Circulating Tumor DNA in Advanced Solid Tumors: Clinical Relevance and Future Directions

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Abstract: The application of genomic profiling assays using plasma circulating tumor DNA (ctDNA) is rapidly evolving in the management of patients with advanced solid tumors. Diverse plasma ctDNA technologies in both commercial and academic laboratories are in routine or emerging use. The increasing integration of such testing to inform treatment decision making by oncology clinicians has complexities and challenges but holds significant potential to substantially improve patient outcomes. In this review, the authors discuss the current role of plasma ctDNA assays in oncology care and provide an overview of ongoing research that may inform real-world clinical applications in the near future. CA Cancer J Clin 2021;71:176-190. © 2020 American Cancer Society.

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Introduction
Molecular characterization of tumors in the clinical care of patients with advanced solid malignancies has enabled rapid advances in precision oncology, with genomic profiling results guiding individualized treatment approaches in an increasing number of cancer types.1-4 Genomic profiling traditionally uses tumor tissue derived from surgical resection or biopsy specimens and primarily focuses on identifying alterations in specific genes that predict for sensitivity to targeted therapies. More recently, genomic profiling assays that, instead, use plasma circulating tumor DNA (ctDNA) have been developed and are in routine clinical use, with multiple commercially available tests. Plasma ctDNA assays present distinct advantages and disadvantages but ultimately have the potential to substantially enhance and broaden the application of genomic profiling to improve cancer care. In this review, we discuss the current and emerging applications of plasma ctDNA assays across advanced solid tumor types and highlight investigational approaches that that have the potential to translate into clinical practice in the near future.

Plasma ctDNA refers to tumor-derived DNA fragments that comprise a subset of plasma cell-free DNA (cfDNA), which also includes DNA in the circulation that originates from other sources, primarily germline DNA resulting from hematopoietic cell death.5-11 Shedding of tumor DNA is highly variable, and the fraction of plasma ctDNA can range from as low as <0.01% to constituting the majority of total plasma cfDNA.12,13 In addition, the half-life of plasma ctDNA is very short, estimated at <2 hours, thus plasma ctDNA levels are subject to dynamic changes.14 Ultimately, the quantity of plasma ctDNA available for testing demonstrates intra- and interpatient variability, with implications toward the interpretation of clinical test results.

Earlier assays used for plasma ctDNA profiling typically used polymerase chain reaction (PCR) to identify somatic mutations in single genes, such as mutations in EGFR to guide the use of EGFR tyrosine-kinase inhibitors (TKIs) in advanced non–small cell lung cancer (NSCLC).15-17 However, the commercially available plasma tests most commonly used in contemporary oncology care use targeted
panel next-generation sequencing (NGS) to assess somatic alterations (including mutations, fusions, and copy number alterations) typically in dozens of genes simultaneously. By comparison, current commercially available genomic profiling assays that use tumor tissue also are based on targeted NGS but are often more comprehensive and typically assess several hundred genes simultaneously. In addition, more comprehensive intronic coverage as well as included or reflexed RNA sequencing may enable improved fusion detection with tumor tissue-based methods.

The relatively low abundance of tumor DNA present in plasma samples compared with tumor tissue poses a substantial challenge for the sensitivity of plasma-based assays. False-negative results can occur both because of insufficient plasma ctDNA content as well as lack of inclusion of altered genes in the targeted NGS panel. Most plasma ctDNA assays do not use matched sequencing of white blood cells, thus false-positive results can occur because of germline variants or the presence of somatic mutations in hematopoietic stem cells because of clonal hematopoiesis as well as sequencing errors and artifacts. Theoretically, false-positive results could also be caused by the identification of variants derived from malignancies other than the cancer of interest (eg, synchronous primaries or undiagnosed cancers). However, the ability to perform comprehensive genomic profiling using plasma samples has multiple inherent advantages. Adequate archival tumor material for NGS is not available for a significant subset of patients, and noninvasive plasma testing may enable the deferral of potentially painful or high-risk biopsies. The turnaround time for plasma NGS can be as little as 7 days compared with typically ≥2 weeks for tumor NGS. This discrepancy can be even greater when tumor NGS is affected by logistical factors related to acquisition and processing of tumor material. Plasma ctDNA may also more accurately assess the current genomic profile of an advanced solid tumor compared with the use of archival tissue specimens, as these can be dated, such as when recurrence occurs many years after resection of an early stage tumor. Furthermore, plasma ctDNA can capture tumor heterogeneity and reflect DNA shed from multiple metastatic sites. Finally, plasma draws can be performed serially at closer time intervals than would be clinically practical with tumor tissue, thus enabling dynamic assessment of tumor evolution throughout the clinical course.

This article will focus on recent developments toward the real-world clinical use of plasma ctDNA assays by oncology practitioners. Tumor DNA can be identified in bodily fluids other than plasma, including cerebrospinal fluid (CSF), urine, saliva, stool, pleural fluid, and ascites. Although testing of these sources has potential clinical relevance in specific disease types, these modalities are beyond the focus of the current review. Liquid biopsy can also refer to tests that assess other tumor-derived material, including RNA and circulating tumor cells, or that analyze epigenetic modifications such as DNA methylation; however, these are also outside the scope of this review. Plasma ctDNA testing also has potential applications in other disease settings, including the detection of minimal residual disease after definitive therapy for early stage cancer and in cancer screening; however, this review will focus on applications relevant to the care of patients with advanced cancer. Additional technical considerations and in-depth methodology related to plasma ctDNA assays have been covered comprehensively in prior reviews.

Summary of the 2018 American Society of Clinical Oncology/College of American Pathologists Joint Review

The American Society of Clinical Oncology (ASCO) and the College of American Pathologists (CAP) convened an expert panel on the clinical use of ctDNA tests in patients with cancer, and the resulting joint review was published in both the Journal of Clinical Oncology and the Archives of Pathology and Laboratory Medicine in March 2018. The literature search used was completed in March 2017. Key topics included preanalytical variables relevant to ctDNA specimens, analytical validity, interpretation and reporting, and clinical validity and utility.

Preanalytical Variables

Plasma is the ideal blood specimen type for ctDNA analysis, as serum (which does not include clotting factors) contains a substantially higher proportion of normal germline DNA derived from leukocyte lysis. Blood is typically drawn from peripheral veins following the manufacturer’s instructions for the tube type used (either cell-stabilizing tubes or EDTA anticoagulant tubes). Cell-stabilizing tubes contain reagents that stabilize cell membranes, decreasing cell lysis and release of cellular genomic DNA from leukocytes and other cells into plasma. Thus EDTA tubes require expedient sample processing within 6 hours, compared with ≥48 hours with cell-stabilizing tubes. Comprehensive data do not exist to inform performing draws from other sites (eg, from ports, arteries, or central veins) or other specimen handling variables (eg, tube inversions, draw order, or fill levels).

Analytical Validity

Multiple ctDNA assays are available and should not be considered interchangeable given variable assay performance characteristics, including differing lower limits of detection and breadth of genomic coverage. However, analytical specificity is typically >95%, and discrepancies in variant
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detection in limited cross-platform comparisons primarily stem from differences in analytical sensitivity. More comprehensive cross-platform comparisons using standardized reference samples are needed to better understand analytical validity in this space, with a goal of establishing optimal sensitivity and specificity thresholds for different clinical applications. Notably, assessment of analytical validity through analysis of concordance between tumor and plasma NGS can be confounded by biologic factors (e.g., time between tissue and plasma collection, tumor heterogeneity, or amount of tumor ctDNA shed), which are independent of analytical factors.

**Interpretation and Reporting**

The use of ctDNA assay results to inform treatment selection should be carefully interpreted in the context of clinical factors, including tumor type, disease burden, and treatment history, as well as the potential identification of germline variants and clonal hematopoiesis. The variant allele fraction (VAF) of alterations identified must be interpreted with caution, as the ratio of ctDNA to total cfDNA can vary dramatically between different patients and timepoints. Somatic variants are typically detected at VAFs well below 50% (the VAF expected for heterozygous germline variants), although somatic variants can also be found at high VAFs, including at genetically amplified loci. Follow-up testing with dedicated germline analysis should be considered in ambiguous cases, for example, to determine whether a BRCA mutation is somatic or germline. Somatic variants related to clonal hematopoiesis are associated with increasing age, starting at approximately age 50 years, with detection in 5% of individuals aged 60 to 69 years, and in 10% of individuals aged >70 years. Clonal hematopoiesis most commonly involves mutations in DNMT3A, TET2, and ASXL1 but can also involve TP53, JAK2, SF3B1, GNB1, PPM1D, GNAS, and BCO1.1. Thus variants in these genes identified by ctDNA analysis should be interpreted cautiously.

Differences in the VAFs of genomic alterations found on the same ctDNA assay report may suggest that certain variants are not found in all tumor cells present in an individual patient (a consequence of tumor heterogeneity), and further research is needed to understand the actionability of potential subclonal variants found at lower VAFs. Lack of detection of somatic variants on ctDNA assays may be related to very low tumor amounts of DNA shed, and potential for discordance with tumor testing should be considered.

**Clinical Validity and Utility**

At the time of the 2018 ASCO/CAP joint review, only one ctDNA test had received regulatory approval in the United States, a PCR-based assay to detect *EGFR* mutations in NSCLC. The authors concluded that the majority of ctDNA assays had yet to demonstrate sufficient clinical validity and clinical utility and that routine use should be limited to those that have demonstrated clinical utility or with regulatory approval. The identification of a genomic biomarker in ctDNA using a well-validated assay may support targeted therapy if clinical utility has been demonstrated for that biomarker when identified by tissue testing. Analyses of ctDNA assays are most reliable when performed at disease progression rather than during response. Given the potential for discordance, testing of tumor tissue is supported to confirm a lack of variant detection by ctDNA analysis, if feasible.

Finally, the authors concluded that there was no evidence of clinical validity and little evidence of clinical utility for treatment monitoring in advanced disease and for the detection of residual disease after definitive therapy for early stage cancer. Neither evidence of clinical validity nor clinical utility was present to support the use of ctDNA for cancer screening outside of a clinical trial. The authors acknowledged the rapid evolution of research toward ctDNA assays in cancer and the need for short-term re-review of the literature.

**Genomic Profiling and Treatment Selection**

Because the ASCO-CAP joint review has yet to be updated, here, we review recent advancements involving standard and emerging clinical applications of ctDNA in advanced solid tumors, with a focus on several key cancer types. Standard clinical applications at this time remain within the context of treatment selection.

**Non–Small Cell Lung Cancer**

NSCLC is the disease type for which plasma ctDNA testing has the most compelling and comprehensive evidence. The identification of genomic alterations in *EGFR, ALK, ROS1, BRAF, MET, RET*, and *NTRK* guide the use of US Food and Drug Administration (FDA)-approved targeted therapies in the first-line advanced disease setting. The National Comprehensive Cancer Network (NCCN) Guidelines for NSCLC (version 4.2020) recommend molecular testing as part of broad molecular profiling in all patients with nonsquamous NSCLC at the time of diagnosis, with repeat biopsy or plasma testing if there is insufficient tissue to permit testing that includes *EGFR, ALK, ROS1, BRAF, MET*, and *RET* (Table 1). Plasma testing is preferred in patients who are medically unfit for invasive repeat biopsy. For patients in whom repeat biopsy could be performed, if an oncogenic driver (e.g., *KRAS* mutations as well as the alterations that guide first-line targeted therapy) is not identified by initial ctDNA testing, subsequent tissue-based analysis should be planned, given the up to 30% false-negative rate of ctDNA assays.
The NCCN Guidelines also support plasma-based testing to identify the acquired resistance mutation EGFR T790M in patients who have progression on a first-generation or second-generation EGFR TKI or to guide treatment with the third-generation EGFR TKI osimertinib. Similarly, tissue-based testing with repeat biopsy is strongly recommended in the setting of negative ctDNA results.58 (Table 1). Note that this clinical scenario is increasingly uncommon in the United States because osimertinib is currently the standard-of-care first-line treatment option for patients with classic sensitizing EGFR mutations.

Two recent publications highlight the potential for plasma ctDNA NGS to improve the detection of guideline-listed genomic biomarkers in real-world practice. The NILE (Noninvasive vs Invasive Lung Evaluation) study (ClinicalTrials.gov identifier NCT03615443) evaluated the completeness and timeliness of molecular profiling in 282 first-line metastatic NSCLC patients who received both plasma ctDNA NGS testing (Guardant360; Guardant Health) and physician discretion standard-of-care tumor testing, which could include NGS, PCR, fluorescence in situ hybridization, immunohistochemistry, or Sanger sequencing-based methods.59 The authors found that the addition of plasma ctDNA NGS increased the positive identification of a guideline-recommended genomic biomarker by 48% (from 60 to 89 patients). Of these, 7 patients had negative tissue results, 6 had insufficient tissue, and 16 did not have the biomarker assessed in tissue (only 12.4% of patients underwent tissue NGS per physician discretion in this cohort). The median turnaround time using the ctDNA assay was significantly faster than tissue testing (9 vs 15 days; \( P < .0001 \)). In another study of 264 first-line patients with advanced NSCLC, plasma ctDNA NGS testing (InVisionFirst; Inivata) identified 26% more actionable alterations (48 vs 38 patients) compared with tissue testing.60 In the latter study, 178 patients had successful tissue-based testing for at least one alteration, and tissue-based tumor NGS was performed in all patients who had sufficient tissue available.

The development of therapies directed at specific molecular subtypes in NSCLC is rapidly evolving, and the breadth of guideline-listed genomic alterations that guide first-line or subsequent-line therapies is likely to expand in the near future. Key emerging biomarkers include HER2 (ERBB2) and KRAS G12C mutations. The HER2-targeting antibody–drug conjugate ado-trastuzumab emtansine61 is listed under “Available Targeted Agents With Activity Against Driver Event in Lung Cancer” under “Emerging Biomarkers” in the NCCN Guidelines, and another antibody–drug conjugate, trastuzumab deruxtecan,62 received US FDA breakthrough therapy designation in May 2020. The KRAS G12C inhibitors AMG51063 and MRTX84964 both demonstrated response rates of 50% in

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**TABLE 1. Key Recommendations From Select National Comprehensive Cancer Network Guidelines (May 2020)**

| Key NCCN recommendations relevant to ctDNA plasma testing in NSCLC |
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| If there is insufficient tissue to allow testing for all of EGFR, ALK, ROS1, BRAF, MET, and RET, repeat biopsy and/or plasma testing should be done. Plasma-based testing should be considered at progression on (first-generation or second-generation) EGFR tyrosine kinase inhibitors for the T790M mutation. If plasma-based testing is negative, tissue-based testing with re-biopsy material is strongly recommended. Practitioners may want to consider scheduling the biopsy concurrently with plasma testing referral. Cell-free DNA/circulating tumor DNA (ctDNA) testing should not be used in lieu of a histologic tissue diagnosis. Studies have demonstrated that cell-free tumor DNA testing generally has very high specificity but significantly compromised sensitivity, with a false-negative rate up to 30%. Standards for analytical performance characteristics of cell-free tumor DNA have not been established, and, in contrast to tissue-based testing, no guidelines exist regarding the recommended performance characteristics of this type of testing. Cell-free tumor DNA testing can identify alterations that are unrelated to a lesion of interest, for example, clonal hematopoiesis of indeterminate potential. The use of cell-free DNA/ctDNA testing can be considered in specific clinical circumstances, most notably: |
| • If a patient is medically unfit for invasive tissue sampling; and |
| • In the initial diagnostic setting, if, after pathologic confirmation of an NSCLC diagnosis, there is insufficient material for molecular analysis, cell-free DNA/ctDNA should be used only if follow-up tissue-based analysis is planned for all patients in which an oncogenic driver is not identified. |

**Key NCCN recommendations relevant to plasma ctDNA testing in invasive breast cancer**

For HR-positive/HER2-negative breast cancer, assess for PIK3CA mutations with tumor or liquid biopsy to identify candidates for alpelisib plus fulvestrant. PIK3CA mutation testing can be done on tumor tissue or ctDNA in peripheral blood (liquid biopsy). If liquid biopsy is negative, tumor testing is recommended.

**Key NCCN recommendations relevant to plasma ctDNA testing in gastroesophageal cancer**

The genomic alterations of solid cancers may be identified by evaluating ctDNA in the blood, hence a form of liquid biopsy. Liquid biopsy is being used more frequently in patients with advanced disease who are unable to undergo a clinical biopsy for disease surveillance and management. The detection of mutations/alterations in DNA shed from gastric (or esophageal and esophagogastric junction) carcinomas can identify targetable alterations or the evolution of clones with altered treatment response profiles. Therefore, for patients who are unable to undergo a traditional biopsy, testing using a validated, next-generation sequencing-based, comprehensive genomic profiling assay performed in a CLIA-approved laboratory may be considered. A negative result should be interpreted with caution because this does not exclude the presence of tumor mutations or amplifications.

**Abbreviations:** CLIA, Clinical Laboratory Improvement Amendments; NCCN, National Comprehensive Cancer Network; NSCLC, non–small cell lung cancer.

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small numbers of patients with NSCLC enrolled in phase 1 trials. Acquired resistance to osimertinib in EGFR-mutant NSCLC is mediated by a diversity of mechanisms, including EGFR C797S, MET amplification, HER2 amplification, BRAF V600E, and various fusions (NTRK, RET, ALK, BRAF), which are potentially detectable by plasma ctDNA NGS and have associated potential standard or investigational treatment approaches. Plasma ctDNA analysis has also contributed to the understanding of acquired resistance mechanisms to other targeted therapies, including inhibitors of ALK, ROS1, and MET.

Breast Cancer

PIK3CA mutations in hormone receptor-positive, HER2-negative metastatic breast cancer guide treatment with the PI3Kα-specific inhibitor alpelisib in combination with fulvestrant as second-line therapy for advanced disease. The NCCN Guidelines for invasive breast cancer (version 2020) recommend assessment for PIK3CA mutations using tumor tissue or ctDNA testing, with reflex tumor testing if ctDNA results are negative (Table 1).

Recent publications have highlighted an important emerging role for ctDNA analysis in guiding other targeted therapy approaches as well as elucidating mechanisms of acquired resistance to breast cancer therapies. The Individualized Molecular Analyses Guide Efforts (IMAGE) trial (ClinicalTrials.gov identifier NCT01939847) in 26 women with progressive, metastatic, triple-negative breast cancer studied the feasibility of obtaining a new metastatic tissue biopsy, performing tissue NGS (FoundationOne; Foundation Medicine, Inc), and providing molecular tumor board recommendations within 28 days, as well as plasma ctDNA NGS. Highlighting the challenges of obtaining tumor tissue NGS results in an expedient timeframe, only 12 of 20 evaluable patients (60%) received recommendations based on tumor tissue NGS within 28 days, and an additional 6 patients did not receive tumor tissue NGS because of insufficient material or lack of an appropriate biopsy site. In contrast, 24 of 26 patients (92.3%) successfully had genomic alterations identified by plasma ctDNA NGS, and 23 of 33 of mutations (70%) were concordant in patients who underwent both tumor tissue and plasma ctDNA NGS. Among all 20 patients who underwent NGS of a metastatic site (including 8 patients who did not receive recommendations within 28 days), the molecular tumor board recommended tumor tissue NGS-informed, targeted treatment as a possible next line of therapy for 13 patients. In a phase 2 trial of the pan-HER inhibitor neratinib in HER2-mutant, nonamplified, metastatic breast cancer, plasma ctDNA NGS identified the same HER2 mutation in 11 of 14 tumor-positive cases (79%) and demonstrated 100% specificity.

The ongoing plasmaMATCH trial (ClinicalTrials.gov identifier NCT03182634) in approximately 1000 patients with advanced breast cancer matched patients into 5 parallel treatment cohorts based on plasma ctDNA mutations (identified using both droplet digital PCR and NGS), and an interim analysis reported that efficacy criteria were met in cohorts studying neratinib for patients with HER2 mutations and the AKT kinase inhibitor capivasertib in patients with AKT1 mutations.

An analysis of 23 patients with metastatic, ER-positive breast cancer who progressed on endocrine therapies demonstrated that ctDNA analysis using droplet digital PCR detected ESR1 mutations at a higher rate than metastatic tissue biopsy and detected additional ESR1 mutations not present in metastatic tissues, possibly identifying tumor heterogeneity. More recently, plasma ctDNA exome sequencing of paired baseline and end-of-treatment samples from 195 patients enrolled on the PALOMA-3 trial (ClinicalTrials.gov identifier NCT01942135) was performed to investigate mechanisms of resistance to the CDK4/CDK6 inhibitor palbociclib plus fulvestrant versus fulvestrant alone. In contrast to earlier findings describing RB1 aberrations as a putative resistance mechanism to CDK4/CDK6 inhibitors, this analysis identified that RB1 mutations are rare (4.7%) and often subclonal (present only in a fraction of cells), suggesting potential activity of subsequent endocrine-based therapy after progression on the combination. Otherwise, no difference in acquired mutation profiles was seen between the groups. New driver mutations in PIK3CA and ESR1 (especially Y537S) emerged after treatment in both arms, suggesting that resistance to fulvestrant is the main driver of resistance to the combination. Emergence at progression of potentially actionable alternations, including HER2 mutations, activating FGFR mutations, and high-level FGFR2 amplifications, was also observed in a small number of patients.

Prostate Cancer

The NCCN Guidelines for prostate cancer (version 2.2020) do not directly address plasma ctDNA testing. The identification of patients for PARP inhibitor (PARPi) therapy is likely the most compelling near-term emerging role for ctDNA analysis in prostate cancer. In May 2020, the PARPi olaparib received US FDA approval for patients with metastatic, castration-resistant prostate cancer (CRPC) with deleterious or suspected deleterious germline or somatic homologous recombination repair (HRR) gene mutations, and rucaparib received approval for patients with metastatic CRPC (mCRPC) who have deleterious germline and/or somatic BRCA mutation. The very high concordance of somatic HRR mutation detection in plasma and tumor tissue in patients with sufficient ctDNA
content suggests that plasma ctDNA testing may be useful to identify the subset of patients eligible for this treatment approach. However, concordance can differ for specific variant types (eg, mutations vs copy number alterations), and commercially available ctDNA tests are not validated for the detection of homozygous loss of HRR genes. Plasma ctDNA has also been shown to identify BRCA reversion mutations that mediate acquired resistance to PARPi treatment and to identify heterogeneity not appreciated by single-site biopsies.

Plasma ctDNA testing has also been studied in understanding primary resistance to abiraterone and enzalutamide as well as in the recognition of neuroendocrine transformation. Pretreatment plasma samples from a study of 202 patients receiving abiraterone or enzalutamide with treatment-naive mCRPC were analyzed with whole-exome and targeted NGS. The study found that ctDNA recapitulated the somatic landscape of mCRPC and identified genomic alteration classes associated with poor clinical outcomes, including defects in BRCA2, ATM, and TP53. AR copy number gain and AR ligand-binding domain missense mutations did not preclude response, but AR gene truncations were associated with primary resistance. In a separate study, matched plasma and tumor biopsies were analyzed in 62 men with metastatic prostate cancer, and the results indicated that genomic and epigenomic features associated with castration-resistant neuroendocrine prostate cancer were detectable by ctDNA. A targeted set combining genomic alterations (TP53, RB1, CYLD, AR) and epigenomic alterations (hypomethylation and hypermethylation of 20 differential sites) applied to ctDNA is capable of identifying patients with neuroendocrine prostate cancer. Detecting neuroendocrine differentiation through a plasma test may guide treatment decisions away from interventions targeting the androgen receptor pathway.

Colorectal Cancer
The NCCN Guidelines for colon cancer (Version 3.2020) do not directly address plasma ctDNA testing.

Tissue-based biomarkers in advanced colorectal cancer (CRC) have been used to predict response to targeted therapies and guide therapy selection. Specifically, patients whose tumors harbor KRAS, NRAS, and BRAF mutations should not be treated with anti-EGFR monoclonal antibodies, such as cetuximab or panitumumab, because their tumors are resistant to EGFR blockade. For patients with BRAF V600E-mutant CRC, treatment with the BRAF tyrosine kinase inhibitor encorafenib in combination with the anti-EGFR monoclonal antibody cetuximab is an effective treatment strategy. Finally, HER2-amplified and RAS and BRAF wild-type CRC responds to HER2-directed therapy with trastuzumab in combination with either laptinib or pertuzumab. Although plasma-based biomarkers have yet to be approved for treatment selection, studies have shown a high concordance between tissue and plasma genomic alterations in advanced CRC. Further prospective studies are needed to confirm the predictive value of plasma-based genomic profiling.

Metastatic CRC was the first disease in which liquid biopsy was used to study mechanisms of acquired resistance to systemic therapy. In 2012, 2 landmark studies revealed the emergence of KRAS mutations as mechanisms of resistance to EGFR blockade. Notably, KRAS-mutant alleles were detectable in patients’ plasma as early as 10 months before radiographic disease progression. Further follow-up of patients after discontinuation of anti-EGFR monoclonal antibodies demonstrated a decline of the resistant KRAS-mutant clones, suggesting that rechallenge of these patients with EGFR-directed therapy may be an effective treatment strategy. In addition to the emergence of KRAS mutations, ctDNA revealed mutations in the extracellular domain of EGFR as a mechanism of acquired resistance to anti-EGFR monoclonal antibodies. As HER2-directed therapy has emerged as a novel treatment strategy in HER2-amplified metastatic CRC, ctDNA analysis in these patients has identified plasma-based biomarkers of response and resistance to HER2-targeted therapy. Plasma from patients treated with trastuzumab and laptinib on the HERACLES study (ClinicalTrials.gov identifier NCT03225937) was collected before treatment, every 15 days during therapy, and at the time of radiographic progression. ctDNA was subjected to NGS using a targeted 73-gene panel. Mutations and/or copy number alterations in RAS, BRAF, PIK3CA, MAP2K1, HER2, and EGFR were associated with either primary or acquired resistance to dual HER2 inhibition. Interestingly, RAS and BRAF mutations were detected in pretreatment plasma samples and conferred primary resistance to HER2 blockade. Although, per protocol, only patients with RAS wild-type were eligible for the study, all patients had received anti-EGFR therapy before enrollment, which led to the emergence of RAS-mutant clones. If ctDNA had been used to determine patient eligibility for HER2-targeted therapy, then 86% of patients, who proved to have primary resistance, would have been spared an ineffective therapy. These data demonstrate the value of liquid biopsy as a noninvasive, real-time molecular tool to monitor clonal evolution and guide subsequent therapies.

Gastroesophageal Cancer
The NCCN Guidelines for gastric cancer (version 2.2020) and esophageal and esophagogastric junction cancers (version 2.2020) support the use of plasma ctDNA NGS to detect targetable alterations or clones with altered treatment sensitivity profiles in patients who are not candidates.
for biopsy and tissue-based NGS, and reinforce that negative results do not exclude the presence of genomic alterations (Table 1).

Recent studies have evaluated the role of ctDNA in gastric and esophageal adenocarcinoma (GEA). In contrast to CRC, GEA is characterized by extensive baseline genomic heterogeneity, by which many cancers harbor discrepant targetable alterations between the primary tumor and the metastatic disease. In this setting, ctDNA profiling may provide a more accurate representation of metastatic disease in GEA. Indeed, tissue-based NGS showed significant discrepancies (36%) between primary and metastatic foci,111-113 which may explain the failure of targeted therapies in GEA. In discordant primary and metastatic lesions, there was 87.5% concordance for actionable alterations in metastatic tissue and ctDNA, suggesting the potential of ctDNA profiling to augment therapy selection to target the metastatic disease.112

Trastuzumab is the only genome-guided therapy approved for the 15% to 20% of patients with HER2-amplified GEA. NGS analysis of ctDNA in patients treated with trastuzumab revealed that, in patients with innate resistance to HER2 blockade, HER2 copy number increased at radiographic progression, whereas, in patients with acquired resistance, HER2 copy number declined during treatment and remained lower at progression compared with baseline. Preexisting or emerging mutations in HER2, ERBB4, PIK3CA, PIK3R1, PIK3C3, and NF1 were associated with resistance to HER2 blockade.114 In a similar study of patients treated with first-line lapatinib, ctDNA profiling revealed that only patients with detectable HER2 amplification in ctDNA responded to HER2-directed therapy. In addition, the emergence of EGFR, MET, FGFR2, MYC, CCNE1 amplification and FGFR1 and PIK3CA mutations was associated with acquired resistance to HER2 inhibition.115 The value of HER2 amplification in ctDNA as a predictor of response to HER2 inhibitors was confirmed in a larger GEA cohort, whereas combined assessment of HER2 amplification in tissue and ctDNA improved the predictive value, suggesting that complementary tissue and ctDNA NGS testing may be the best approach to guide therapy selection.113

Pancreatobiliary Cancers

The NCCN Guidelines for pancreatic adenocarcinoma (version 1.2020) and hepatobiliary cancers (version 3.2020) do not directly address plasma ctDNA testing for advanced disease, but they do mention that plasma ctDNA is being investigated as a possible biomarker for pancreatic cancer screening.

In pancreatic and biliary cancers, liquid biopsy has been shown to mirror the genetic alterations that are present in patients’ tumors. In a study that compared tissue and ctDNA NGS in 26 patients with advanced pancreatobiliary carcinomas, there was high concordance between the 2 specimens, with 90.3% of mutations detected in both biopsy and ctDNA.116 As biopsies in these cancers are often inadequate for genomic profiling, ctDNA NGS may enable molecular profiling and guide genome-directed therapies. Olaparib has been approved as maintenance treatment for adult patients with deleterious or suspected deleterious germline BRCA-mutated metastatic pancreatic adenocarcinoma, but targeted therapeutic approaches based on somatic genetic alterations that would be detected in a ctDNA assay have yet to be validated or approved.

In contrast to pancreatic cancer, biliary cancers often harbor actionable alterations. An emerging target is FGFR2 fusion or rearrangement in advanced intrahepatic cholangiocarcinoma (ICC). Several FGFR inhibitors are being developed, and one of these TKIs, pemigatinib, was recently approved for the treatment of metastatic ICC harboring FGFR2 fusion or rearrangement.117 Serial analysis of ctDNA in patients with FGFR2 fusion-positive ICC who received different FGFR inhibitors revealed the emergence of multiple point mutations in the FGFR2 kinase domain at disease progression.118,119 These data highlight the dependence of these cancers on the FGFR pathway and can inform the development of treatment strategies to overcome resistance. Another emerging target in ICC is IDH1 mutation. In a recently published phase 3 study that compared the IDH1 inhibitor ivosidenib versus placebo in patients with advanced, previously treated IDH1-mutant cholangiocarcinoma, ivosidenib led to a significant improvement in progression-free survival (PFS) compared with placebo.120 Although tissue-based NGS was used to determine IDH1 mutation status in that study, a retrospective analysis of ctDNA in those patients showed 92% concordance of IDH1 mutation between plasma and tissue, suggesting that liquid biopsy may be a valid method of patient selection for IDH1-directed therapy.121

Melanoma

The NCCN Guidelines for cutaneous melanoma (version 3.2020) do not directly address plasma ctDNA testing; however, they point out that improved assays for the detection of BRAF and KIT mutations are in development and reference the literature on plasma ctDNA detection of BRAFV600 mutations.122-124 In a study of 732 patients who had metastatic melanoma with known BRAF V600E or V600K mutations enrolled across 4 clinical trials of dabrafenib or trametinib, the respective mutation was detectable in pretreatment plasma in 76% and 81% of patients, respectively.124

Tissue-Agnostic Biomarkers

Current tissue-agnostic US FDA approvals include the tropomyosin receptor kinase inhibitors entrectinib
and larotrectinib for NTRK gene fusion tumors and the anti–PD-1 antibody pembrolizumab in microsatellite instability-high or mismatch repair deficient tumors as well as tumor mutational burden-high tumors. National guidelines do not address plasma-based testing these biomarkers; however, plasma ctDNA may be useful in select scenarios. The gene list and methodology of commercial ctDNA NGS assays should be carefully considered when interpreting results because the inclusion of NTRK genes\textsuperscript{125} and microsatellite instability-high detection\textsuperscript{126,127} may vary. Blood tumor mutational burden is being studied as a potential predictive biomarker for immunotherapy efficacy in NSCLC but is not included in most current commercial plasma ctDNA NGS assays.\textsuperscript{128,129} The detection of acquired resistance mutations after treatment with first-generation TRK inhibitors using ctDNA has been described, and next-generation TRK inhibitors are in development.\textsuperscript{130,131}

**Risk Stratification, Response Assessment, and Resistance Monitoring**

Although most of the existing literature on plasma ctDNA analysis describes studies to support the selection of precision cancer therapies, the increasing availability of this noninvasive diagnostic motivates an investigation of newer applications. We conceptualize the key potential applications of plasma ctDNA beyond treatment selection in advanced solid tumors as 3 distinct topics: risk stratification, response assessment, and resistance monitoring (Fig. 1).

**Risk Stratification**

Plasma ctDNA levels have been examined as a prognostic biomarker across multiple cancer types. In a study of 164 patients with metastatic, triple-negative breast cancer, a ctDNA fraction \( \geq 10\% \) of total plasma (determined using low-coverage whole-genome sequencing\textsuperscript{88}) was associated with significantly shorter survival (median, 6.4 vs 15.9 months; log-rank \( P < .001 \)).\textsuperscript{132} In the study of pretreatment plasma ctDNA from \( B R A F \text{ V600}- \text{mutation–positive metastatic melanoma} \) treated on the dabrafenib or trametinib clinical trials referenced above, patients with \( B R A F \text{ V600} \) mutations detected in plasma had increased tumor burden and exhibited shorter PFS and overall survival (OS) than those who did not.\textsuperscript{124} Several studies have established the prognostic significance of ctDNA in advanced pancreatic ductal adenocarcinoma. A high pretreatment ctDNA level was strongly correlated with large tumor burden and was an independent predictor of poor OS.\textsuperscript{133-135} It is intuitive that these poorer risk cancers with high ctDNA shed may require more aggressive treatment strategies. Thus we envision that plasma ctDNA as a prognostic biomarker could be used for risk stratification, such as in a trial design in which patients with high plasma ctDNA levels are directed toward more intensive therapy and patients with low plasma ctDNA levels are directed toward less intensive therapy (Fig. 2). Such an approach is not dissimilar from ongoing studies of minimal residual disease in which ctDNA-positive patients receive ongoing therapy whereas ctDNA-negative patients are permitted a treatment break.\textsuperscript{136}

**Response Assessment**

Response assessment using plasma ctDNA was first described in the context of improved clinical outcomes with decreased levels of oncogenic driver alterations after treatment initiation with targeted therapies active against the respective alteration. Examples include \( E G F R \)-mutant or
ctDNA in Advanced Solid Tumors

Risk Stratification

- Pretreatment plasma ctDNA assessment
  - High ctDNA levels → More intensive therapy
  - Low ctDNA levels → Less intensive therapy

Response Assessment

- Pretreatment plasma ctDNA assessment
- All patients start with less intensive therapy
- On-treatment plasma ctDNA assessment
  - Partial/Complete Response (PR/CR) by imaging (RECIST)
  - Stable Disease (SD) by imaging (RECIST)
  - Progressive Disease (PD) by imaging (RECIST)
  - No plasma response
  - Continue less intensive therapy
  - Change treatment to more intensive therapy

Resistance Monitoring

- Pretreatment plasma ctDNA assessment
- All patients start systemic therapy #1
- Progressive Disease (PD) determined by resistance by plasma ctDNA or imaging (RECIST)
- Change to systemic therapy #2
- Progressive Disease (PD) determined by imaging (RECIST) only
- Change to systemic therapy #2

FIGURE 2. Trial Designs to Establish the Clinical Utility of Emerging Applications of Plasma Circulating Tumor DNA (ctDNA) Analysis in Advanced Solid Tumors. RECIST indicates Response Evaluation Criteria in Solid Tumors.

RET fusion-positive NSCLC treated with EGFR or RET inhibitors, BRAF V600E-positive metastatic melanoma treated with BRAF inhibitors, and AKT E17K-mutant solid tumors treated with an AKT inhibitor on a basket study. However, recent publications have demonstrated the potential utility of plasma ctDNA-based response assessment across numerous systemic therapy and disease types. In the BEECH trial of ER-positive metastatic breast cancer treated with paclitaxel plus placebo versus paclitaxel plus capivasertib (ClinicalTrials.gov identifier NCT01625286), suppression of likely driver mutations in ctDNA between baseline and week 4 was associated with superior median PFS (11.1 vs 6.4 months; hazard ratio [HR], 0.20; 95% CI, 0.083–0.50; \( P < .0001 \); log-rank test). Suppression of ctDNA could be observed as early as 8 days on-treatment. In a study of metastatic CRPC, a decline in ctDNA (when the baseline level was >7%) was observed with all treatments associated with a prostate-specific antigen decline ≥30%. Reduction in ctDNA was associated with OS in the TOPARP-A trial of olaparib (ClinicalTrials.gov identifier NCT01682772) and with radiological PFS and best overall response in the A.MARTIN trial of ipatasertib (ClinicalTrials.gov identifier NCT01485861). In a study of patients with metastatic CRC receiving first-line chemotherapy, the vast majority of patients (98.1%) had detectable ctDNA at baseline, and significant reductions in ctDNA before cycle 2 were associated with a radiographic response at 8 to 10 weeks (odds ratio, 5.25 with a 10-fold ctDNA reduction; \( P = .016 \)). Similarly, the PLACOL study also demonstrated that an early change in ctDNA concentration after one or two cycles of chemotherapy can predict therapeutic efficacy in patients with metastatic CRC (ClinicalTrials.gov identifier NCT01983098). In longitudinal studies of ctDNA in advanced pancreatic cancer, a decrease in plasma levels of mutant KRAS 2 weeks after the initiation of systemic therapy was an early indicator of response to chemotherapy and was more strongly associated with clinical outcomes than changes in protein tumor markers. Among patients with advanced NSCLC receiving immune checkpoint blockade, the ctDNA response (defined as a >50% relative decrease in mutant allele fraction from baseline, for the mutation with the highest mutant allele fraction at baseline) strongly agreed with the radiographic response (Cohen \( \kappa \), 0.753) and was associated with superior PFS (HR, 0.29; 95% CI, 0.09–0.89; \( P = .03 \)) and OS (HR, 0.17; 95% CI, 0.05–0.62; \( P = .007 \)). The median time to initial ctDNA response was 24.5 days compared with 72.5 days by imaging. Building on these findings, our center is leading an adaptive clinical trial that leverages plasma ctDNA response assessment in patients with advanced NSCLC who are receiving pembrolizumab monotherapy to prospectively guide intensification to pembrolizumab plus doublet chemotherapy (ClinicalTrials.gov identifier NCT04166487). Patients with NSCLC expressing PD-L1 and without biomarkers supporting first-line targeted therapy are offered an
El-Deiry WS, Goldberg RM, Lenz HJ, did not permit the detection or monitoring of intracranial melanoma with brain metastases, plasma ctDNA analysis. Notably, in a recent study of patients who had metastatic melanoma with brain metastases, plasma ctDNA analysis did not permit the detection or monitoring of intracranial disease activity, highlighting the restriction of ctDNA by the blood-brain barrier.

Resistance Monitoring
Although response assessment is directed toward evaluating the efficacy of a recently initiated systemic therapy, resistance monitoring is aimed at identifying disease progression during or after systemic therapy ahead of clinical or radiographic indicators. The literature on plasma-based detection of acquired resistance is reviewed above (see Genomic Profiling and Treatment Selection). The monitoring of components of cfDNA other than ctDNA, such as plasma human papillomavirus (HPV) cfDNA in HPV-associated oropharyngeal cancer and cervical cancer, also has been investigated in observational and retrospective studies, demonstrating an association of plasma HPV cfDNA levels with tumor burden and treatment response. Earlier detection of resistance has the potential to inform changes in the treatment approach or the frequency of imaging; however, as with response assessment, prospective studies are needed to investigate whether changes in therapy driven by ctDNA findings will lead to improved outcomes (Fig. 2). The APPLE trial in EGFR-mutant NSCLC (ClinicalTrials.gov identifier NCT02856893) randomizes patients (1:1:1) to first-line osimertinib versus the first-generation EGFR TKI gefitinib, followed by osimertinib at the time of progression (determined by EGFR T790M detection by plasma ctDNA testing) versus gefitinib, followed by osimertinib at the time of progression (determined by imaging). Numerous additional questions remain, including the optimal interval with which to monitor using plasma draws and which variants should be followed. Clinical decision making based on plasma ctDNA results will likely need to integrate both increases in the levels of oncogenic driver alterations identified at baseline as well as the emergence of new variants that are known to or may mediate acquired resistance.

Conclusions
Clinical decision making in contemporary cancer care is increasingly defined by molecular stratification, with targeted therapies and other systemic therapies driven by the identification of specific genomic biomarkers across numerous cancer types. Indeed, our therapeutic armamentarium has never been richer, requiring the availability of nimble diagnostics that allow us to guide each patient to the best treatment approach. Plasma ctDNA testing has the potential to enhance successful genomic profiling, especially in patients with limited available tumor material, and robust commercial assays are now available for routine clinical use. Clinicians should understand the respective advantages and disadvantages of plasma ctDNA assays versus tumor tissue assays and evaluate the potential for false-positives and false-negatives when interpreting assay results. Although the standard clinical uses of plasma ctDNA testing currently are limited to treatment selection, the ability to longitudinally profile patients at regular intervals and capture dynamic changes in ctDNA will likely translate into expanded clinical applications for the care of patients with advanced solid tumor in the near future. We believe the key next applications are toward response assessment and resistance monitoring. We envision that longitudinal plasma ctDNA testing will enable the recognition of response or progression more nimbly than imaging and also will augment the interpretation of equivocal scan results, thus permitting more timely systemic treatment changes for individual patients. However numerous questions still exist, including the optimal timepoints, assays, and VAF thresholds with which to make clinical decisions. Innovative clinical trials will be critical in establishing the clinical utility of plasma ctDNA analysis beyond treatment selection.

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