Review Article

Clinical applications of liquid biopsies in gastrointestinal oncology

Jason Zhu, John H. Strickler

Department of Medicine, Division of Medical Oncology, Duke University Medical Center, Durham, NC 27710, USA

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Correspondence to: John H. Strickler, MD. Assistant Professor of Medicine, Department of Medicine, Division of Medical Oncology, Duke University Medical Center, DUMC 2823, Durham, NC 27710, USA. Email: john.strickler@dm.duke.edu.

Abstract: "Liquid biopsies" are blood based assays used to detect and analyze circulating tumor products, including circulating tumor cells (CTCs), circulating tumor DNA (ctDNA), circulating messenger RNA (mRNA), circulating microRNA (miRNA), circulating exosomes, and tumor educated platelets (TEP). For patients with gastrointestinal (GI) malignancies, blood based biopsies may offer several advantages. First, tumor tissue samples are often challenging to procure, and when obtainable, are often insufficient for genomic profiling. Second, blood based assays offer a real-time overview of the entire tumor burden, and allow anatomically unbiased genomic profiling. Third, given the convenience and relative safety of liquid biopsies, this technology may facilitate identification of genomic alterations that confer sensitivity and resistance to targeted therapeutics. This review will assess the clinical applications of circulating tumor products for patients with GI tumors.

Keywords: Liquid biopsies; circulating tumor cells (CTCs); circulating tumor DNA (ctDNA); cell free DNA (cfDNA)

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Introduction

In recent years, interest in blood-based "liquid biopsies" to diagnose, monitor, and characterize solid tumors has surged. With the arrival of several commercially available blood-based assays, the use of these assays in clinical practice is increasingly routine. Although many of these assays are analytically sensitive, specific, and accurate, prospective evidence linking results with clinical utility remains limited. In the absence of this prospective evidence, the increased use of blood-based biopsies reflects a desire to minimize procedural risk to the patient, while applying therapies tailored to a patient's specific tumor characteristics.

Liquid biopsies can be used to identify a range of circulating tumor products, including circulating tumor cells (CTCs), circulating tumor DNA (ctDNA), circulating messenger RNA (mRNA), circulating microRNA (miRNA), circulating exosomes, and tumor educated platelets (TEPs). Of these circulating tumor products, CTC and ctDNA assays are orderable through commercial vendors, and are most broadly incorporated into clinical practice. The detection of circulating tumor products has been well described for decades (1,2), but the assays used to detect these products have only recently been technically validated for routine clinical practice.

For patients with gastrointestinal (GI) malignancies, the use of blood-based biopsies to screen, diagnose, and genotype tumors may be particularly beneficial. First, tumor tissue samples—which are often limited to cytology or fine needle aspiration (FNA)—may be of insufficient quantity or tumor content. As a result, genomic profiling for clinical trial participation and personalized treatment strategies may not be possible. When tumor tissue is unavailable, clinicians must choose between a repeat tumor biopsy or treatment in the absence of genomic data. In some cases, metastatic tumor lesions are only available through diagnostic

laparoscopy or other invasive procedures. Second, blood-based assays offer a dynamic "snapshot" of the entire tumor burden at a specific point in time. In addition to showing dominant mutations, this anatomically unbiased profiling often reveals clinically relevant subclones. These subclones may not be detected in primary tumor tissue, but may increase under the selective pressure of targeted therapy. Third, blood-based biopsies capture both inter and intratumoral heterogeneity. In some cases this heterogeneity may be substantial (3-5). Finally, given the relative ease and speed with which blood can be obtained, blood-based assays can be utilized to identify genomic changes associated with sensitivity and resistance to novel therapeutics. This can accelerate efforts to discover relevant resistance-conferring alterations, and may support efforts to further personalize treatment.

This review will examine current liquid biopsy technologies for patients with GI malignancies, and will assess the ability of circulating tumor products to detect new cancers, diagnose recurrent disease, identify genomic biomarkers of sensitivity and resistance, and predict prognosis.

Circulating tumor products: overview of current technologies

CTCs

CTCs originate from the primary tumor and metastatic sites. Most CTC assays rely on selection of tumor cells based on their biological (protein expression) and physical properties (size, density, deformability, electric charge). Similar to ctDNA, CTCs have been used to help determine tumor mutation status (6) and prognosis (7). There currently exists a FDA-approved CTC assay, CellSearch (Veridex, New Jersey, USA), for the enumeration of CTCs. Although commercially available, the use of CTCs in GI cancers is limited due to low rates of detection, and its prognostic, rather than predictive value (8). While CTCs can be detected in over 60% of patients with metastatic breast cancer and prostate cancer, CTCs can only be detected in 30–40% of patients with metastatic CRC (mCRC) (8-11). When CTCs are detected in mCRC, they are typically at a lower absolute number (median 1–2 CTC/7.5 mL) than when they are detected in metastatic breast and prostate cancer (median 6–7 CTC/7.5 mL) (12-14). The reason for this discrepancy is multifactorial. First, CRC CTCs often travel in adherent clusters due to increased surface adhesion molecules (15). Second, the circulation anatomy may affect cell collection (16). Specifically, hepatic drainage of portal blood may act as a filter, resulting in fewer peripheral blood CTCs. Tumor location also plays a role. For example, lower rectal tumors have increased CTCs in the central venous blood, compared with tumors in the upper rectum and colon. Lastly, as CTCs undergo epithelial-mesenchymal transition (EMT), cells lose their epithelial markers and become more difficult to detect. CellSearch relies on expression of EpCAM, a marker of epithelial phenotype (17). Even when the cells do express EpCAM, it has been shown that the specific EpCAM antibody used can greatly change the detection rate (18). There are currently efforts underway to detect CTCs lacking EpCAM expression (17,19-21). One example is capturing cells which express the tyrosine kinase receptor c-MET (21).

ctDNA

ctDNA is comprised of fragments of cell-free, tumor-derived DNA from necrotic or apoptotic tumor cells (22). ctDNA can originate from CTCs, metastases, or the primary tumor. There are two challenges facing detection of ctDNA. First, a tumor-encoded somatic aberration (e.g., mutation, translocation, or methylation event) must be present in the tumor genome if it is ever to be detected in the blood. While genes like *APC*, *TP53* and *KRAS* are frequently mutated in GI malignancies, none is mutated in 100% of them, and a tumor suppressor gene has several mechanisms by which it can be inactivated, making for an unacceptably large potential search space for some technologies. Second, while poorly understood, quantity of ctDNA is likely related to tumor burden in a non-linear manner, meaning ctDNA is present in small amounts in early stage disease, sometimes occupying only 0.01% of total cell free DNA (cfDNA) (23). For example, in patients with localized GI tumors [colorectal cancer (CRC), gastric or gastroesophageal (GE) cancer, and pancreatic cancer] ctDNA can be detected in 48–73% of patients (24). However, as tumor burden increases, deep surveillance of sufficient genomic space increases ctDNA detection to nearly 100% (25). For this reason—and because tumor genotyping is currently less likely to influence early stage treatment decisions—ctDNA is now most useful in patients with advanced metastatic disease. The standard DNA sequencing approaches such as Sanger sequencing or pyrosequencing are only able to detect ctDNA in patients with significant tumor burden. To improve the ability to detect and analyze ctDNA, a variety of technologies

have been developed, including digital PCR (26), digital NGS (27), beads, emulsion, amplification, and magnetics (BEAMing) (28), pyrophosphorolysis-activated polymerization (PAP) (29), cancer personalized profiling by deep sequencing (CAPPSeq) (30), and tagged-amplicon deep sequencing (TAm-Seq) (31).

The optimal liquid biopsy assay remains an area of active investigation. Nonetheless, compared with traditional CTC assays, detection of ctDNA is arguably more sensitive (32,33). For example, in a recent study of patients with advanced solid tumors, ctDNA was always detected when CTCs were present (25). On the other hand, ctDNA was often detected when CTCs were absent. However, it should be noted that this study did not utilize enrichment methods prior to detection of CTCs, something which is now standard amongst modern CTC tests.

In addition to the ongoing research regarding early detection of malignancy, ctDNA is also being developed to detect clinically actionable somatic point mutations or deletions (34), cancer surveillance after definitive surgery (24), and monitoring for the development of molecular resistance to targeted therapies (35).

Circulating exosomes

Exosomes are extracellular vesicles—secreted by all cells—which contain proteins and nucleic acids. Cancer specific exosomes may have unique cell surface proteins which are distinguishable from normal exosomes (36). Glypican-1 (GPC1), a membrane anchored protein overexpressed in breast (37) and pancreatic cancer (38), was recently discovered to be detected exclusively in cancer exosomes (39). In a validation cohort of 56 patients with pancreatic ductal adenocarcinoma (PDA) (with patients from all four stages of cancer including carcinoma in situ), 6 patients with benign pancreatic disease such as chronic pancreatitis, and 20 healthy patients, $GPC1^{+}$ circulating exosomes (crExos) were 100% sensitive and specific for distinguishing patients with PDAC (39). Additional validation studies are needed for this novel approach.

Circulating mRNA

Several circulating mRNA candidates have been studied for CRC screening and prognosis after surgical resection (40-42). Blood mRNA biomarkers such as CEA, CK20, CK19, human telomerase reverse transcriptase (hTERT), and guanylyl cyclase C (GCC) have all been examined in the perioperative setting (42). In most cases, persistence of tumor associated mRNA within 24 hours of tumor resection has been predictive of relapse (43). In a meta-analysis of nine studies with patients undergoing curative surgery for CRC, CTC detection based on CEA, CK19 and CK20 mRNA correlated with the development of hepatic metastases and decreased disease-free survival (44). Recently, Rodia *et al*. utilized a novel Transcriptome Mapper (TRAM) to identify TSPAN8, LGALS4, COL1A2, and CEACAM6 as candidate mRNAs for detection of CRC (40).

However, circulating mRNA also has limitations. Extracellular mRNA is susceptible to degradation by RNase in plasma (45). Attempts to use RNase inhibitors have been unsuccessful at protecting mRNA from degradation (45). Thus, the use of mRNA in cancer screening and prognosis remains investigational.

Circulating miRNA

miRNAs are small noncoding RNA molecules that regulate cellular processes (46). miRNAs are stable in plasma and serum, properties which make them an excellent candidate as a blood-based tumor diagnostic (47). A recent meta-analysis of 16 studies examined the diagnostic accuracy of circulating miRNA in CRC. This study found that miRNA-21, as opposed to miRNA panels, was promising as a diagnostic biomarker (47). Nonetheless, there are several technical limitations with circulating miRNA. First, there is no standard approach for miRNA isolation. Studies report using serum, plasma, peripheral blood mononuclear cells, and whole blood (48). Second, it has been shown that hemolysis via centrifugation can impact miRNA levels, highlighting the need for consistent enrichment techniques (49). Further standardization is needed before miRNA is appropriate for routine clinical use.

TEPs

Platelets are involved in hemostasis and wound healing. While platelets are anucleate, they contain cytoplasmic pre-mRNA which may be translated into protein in response to external stimuli (50). Tumor cells may influence ("educate") neighboring platelets to undergo specific splicing of pre-mRNA, thereby altering the RNA profile of blood platelets (51). Using the mRNA of TEPs, Best *et al*. were able to distinguish between patients with malignancies versus healthy controls with 96% accuracy, and were able to determine the location of the primary tumor with 71%

accuracy (52). This technology remains investigational.

Screening

Current cancer screening modalities include direct visualization, imaging, histologic evaluation, and blood protein tests. While current screening methods help identify patients with potentially curable early stage malignancies, there still exists several barriers to cancer screening (53,54). Many tests are uncomfortable (colonoscopies, pap smears, mammograms), expose patients to radiation (CT scans, mammography), are unpleasant to accomplish (stool studies), and almost all require scheduling additional healthcare visits. Thus, the optimal cancer screening assay remains an area of active research.

Two new non-invasive techniques were recently approved for CRC screening. In 2014, the FDA approved fecal DNA testing with Cologuard (Exact Sciences Corporation, Madison, WI, USA). In 2016, the FDA approved Epi proColon (Epigenomics, Berlin, Germany), a blood-based test that detects methylated Septin9 (*SEPT9*) DNA to identify CRC in patients who choose not to undergo colonoscopy and stool-based fecal immunochemical tests (55). Other assays to detect early stage GI malignancies are currently under development.

Already, liquid biopsies have been utilized to detect clinically silent malignancies. In a study of 125,426 women undergoing non-invasive prenatal testing (NIPT) for fetal aneuploidy via *verifi Prenatal Test* (Illumina, San Diego, California, USA), 3,757 women tested positive for one or more aneuploidies involving chromosome 13, 18, 21, X, or Y. Of these women, 39 had multiple aneuploidies, and seven (18%) were diagnosed with an occult malignancy (56,57). Three other women with single aneuploidy were also diagnosed with malignancies. In three of the ten cases, the discordant NIPT test prompted the evaluation for malignancy. One limitation of this approach to cancer screening is that it requires a comparison between two different types of tissue—in this case mother and fetus. One approach to overcome this barrier is the use of a "nucleosome footprint"—ctDNA nucleosome spacing is unique with respect to the nuclear architecture and gene expression and thus may inform the cell type (58).

Another method to overcome the use of a comparator is digital karyotyping (DK). Abnormal chromosomal content in the form of losses and gains of entire chromosomes, modification of chromosome arms, amplifications and deletions, and chromosomal rearrangements can all be analyzed via next generation sequencing (NGS) (59). Using the DK approach and NGS data from 81 cancer patients and 10,000 simulated controls, Leary *et al*. hypothesized that they could reach a sensitivity of >90% and 99% specificity with detection of breast cancer and CRC when ctDNA concentrations reached more than 0.75%. While this test may be sensitive and specific, the required ctDNA concentration is still greater than 0.10%—the sensitivity needed to detect tumors based on BEAMing assays in early stage CRC (23,24). Another challenge will be the detection of false positives, due to constitutional germline or mosaic structural alterations, which can occur in 1.9% of patients older than 75 (60). Given the current challenges identifying ctDNA in patients with low tumor burden (23,25), colonoscopy should remain the standard of care for CRC screening.

Surveillance for recurrent disease

ctDNA may be effective for detecting tumor recurrence. In breast cancer, for example, ctDNA has been shown to be reliable in detecting tumor progression or metastatic disease, often with clinical lead time of up to almost 1 year (61). Similar studies have found that liquid biopsies can also be used for surveillance of GI malignancies (62,63). In addition to detection of recurrent disease, the presence of circulating tumor products may eventually guide adjuvant chemotherapy decisions.

CRC

In patients with CRC, the BEAMing and patient specific somatic structural variants (SSVs) techniques have been investigated as tools to identify cancer recurrence (23,62). In both methods, ctDNA was found to be more reliable and sensitive at detecting tumor recurrence than CEA. Diehl *et al*. measured ctDNA in patients after both complete and incomplete resections. Those patients with undetectable ctDNA had 100% recurrence free survival (RFS) at 2 years, compared to less than 15% RFS for patients with detectable ctDNA (23). Frattini *et al*. reported similar findings in a cohort of 70 CRC patients who underwent surgery and then had plasma DNA and CEA levels followed for 16 months (64). Prior to surgery, circulating plasma DNA was elevated in all patients, whereas only 30% of patients had an elevated CEA. At follow up, plasma DNA levels increased in all patients who ultimately developed metastases or loco-regional relapse—however, CEA levels

did not increase in all patients with recurrent disease, demonstrating the increased sensitivity of plasma DNA over CEA.

In patients with stage II CRC, ctDNA is being evaluated in the post-operative setting to detect disease recurrence. Preliminary data on 190 patients demonstrate cancer recurrence in 5 of 6 patients with detectable ctDNA and 5 of 72 patients with undetectable ctDNA (63). Patients with detectable ctDNA had a shorter recurrence-free survival (median 234 days *vs.* undefined, HR 23.09, P<0.0001). For patients with stage II colon cancer—where overall prognosis is good and the benefit of adjuvant chemotherapy is limited, ctDNA might eventually be used to identify patients with the greatest risk of recurrence, and guide adjuvant chemotherapy decision-making (65-67).

Gastric cancer

ctDNA is also being studied as a tool to monitor disease recurrence following gastrectomy. Hamakawa *et al.* collected gastric cancer tissue and blood from 42 patients undergoing gastrectomy (68). Ten patients had a detectable P53 mutation. Of those 10 patients, pre-operative cfDNA was available in 6 cases and ctDNA was measurable in only 3 cases. ctDNA correlated with disease progression in all three patients, whereas cfDNA did not. To enhance the feasibility of this approach, future efforts will need to include a larger panel of detectable oncogenes.

Pancreatic cancer

Only a minority of patients with pancreatic cancer are candidates for surgical resection. Even when patients undergo resection, recurrence rates range from 70–90% (69). Current post-operative surveillance for pancreatic cancer includes CA19-9 and imaging, both of which lack sensitivity and specificity. Thus, the use of circulating tumor products may be helpful in this setting. A study showed that 14 of 46 patients who had undetectable ctDNA following resection had a longer time to disease recurrence (median time 545 *vs.* 471 days), but this difference was not statistically significant (HR =0.58; P=0.3) (70). Similar findings were reported by Sausen *et al.*—patients with detectable ctDNA after surgical resection were more likely to relapse compared with those with undetectable ctDNA (71). Additional larger studies are needed to validate the use of ctDNA as a biomarker for recurrence in resected pancreatic cancer.

Predictive biomarkers—molecular profiling of blood to identify markers of sensitivity and resistance

Precision medicine is based on the notion that molecular profiles (tumor, stroma, immune) can be used to personalize cancer therapy (72). Until recently, most precision medicine clinical trials utilized tissue biopsies—either from primary tumors or at progression—to identify potential predictors of sensitivity and resistance (73-75). While these precision medicine initiatives represent advancements in the treatment of cancer, the technical and operational challenges of relying exclusively on tumor tissue are increasingly recognized. For example, the International Working Group on Multidisciplinary Lung Adenocarcinoma Classification estimated that only 57% of such biopsies had sufficient tissue for genomic analysis after initial pathology diagnosis and staining (76). Since 2009, the NCCN has recommended that all patients with mCRC be tested for *KRAS* mutations upon diagnosis (65). Yet, in 2010, only 23% of patients with mCRC received guideline based genomic analysis (77,78). While many factors contribute to lack of testing (77), the use of liquid biopsies may ease the practical burden of genomic testing, and facilitate the use of predictive biomarkers in clinical practice.

CRC

One application of ctDNA in CRC is to identify predictive biomarkers of primary treatment resistance. Both *KRAS* and *NRAS* (RAS) mutations are well established biomarkers that predict resistance to anti-epidermal growth factor receptor (anti-EGFR) monoclonal antibody therapy (either cetuximab or panitumumab) (79-81). Current NCCN practice guidelines recommend expanded RAS tissue testing in all patients with mCRC (65). Bettegowda *et al.* evaluated *KRAS* mutation status in the plasma and tumors of 206 patients with mCRC (25). Of the 206 patients, ctDNA correctly identified 127 of the 128 patients with *KRAS* wild-type tumors and 69 of 79 patients with *KRAS* mutations, yielding a sensitivity of 87% and specificity of 99%. Theirry *et al.* tested 106 patient plasma samples and companion tumor samples for seven *KRAS* point mutations and yielded 98% specificity and 92% sensitivity with a concordance value of 96% (82). It is unlikely that liquid biopsies will soon supplant standard of care tissue testing for RAS and *BRAF* mutations. However, in cases where tissue is not safely accessible or is not available, blood-based genotyping

may provide a surrogate for tissue.

A second application of ctDNA in CRC is to interrogate the genomics of acquired treatment resistance. Anti-EGFR therapies improve survival in patients with RAS WT mCRC (83-87), but nearly all patients develop resistance. Blood based assays—in particular ctDNA—could help identify acquired mutations that confer treatment resistance. Already, ctDNA has identified *KRAS* and *NRAS* mutations as the dominant drivers of acquired resistance to anti-EGFR therapies (88). These mutations may initially exist as rare subclones within a metastatic lesion, but increase under selective pressure (35). Diaz *et al*. examined the blood of 24 patients with *KRAS* WT tumors who received anti-EGFR therapy (35). Of the 24 patients, 9 patients were found to develop *KRAS* mutations in codons 12 and 13 during the course of therapy. The detection of *KRAS* mutations in blood preceded radiographic evidence of progression in 3 of 9 cases, with an average lead-time of 21 weeks. Additionally, Morelli *et al*. examined 62 patients who developed resistance to EGFR therapy. Of these 62 patients, 27 patients developed acquired *KRAS* mutations. In this same study, EGFR-ectodomain (ECD) mutations were detected in five patients treated with cetuximab but none of those treated with panitumumab (88). Since some EGFR ECD mutations do not confer cross-resistance to panitumumab, they are potentially targetable with panitumumab or other novel therapeutics (89-91).

Recently, ctDNA profiling has been utilized to identify other rare or overlapping genomic alterations associated with EGFR resistance. Bettegowda *et al*. performed ctDNA profiling on 24 patients who initially responded to EGFR therapy. The panel included not only *KRAS* codons 12 and 13, but also *NRAS*, *BRAF*, *EGFR*, and *PIK3CA* (25). Of these 24 cases, they discovered at least one mitogen-activated protein kinase pathway gene mutation in 23 patients (96%). The average number of mutations per each patient was 2.9 (range, 0–12). In total, they observed 70 somatic mutations, which had not been detected in the tumor or plasma prior to EGFR blockade.

The combination of ctDNA and tissue-based NGS may provide further insights into mechanisms of EGFR resistance. Siravegna *et al*. evaluated matched tissue and blood samples from 100 patients with *KRAS* WT mCRC (92). Ten out of 100 patients received an EGFR antibody but did not have clinical benefit. Of these ten patients, ctDNA revealed *NRAS* mutations in two patients, which had not been tested prior to treatment. In the remaining eight patients, NGS revealed *ERBB2* amplification in four patients, an alteration associated with primary EGFR resistance (93). Another patient had a somatic variant of the *MAP2K1* gene encoding the MEK protein, a rare mutation present in 1.5% of CRC tumors, and not yet associated with EGFR resistance.

CTCs have also been utilized to identify actionable targets in patients with CRC. Heitzer *et al*. isolated CTCs from patients with mCRC and compared 68 CRC-associated genes between primary tumors, metastases, and the corresponding CTCs (94). They found many mutations exclusively within the CTCs but not initially identified in the original tumor tissue.

Pancreatic and biliary cancers

For patients with pancreatic and biliary cancers, the use of tumor tissue to identify markers of sensitivity and resistance has proven challenging. First, these malignancies are often diagnosed by cytology or FNA, leaving limited tissue for molecular studies (95). Second, even when tissue is available, tumor cellularity is often less than 30%, making genomic analyses difficult (96). As a result of these challenges, genotype-directed clinical trials that rely on tissue testing have failed to demonstrate clinical benefit (97).

For patients with pancreatic cancer, ctDNA may offer advantages for genomic profiling. Over 75% of patients with metastatic PDA have detectable ctDNA (25). Zill *et al*. studied the sensitivity and specificity of ctDNA by analyzing ctDNA and primary tumor tissue of 26 patients with PDAs. They found that ctDNA had 92% sensitivity and 100% specificity in detecting genetic mutations when tumor tissue was used as the gold standard (98). Seven of nine patients (78%) in this study had clinically actionable mutations discovered in ctDNA but not in tumor tissue. One patient had an activating EGFR mutation discovered in blood, which was not seen in tumor until repeat biopsy 7 months later. That patient was treated with capecitabine and erlotinib with an exceptional response. Another patient found to have a *FGFR2* mutation was enrolled in a FGFR inhibitor clinical trial. This is in stark contrast to the recent IMPaCT trial, which relied upon tumor tissue profiling, and enrolled no patients in genotype directed therapies (97). Future applications for ctDNA in pancreatic cancer may include identification of BRCA pathway mutations (99) and other potentially actionable genomic markers of DNA mismatch repair deficiency (100).

In addition to ctDNA, investigators have also used exosomal DNA (exoDNA) to assess the genomic profiles of

pancreatic cancer (36). One advantage of exoDNA is that it is shielded from degradation in circulation (101). In a small proof-of-concept study, San Lucas *et al*. used whole exome sequencing to analyze three patients with pancreaticobiliary cancer and were able to find actionable mutations, including *ERBB2* and *NOTCH1* (102). In one of the three patients, the patient's pleural fluid was used for analysis. His pleural effusion had fewer than 1% malignant cells on cytospin and a NGS assay was unable to detect tumor DNA. Nonetheless, there were numerous tumor-derived exosomes present for analysis, demonstrating the utility of this approach.

Gastric cancer

Up to one sixth of patients with gastric cancer have *HER2* overexpression or amplification (103). Detection of *HER2* gene amplification in ctDNA can be serially monitored in patients undergoing treatment (104). In addition to ctDNA, CTCs have also been utilized to predict tumor progression in patients treated with palliative chemotherapy (105).

Prognostic biomarkers

Cancer prognosis has traditionally relied on pathologic stage at the time of diagnosis. Because the presence or absence of circulating tumor products correlates with absolute tumor burden, it may represent another independent prognostic metric. CTCs and ctDNA have been found to correlate with survival in many GI cancers, including colon, pancreatic, and gastric cancer. A recent systematic review of 39 studies and 4,052 patients found that detection of ctDNA is associated with significantly worse overall survival (106).

CRC

In patients with mCRC, there is an inverse relationship between level of cfDNA and survival. In a study by Spindler *et al*., patients with cfDNA below the median had an overall survival of 12.2 months, versus only 4.5 months in patients with levels above the median (107). In addition to total levels of cfDNA, they were able to correlate plasma mutant *KRAS* (mutKRAS) levels with prognosis. Patients with high plasma mutKRAS levels had a 0% rate of disease control (stable disease or response), compared with 42% in patients with low plasma mutKRAS levels (108).

In addition to cfDNA and ctDNA, CTCs have also been found to be prognostic in patients with CRC. In a study using CellSearch to detect CTCs, 430 patients with mCRC were split into favorable and unfavorable groups based on the number of CTCs—those patients with more than 3 CTCs per 7.5 mL of blood were classified as unfavorable (109). Those patients with a low CTC count had nearly twice as long progression free survival and overall survival compared to those with a high CTC count. CTC count was prognostic in all subgroups analyzed, including age, ECOG status, liver involvement, receipt of first line chemotherapy, and receipt of various chemotherapy regimens. As technology emerges to detect CTCs in a greater percentage of patients, the utility of CTCs in clinical practice may increase.

Pancreatic cancer

The detection of CTCs has been found to be prognostic in pancreatic cancer. In a randomized controlled trial in patients with locally advanced pancreatic cancer, CTC detection with CellSearch was associated with inferior survival (110). These results have been replicated by several other studies, and a meta analysis of nine independent cohort studies concluded that detection of CTCs is associated with worse progression free survival and overall survival (111).

In addition to CTCs, ctDNA may also predict prognosis in patients with pancreatic cancer (71,112,113). In a pilot study of 45 patients with exocrine pancreatic cancer, patients with *KRAS* mutated ctDNA had significantly decreased 60 day overall survival compared with those with *KRAS* WT ctDNA (60 *vs*. 772 days) (114). In this same study, the detection of CTCs also strongly correlated with survival—however, one major difference was that CTCs were only detectable in patients with metastatic disease, whereas *KRAS* mutated ctDNA was detected in patients with both resectable and locally advanced disease. The lack of detection of CTCs in early stage disease may be due to the detection technique as well as tumor physiology. A mouse model by Rhim *et al*. found that only 27% of circulating epithelial cells express EpCAM (115). The use of CTCs and ctDNA to predict prognosis in patients with pancreatic cancer remains investigational.

Gastric cancer

A recent meta-analysis of 26 studies involving 1,950 patients examined the prognostic significance of CTCs in gastric cancer (116). Zhang *et al*. found that CTCs are associated with worse RFS and overall survival. The authors found

that the time at which CTCs were collected was an important factor in predicting overall survival. CTCs collected prior to chemotherapy or surgery were predictive of metastases. CTCs collected immediately after surgery did not impart such prognostic information, as there is a transient increase in CTC detection after surgery, which has been demonstrated both in human and mouse models (117). CTCs collected in the surveillance period after surgery may be useful in indicating tumor recurrence. Thus, when using CTCs as a prognostic biomarker, it is important to specify when CTCs are collected, as blood collected prior to surgery and chemotherapy is likely more meaningful for overall survival, but CTCs collected after surgery are important for monitoring tumor recurrence.

Conclusions

Liquid biopsies have received widespread attention because of the implications for personalized medicine. From cancer screening and surveillance to molecular analysis of metastatic lesions, a liquid biopsy may obviate the need for invasive biopsies and help guide therapeutic decision making. Numerous studies have shown that circulating tumor products can predict prognosis and recurrence risk after surgery. However, there are still barriers that must be overcome before liquid biopsy technology replaces the current tissue-based gold standard. First, the most sensitive and specific methodology of detecting circulating tumor products remains an area of active investigation. Second, there is a paucity of prospective studies linking results from liquid biopsy assays to clinical benefit. Finally, while liquid biopsies may give us the ability to detect mutations, we still lack effective drugs for many genomic alterations. Despite these limitations, liquid biopsy technologies represent a promising step forward in the detection and monitoring of GI cancers.

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Footnote

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