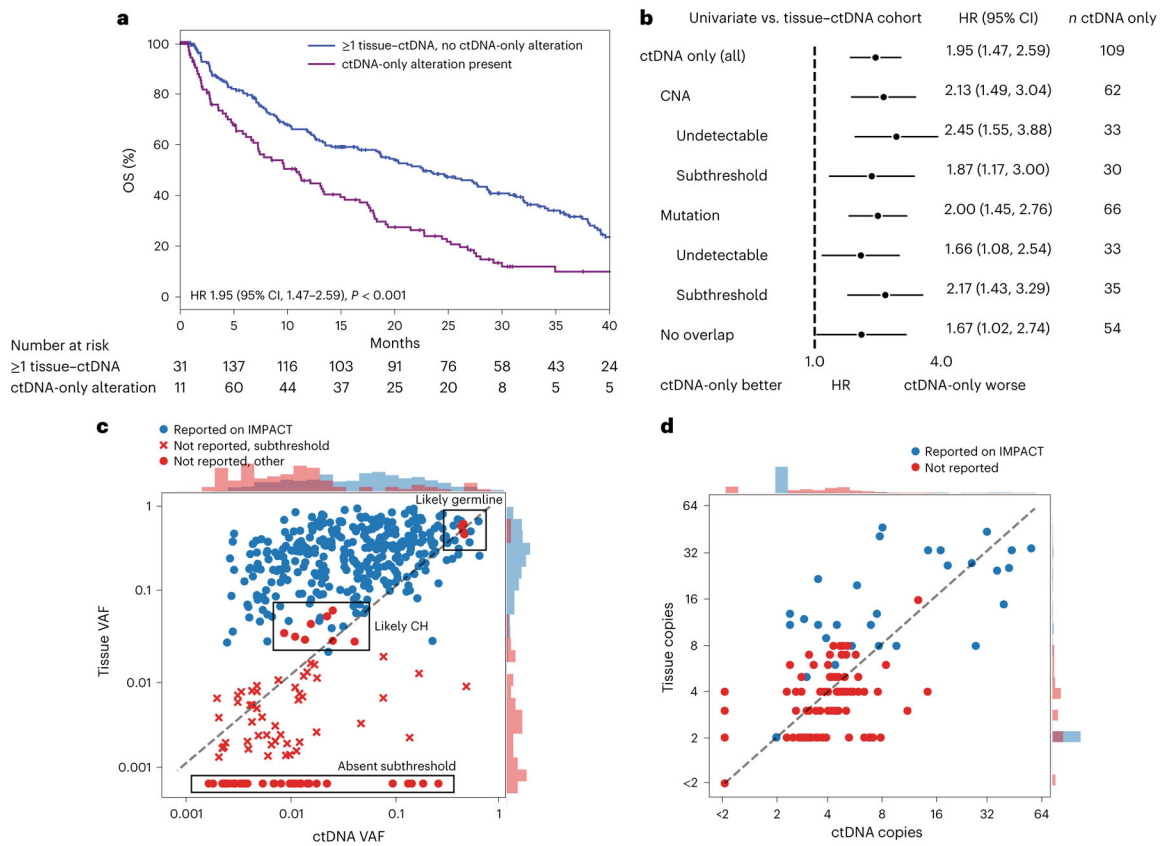


Fig. 4 | ctDNA mutations not detected on time-matched tissue sequencing (MSK-IMPACT).
a, Kaplan–Meier survival curves for patients without ctDNA alterations (black) or with ctDNA-only alterations (purple), compared by two-sided Cox proportional hazards model ($P = 1 \times 10^{-5}$). **b**, Forest plots comparing patients with ctDNA-only alterations versus those with matched ctDNA–tissue alterations only in the listed subgroups (the median OS and number of independent patients in each arm are given in Supplementary Table 8). Error bars represent 95% CI. **c,d**, Scatterplots of mutations (**c**) and CNAs (**d**) detected in ctDNA. Red dots correspond to ctDNA-only alterations. The y axis in **d** shows CNA levels calculated algorithmically from tissue MSK-IMPACT. Histograms at the top and right show the density of points of each category along the respective axis. Raw values of zero were set to the minimum value of the log axis.

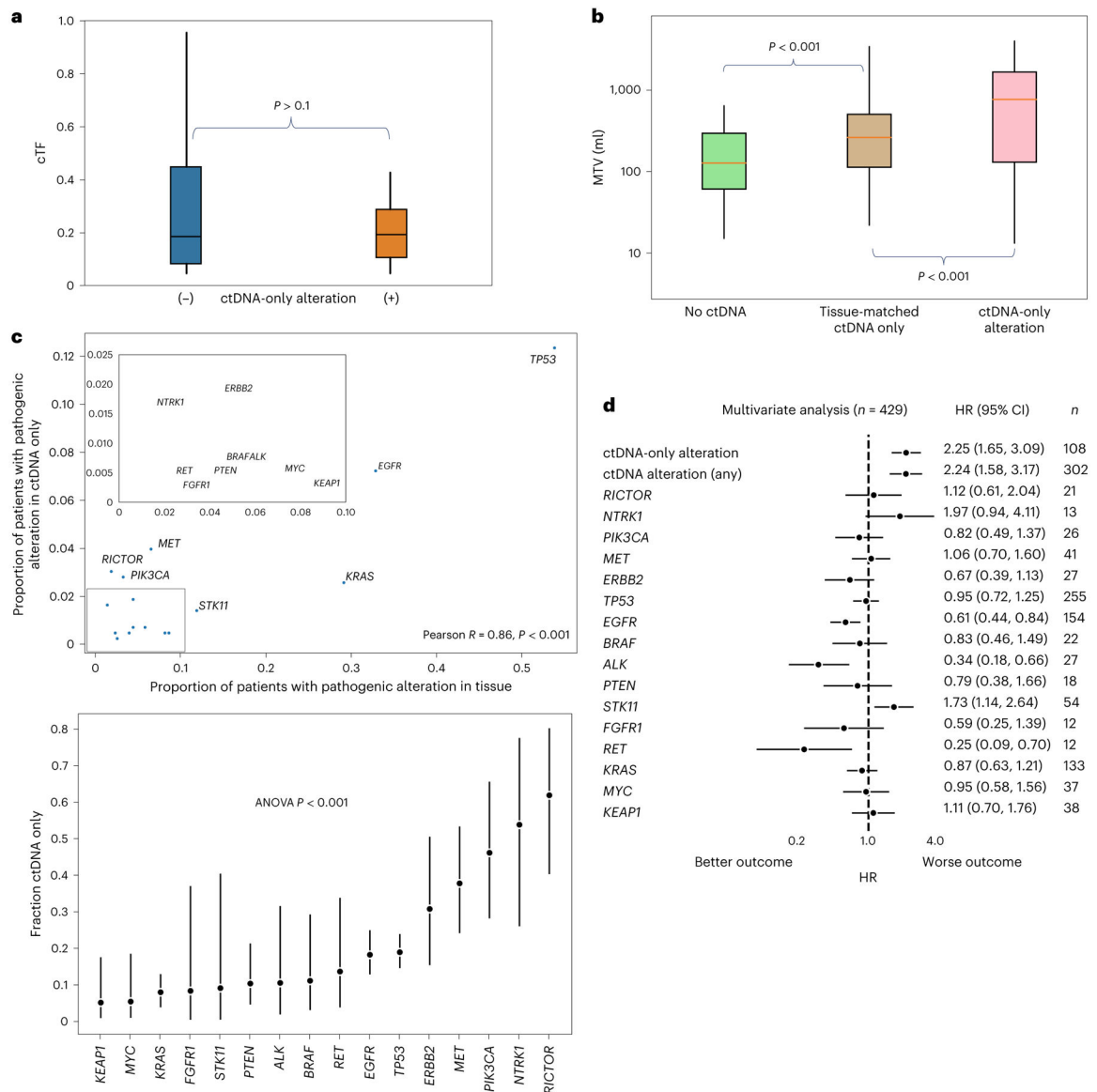


Fig. 5 | Correlates of plasma-tissue divergence.

a,b, Boxplots showing cTF for patients with ($n = 102$ independent patients) and without ($n = 30$ independent patients) ctDNA-only alterations (two-sided Mann-Whitney U test, $P = 0.5$) (**a**) and MTV on time-matched PET imaging (segmented independent patients only, $n = 129$) for patients without ctDNA ($n = 41$), with tissue-matched ctDNA only ($n = 62$) and with ctDNA-only alterations ($n = 25$) (**b**). Boxes denote medians \pm IQRs. Whiskers denote 1.5 times IQRs. P values are from two-sided Mann-Whitney U tests (P value for tissue-matched versus no ctDNA, 8×10^{-10} ; ctDNA-only versus tissue-matched, 1×10^{-5}). **c,** Top, scatterplot showing the proportion of patients with an oncogenic driver in a given gene as detected in either tissue (\pm ctDNA) or in ctDNA only (that is, not in tissue). Pearson's two-sided P value = 1×10^{-5} . Bottom, fraction of the total number of patients with an oncogenic driver in the indicated gene detected only in ctDNA. Center points are fractions; whiskers are binomial 95% CI. Only genes with at least 10 patients possessing

such a driver by either method are shown. ANOVA, P value = 4×10^{-13} . **d**, Multivariate Cox proportional hazards model with the listed variables. Oncogenic driver gene alterations refer to those found in either tissue or ctDNA.

Table 1 |

Baseline patient characteristics

Characteristic	MSK (<i>n</i> =1,002)	Sydney (<i>n</i> =125)	Total (<i>n</i> =1,127)
Age, median (IQR), years	68 (59–75)	67 (57–74)	68 (59–75)
Sex, <i>n</i> (% total)			
Men	432 (43%)	48 (38%)	480 (43%)
Women	570 (57%)	77 (62%)	647 (57%)
Race, <i>n</i> (% total)			
White	730 (73%)	37 (30%)	767 (68%)
Asian	164 (16%)	34 (27%)	196 (17%)
Black	53 (5%)	0 (0%)	53 (5%)
Other	20 (2%)	0 (0%)	20 (2%)
Unknown	35 (3%)	54 (43%)	89 (8%)
Histology, <i>n</i> (% total)			
Adenocarcinoma	849 (85%)	120 (96%)	969 (86%)
Squamous	84 (8%)	4 (3%)	88 (8%)
Other ^a	69 (7%)	1 (1%)	70 (6%)
Smoking history, <i>n</i> (% total)			
Current/former	553 (55%)	42 (34%)	595 (53%)
Never	449 (45%)	66 (53%)	515 (46%)
Unknown	0 (0%)	17 (14%)	17 (2%)
Treatment history, <i>n</i> (% total)			
Treatment naive	677 (68%)	51 (41%)	728 (65%)
Previous treatment	325 (32%)	74 (59%)	399 (35%)
Previous targeted therapy	152 (15%)	49 (39%)	201 (18%)
Previous nontargeted therapy	173 (17%)	25 (20%)	198 (17%)
Additional sequencing, <i>n</i> (% total)			
MSK-ACCESS	230 (23%)	NA	230 (20%)
Within 30 days	163 (16%)	NA	163 (14%)
MSK-IMPACT	676 (67%)	NA	676 (60%)
Within 30 days	429 (43%)	NA	429 (38%)
Other tissue sequencing	129 (13%)	112 (90%)	241 (21%)
No tissue sequencing ^b	197 (20%)	12 (10%)	209 (19%)
Concurrent PET, <i>n</i> (% total)			
MSK (<i>n</i> =337)		Sydney (<i>n</i> =120)	Total (<i>n</i> =457)
Extrapulmonary disease	252 (75%)	83 (69%)	335 (73%)
No extrapulmonary disease	85 (25%)	37 (31%)	122 (27%)

^aIncludes dedifferentiated, neuroendocrine and mixed histologic subtypes.

^bReasons included an inability to collect samples from the patient or an outside institution, insufficient material or sequencing failure.