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Hybrid Capture-Based Genomic Profiling of Circulating Tumor DNA from Patients with Advanced Cancers of the Gastrointestinal Tract or Anus

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Disclosure of Potential Conflicts of Interest

S.J. Klempner reports receiving speakers bureau honoraria from Foundation Medicine and is a consultant/advisory board member for Boston Biomedical, Lilly Oncology, and Merck. B. Leyland-Jones reports receiving speakers bureau honoraria from Genentech. R.D. Carvajal is a consultant/advisory board member for AstraZeneca, Aura Biosciences, Bristol-Myers Squibb, Castle Biosciences, Chimeron, Foundation Medicine, Iconic Therapeutics, Immunocore, Incyte, Janssen, Merck, Novartis, Rgenix, and Roche/Genentech. R. Kurzrock is an employee of and holds ownership interest (including patents) in CureMatch, Inc.; reports receiving other commercial research support from Foundation Medicine, Genentech, Guardant Health, Incyte, Merck Serono, Pfizer, and Sequenom, and speakers bureau honoraria from Roche; and is a consultant/ advisory board member for Actuate Therapeutics, Genentech, Loxo Oncology, and Sequenom. J.K. Sicklick reports receiving other commercial research support from Foundation Medicine and Novartis Pharmaceuticals and is a consultant/advisory board member for Loxo Oncology. J.S. Ross and P.J. Stephens hold ownership interest (including patents) in Foundation Medicine. V.A. Miller is a consultant/advisory board member for Revolution Medicines. No potential conflicts of interest were disclosed by the other authors.

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Abstract

Purpose: Genomic profiling of tumor biopsies from advanced gastrointestinal and anal cancers is increasingly used to inform treatment. In some cases, tissue biopsy can be prohibitive, and we sought to investigate whether analysis of blood-derived circulating tumor DNA (ctDNA) may provide a minimally invasive alternative.

Experimental Design: Hybrid capture-based genomic profiling of 62 genes was performed on blood-based ctDNA from 417 patients with gastrointestinal carcinomas to assess the presence of genomic alterations (GA) and compare with matched tissue samples.

Results: Evidence of ctDNA was detected in 344 of 417 samples (82%), and of these, 1 reportable GA was detected in 89% (306/344) of samples. Frequently altered genes were *TP53* (72%), *KRAS* (35%), *PIK3CA* (14%), *BRAF* (8%), and *EGFR* (7%). In temporally matched ctDNA and tissue samples available from 25 patients, 86% of alterations detected in tissue were also detected in ctDNA, including 95% of short variants, but only 50% of amplifications. Conversely, 63% of alterations detected in ctDNA were also detected in matched tissue. Examples demonstrating clinical utility are presented.

Conclusions: Genomic profiling of ctDNA detected potentially clinically relevant GAs in a significant subset of patients with gastrointestinal carcinomas. In these tumor types, most alterations detected in matched tissue were also detected in ctDNA, and with the exception of amplifications, ctDNA sequencing routinely detected additional alterations not found in matched tissue, consistent with tumor heterogeneity. These results suggest feasibility and utility of ctDNA testing in advanced gastrointestinal cancers as a complementary approach to tissue testing, and further investigation is warranted.

Introduction

The treatment of gastrointestinal cancers is influenced by the presence or absence of prognostic and predictive genomic alterations (GA), following precedents set in non-small cell lung cancer and other solid tumors. Extended *RAS* and *BRAF* testing is widely performed in colorectal carcinoma to guide the use of anti-EGFR antibody therapies, which have shown particular efficacy in patients with tumors wild type for *KRAS*, *NRAS*, and *BRAF* mutations (1, 2). Similarly, *MET* and *HER2 (ERBB2)* amplification have each emerged as negative predictors of response to EGFR antibodies, and simultaneously as putative molecular targets themselves in tumor types, including gastric cancer and colorectal carcinoma (3–6). Testing gastroesophageal adenocarcinomas for HER2 overexpression by IHC and/or gene amplification by FISH is currently considered a standard of care for advanced disease (7). Additional molecular targets both predicting lack of benefit from EGFR antibodies and predicting sensitivity to matched targeted therapies continue to be elucidated in tumors of the gastrointestinal tract and anus (8, 9), necessitating methods for comprehensive assessment.

Currently, tissue-based genomic testing of cancer specimens remains the gold standard, but assays utilizing sequencing of circulating tumor DNA (ctDNA) isolated from blood samples have recently been introduced into clinical care. Clonal evolution and tumor heterogeneity are known to impact the response and resistance to targeted therapies in gastrointestinal cancers, but serial or simultaneous tissue sampling of multiple metastatic lesions is problematic (10, 11). Blood-based genomic profiling assays provide a noninvasive alternative to traditional tissue biopsies and may provide complementary genomic information to tissue-based testing. As more patients receive targeted therapies, sequencing pretreatment and postprogression samples will become increasingly important. In the current study, we analyze results from ctDNA-based genomic profiling using a 62-gene assay from over 400 patients with carcinomas of the gastrointestinal tract and anus. Comparison with temporally matched tissue samples and large tissue databases, as well as examples of clinical utility, is described.

Materials and Methods

Blood samples were obtained for 417 patients with primarily late-stage carcinomas of the gastrointestinal tract or anus with consecutive genomic profiling results reported. Genomic profiling of ctDNA was performed on samples submitted by clinicians as part of routine clinical care between May 2016 and June 2017. Testing was performed in a CLIA-certified/CAP-accredited laboratory (Foundation Medicine Inc.). Approval for this study, including a waiver of informed consent and a HIPAA waiver of authorization, was obtained from the Western Institutional Review Board (Protocol No. 20152817).

Twenty milliliters of peripheral whole blood was collected in Cell-Free DNA BCT tubes (Roche/Ariosal or Streck) for genomic profiling of ctDNA. Plasma was isolated, and 20 ng ctDNA was extracted to create adapted sequencing libraries before hybrid capture and sample-multiplexed sequencing (Illumina HiSeq 2500 or 4000) to a median unique coverage depth of $7,587\times$ for 62 genes (Supplementary Table S1; ref. 12). Results were analyzed for alterations at low allele frequencies (AF), including substitutions (AF $\geq 0.1\%$), short insertions/deletions (indels AF $\geq 0.1\%$), rearrangements, and copy number amplification. AF represents the percentage of mutant DNA allele reads relative to total DNA allele reads (mutant plus wild type). Custom filtering was applied to report GAs and remove benign germline events as described previously (13). Maximum somatic allele frequency (MSAF) measures the AF of all somatic alterations (including reportable GAs, variants of unknown significance, and synonymous mutations) identified per sample; alterations in the ExAC database are removed from the alteration list for MSAF calculation as they are likely germline, and dbSNP variants are also excluded. The maximum AF identified out of all alterations measured is defined as the MSAF, which can provide an estimate of the ctDNA fraction in blood. Clinically relevant GAs were defined as alterations that are targetable by anticancer drugs currently available on the market or in registered clinical trials.

Twenty-five patients in this series had a paired tissue sample that was sequenced using hybrid capture-based genomic profiling (FoundationOne) according to previously published methods (13, 14). For comparative analyses with prior studies, data from the FoundationCORE database (tissue samples analyzed using the FoundationOne assay),

TCGA (15), and the Giannakis and colleagues' study (16) were extracted from cBioPortal (17) in July 2017. Ordinal relationships were examined using the Mann-Whitney U test; categorical relationships were examined using Pearson χ^2 test with Yates' continuity correction when applicable.

Results

Patient characteristics

Hybrid capture-based genomic profiling was performed on ctDNA isolated from blood samples collected from 417 patients with primarily late-stage carcinomas of the gastrointestinal tract or anus. The disease histologies included colorectal adenocarcinoma (72%), gastroesophageal junction adenocarcinoma (GEJ, 11%), gastric adenocarcinoma (8.9%), anal squamous cell carcinoma (3.1%), esophageal carcinoma (2.4%), small-bowel adenocarcinoma (1.6%), or high-grade gastrointestinal neuroendocrine carcinoma (0.5%). The median patient age was 60 years (range, 27–93), and 43% of patients were women (Table 1).

GAs identified in the ctDNA of patients with gastrointestinal cancers

Evidence of ctDNA in the blood, as approximated using a MSAF >0, was detected in 82% (344/417) of cases. The median MSAF across all cases was 2.36% (range, 0%–89.9%), and among cases with evidence of ctDNA present, at least one reportable GA was detected in 89% (306/344) of cases, for an average of 2.28 GA per case. However, both the fraction of cases with detectable ctDNA and consequently the median MSAF varied across disease histologies, with the highest average number of GA detected in colorectal carcinoma (Fig. 1; Table 1).

Among 344 cases with evidence of ctDNA in the blood, the most frequently altered genes were *TP53* (72%), *KRAS* (35%), *PIK3CA* (14%), *BRAF* (8%), and *EGFR* (7%; Fig. 2A). Potentially actionable GAs informing selection of matched targeted therapies and clinical trials or predicting lack of response to EGFR antibody therapies were identified (Fig. 2B and C). RAS/RAF/MEK pathway alterations (48%), PI3K pathway alterations (17%), point mutation, amplification or fusion of receptor tyrosine kinases (RTK, 18%), and *BRCA1/2* alterations (5%) were present across histologies, with a total of 66% (228/344) of cases harboring at least one alteration in one or more of these pathways. *HER2 (ERBB2)* alterations were observed in 5% (17/344) of cases and included copy number amplification in 2.3%, activating point mutation in 2.0%, or both in 0.6%. EGFR extracellular domain (ECD) mutations within the cetuximab and panitumumab-binding domains, including V411D/G, S464L, S465E/R, and S492R, were found in 3.2% (11/344) of cases, all of which were colorectal carcinomas. Three cases harbored multiple EGFR ECD mutations and the *cis/trans* relationship could be evaluated for 2 cases, both of which showed that all the mutations were in *trans* (Supplementary Fig. S1). Eight potentially targetable RTK fusions were detected in 7 cases and included *GOPC-ROS1* (2 cases), *SLC24A2-ROS1*, *STRN-ALK*, *CCDC6-RET*, *KIF5B-PDGFR*, *EGFR-SEPT14*, and *FGFR3-TACC3*.

Comparison of GAs identified in ctDNA with paired tissue samples and prior genomic studies of tissue

Frequencies of short variant alterations in commonly mutated genes observed in the 344 ctDNA samples included in this study were largely similar to those observed among 15,948 tissue samples included in the FoundationCORE database for patients with matched disease histologies (Fig. 3A). These included *TP53* (72.1% vs. 73.6%), *KRAS* (34.0% vs. 41.5%), *PIK3CA* (14.0% vs. 15.5%), and *BRAF* (8.1% vs. 6.7%). However, gene amplification was less frequently detected in ctDNA samples from this study compared with samples from similar patients in the FoundationCORE database (Fig. 3B). *FLT3*, *HER2*, and *MYC* were amplified in 3.5%, 2.9%, and 2.3% of ctDNA samples, respectively, and in 5.5%, 5.2%, and 8.6% of tissue samples in the FoundationCORE database, respectively ($P = 0.13$, $P = 0.07$, and $P < 0.001$).

For 25 patients included in this study, DNA from matched tissue and blood samples collected within 30 days was available, and samples were analyzed using similar hybrid capture-based genomic profiling assays. Matched samples without evidence of ctDNA in the blood were excluded from analysis. In total, 57 alterations across the 25 cases were detected in tissue samples, of which 49 of these alterations (86%) were also detected in temporally matched ctDNA. This included 95% (42/44) of short variant alterations, 50% (6/12) of amplifications, and 1/1 rearrangements (Fig. 4; Supplementary Table S2). Conversely, 63% (49/78) alterations detected in ctDNA samples were also detected in DNA isolated from temporally matched tissue samples. Notably, 4/4 EGFR ECD mutations and 4/5 kinase rearrangements were found in ctDNA but not in paired tissue samples (Fig. 4A). Only alterations covered by both assays were assessed. No significant difference in concordance was observed when primary versus metastatic tissue biopsies was compared with temporally matched ctDNA. For 18 metastatic tissue biopsies and 7 primary tissue biopsies, 85% (33/39) and 89% (16/18) of alterations detected in tissue, respectively, were also found in matched ctDNA samples. Of alterations detected in ctDNA, 61% (33/54) and 67% (16/24), respectively, were also detected in matched metastatic or primary tissue biopsies. Similarly, among tissue samples from the liver ($n=10$), 20 of 24 (83%) alterations found in tissue were also found in ctDNA, and 20 of 31 (65%) alterations found in ctDNA were also found in tissue.

Case studies supporting clinical utility of ctDNA testing in patients with gastrointestinal and anal cancers

Clinical outcomes were sought for cases with potentially actionable alterations detected in ctDNA. Follow-up was available from the treating physician for 13 patients (Table 2). Of these, 2 (*ROS1* fusion, *FGFR2* amplification) are responding to current systemic therapy and will pursue trial enrollment at progression, 5 received matched targeted therapies, approved in other tumor types or through clinical trials, based on results from ctDNA profiling, and 2 had ctDNA testing done after targeted therapy, and a potential mechanism of acquired resistance was discovered. One patient was lost to follow-up, and 3 passed away without receiving targeted therapy.

The 5 patients of whom we are aware received matched targeted therapy each had stage IV disease at the time CGP was performed. In the first case (Table 2, case 3), a *STRN-ALK* fusion was detected by both genomic profiling of tissue and ctDNA, despite the same tumor tissue being initially negative for ALK by local IHC testing. Details of this case have been published (18); however, since the original report, the patient relapsed on standard therapy and has enrolled on a clinical trial of a kinase inhibitor targeting ALK. In the second case (Table 2, case 4), an FGFR2 C382R activating mutation was detected and the patient subsequently initiated treatment with pazopanib, a multikinase inhibitor with anti-FGFR2 activity. In the third case (Table 2, case 5), ctDNA testing detected multiple activating *HER2* mutations, and based on this result, the patient is now enrolled on a clinical trial and receiving matched targeted therapy with trastuzumab plus pertuzumab. In the fourth case (Table 2, case 6), ctDNA testing revealed a *GOPC-ROS1* fusion. The patient began treatment with the ALK/ROS1 inhibitor crizotinib and had clinical improvement and shrinkage of multiple lesions within 3 months of initiating therapy (Supplementary Fig. S2). Breaks in treatment due to infection requiring hospitalization and later due to compliance issues correlated with increased CEA levels and progressive disease; however, resumption of crizotinib resulted in symptom improvement. Because of persistent progressive disease, the patient was then switched to ceritinib, a second-generation ALK/ROS1 inhibitor, with a clinical response, but discontinued due to gastrointestinal toxicity and later expired. In the fifth case (Table 2, case 7), ctDNA testing detected an *HER2*-activating mutation and coamplification. Notably, the patient was originally diagnosed 15 months prior to ctDNA testing, and *HER2* IHC performed on a previous biopsy had been negative, and therefore, the patient did not receive *HER2*-targeted therapy at that time. When ctDNA testing was performed, the patient was declining rapidly but derived symptomatic improvement and clinical benefit on palliative trastuzumab monotherapy.

Two patients underwent treatment with targeted therapy following tissue-based genomic profiling (FoundationOne), and later had ctDNA testing performed at progression. The first patient (Table 2, case 8) was treated on a clinical trial of trastuzumab + pertuzumab. Following a 2-year response, a blood sample was collected upon progression due to difficulty in obtaining a biopsy in the setting of bone-only disease. Genomic profiling of ctDNA revealed the initial *HER2* amplification as well as a *PIK3CA* mutation not present in the prior tissue sample. *PIK3CA* mutation is a known resistance mechanism to *HER2*-targeted therapy, particularly in breast cancer (19, 20). Of note, no outlier lesions concerning for second primary lesions, such as a breast primary, were identified in this patient, and the bone disease was determined to be metastatic colorectal adenocarcinoma. The second patient (Table 2, case 9) was treated with first-line trastuzumab, followed by paclitaxel and ramucirumab. At progression on first-line therapy, ctDNA testing revealed the initial *HER2* amplification as well as a focal *MET* amplification not present in the prior tissue sample.

Discussion

Herein, we report results from genomic profiling of ctDNA from 417 patients with carcinomas of the gastrointestinal tract or anus. Evidence of ctDNA in the blood, as estimated by MSAF > 0, was detected in 82% of cases, similar to recent studies in colon cancer (21, 22). Among cases with evidence of ctDNA, at least one reportable GA was

detected in 89% of cases with an average of 2.28 reportable alterations per case. Potentially actionable alterations in the RAS/RAF/MEK pathway (48%) and PI3K pathway (17%) were common, and inactivating DNA repair pathway mutations in *BRCA1/2* (5%) were observed across anatomic tumor types. Activating alterations in RTKs, including *EGFR*, *HER2*, *MET*, *FGFR1/2/3*, *ALK*, *ROS1*, *RET*, and *PDGFRA*, were observed in 18% of cases, including *HER2* amplification or point mutation in 5% of cases. RTK fusions, rare potentially targetable driver events in gastrointestinal cancers (18, 23, 24), were detected in 2% of cases, which is notably higher than in previously published frequencies in gastrointestinal tissue samples (9, 25). Examples of clinical utility were observed in a significant fraction of patients in whom potentially actionable alterations were detected in ctDNA and follow-up was available (Table 2). However, it should be noted that the role of ctDNA testing in gastrointestinal cancers remains largely investigational, and alterations identified herein are largely utilized to identify matched off-label therapies or genomically matched clinical trials.

The frequency of gene amplification detection was notably lower in ctDNA samples compared with that detected in tissue samples from both the FoundationCORE tissue database (representing similar disease ontologies), as well as to published tissue studies in colorectal carcinoma (15, 16). In contrast, the spectrum and incidence of genes with short variant alterations was similar to that observed in tissue samples from patients with gastrointestinal cancers, supporting the ability of ctDNA profiling to reflect tissue-based characterization. In recent studies comparing ctDNA and tissue samples in colorectal carcinoma patients, similar rates of short variant alterations to those seen here were also observed for frequently altered genes (21, 22). However, in one study (21), reported frequencies of *EGFR* and *MET* amplification in ctDNA samples were notably enriched compared with ctDNA results observed here and relative to published tissue studies of colorectal carcinoma (21). In another study (22), although short variant alteration frequencies were reported, the prevalence of gene amplifications and fusions detected in cell-free DNA samples were not included for the majority of cases (22).

Although concordance for short variant alterations detected in tissue samples with those detected in temporally matched ctDNA was quite high (95%), only 50% (6/12) of gene amplifications detected in tissue were detected in matched ctDNA samples. This is similar to what has been observed in other ctDNA studies in solid tumors (26, 27). In gastrointestinal cancers, detection of amplification of potentially targetable RTKs, including *HER2* and *MET*, is likely to be clinically relevant, as these events have been shown to mediate acquired resistance and are themselves targetable using therapies currently approved in other tumor types or in active clinical trials, including the NCI-MATCH and TAPUR studies (3, 6, 28). In fact, in a recent study of 22 pan-RAS/BRAF/HER2/MET wild-type colorectal carcinoma cases progressing on EGFR antibody therapeutic regimens, tissue and ctDNA analyses at cetuximab or panitumumab progression supported *KRAS* mutations and *HER2* or *MET* amplification as the dominant mediators of clinical resistance (27). Furthermore, *EGFR* amplification is an emerging target in gastric and esophageal cancers (29–31). However, in the relatively small analysis of paired samples in this study, no *HER2* or *MET* amplifications (and just one *EGFR* amplification, found in tissue only) were observed in either subset, so concordance analysis was limited for these specific gene amplifications. Eight GAs across 6 patient cases were identified in tissue and not in paired ctDNA, including 6 gene

amplifications (*PIK3CA*, *CDK6*, *FLT3*, *KRAS*, *EGFR*, and *MYC*) and two short variant mutations (Supplementary Table S2). However, in the majority of these cases, the amplifications not detected in ctDNA were identified in the setting of one or more other known and potentially targetable driver alterations, so the clinical significance of these gene amplifications is unclear. For the two cases with short variant mutations not detected in ctDNA, one case harbored an *NF1* truncating alteration was identified only in matched tissue, which is potentially actionable through clinical trial enrollment or treatment with off-label therapies; in the second case, an activating *NRAS* G60E mutation was detected in paired tissue only. However, the actionability of this alteration in GEJ adenocarcinoma is not established. Notably, 7 of 7 *KRAS* mutations detected in tissue were also detected in matched ctDNA.

When we compared the ctDNA fraction, as estimated by MSAF, in samples where at least one amplification event was detected versus those with no amplification, we found that cases with amplification present had significantly higher MSAF than those without (Supplementary Fig. S3A). This may suggest that, similar to what has recently been reported in a ctDNA breast cancer study (32), higher levels of ctDNA in a blood sample may be necessary to reliably detect gene amplifications. Interestingly, the predicted copy number for amplified genes in paired tissue samples was not significantly different for amplifications found in both tissue and blood compared with amplifications found only in the paired tissue sample. We acknowledge that this observation is preliminary as the number of samples and amplification events available for analysis was relatively small (Supplementary Fig. S3B).

EGFR mutations were notably more common in ctDNA (5.8%) samples compared with tissue samples in the FoundationCORE database (0.8%) for gastrointestinal carcinomas, and the majority of *EGFR* mutations detected were in the cetuximab/panitumumab-binding region of the ECD, which have been shown to mediate resistance to these anti-*EGFR* therapies (10) that are approved and commonly used for the treatment of *KRAS* wild-type colorectal carcinoma. This observed enrichment in ctDNA versus tissue biopsies likely reflects the subclonal nature of these mutations, although other factors may also contribute to the discrepancy. Of note, *EGFR* mutations were more common in the other two published tissue studies examined relative to FoundationCORE; however, this may be expected as these datasets included only colorectal carcinoma cases and excluded other gastrointestinal-type malignancies less likely to have received anti-*EGFR* therapies. Similar observations regarding subclonal *EGFR* ECD mutations were noted in a recent large study of blood-based cell-free DNA samples in colorectal carcinoma, including recurrent mutations at *EGFR* V441D/G, which had not been previously reported (22). This study also noted treatment history with *EGFR* antibodies in all patients with samples harboring these mutations for whom clinical data were available, further suggesting a role for ECD mutations in cetuximab/panitumumab resistance.

Although 95% (42/44) short variant alterations detected in tissue were also detected in temporally matched ctDNA samples (restricting to genes represented in both panels), only 63% (49/78) short variant alterations detected in ctDNA were also detected in matched tissue. Furthermore, only 1 of 5 kinase rearrangements detected in ctDNA were detected in matched tissue. Therefore, blood-based ctDNA testing may provide added value beyond

tissue testing to detect multiple primary drivers or diverse mechanisms of acquired resistance in a single patient. However, large collaborative efforts with clinically annotated tissue and ctDNA are critical to determine the relative importance of multiple potential drivers, and to establish how these genomic data may be useful in making clinical treatment decisions. Tissue biopsy done in parallel with ctDNA may be useful to determine which heterogenous alterations are present in a given tumor, or to determine the genomic profile of a given lesion of particular clinical concern (11). The optimal method to comprehensively assess tumoral evolution and acquired resistance is not known, but our data suggest there is likely additional value in utilizing ctDNA for repeat genomic profiling to capture dynamic changes in a patient's tumor genomic profile over time, particularly during the course of treatment with targeted therapy (28, 33).

Other studies of ctDNA profiling in gastrointestinal cancers have focused on colon cancer, and most have been limited in size, and in some cases restricted to hotspot alterations in a limited set of genes (21, 28, 34). Strickler and colleagues recently reported cell-free DNA genomic profiling results for over 1,000 colorectal carcinoma patients, but analysis was largely focused on short variant alterations, and no comparison with paired tissue samples was available (22). Alternative applications for ctDNA, including detection of residual disease and early recurrence, rather than identification of actionable GAs, have been the focus of other studies and are not formally examined in our analysis (35, 36). Despite the promise of ctDNA, there are clear disadvantages in relying solely on ctDNA testing, including lack of sufficient ctDNA in approximately 18% of samples, low purity (MSAF) of ctDNA, which can lead to diminished ability to reliably detect gene amplifications, and overall smaller gene panels with ctDNA versus tissue, which limit the total number of assessable alterations. A recent study in colorectal carcinoma noted that in over 25% of patients studied, a potentially actionable alteration was identified in tissue that was not detected in ctDNA (21). Comprehensive tissue-based genomic profiling assays have the capability to report additional GAs and signatures in gastrointestinal cancers, specifically microsatellite instability, tumor mutational burden, and PD-L1 IHC, all of which have implications for the role of immunotherapies in gastrointestinal cancers. We anticipate improving technological methods will allow more comprehensive assessment from ctDNA in the future, but currently available ctDNA assays remain limited.

The limited accessibility of tumor tissue in advanced cancer patients represents a significant clinical challenge. Blood-derived ctDNA may provide an alternative approach for genomic profiling in cases where tissue biopsy is prohibitive, and ctDNA testing may have the additional advantage of identifying heterogenous alterations not present in a single tumor site; however, the clinical implications of detection of such alterations requires further investigation. ctDNA testing is currently limited relative to tissue testing in the detection of gene amplification, and currently available ctDNA assays are typically less comprehensive than available tissue-based assays. Thus, we conclude that ctDNA testing should be utilized as a complementary methodology to tissue-based genomic profiling and may be particularly useful in the setting of acquired resistance, or in patients when tissue biopsy is challenging.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Translational Relevance

Genomic testing to gain predictive and prognostic information has increasingly become part of clinical care for patients with advanced gastrointestinal cancers. Tissue testing remains the gold standard; however, blood-derived circulating tumor DNA (ctDNA) has emerged as a minimally invasive alternative. In this series, we present results from a large cohort of patients with advanced gastrointestinal malignancies whose samples were tested prospectively during routine clinical care using hybrid capture-based ctDNA profiling. For a subset of cases, temporally matched tissue samples assayed using a similar platform were analyzed for concordance and comparison. This study highlights the utility of noninvasive ctDNA testing in gastrointestinal cancers, particularly in the setting of acquired resistance to targeted therapies. The limitations of ctDNA versus tissue-based genomic profiling are discussed.

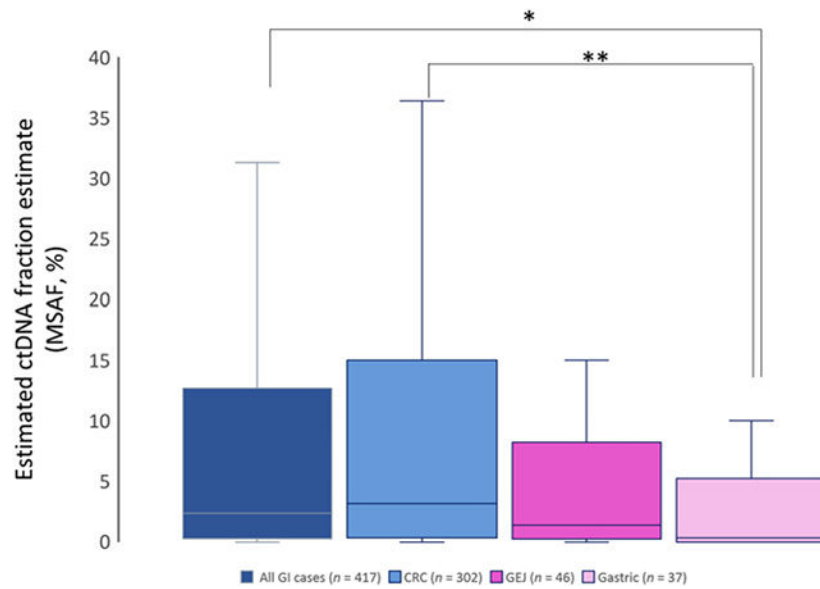
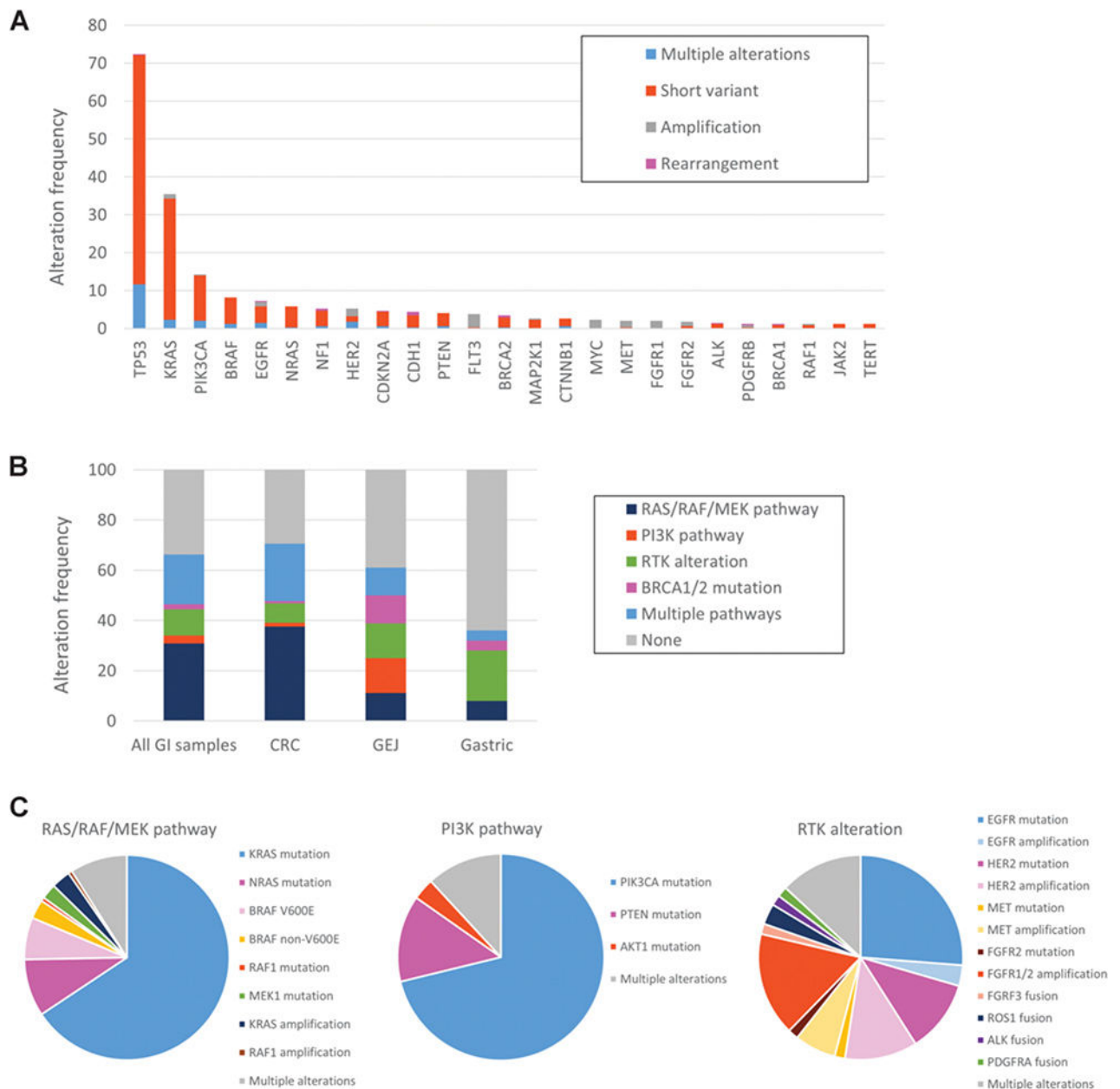


Figure 1. ctDNA fraction estimated across gastrointestinal disease histologies. MSAF was used to estimate the ctDNA fraction in a given sample. MSAF was significantly greater for all gastrointestinal ctDNA cases versus gastric carcinoma ctDNA cases (*, $P=0.011$) and in colorectal carcinoma ctDNA cases versus gastric ctDNA cases (**, $P=0.003$). Box-and-whisker plots: box spans first and third quartiles, the median is denoted by the horizontal line in the box, and whiskers indicate maximum and minimum values within $1.5\times$ the interquartile range.

**Figure 2.**

GAs identified in ctDNA from patients with gastrointestinal or anal carcinomas. Includes samples with evidence of ctDNA in the blood (MSAF >0). All gastrointestinal samples ($n = 344$), colorectal carcinoma ($n = 258$), GEJ ($n = 36$), gastric adenocarcinoma ($n = 25$). **A**, Longtail of frequently altered genes in carcinomas of the gastrointestinal tract or anus. **B**, Frequency of major pathway alterations across gastrointestinal disease histologies. RTK alterations include mutation, amplification, or fusion of *EGFR*; amplification or mutation of *MET*, *ERBB2*, *FGFR1*, and *FGFR2*; and fusion only of *ALK*, *ROS1*, *RET*, *PDGFRA*, and *FGFR3*. RAS/RAF/MEK pathway alterations include mutation or amplification of *KRAS*, *NRAS*, *BRAF*, *RAF1*, or *MEK1*. PI3K pathway alterations include amplification or

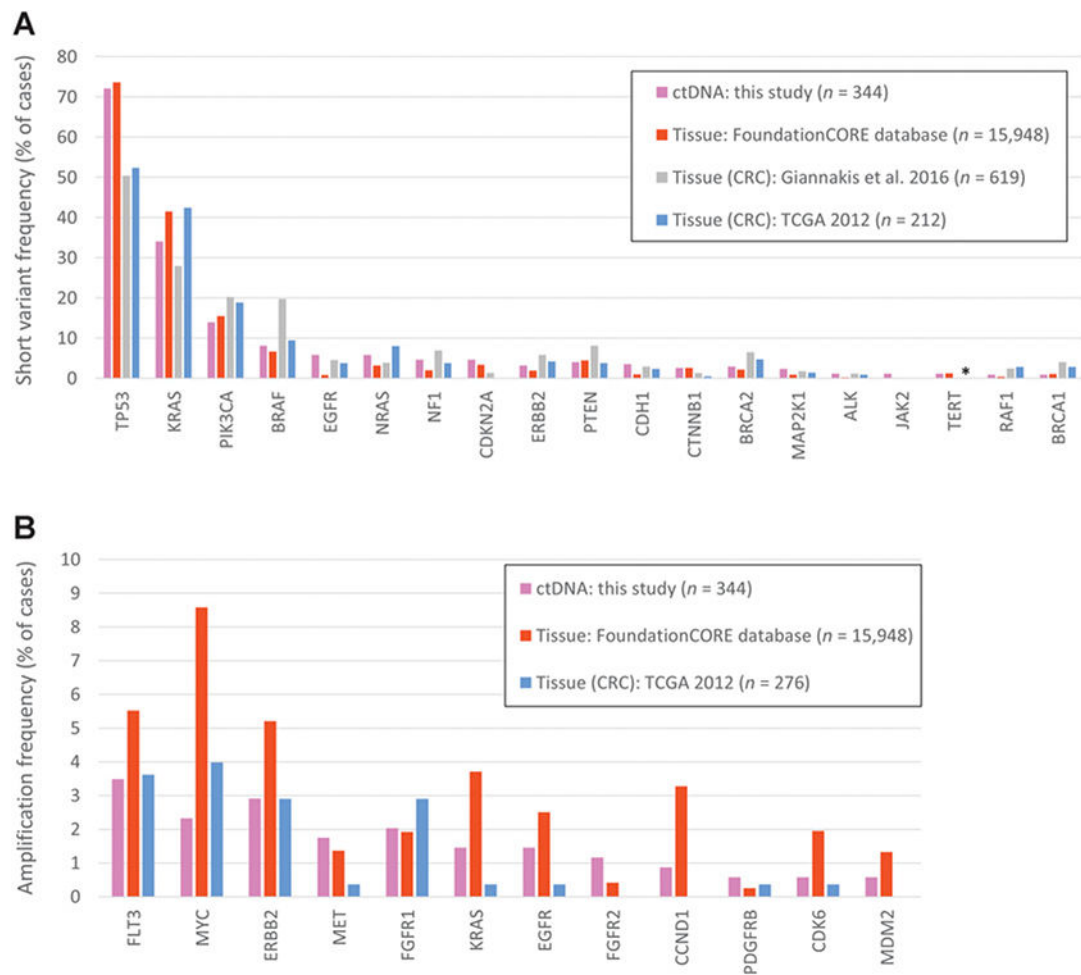
mutation of *PIK3CA*, and mutation of *AKT1* or *PTEN*. **C**, Distribution of individual gene alterations within pathways defined in **B**.

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**Figure 3.**

GAs in ctDNA from patients with carcinomas of the gastrointestinal tract or anus compared with tissue. Comparison of the most frequently mutated (**A**) or amplified (**B**) genes observed in ctDNA in this study with tissue-based genomic profiling of carcinomas of the gastrointestinal tract of anus (FoundationCORE database) or with published tissue-based genomic profiling studies of primarily early-stage colorectal carcinoma (TCGA 2012 and Giannakis and colleagues 2016). Copy number data were not available in the Giannakis and colleagues study. Data from the TCGA and Giannakis studies were extracted from cBioPortal. *, TERT alterations were not assessed in the Giannakis (gray bars) or TCGA (blue bars) series.

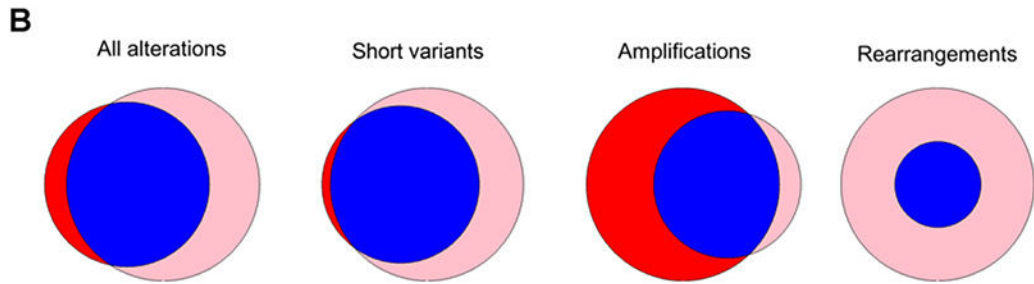
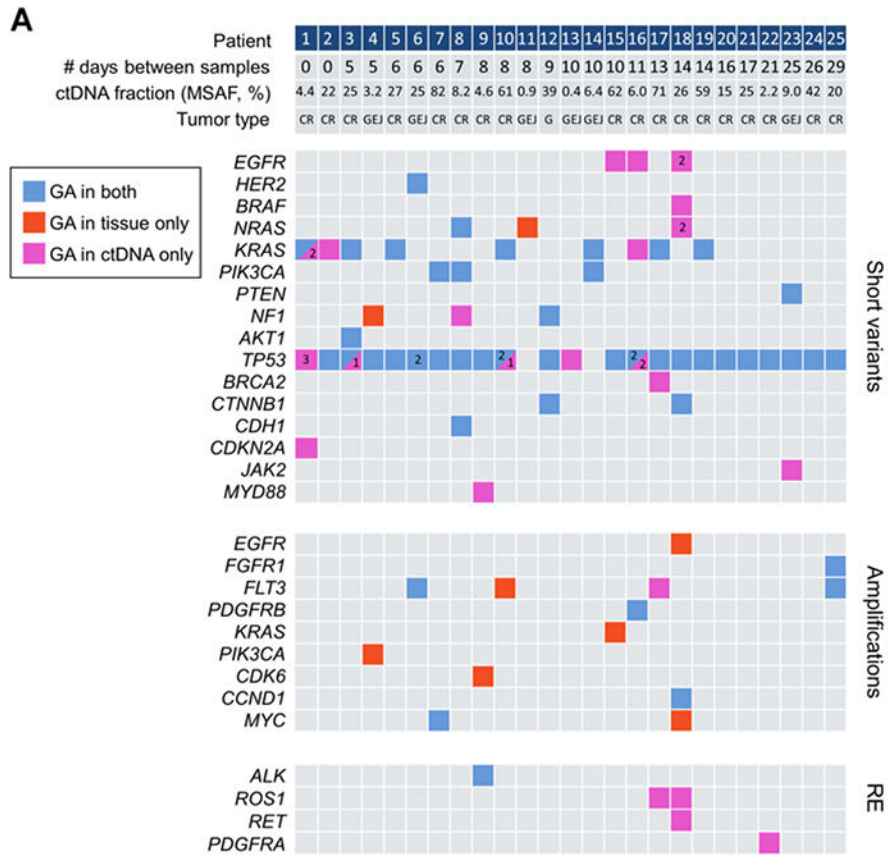


Figure 4. Concordance between GAs found in ctDNA and matched tissue from 25 patients with gastrointestinal carcinomas. **A**, Days between ctDNA and tissue collection, MSAF, and disease histology are shown. CR, colorectal adenocarcinoma; G, gastric adenocarcinoma; RE, rearrangement. Concordant/shared GAs are in blue, GAs found only in tissue are in red, and GAs found only in ctDNA are in pink. For samples with multiple unique mutations in a given gene, the number of mutations is indicated. **B**, Venn diagrams of concordant and discordant alterations by class. Blue, GAs found in both ctDNA and tissue; pink, GAs found only in ctDNA; red, GAs found only in tissue.

Table 1.

Characteristics of gastrointestinal ctDNA samples by anatomic tumor location

	All cases	CRC	GEJ	Gastric	Anus SCC	Esophagus	SBA	Neuroendocrine
Cases, <i>n</i>	417	302	46	37	13	10	7	2
Median patient age (range), years	60 (27–93)	59 (27–92)	64 (31–93)	60 (33–89)	61 (50–83)	66 (53–82)	61 (50–90)	48 (40–56)
Gender	43F: 57M	57F: 43M	24F: 76M	38F: 62M	62F: 38M	30F: 70M	57F: 43M	50F: 50M
MSAF >0 (%)	344 (82)	256 (85)	36 (78)	25 (68)	9 (69)	10 (100)	5 (71)	1 (50)
Median MSAF	2.36%	3.19%	1.38%	0.35%	0.54%	2.54%	0.42%	43.0%
Avg. GA/case ^a	2.28	2.37	1.94	1.76	2.44	2.3	2.2	0.5

Abbreviations: CRC, colorectal carcinoma; SBA, small-bowel adenocarcinoma.

^aIncludes only cases with MSAF >0.

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Table 2.

Characteristics of patients with clinical follow-up available

Patient	Disease histology	Age	Gender	Stage	Potentially targetable GAs identified in ctDNA	Treatment information
1	Rectum adenocarcinoma	41	M	IV	<i>SLC34A2-ROS1</i> fusion	Currently receiving first-line SOC therapy, will consider trial of ROS1 inhibitor at progression
2	Gastric adenocarcinoma	64	M	IV	<i>FGFR2</i> amplification	Currently responding to immune checkpoint inhibitor therapy, will consider trial of FGFR2 inhibitor at progression
3	Colon adenocarcinoma	62	F	IV	<i>STRN-ALK</i> fusion	Enrolled in clinical trial of ALK inhibitor
4	Anus SCC	75	F	IV	FGFR2 C382R	Began treatment with pazopanib based on FGFR activity
5	Colon adenocarcinoma	59	M	IV	HER2 D769Y, R678Q, S310Y	Enrolled in clinical trial of matched targeted therapy with trastuzumab + pertuzumab
6	Small-bowel adenocarcinoma	54	F	IV	<i>GOPC-ROS1</i> fusion	Clinical response to crizotinib, then ceritinib upon progression of disease
7	Esophagus adenocarcinoma	53	M	IV	<i>HER2</i> amplification, HER2 D769Y	Symptomatic improvement during 7-week treatment with trastuzumab, passed away due to advanced disease before scans were performed
8	Rectum adenocarcinoma	37	F	IV	<i>HER2</i> amplification, PIK3CA N345K	ctDNA testing postprogression on trastuzumab + pertuzumab trial detected PI3K mutation not present in tissue tested pretreatment. ^a Treated on a trastuzumab emtansine (TDM1)-based protocol and passed away 8 months after ctDNA testing.
9	GEJ adenocarcinoma	78	M	IV	<i>HER2</i> amplification, <i>MET</i> amplification	ctDNA testing postprogression on trastuzumab-containing therapy detected <i>MET</i> amplification not present in tissue tested pretreatment. ^a Developed a malignant biliary obstruction; had a rapid clinical decline and was unable to receive a MET-directed or dual-targeted therapy.
10	Rectum adenocarcinoma	70	M	IV	HER2 S310Y	Lost to follow-up
11	Duodenum adenocarcinoma	77	F	IV	HER2 D769N, S310F, V777M	Passed away before receiving targeted therapy
12	Esophagus carcinoma	75	M	IV	<i>MET</i> amplification	Passed away before receiving targeted therapy
13	Gastric adenocarcinoma	43	F	IV	<i>MET</i> amplification	Passed away before receiving targeted therapy

NOTE: Age and stage indicate time when ctDNA testing was performed.

Abbreviation: SOC, standard of care.

^aPretreatment testing of DNA isolated from a tissue biopsy was performed using the parallel FoundationOne platform.

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