The Pan-Tumor Landscape of Targetable Kinase Fusions in Circulating Tumor DNA

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ABSTRACT

Purpose: Oncogenic kinase fusions are targetable with approved and investigational therapies and can also mediate acquired resistance (AR) to targeted therapy. We aimed to understand the clinical validity of liquid biopsy comprehensive genomic profiling (CGP) to detect kinase fusions pan tumor.

Experimental Design: CGP was performed on plasma and tissue samples during clinical care. All exons plus selected introns of 16 kinases involved in oncogenic fusions (*ALK, BRAF, EGFR, ERBB2, FGFR1/2/3, MET, NTRK1/2/3, PDGFRA/B, RAF1, RET,* and *ROS1*) were sequenced to capture fusions, including well-characterized and novel breakpoints. Plasma circulating tumor DNA (ctDNA) fraction was estimated to inform sensitivity.

Results: Of 36,916 plasma cases, 32,492 (88%) had detectable ctDNA. Kinase fusions were detected in 1.8% of ctDNA-positive cases (571/32,492) and were most prevalent in patients with cholangiocarcinoma (4.2%), bladder cancer (3.6%), and non–small cell lung cancer (NSCLC; 3.1%). Of the 63 paired patient samples that had tissue and ctDNA specimens collected within 1 year and with estimated plasma ctDNA fraction >1%, fusions were detected in 47 of 51 (92%) liquid specimens with a fusion in the tissue sample. In 32 patients with fusions detected in liquid but not in tissue, 21 (66%) had evidence of putative acquired resistance.

Conclusions: Targetable kinase fusions are identified in ctDNA across cancer types. In pairs with tissue-identified fusions, fusion detection in ctDNA is reliable with elevated ctDNA fraction. These data support the validity of CGP to enable ctDNA-based fusion detection for informing clinical care in patients with advanced cancer.

Introduction

Kinases activated by gene fusions are established oncogenic drivers and therapeutic targets associated with both hematopoietic malignancies and solid tumors. A number of tyrosine kinase gene fusions (i.e., the *NTRK* and *FGFR* gene families) have been identified across several different cancers, while recent approvals of *NTRK* inhibitors have led to routine diagnostic testing for *NTRK* fusions across many cancer types (1). Kinase fusions have also been observed in patients following initial treatment with targeted therapy, suggesting that kinase fusions may be an acquired resistance (AR) mechanism in *EGFR*-mutant lung cancers, potentially benefitting from combination strategies targeting EGFR and the acquired kinase fusion (2–5).

The emergence of high-quality, validated diagnostic tools for genomic analysis of circulating tumor DNA (ctDNA) has created new opportunities to test for kinase fusions across tumor types, including in patients with targeted therapy resistance. Liquid biopsies have the advantage of being less invasive than traditional tissue biopsies, while potentially generating insights into tumor heterogeneity (6–11).

Liquid biopsy using next-generation sequencing (NGS) of ctDNA is becoming a more widely used diagnostic tool, now with FDA approval of multigene panels offering robust and simplified profiling across solid tumors (12, 13). To date, tissue-liquid concordance has varied widely, particularly with regard to fusion detection on some platforms (14–17), potentially related to the design of some assays. Given their growing role in treatment selection at both the time of initial diagnosis and at relapse, there is a particular need for data supporting liquid biopsy sensitivity for kinase fusions. We hypothesize that liquid biopsy is a reliable method for sensitive pan-tumor detection of oncogenic kinase fusions in specimens with adequate ctDNA shed.

In this study, we analyzed a real-world dataset consisting of high-quality, validated hybrid capture-based NGS results generated during the course of routine clinical care to characterize the pan-cancer landscape of kinase fusions in 36,916 ctDNA samples [FoundationACT (FACT), FoundationOne Liquid (F1L), or FoundationOne Liquid CDx (F1LCDx)] and 368,931 tumor tissue samples (FoundationOne or FoundationOne CDx). This study was designed to determine whether a ctDNA assay could reliably identify fusions that were detected in tissue samples from the same patient. To our knowledge, this is the largest correlative study of the genomic sequencing of tissue and liquid biopsy specimens in a pan-cancer patient population.

Materials and Methods

Patient population

Approval for this retrospective study, including a waiver of informed consent and a Health Insurance Portability and Accountability Act waiver of authorization, was obtained from the Western Institutional Review Board (protocol No. 20152817). Studies were performed in accordance with the Declaration of Helsinki. Liquid biopsy results with no evidence of ctDNA variants were excluded.

Translational Relevance

Kinase fusions are an important class of targetable oncogenic driver variants detectable using hybrid-capture DNA sequencing but can be challenging to detect in circulating tumor DNA (ctDNA). In this study, we report on the detection of kinase fusions in 1.8% of ctDNA-positive cases (571/32,492) spanning a diversity of cancer types, oncogenes, and breakpoints. Tissue/plasma discordance was primarily related to plasma ctDNA fraction and heterogeneity following putative treatment resistance. Overall, we find kinase fusion detection via ctDNA offers an emerging opportunity for minimally invasive characterization of pan-tumor driver variants.

Detected fusions were considered in this report if the fusion product included the full kinase domain of the target oncogene (*ALK*, *BRAF*, *EGFR*, *ERBB2*, *FGFR*, *MET*, *NTRK*, *PDGFRA/B*, *RAF1*, *RET*, *ROS1*) and the predicted chimeric protein included both an N-terminus and a C-terminus. For the fusion concordance analysis, patients were identified with at least one tumor NGS result (FoundationOne or FoundationOne CDx) and at least one liquid biopsy NGS result (FoundationACT, FoundationOne Liquid, or FoundationOne Liquid CDx, Supplementary Table S1) in the same disease, both with sufficient tumor content and coverage. Only fusions involving genes and exons covered by the tissue assay and the same exons and genes in the liquid assay were included in the concordance analysis. For patients with more than one liquid specimen available for concordance analysis, the liquid sample with the highest estimated ctDNA fraction was chosen. For patients with more than one tissue specimen available, the tissue specimen collected closest to the liquid specimen was chosen. Manual review of submitted pathology reports was also performed to collect patient treatment history if available.

Liquid and tissue NGS

Hybrid-capture based NGS was performed in formalin-fixed paraffin-embedded (FFPE) tumor tissue or blood samples prospectively collected from 405,847 patients in the course of clinical care. For tumor tissue samples, DNA was extracted from FFPE specimens and NGS was performed by hybridization capture, adaptor ligation-based libraries to high, uniform coverage (>500×) for all coding exons of up to 324 cancer-related genes plus selected introns (18). NGS of ctDNA was performed on ≥20 ng of ctDNA extracted from blood plasma to create adapted sequencing libraries before hybrid capture and sample-multiplexed sequencing to a median unique exon coverage depth of >6,000× for up to 324 genes (19, 20). Results were analyzed for base substitutions, short insertions and deletions (indels), copy-number alterations, and rearrangements. *NTRK* was only baited for on F1LCDx and not included in older liquid assays. All liquid biopsy specimens run on a historical version of the assay (FACT, F1L) were reanalyzed on an updated pipeline for consistency. Testing was performed in a CLIA-certified/CAP-accredited laboratory (Foundation Medicine Inc.).

Determination of ctDNA content in cell-free DNA fraction

The ctDNA fraction in the plasma cell-free DNA was estimated using two complementary methods. When ctDNA fraction is sufficient, a tumor fraction (TF) estimate is calculated based on a measure of tumor aneuploidy that incorporates observed deviations in coverage across the genome for a given sample. Calculated values for this metric are calibrated against a training set based on samples with well-defined TFs to generate an estimate of TF. When ctDNA content is lower, the maximum somatic allele frequency (MSAF) is determined by calculating the allele fraction for all known somatic, likely somatic, and variant of unknown significance (VUS) base substitutions, excluding certain common and rare germline variants. The estimated ctDNA fraction is based on the TF estimate when available and is generated from MSAF when lack of tumor aneuploidy limits the ability to return an informative estimate of TF.

Statistical and data analysis

The Fisher exact test was used to assess significance of categorical relationships, and the Benjamin–Hochberg procedure was used to control the false discovery rate. Differences between continuous variables were assessed by the Mann–Whitney U test. For sensitivity of kinase fusion detection between tissue and liquid biopsies, positive percent agreement (PPA) was calculated as true positives/true positives + false negatives, using tissue CGP as the reference. Negative percent agreement (NPA) was calculated as true negatives/true negatives + false positives.

Results

Patient characteristics

Foundation Medicine test results were available from 36,916 patients with liquid biopsy and 368,931 patients with tissue biopsy. The median patient age was 68 years, and 50% of patients were women (**Table 1**). Of 32,492 plasma samples with detectable ctDNA, at least one reportable genomic alteration (GA) was detected in 93% (30, 348), for an average of 3.3 GAs per case (**Table 1**).

Table 1. Clinico-genomic characteristics of the overall cohort and of select cancer types.

	All ctDNA cases	NSCLC	Breast	CRC	CUP	Cholangiocarcinoma	Other cancer types
Total cases	36,916	10,754	5,148	2,742	1,918	901	15,453
Median age (y)	68	70	64	63	69	67	69
Gender (M:F)	50%:50%	47%:53%	0.84%:99%	57%:43%	50%:50%	50%:50%	66%:34%
Median ctDNA fraction	1.6%	1.5%	2.2%	3.3%	1.6%	0.96%	1.5%
Cases with ctDNA fraction > 0 (%)	32,492 (88%)	9,604 (89%)	4,617 (90%)	2,471 (90%)	1,667 (87%)	767 (85%)	13,366 (86%)
Cases with ≥1 GA (%)	30,348 (93%)	8,907 (93%)	4,413 (96%)	2,388 (97%)	1,569 (94%)	702 (92%)	12,369 (93%)
Avg GAs/case	3.3	3.0	3.8	4.2	3.1	2.9	3.2
Fusion-positive cases	571 (1.8%)	296 (3.1%)	44 (0.95%)	37 (1.5%)	29 (1.7%)	32 (4.2%)	133 (1.0%)

Kinase fusions detected in ctDNA from a diverse range of cancer types

Overall, 571 of 32,492 cases studied (1.8%) had kinase fusions detected in ctDNA (131 FACT, 237 F1L, 203 F1LCDx). Cholangiocarcinoma samples showed the highest frequency of kinase fusions (4.2%) followed by bladder cancer (3.6%), and NSCLC (3.1%; **Fig. 1A**; **Table 1**). Among 297 fusions detected in 296 NSCLC cases, the most frequently rearranged kinases were *ALK*

(171, 58%), *RET* (54, 18%), and *ROS1* (38, 13%; **Fig. 1B**). In NSCLC, kinase fusions involved diverse partners, most commonly *EML4-ALK* ($n = 159$), *KIF5B-RET* ($n = 34$), and *CD74-ROS1* ($n = 22$; **Fig. 2A**). In cholangiocarcinoma, *FGFR2* (85%, 28/33) was the most commonly rearranged kinase (**Fig. 1B**), most commonly fused to *BICC1* ($n = 8$; **Fig. 2B**). *ALK* fusions including *EML4-ALK* have been identified in multiple tumor types (21–24). Consistent with previous studies, we observed that 9.6% (20/209) of *ALK* fusion-
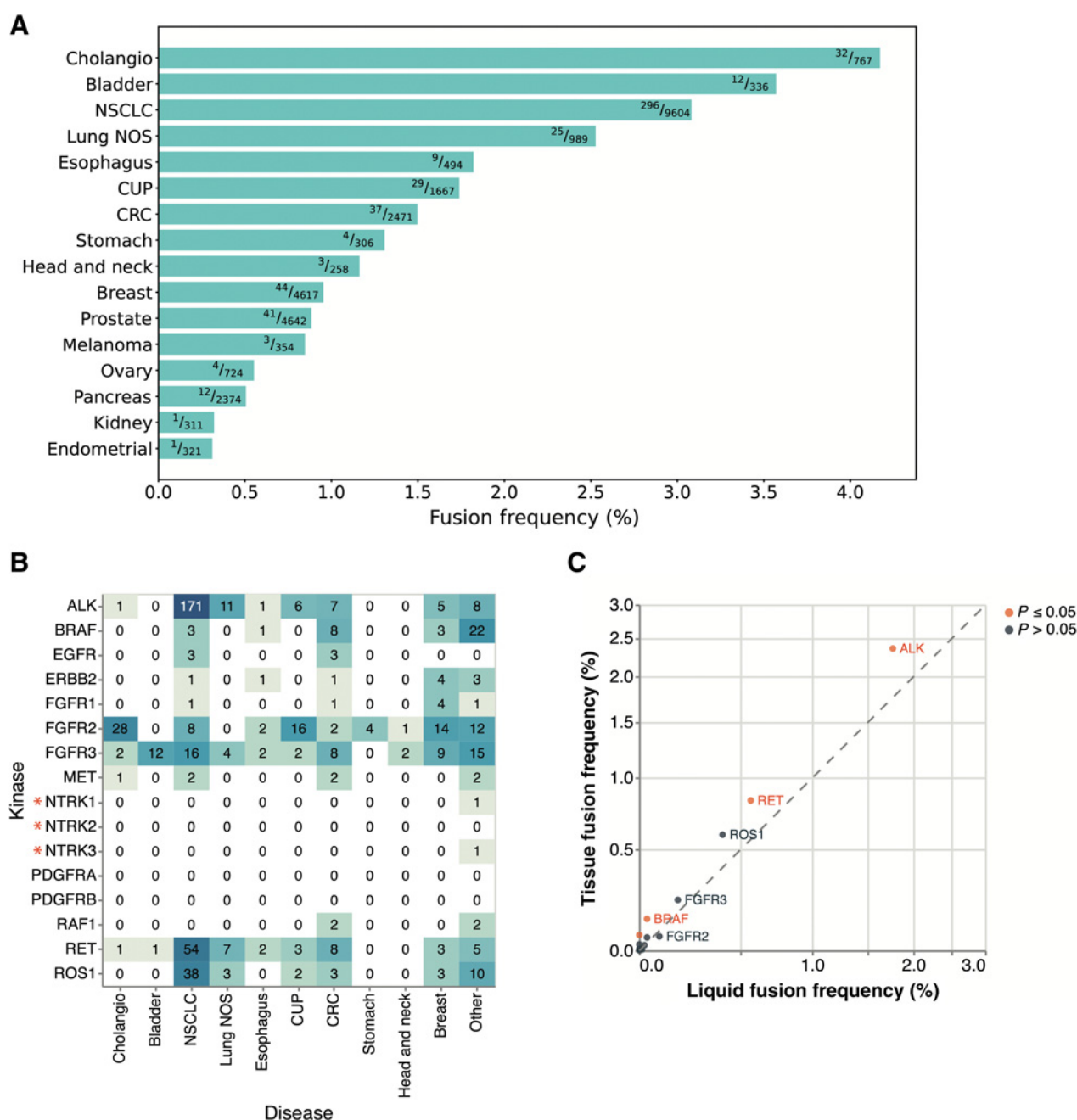

Figure 1.
Kinase fusions are detected in ctDNA across diverse cancer types. **A,** Prevalence of kinase fusions detected in ctDNA by cancer type. **B,** Heatmap of kinase fusions by cancer type. *, Genes that are baited only on the current version of the liquid assay (F1LCDx). **C,** Prevalence of kinase fusions in NSCLC in tissue and ctDNA. Genes with a statistically significant ($P \leq 0.05$) difference in prevalence between tissue and ctDNA are displayed in orange.
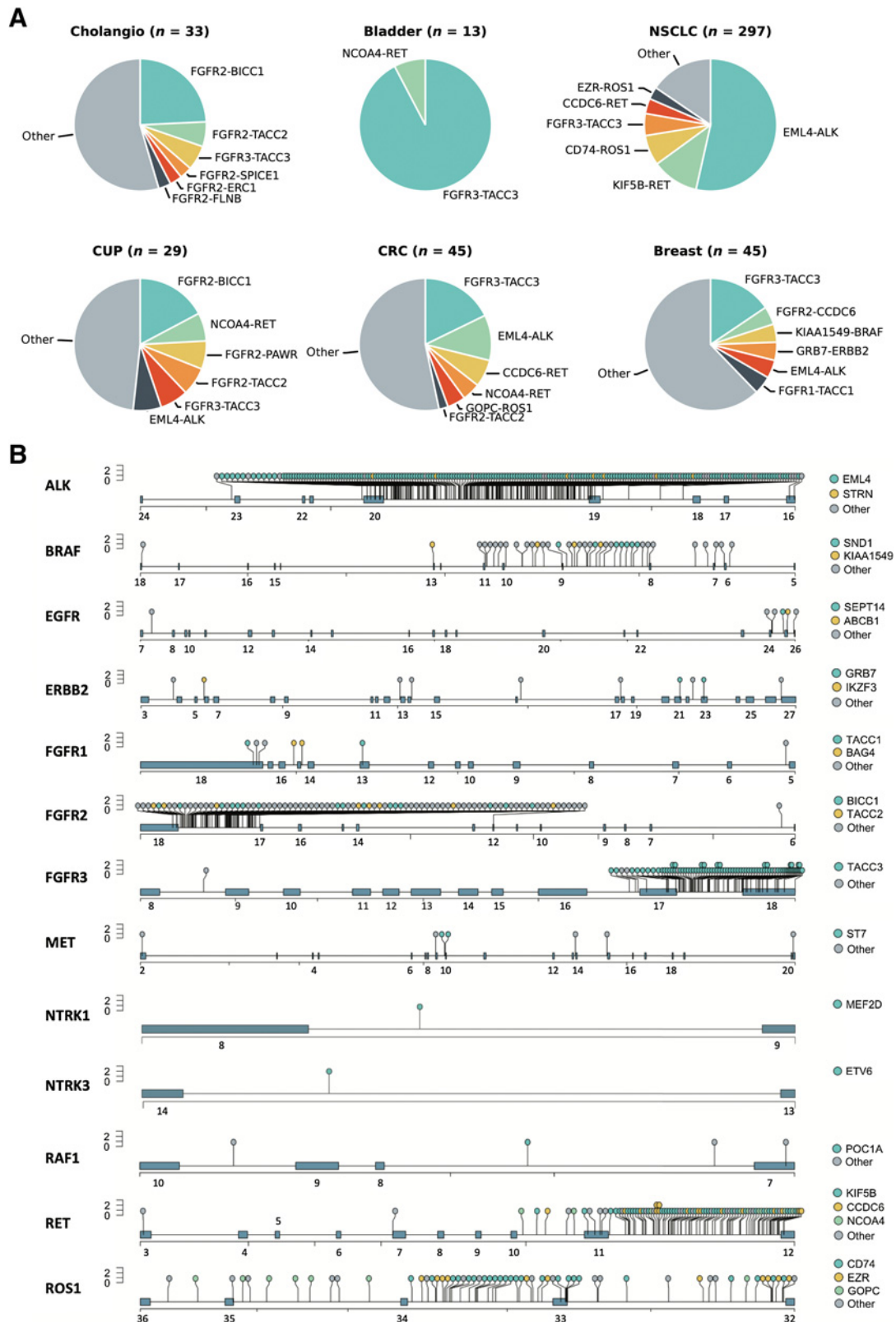
Figure 2.
Kinase fusions in ctDNA can be detected with a diversity of partner genes and breakpoint locations. **A,** Most frequent fusion partners by cancer type. **B,** Lollipop plot of fusion breakpoint locations.

positive cases were non-lung (Supplementary Table S2), including one cholangiocarcinoma with an *EML4-ALK* fusion. Among 210 *ALK* fusions, 188 (90%) occurred with a breakpoint in intron 19. Breakpoints were also observed in exon 20 (4.8%), intron 18 (1.9%), and exon 19 (1.9%; **Fig. 2B**). In bladder cancer, *FGFR3* was the most common kinase fusion observed (92%, 12/13; **Fig. 1B**), exclusively partnered with *TACC3* (**Fig. 2A**).

We compared the frequency of 16 kinase fusions in the NSCLC ctDNA dataset ($n = 296$) with the frequency of the same fusions in our tissue database for patients with matched disease ontology. The overall prevalence of kinase fusions was slightly elevated in tissue samples compared with the prevalence in ctDNA samples for several kinases including *ALK* (2.4% vs. 1.8%, $P = 0.003$), *RET* (0.83% vs. 0.56%, $P =$

0.02), *BRAF* (0.14% vs. 0.03%, $P = 0.02$), and *NTRK1* (0.07% vs. 0%, $P = 0.02$; **Fig. 1C**).

High concordance for kinase fusions between tissue and liquid biopsy specimens from the same patient

To determine how closely liquid biopsy sequencing recapitulated the kinase fusions identified in tissue, we examined 4,722 cases with both tissue and liquid results available, of which 169 pairs harbored a fusion in either the tissue or liquid specimen (Supplementary Fig. S1). In 137 cases with a fusion detected in tissue, the fusion was also detected in ctDNA in 96 cases (PPA = 70%). Sensitivity for *ALK* and *RET* fusion detection was 73% and 71%, respectively. *ROS1* is a fusion that has been historically challenging to detect in ctDNA, but in our
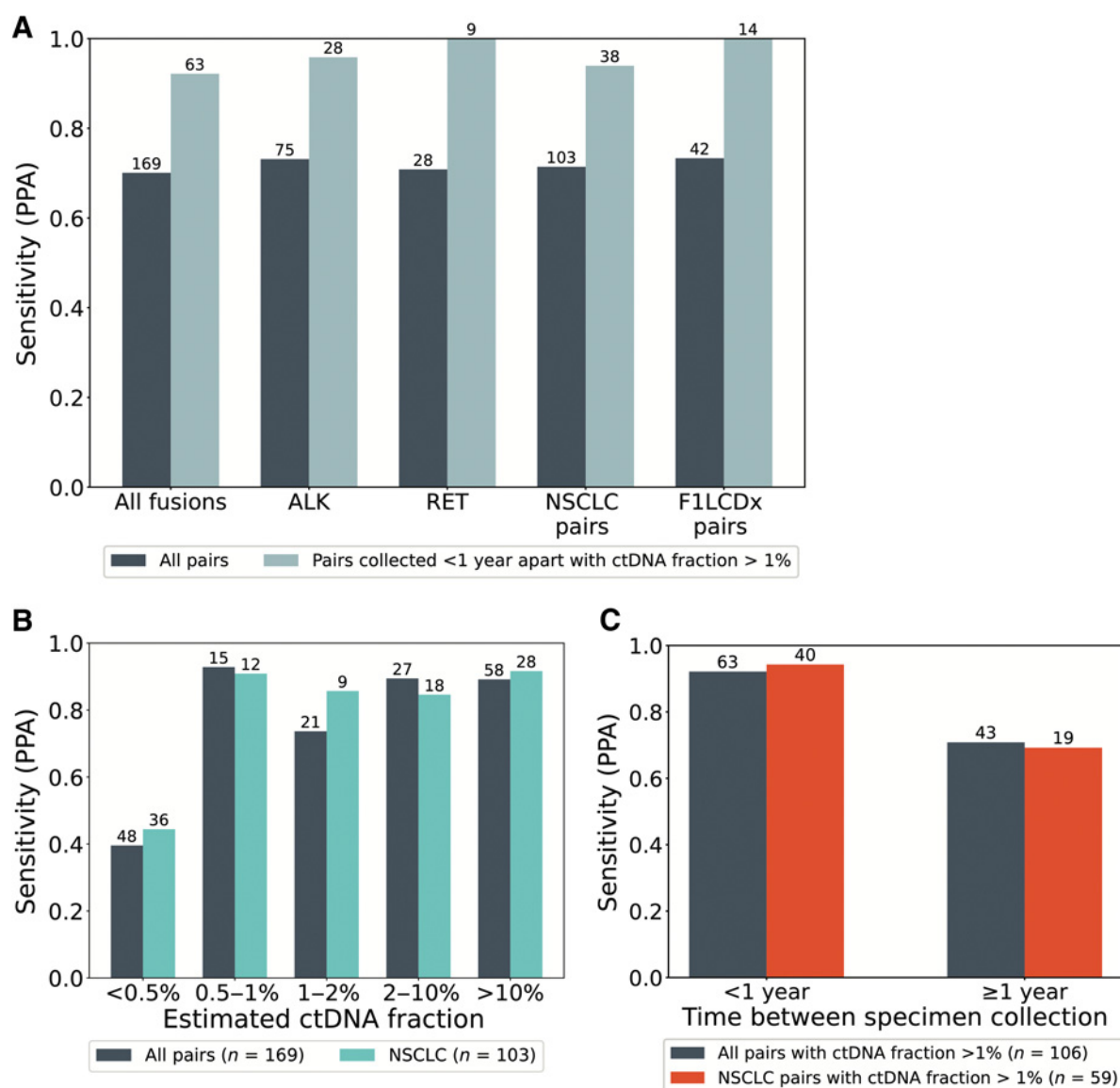

Figure 3.
Sensitivity (PPA) for kinase fusion detection in cases with both tissue and ctDNA results. **A,** Sensitivity (PPA) of fusion detection between patient-matched tissue and ctDNA specimens. Of 4,722 tissue–ctDNA pairs, 169 pairs harbored a fusion in either the tissue or liquid specimen. PPA for disease and kinase-specific subsets with at least 20 pairs are shown. **B,** Sensitivity of kinase fusion detection is increased with higher plasma ctDNA fraction. **C,** Focusing on pairs where the estimated plasma ctDNA fraction is >1%, sensitivity (PPA) of kinase fusion detection in ctDNA is higher with less time between collection of the tissue and ctDNA specimens.

analysis, we identified 10 of 13 overall and five of five with >1% estimated ctDNA fraction (**Fig. 3A**; Supplementary Table S3). Because variable ctDNA shed is known to affect the sensitivity of ctDNA genotyping (25, 26), we evaluated whether an estimation of ctDNA fraction influenced sensitivity. Median ctDNA fraction was higher in concordant ctDNA samples (2.2%) than in discordant ctDNA samples (0.37%, $P < 0.001$; Supplementary Fig. S2A). We also observed a direct relationship between PPA and ctDNA fraction of the liquid biopsy specimen. In liquid specimens with estimated ctDNA fraction <1%, fusions were detected in 32 of 62 samples with a fusion detected in tissue (PPA = 52%), compared with 64 of 75 (PPA = 85%) for ctDNA specimens with ctDNA fraction ≥1% ($P < 0.001$; **Fig. 3B**).

Prolonged time between CGP analysis also has the potential to influence assay concordance (26). Median time between sample collection was 206 days (interquartile range, 26–696 days). Among pairs where the estimated plasma ctDNA fraction was >1%, PPA for pairs collected <1 year apart was higher than pairs collected ≥1 year apart (92% vs. 71%, $P = 0.03$; **Fig. 3C**), and the median time between specimen collection for concordant and discordant pairs was 110 and 426 days ($P < 0.001$; Supplementary Fig. S2B). Among temporally matched pairs collected <1 year apart with ctDNA fraction >1%, fusions were detected in 47 of 51 samples with a fusion in tissue (PPA = 92%; **Fig. 3A**; Supplementary Table S3).

We further analyzed the 41 cases with a fusion detected in tissue but not liquid (Supplementary Fig. S1). Thirty of 41 (73%) had likely impaired sensitivity for fusion detection with an estimated tumor fraction of <1%. Five of 41 (12%) fusions were absent in samples with suspected false-positive estimated tumor fractions of >1% due to use of older assays or the presence of possible clonal hematopoiesis. Four of 41 (9.8%) discordant fusions were likely due to intratumoral or temporal heterogeneity. Three of these fusions were detected at low read count (<70 reads) in high-tumor-content tissue specimens (>50% tumor) and thus may be subclonal events beneath the limit of detection in the liquid biopsy. Furthermore, two fusions were not detected in liquid specimens collected >3 years after the primary tissue biopsy. Two of 41 (4.9%) fusions were putative AR mechanisms detected in tissue after a primary EGFR-mutated liquid specimen (Supplementary Table S4).

To better understand the risk of false-negative results for kinase fusions, we queried our database for cases harboring ALK resistance mutations, which should invariably be detected in the presence of a driver ALK fusion. Some reports in the literature have described recurring cases with ALK resistance mutations detected in ctDNA in the absence of the kinase fusion, suggesting gaps in detection of the required prior driver fusion events (27–29). We identified 50 cases with a suspected ALK resistance mutation detected [variant allele frequency (VAF), 0.10%–97%; Supplementary Fig. S3; Supplementary Table S5], and in 48 (96%) we were also able to identify a corresponding ALK kinase fusion. In one case where the fusion was not detected, an EGFR driver mutation was detected, potentially suggesting a subclonal ALK rearrangement event.

Acquired kinase fusions in ctDNA may mediate drug resistance

We next investigated the 32 specimens where a kinase fusion was found exclusively in the liquid biopsy (Supplementary Fig. S1). In six of 32 (19%) of these cases, comparison of the tissue and liquid results raised the possibility of multiple primary tumors as a potential mechanism for discordance. For example, one subject with a clinical diagnosis of perineal sarcoma had tissue testing that revealed an EWSR1-ATF1 fusion, a driver event strongly associated with clear

cell sarcomas, whereas liquid testing showed a high confidence EML4-ALK fusion, most frequently found in NSCLC. Four of 32 (13%) fusions were observed at a low read count (<10 reads) and may represent intratumoral heterogeneity captured by liquid biopsy. One of 32 (3.1%) cases was a prostate cancer liquid specimen harboring a RET fusion collected >2 years after the primary tissue specimen and may represent temporal heterogeneity and evolution to neuroendocrine prostate cancer (30). In the remaining 21 of 32 (66%), there was evidence that the fusion represented putative AR based on the detection of a co-occurring EGFR ($n = 10$) or other driver mutation ($n = 3$) or other co-occurring established resistance mechanisms ($n = 8$; **Table 2**). Such acquired kinase fusions have been described as resistance mechanisms to targeted therapies including EGFR tyrosine kinase inhibitors in patients with NCSLC harboring EGFR driver mutations (2–5).

In 18 of 21 of these cases, the tissue predated the ctDNA specimen collection date (median 813-day difference; range, 75–2,959; **Table 2**, lines 1–5, 7, 8, 10, 11, 13–21). The remaining three of 21 cases had a liquid specimen collected prior to the paired tissue biopsy (**Table 2**, lines 6, 9, 12) and harbored kinase fusions that co-occurred with an activating driver alteration that was detected by both tissue and liquid CGP. These fusions were all detected at low read count (range, 3–62) and may represent subclonal AR that was unable to be detected by tissue CGP.

Ten of 21 cases were NSCLC specimens where an activating EGFR mutation co-occurred with a range of ctDNA-detected kinase fusions, including ALK ($n = 4$), FGFR3 ($n = 3$), RET ($n = 2$), and FGFR2 ($n = 1$; **Table 2**, lines 1–10; refs. 4, 31). Moreover, we found that these fusions frequently co-occurred with other mechanisms of resistance; two patients harbored well-characterized EGFR resistance mutations T790M with and without C797G, which co-occurred with FGFR3 and FGFR2 fusions, respectively. One sample additionally acquired BRAF V600E co-occurring with an FGFR3 fusion in the posttreatment sample. Seven of 21 of these pairs were KRAS wild-type (WT) colorectal carcinoma samples. ALK, BRAF, EGFR, FGFR3, MET, and RAF1 fusions were identified as possible AR to anti-EGFR therapy and occurred alongside other putative resistance alterations including EGFR alterations and RAS mutations (**Table 2**, lines 11–13, 15–17, 19; refs. 32–34). Two of 21 specimens were KRAS-mutated colorectal cancer samples with possible acquired BRAF, FGFR3, and RAF1 fusions to regorafenib and adagrasib (**Table 2**, lines 14, 18). Two of 21 specimens were breast samples with acquired RET and FGFR3 fusions (**Table 2**, lines 20–21). The FGFR3 fusion was present in a hormone receptor positive specimen and seen alongside two ESR1 mutations and may represent heterogeneous AR to hormonal therapy (35). The RET fusion was observed alongside an activating PIK3CA mutation and may similarly confer resistance to PI3K-targeted therapy. Collectively, these findings highlight the clinical validity of liquid biopsy CGP to identify potentially targetable mechanisms of AR.

Discussion

Genomic profiling of ctDNA has the potential to define the molecular drivers of primary tumors and to detect mechanisms of AR to therapy, thus providing a complementary approach to tissue biopsy. However, to confidently use these results in clinical care, it is critical to define how accurately liquid biopsy assays recapitulate tissue-based diagnostics, especially for technically challenging, but therapeutically important targetable drivers such as kinase fusions. We describe pan-cancer genomic profiling of kinase gene fusions in ctDNA from 32,492

Table 2. Fusions detected in ctDNA but not in tissue may represent acquired resistance mechanisms.

Case	Disease	Days between specimen collection	Tissue biopsy	Liquid biopsy	Pre–liquid biopsy treatment and response
1	NSCLC	75	EGFR ex19del, C797S	EGFR ex19del **EML4-ALK fusion**	Gefitinib
2	NSCLC	196	EGFR L858R	EGFR L858R **EML4-ALK fusion**	NA
3	NSCLC	775	EGFR ex19del	EGFR ex19del **EML4-ALK fusion**	NA
4	NSCLC	894	EGFR L858R	EGFR L858R **PLEKHA7-ALK fusion**	Erlotinib (12 mo) – PR Afatinib (2 mo) Osimertinib (12 mo) – PR[a]
5	NSCLC	454	EGFR L858R, L833V	EGFR L858R, L833V, T790M **FGFR2-CCDC6 fusion**	Erlotinib
6[b]	NSCLC	133	EGFR ex19del	EGFR ex19del **FGFR3-TACC3 fusion**	NA
7	NSCLC	426	EGFR L858R, E709K	EGFR L858R **FGFR3-TACC3 fusion**	Afatinib + cetuximab (10 mo) – SD[a]
8	NSCLC	464	EGFR ex19del, T790M	EGFR ex19del, T790M, C797G BRAF V600E **FGFR3-ADD1 fusion**	Osimertinib (7 mo) - SD
9[b]	NSCLC	13	EGFR ex19del	EGFR ex19del **ERC1-RET fusion**	NA
10	NSCLC	686	EGFR ex19del, T790M	EGFR ex19del **NCOA4-RET fusion**	Osimertinib (8 mo)
11	CRC	850	KRAS WT	EGFR amp MAP2K1 I103_K104del NF1 truncation **EML4-ALK fusion**	FOLFOX, capecitabine, Lonsurf, ramucirumab, bevacizumab, Panitumumab
12[b]	CRC	31	KRAS WT MET amp	EGFR V441G, G465E/R NRAS G13D, Q61K KRAS G12C **ZC3HAV1-BRAF fusion**	NA
13	CRC	615	KRAS WT	EGFR S492R KRAS G12V, Q61H NRAS Q61K/L MAP2K1 Q58del, I111T MAP2K2 F57V NF1 F945fs*9 **MKRN1-BRAF fusion**	NA
14	CRC	1,014	KRAS G13D	KRAS G13D **DENND2A-BRAF fusion**	FOLFOX, 5FU maintenance, FOLFIRI, regorafenib
15	CRC	2,959	KRAS WT	NRAS Q61K **TRIM24-BRAF fusion**	NA
16	CRC	150	KRAS WT	KRAS G12A, Q61H NRAS G12D MAP2K1 E102_I103del **PDE7A-EGFR fusion**	NA
17	CRC	854	KRAS WT	BRAF V600E EGFR V441G, S492R HRAS Q61L MAP2K1 K57T, E102_I103del NRAS Q61K **FGFR3-TACC3 fusion** **GOLGA3-BRAF fusion** **SYN2-RAF1 fusion**	FOLFIRI + bevacizumab (4 mo) FOLFIRI + cetuximab (6 mo) FOLFOX + bevacizumab (11 mo) Pembrolizumab + regorafenib
18[c]	CRC	1,912	KRAS G12C	KRAS G12C, G13D MAP2K1 E102_I103del NRAS Q61K **AKAP9-BRAF fusion** **FGFR3-TACC3 fusion** **RAF1-TRAK1 fusion** **RAF1-CCDC176 fusion**	Adagrasib Adagrasib + cetuximab

(Continued on the following page)

Table 2. Fusions detected in ctDNA but not in tissue may represent acquired resistance mechanisms. (Cont'd)

Case	Disease	Days between specimen collection	Tissue biopsy	Liquid biopsy	Pre–liquid biopsy treatment and response
19	CRC	1,236	KRAS WT	KRAS Q61H EGFR amp **MET-CAPZA2 fusion**	NA
20	Breast	1,269	ER+/PR+	ESR1 Y537N, D538G AKT1 E17K **FGFR3-TACC3 fusion**	Everolimus, denosumab, fulvestrant
21	Breast	1,591	PIK3CA E542K	PIK3CA E542K ESR1 E380Q KRAS G12C PTEN S59*, M134I **BAIAP2L1-RET fusion**	NA

[a]Schrock AB, Zhu VW, Hsieh WS, et al. Receptor tyrosine kinase fusions and BRAF kinase fusions are rare but actionable resistance mechanisms to EGFR tyrosine kinase inhibitors. J Thorac Oncol 2018;13:1312–23.
[b]These patients had their liquid specimen collected before the tissue specimen and the fusions may represent subclonal resistance not captured in the subsequent tissue biopsy. All other patient pairs had the fusion-negative tissue collected first.
[c]Awad MA, Liu S, Rybkin II, et al. Acquired resistance to KRAS[G12C] inhibition in cancer. 2021 Jun 24;384(25):2382–2393.

patients and tumor tissue samples from 368,931 patients with Foundation Medicine testing. Tissue genomic profiling has demonstrated that kinase fusions can be found at low frequencies in a broad spectrum of tumor types (19, 36–39). This study confirms these results using ctDNA, with diverse kinase fusions detected even in tumor types where these are not commonly found, thus demonstrating the importance of validated broad-panel genomic profiling to identify uncommon cancer drivers. Importantly, responses to targeted therapies have been documented in patients with kinase fusions in new tumor types where they have not been previously observed, suggesting some kinase fusions may be evaluated for pan-tumor indications (40–42). Analysis of ctDNA also identified a large diversity of fusion partners, highlighting the ability to detect diverse kinase fusions with DNA sequencing alone. While some have advocated for targeted RNA sequencing to improve tissue-based detection of atypical noncanonical fusions, circulating tumor RNA analysis remains investigational, suggesting ctDNA CGP will remain the preferred approach for liquid biopsy–based fusion detection in the near-term.

Genomic profiling of ctDNA closely recapitulated the results of tissue-based testing, and the majority of discordances can be attributed to a combination of biological and/or analytical factors (e.g., low ctDNA fraction, temporal factors, evolution of AR, subclonal mutations, multiple primary tumors). The overall prevalence of fusions was slightly elevated in tissue NSCLC samples compared to liquid, and this may be partially attributable to differences in intron baiting between assay versions. The ctDNA assays used in this study included intronic baiting for only nine genes (*ALK*, *RET*, *ROS1*, *FGFR2*, *FGFR3*, *EGFR*, *NTRK1/2*, and *PDGFRA*) such that rearrangements with noncanonical breakpoints in other introns or involving genes without intron baiting may be underrepresented here. Studying patients with both tissue and liquid results and using tissue as reference, the PPA of ctDNA profiling was 70% and increased with higher ctDNA fraction and with specimens collected within a shorter time window. Prior studies have found that low ctDNA shed is an important cause of failure to identify genomic alterations in ctDNA that were detected by tissue testing (25, 26). ctDNA fraction can be affected by factors that influence tumor DNA shedding including tumor type, location, size, stage, or vascularity, which can affect the accessibility of the tumor to circulation (6, 43, 44). Hence, these biological factors can affect the release of tumor DNA in the blood, impacting their representation and detect-

ability in ctDNA (45, 46). In 50 cases where detection of an ALK putative resistance mutation confirmed the presence of ctDNA, the underlying driver ALK fusion was detected 96% of the time.

We observed that cases of genomic rearrangements exclusively detected in ctDNA appeared mostly in diseases where fusions are a known recurring phenomenon (38% in NSCLC, 34% in colorectal cancer). These kinase fusions detected only in liquid, but not in tissue samples from the same patient, were manually reviewed and confirmed to be of high confidence. Furthermore, a large proportion of the discordant cases occurred with *EGFR* mutations, and most of these are putative cases of AR to anti-EGFR therapies (**Table 2**), as described previously (4). The evaluation of the discordant pairs that exclusively harbored a fusion in the liquid sample revealed that 10 patients with NSCLC had pretreatment tissue samples with a driver mutation and had acquired a kinase fusion (four *ALK*, three *FGFR3*, two *RET*, one *FGFR2*) in the subsequent ctDNA specimen (**Table 2**, patients 1–10). Importantly, these acquired fusions were detected after treatment with first-, second-, or third-generation EGFR TKIs (4). We further found that in six cases with ctDNA-only fusions, the kinase fusions were indicative of a possible separate primary, highlighting that ctDNA testing may provide added value beyond tissue testing to detect multiple primary tumors coexisting in a single patient (Supplementary Fig. S1).

Overall, these results provide compelling evidence that high quality, broad-panel CGP of ctDNA can detect a broad range of fusions, similar to tissue CGP results, and across multiple tumor types. In particular, ctDNA fusion status was highly concordant with tissue testing when the liquid specimen had a higher ctDNA fraction, suggesting that clinical decision making, especially for negative results, should depend on confirmation of adequate sampling of tumor in the liquid sample. The size of the database of patient genomic profiles ($n = 405,847$ cases) is a clear strength of our study. However, we acknowledge limitations of our findings, largely owing to lack of clinical annotation but also the relatively low number of paired and serial patient samples, as well as the descriptive nature of this landscape analysis. Nevertheless, our study offers unique insights into therapeutic resistance. Kinase fusions found only by liquid, not tissue testing, were often associated with AR. The gold standard method to comprehensively assess tumoral evolution and AR has not yet been identified, but liquid biopsy accurately captures dynamic changes in a patient's tumor over time and illuminates the challenges of overcoming resistance to targeted therapy.

Taken together, these results support the clinical validity of ctDNA profiling for detecting oncogenic fusions with compelling sensitivity, understanding that negative results should be confirmed with tissue testing due to the possibility of variable ctDNA shed, as with other biomarkers in ctDNA.

Authors' Disclosures

Authors' Contributions

J.K. Lee: Conceptualization, formal analysis, investigation, visualization, methodology, writing–original draft. **M. Hazar-Rethinam:** Conceptualization, formal analysis, investigation, writing–original draft. **B. Decker:** Supervision, methodology, writing–review and editing. **O. Gjoerup:** Conceptualization, project administration, writing–review and editing. **R.W. Madison:** Conceptualization, data curation, formal analysis, investigation, visualization. **D.S. Lieber:** Resources, software, supervision. **J.H. Chung:** Conceptualization, supervision. **A.B. Schrock:** Conceptualization, supervision, writing–review and editing. **J. Creeden:** Conceptualization, supervision. **J.M. Venstrom:** Conceptualization, supervision. **B.M. Alexander:** Conceptualization, supervision. **G.R. Oxnard:** Conceptualization, supervision, writing–review and editing.

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