The Role of Circulating Tumor DNA in Evaluating Minimal Residual Disease in Luminal Gastrointestinal Malignancies

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Abstract: The analysis of circulating tumor DNA (ctDNA) has multiple uses in oncology. In the past few years, studies with varying designs, methods, and quality have emerged that show promise for the use of ctDNA as a tool to detect minimal residual disease (MRD) across luminal gastrointestinal malignancies. This review of the current literature looks at ctDNA in relation to detecting MRD, predicting patient prognosis, and assessing risk for recurrence.

Keywords
Circulating tumor DNA, gastrointestinal cancer, MRD, prognosis, residual disease

Introduction

Gastrointestinal (GI) malignancies account for a significant number of new cancer diagnoses every year. In 2020, GI malignancies were diagnosed in approximately 280,000 individuals in the United States. These accounted for approximately 18% of all new cancer diagnoses, a percentage second only to that of genitourinary malignancies across both sexes.[1] Furthermore, GI malignancies accounted for 27.8% of cancer deaths in the United States in 2020, the largest proportion of deaths from cancer in any organ system. GI malignancy is a broad heading and encompasses subtypes with high mortality rates, such as pancreatic carcinoma, and subtypes with the potential for cure, such as early-stage colorectal carcinoma (CRC). Novel approaches to therapy and prognostic testing are often studied in CRC, given its high prevalence. Despite their lower prevalence, esophagogastric malignancies often present an opportunity for innovation, given their generally advanced stage at presentation and high mortality rates.[2-4]

For many of these GI tumors, even those with a potential for cure, such as early-stage CRC, recurrence rates are high despite well-established treatment guidelines. Current methods of prognostication rely largely on tumor staging, particularly according to the American Joint Committee on Cancer (AJCC) TNM (tumor, node, metastasis) staging system, and the use of histopathologic characteristics to stratify patients' risk for recurrence.[5-7] Unfortunately, despite

the widespread use of these methods, real-world results reveal recurrence rates upwards of 50% for certain stages of these malignancies. Tests that can be trusted to identify reliably patients whose disease is likely to recur after curative treatment are lacking.[8-10]

Opportunities for lowering recurrence rates in GI malignancies are topics of ongoing study.[11-15] One such opportunity for innovation has been the concept of minimal residual disease (MRD) in GI malignancies, especially its use as a means to predict recurrence and tailor treatment when its presence is suspected despite standard-of-care treatment.[16,17] Since the advent of widely available blood-based circulating tumor DNA (ctDNA) assays, significant effort has gone into evaluating their use for detecting MRD across a multitude of malignancies, with varying degrees of success.[16,18-21] Success in this arena has perhaps been greatest with GI malignancies, and this review aims to outline the current state of ctDNA assays in detecting MRD, aiding prognosis, and informing treatment decisions across luminal GI malignancies: esophageal, gastric, and colorectal carcinomas.

Search Strategy

Pertinent articles were identified by searching the PubMed and Google Scholar databases through July 15, 2021. The following search terms were used in permutation with one another: ("circulating tumor DNA," "ctDNA," "cell-free DNA," "cfDNA") AND ("gastrointestinal cancer," "GI cancer," "gastrointestinal malignancy," "GI malignancy," "esophageal carcinoma," "esophageal cancer," "esophageal adenocarcinoma," "esophageal squamous cell carcinoma," "esophagogastric carcinoma," "esophagogastric cancer," "gastric cancer," "gastric carcinoma," "gastric adenocarcinoma," "colon cancer," "colon carcinoma," "colon adenocarcinoma," "rectal carcinoma," "rectal adenocarcinoma," "rectal cancer," "colorectal cancer," "colorectal carcinoma," "colorectal adenocarcinoma" AND ("minimal residual disease," "molecular residual disease," "MRD," "postoperative," "post-operative," "postsurgical," "post-surgical," "prognosis," "prognostic," "monitoring"). Included articles were those pertaining to esophageal adenocarcinoma or squamous cell carcinoma, gastric carcinoma, and colon/rectal/colorectal carcinoma. This review focuses on luminal GI carcinomas, so pancreaticobiliary carcinomas, hepatocellular carcinoma, and neuroendocrine tumors of the GI tract are outside its scope. Of note, any studies with curative intent of treatment, even those of oligometastatic disease including metastectomy, were included, although the majority pertained to nonmetastatic disease amenable to surgical resection. Articles specific to metastatic disease alone with only palliative intent of treatment were not included. For the purposes of this review, both ctDNA and cell-free DNA were reviewed and evaluated similarly. Articles were reviewed by the first author (BU) and principal investigator (TBS) for appropriate fit with scope. Excluded articles were review articles, articles without application to the clinical setting (eg, genomic sequencing without survival outcomes), and articles regarding circulating tumor cells only. The final number of articles for initial review was 24. An abstract with emerging data was added during the review period.

Colorectal Carcinoma

ctDNA has been studied better in CRC than in any other GI malignancy, which is unsurprising, given the high prevalence of CRC worldwide. Multiple studies have correlated preoperative ctDNA with prognosis, and many of these have shown ctDNA detection outperforming conventional prognostic tools, such as AJCC staging or measurement of carcinoembryonic antigen (CEA) blood levels.[22,23] As ctDNA assays became commercially available and the idea of serial monitoring, such as with CEA, came about, the idea of using assays to evaluate patients' recurrence risk and prognosis in the post-treatment and surveillance setting began to arise.[24,25] Below, we review pertinent articles regarding the role of ctDNA in detecting MRD and correlating detection with prognosis (Table 1).

Tie and colleagues prospectively evaluated postoperative ctDNA via the safe-sequencing system (Safe-SeqS, Illumina) in 230 patients with stage II CRC.[19] ctDNA positivity after resection was associated with significantly shorter recurrence-free survival (RFS) in comparison with ctDNA negativity after surgery, at 7.9 vs 40 months, respectively (P<.0001). Of 14 patients with postoperative ctDNA positivity, 11 had recurrence at the 3-year mark, in comparison with only 10% of those without detectable ctDNA. The investigators reported a sensitivity of 48% and a specificity of 100% for ctDNA positivity in predicting recurrence by the 3-year mark for all patients in the study. Among 52 patients treated with adjuvant chemotherapy (ACT), ctDNA positivity immediately after ACT was associated with shorter RFS (1.8 vs 68 months; P=.001). In addition, ctDNA outperformed CEA in regard to both positivity at the time of radiologic recurrence (85% vs 41%; P=.002) and time between detection and radiologic recurrence (median, 167 vs 61 days; P=.04). These results suggest that ctDNA testing may be useful both after surgery and after ACT for its ability to stratify patients according to risk for recurrence months earlier than conventional methods. On the other hand, given the poor sensitivity and thus negative predictive value (NPV) of ctDNA detection, a negative test result should not be considered a reason to alter or withhold treatment that is otherwise indicated.

Table 1. Summary of Studies of ctDNA MRD in Colorectal Carcinoma

Author (Y)	Study Design (N)	Summary of Findings
Tie (2016)[19]	Prospective study evaluating postop detection of ctDNA for predicting recurrence in patients with stage II CRC undergoing definitive resection +/- ACT (230)	- Detectable ctDNA was associated with shorter RFS after resection (7.9 vs 40 mo; *P*<.0001) and after ACT (1.8 vs 68 mo; *P*=.001). - ctDNA was detectable 167 d before radiologic recurrence vs 61 d for CEA (*P*=.04).
Chen (2021)[26]	Prospective study evaluating detection of postop and post-ACT ctDNA to predict recurrence in patients with stage II/III disease (240)	- At all time points (postop, before ACT, after ACT), detectable ctDNA was associated with higher risk for recurrence. - HR for recurrence after ACT was 12.76 in patients who did not achieve ctDNA clearance vs those who did. - ctDNA was most significant risk factor for recurrence in multivariate modeling. - Mean lead time for ctDNA detection vs imaging detection of recurrence was 5.01 mo.
Tarazona (2019)[27]	Prospective study of use of tumor-matched ctDNA testing in the immediate postop setting to predict recurrence in "localized" CRC (94)	- Presence of immediate postop ctDNA was associated with poorer DFS (HR, 6.96; *P*=.0001). - ctDNA was strongest negative predictor of DFS (HR, 11.6; *P*<.0001) in multivariate analysis with CEA, TNM stage. - Detectable ctDNA after ACT had HR for recurrence of 10.02 (*P*<.0001).
Reinert (2019)[28]	Prospective study of patients with stage I-III CRC undergoing curative treatment, monitoring ctDNA 30 d after resection, then after ACT, and serially thereafter (125)	- At postop d 30, detectable ctDNA was associated with heightened risk for recurrence (HR, 7.2; *P*<.001). - After ACT, detectable ctDNA had an even greater association with recurrence (HR, 17.5; *P*<.001). - Development of detectable ctDNA during surveillance had a lead time of 8.7 mo vs imaging for detecting recurrence.
Suzuki (2020)[29]	Prospective study of patients with mixed CRC and GC (nonmetastatic), assessing use of postop ctDNA for detecting recurrence (77, CRC and GC)	- Patients with detectable postop ctDNA had shorter RFS (HR, 14.9; *P*<.0001). - All patients with recurrence had detectable ctDNA mutation(s). - Minimum lead time for ctDNA vs imaging and/or serologic recurrence was 2 mo.
Parikh (2021)[30]	Prospective study using commercially available ctDNA testing and a tumor mutation–agnostic approach, including patients with stage IV disease treated with curative intent; ctDNA checked 1 mo after completion of therapy with resection +/- ACT (70)	- All 15 patients with detectable ctDNA after definitive therapy had recurrence (HR, 11.28; *P*<.0001). - Sensitivity and specificity for recurrence across all patients were 55.6% and 100%, respectively. - CEA did not predict recurrence in this population, whereas ctDNA did. - This is perhaps the most accessible methodology, given the tumor-agnostic approach and use of a commercially available ctDNA assay.
Diehl (2008)[31]	Prospective study using BEAMing ctDNA technology in the postop setting to predict recurrence (20)	- Of 16 patients with detectable ctDNA, 15 had recurrence, whereas none of the 4 patients without ctDNA had recurrence at first follow-up surveillance (*P*=.006). - ctDNA was more likely than elevated/rising CEA to be present at time of recurrence (*P*=.03). - In vitro study estimated ctDNA half-life to be 2 h, suggesting detection indicates MRD.
Schøler (2017)[32]	Prospective study with serial monitoring of ctDNA, CEA, and imaging after definitive treatment, comparing the 3 for detecting recurrence in 3-y follow-up period (45)	- ctDNA was detected in all 14 patients who had recurrence during follow-up period. - ctDNA detection within 3 mo of resection was associated with increased risk for recurrence (HR, 37.7; *P*<.001). - Detectable ctDNA never developed in any of the patients who remained disease-free through follow-up. - Average lead time for ctDNA detection of recurrence vs CT was 9.4 mo.

Table 1. *(Continued)* Summary of Studies of ctDNA MRD in Colorectal Carcinoma

Author (Y)	Study Design (N)	Summary of Findings
Sun (2018)[33]	Prospective study of patients with surgically treated CRC monitored for recurrence specifically with *TP53* ctDNA testing (11, including 1 patient with stage IV disease)	- This was largely a proof-of-concept study without statistical analysis, a significant limitation. - Of note, progressive increases in quantitative *TP53* mutation rate on ctDNA testing were observed in one patient despite normal CEA measurements, and eventual recurrence and death of the patient suggest a possible quantitative approach to ctDNA mutations.
Benešová (2019)[34]	Prospective study of patients with oligometastatic CRC undergoing resection with curative intent, correlating postop ctDNA levels with histopathologic success of resection, imaging findings, and serologic testing results to predict recurrence (50)	- Study used a custom ctDNA panel of mutations/genes (*KRAS, TP53, APC, PIK3CA, BRAF, CTNNB1*). - Detectable ctDNA correlated with histopathologic grade as follows: o R0: 2/28 patients with postop ctDNA o R1: 4/7 patients with postop ctDNA o R2: 15/15 patients with postop ctDNA - All 22 patients with R0 resection and recurrence had detectable ctDNA, making it the most sensitive tool for detecting recurrence in this study.
Kidess (2015)[35]	Observational study including patients with oligometastatic CRC undergoing hepatectomy for cure (38)	- Study lacked statistical analysis, a significant limitation. - Patients with increasing quantitative ctDNA after hepatectomy had disease recurrence, and ctDNA increase preceded rising CEA levels.
Boysen (2020)[36]	Prospective study evaluating use of ctDNA to monitor for recurrence in patients with oligometastatic disease undergoing curative treatment of metastases (35 total, 5 with detectable ctDNA)	- Median time to recurrence was 273 d in patients with detectable ctDNA after treatment. - Median time to recurrence was not reached in patients with undetectable ctDNA after treatment. - *P*=.03 for difference between rates of recurrence in the 2 groups. - Study was limited by small sample size, particularly the group with detectable ctDNA.
Tie (2021)[37]	Prospective study of use of ctDNA after treatment to detect recurrence in patients undergoing hepatectomy for oligometastatic CRC (54)	- Treatment with NAC, ACT, or both was left to discretion of treating physician, introducing some heterogeneity in treatment as a limitation. - Detection of postop ctDNA was associated with shorter RFS (HR, 6.3; *P*<.001) and OS (HR, 4.2; *P*<.001).
Kotaka (2022)[38]	Association of ctDNA dynamics with clinical outcomes in the adjuvant setting among patients with CRC in an observational GALAXY study in CIRCULATE-Japan (1365)	- Patients were assessed for ctDNA at 4 and 12 wk after definitive resection and stratified into positive-positive, positive-negative, negative-positive, and negative-negative groups. - DFS was significantly shorter in positive-positive group than in positive-negative group (HR, 52.3; *P*<.001). - In a subgroup of patients with ctDNA positivity at 4 wk (n=188), ctDNA clearance rate was significantly higher in those who received ACT than in those who did not (57% vs 8%; *P*<.001).

ACT, adjuvant chemotherapy; BEAMing, beads, emulsions, amplification, and magnetics; CEA, carcinoembryonic antigen; CRC, colorectal carcinoma; CT, computed tomography; ctDNA, circulating tumor DNA; d, day(s); DFS, disease-free survival; GC, gastric carcinoma; h, hour(s); HR, hazard ratio; mo, month(s); MRD, minimal residual disease; NAC, neoadjuvant chemotherapy; OS, overall survival; RFS, recurrence-free survival; TNM, American Joint Committee on Cancer tumor, node, metastasis staging system; wk, weeks.

In a prospective randomized trial of 240 patients by Chen and colleagues, a commercial targeted-sequencing ctDNA panel (GeneseeqPrime, Geneseeq) was used to detect ctDNA in patients with stage II or III CRC who underwent primary resection and ACT.[26] ctDNA testing was performed postoperatively before ACT and then again after ACT. At all times, the presence of ctDNA was significantly associated with a risk for recurrence, including a hazard ratio (HR) of 12.76 for failure to clear ctDNA after ACT. ctDNA positivity was the most significant risk factor for recurrence in a multivariate model of patient-specific risk factors and serologic testing. This study reported a mean lead time of 5.01 months for the detection of recurrence by ctDNA testing in comparison with detection by conventional imaging. As indicated by the study of Tie and colleagues, ctDNA detection may be useful not only postoperatively but also after ACT for assessing the potential for MRD.

Tarazona and colleagues used a tumor-matched approach to follow ctDNA in the serum after resection (n=94).[27] Of note, ACT was left to the discretion of the treating provider, who was blinded to the ctDNA results. The presence of ctDNA in the serum immediately after surgery was associated with poorer disease-free survival (DFS; HR, 6.96; *P*=.0001). On multivariate analysis, ctDNA positivity remained the strongest negative predictor of DFS (HR, 11.6; *P*<.0001). Finally, ctDNA positivity after completion of ACT had a HR of 10.02 (*P*<.0001) for DFS/recurrence, further strengthening the evidence of the 2 preceding studies regarding the detection of MRD after surgical treatment and ACT.

Reinert and colleagues prospectively evaluated 125 patients with stages I through III CRC undergoing curative treatment, integrating tumor-derived mutations into a panel of 16 specific mutations via ctDNA.[28] ctDNA mutation was present in 14 of 16 patients with relapse. At postoperative day 30, the ctDNA-positive patients were significantly more likely to have had recurrence than those who were not ctDNA-positive (HR, 7.2; *P*<.001). Recurrence was even more likely in the patients who were ctDNA-positive after ACT than in those who were not ctDNA-positive after ACT, with an HR of 17.5 (*P*<.001); all patients who were ctDNA-positive after ACT had recurrence. Finally, during surveillance after ACT, the detection of ctDNA demonstrated an HR of 43.5 (*P*<.001) for recurrence. Serial ctDNA analysis predicted recurrence with a mean lead time of 8.7 months in comparison with standard-of-care radiologic surveillance. Further study will elucidate whether this finding represents lead-time bias or is a reason for actionable alterations in a patient's care on the basis of positive ctDNA testing. It is also important to note that the timing of the ctDNA draw influences its sensitivity and specificity; furthermore, given the poor sensitivity (43% for postoperative sampling), a negative result should not otherwise alter treatment and/or further surveillance.

In a combined study of patients with gastric cancer and CRC, Suzuki and colleagues matched postoperative serum ctDNA via digital droplet polymerase chain reaction (ddPCR) with preoperative serum ctDNA mutations, whereas in most instances, serum mutations are matched with tumor tissue mutations.[29] This study filtered and excluded mutations of clonal hematopoiesis of indeterminate potential (CHIP), a key approach to eliminating mutations potentially without relevance to the GI malignancy. Although exact methods vary, commercially available assays generally perform CHIP filtering. Of the 77 evaluated patients (across gastric cancer and CRC), 6 had postoperative recurrence, and all had ctDNA mutations detected at a minimum of 2 months before imaging or serologic recurrence. Patients with ctDNA detected postoperatively had significantly shorter RFS (HR, 14.9; *P*<.0001).

Parikh and colleagues,, using a commercially available next-generation sequencing (NGS) multigene panel coupled with the Guardant Reveal methylation assay from Guardant Health, recently evaluated a tumor tissue–uninformed approach in a prospective series of 70 patients.[30] Patients were treated with standard-of-care therapy according to traditional staging and guidelines, and ctDNA was drawn 1 month after definitive therapy whether that was surgery alone or surgery plus ACT. Of note, patients with stage IV disease were included if surgery and ACT were administered with curative intent. All 15 patients with detectable ctDNA after definitive therapy and at least 1 year of follow-up had recurrence (HR, 11.28; *P*<.0001). Sensitivity and specificity for recurrence across all patients were 55.6% and 100%, respectively. This appears to be a rather novel approach and likely one of the most accessible, given the ease of sending serologic samples to commercial entities for such testing.

Diehl and colleagues presented a novel approach, combining real-time PCR with a process called beads, emulsions, amplification, and magnetics (BEAMing), which obtains a relative amount of mutant-to-somatic DNA alterations from serum testing with tumor matching.[31] Interestingly, they used this technique to examine preoperative and postoperative ctDNA levels and estimated the half-life of ctDNA to be approximately 2 hours in this setting, which suggests that ctDNA should rapidly dissolve if MRD has been fully eradicated. Follow-up ctDNA testing was carried out thereafter at a range of 13 to 56 days across a cohort of 20 patients. Of the 16 patients with detectable ctDNA, 15 of had recurrence, whereas none of the 4 subjects with undetectable ctDNA had recurrence at first follow-up (*P*=.006). The authors also reported a significantly better ability to predict recurrence with ctDNA than with CEA when patients were checked 24 to 48 days after surgical treatment (*P*=.03).

Schøler and colleagues provided further evidence of the earlier detection of recurrence with ctDNA than with imaging and/or CEA after curative therapy.[32] In this longitudinal cohort study, 45 patients underwent serial massively parallel sequencing of serum ctDNA (with ddPCR) matched to tumor tissue mutations through a 3-year follow-up period. ctDNA was detected during the follow-up period in all 14 of the patients who had relapse, with an average lead time of 9.4 months in comparison with computed tomography (CT). All patients who remained disease-free through the follow-up period never had detectable ctDNA. Furthermore, detection of ctDNA within 3 months of surgical resection for localized disease was associated with significantly increased risk for recurrence (HR 37.7; *P*<.001).

Sun and colleagues enrolled a cohort of 11 patients in a broad study of ctDNA in surgically treated CRC, including 10 patients with stages I through III CRC and 1 patient with stage IV disease.[33] Although this study focused more on proof-of-concept, it was notable for one patient with post-treatment disease progression who had a progressively increasing *TP53* mutation rate on ctDNA testing despite multiple normal CEA measurements in the setting of progressive disease and eventual death. This finding further suggests that ctDNA-based evidence often precedes CEA-based evidence of progression, and specific driver mutations and their quantification may warrant further investigation.

With regard to potentially curable oligometastatic disease, Benešová and colleagues evaluated ctDNA monitoring in patients with oligometastatic CRC who underwent resection of metastatic disease with curative intent, using a custom panel detecting *KRAS*, *TP53*, *APC*, *PIK3CA*, *BRAF*, and *CTNNB1* mutations.[34] Postoperative ctDNA detectability correlated with the histopathologic success of resection; only 2 of 28 patients with an R0 resection, 4 of 7 with an R1 resection, and 15 of 15 with an R2 resection had detectable ctDNA. In the 22 patients with an R0 (curative) resection and recurrence, 4 recurrences were detected by the presence of ctDNA alone, while the imaging findings and serologic testing results were negative despite eventual recurrence. ctDNA mutations were detected in all 22 instances of recurrence in patients with an R0 resection, making it the most sensitive tool for detecting recurrence in this study. Interestingly, the Schøler study summarized previously mentioned a correlation between lower ctDNA levels and treatment for hepatic metastatic disease vs no treatment, and another, older study by Kidess and colleagues mentioned a similar finding of a correlation between post-hepatectomy increases in ctDNA and disease recurrence, with ctDNA increases appearing earlier than CEA increases.[35]

Boysen and colleagues presented a small study of definitive treatment for liver and/or lung oligometastatic disease in which detectable ctDNA after treatment for oligometastatic disease was associated with shorter time to recurrence.[36] Finally, Tie and colleagues addressed a similar topic, performing a prospective study of patients undergoing hepatectomy for oligometastatic disease, some of whom received neoadjuvant chemotherapy (NAC), ACT, or both.[37] As in other studies, patients with detectable postoperative ctDNA had significantly shorter RFS (HR, 6.3; $P<.001$) and overall survival (OS) (HR, 4.2; $P<.001$) in comparison with those with undetectable ctDNA. The patients who received ACT underwent serial ctDNA monitoring, and all 8 patients with persistent ctDNA detection had progression, whereas two-thirds of the patients without detectable ctDNA after treatment remained disease-free through follow-up. These studies indicate that ctDNA appears to have a role in detecting MRD in patients with oligometastatic disease undergoing treatment with curative intent, as it does in patients with locoregional disease.

Most recently, Kotaka and colleagues presented results from the GALAXY study.[38] A commercially available, personalized, tumor-informed ctDNA assay was used to divide patients (n=1365) into 4 groups according to ctDNA detection at 4 and 12 weeks after definitive resection (positive-positive, positive-negative, negative-positive, negative-negative). The 6-month DFS rate was significantly lower in the positive-positive group than in the positive-negative group (HR, 52.3; $P<.001$). Of 188 patients with ctDNA positivity at 4 weeks, 95 received ACT and the remainder did not; the rate of ctDNA clearance (positive-to-negative ctDNA) was significantly higher with ACT than without ACT (57% vs 8%; $P<.001$). When ctDNA monitoring to as far as 24 weeks postoperatively was included, the ctDNA clearance rate was significantly higher in those receiving ACT (67% vs 7%; HR, 17.1; $P<.001$). This study provides many novel insights, in particular early evidence that ctDNA may serve as a minimally invasive surrogate for the efficacy of ACT. CIRCULATE-Japan and CIRCULATE-US are related studies that plan to alter adjuvant treatment according to a similar postoperative ctDNA model.

In summary, ctDNA has repeatedly been shown to be detectable on blood-based assays months before imaging and/or CEA evidence of recurrence, and this finding suggests that ctDNA testing in the postoperative setting, during ACT, and/or during surveillance may be a valuable tool for detecting recurrence before imaging and/or CEA testing can do so. Particularly in CRC compared with the other malignancies discussed in this review, there is strong support that ctDNA detection after ACT indicates ongoing disease progression and an incomplete response to treatment despite negative imaging. Furthermore, ctDNA detection has a strong positive predictive value (PPV) across multiple studies for predicting recurrence after definitive therapy, although the absence of ctDNA cannot be relied on to rule out recurrence, given the poor NPV reported above. It is worth considering the different modalities previously discussed—broad NGS detection of mutations, quantitative cutoff of variant allele frequency (VAF) or mutation allele frequency (MAF), tumor-informed vs tumor-uninformed approaches, epigenetic methylation, and quantification of specific driver mutations—and how these may work both in isolation and potentially in integrated platforms of ctDNA. Although a tumor-uninformed approach does not require tumor genotyping, the studies discussed previously show promise with regard to matching serum mutations with tumor

mutations for post-treatment prognosis, and an evaluation of these approaches in head-to-head fashion warrants discussion. Outcomes data, especially for recurrence, are becoming more robust with the growing pool of studies; however, prospective data on the use of ctDNA to select patients who may benefit from more intense therapy (either ACT if the plan was to treat with surgery alone, or more intense/longer ACT) are scant, and further study in this arena is warranted.

Esophageal Carcinoma

Esophageal carcinoma—both adenocarcinoma (EAC) and squamous cell carcinoma (ESCC)—is also relatively well studied regarding ctDNA and MRD. The prognosis for stages I through III ESCC, like that of many of the other malignancies discussed here, depends on presurgical factors, particularly AJCC TNM stage, which also typically guides the indication for NAC and/or ACT. In both ESCC and EAC, 5-year survival rates range from approximately 50% for localized disease to 25% or lower for locally invasive disease and/or regional lymph node involvement.[3,39] Despite protocolized treatment based on stage, recurrence rates remain quite high, with reports of up to 50% of patients with EAC having recurrence after resection and similar rates for patients with ESCC.[11,40] ctDNA has thus gained traction in predicting recurrence for both subtypes of esophageal malignancy, and pertinent studies are reviewed here (Table 2).

Azad and colleagues used CAPP-Seq (cancer personalized profiling by deep sequencing) ctDNA detection to study 45 patients undergoing definitive chemoradiation therapy (CRT), esophagectomy, or a combination of neoadjuvant CRT followed by resection. The cases included a mix of EAC and ESCC.[41,42] The authors first examined a subgroup of 31 patients who had localized disease treated with CRT alone (no resection). Detectable ctDNA after CRT was associated with significantly increased risk for recurrence/progression (HR, 18.7; *P*<.0001) and disease-specific death (HR, 23.1; *P*<.0001). Next, they examined the emergence of new ctDNA mutations after neoadjuvant CRT and before resection. The patients who ultimately had recurrence were significantly more likely to have acquired novel, detectable ctDNA mutations after neoadjuvant CRT (*P*=.027). The pre- and postoperative ctDNA samples of each patient undergoing resection were analyzed to match the postoperative ctDNA mutations with the patient's tumor tissue mutations analyzed at resection. When a tumor-matched approach was used, ctDNA detection after resection had mean lead time of 114.9 days vs standard-of-care positron emission tomography (PET)/CT in patients with recurrence (*P*=.0026). Thus, for patients undergoing definitive CRT or primary tumor resection with or without CRT, ctDNA detection was able to predict recurrence on average nearly 4 months before conventional imaging.

Ococks and colleagues followed 97 patients who had resectable EAC treated with NAC and surgical resection. After treatment, they performed serial ctDNA testing with Roche AVENIO NGS assays.[43] This was a tumor mutation–agnostic approach with CHIP (clonal hematopoiesis of indeterminate potential) filtering. Sufficient ctDNA and follow-up data were available for 63 patients. The investigators found that the ctDNA-positive patients had significantly shorter DFS (8.7 vs 26.7 months; *P*=.001), and DFS remained shorter in multivariate analysis (*P*<.001). Overall, this was a well-designed study that supports the role of tumor-uninformed, CHIP-filtered ctDNA detection for predicting recurrence of surgically treated EAC.

Liu and colleagues evaluated the ctDNA of 23 patients undergoing resection of stages I through III ESCC via capture-based NGS assay with a tumor-matched approach, specifically using an MAF of more than 5% to define ctDNA positivity.[44] DFS was significantly shorter in the patients with postoperative ctDNA detection than in those without (HR, 27.5; *P*=.005), and OS was also shorter (HR, 27.6; *P*=.004). Both results remained significant in multivariate analysis. This study supports the use of tumor-matched ctDNA positivity with defined MAF/VAF levels as a means for assessing MRD, and thus DFS and OS.

In another study limited to ESCC, Hsieh and colleagues examined 81 patients undergoing esophagectomy for stages I through III disease.[45] They matched patient cell-free DNA (cfDNA) mutational burden with the cfDNA of healthy controls without specific mention of the subtype of cfDNA. On the basis of the median cfDNA copy number in the cohort, they divided the patients into a "low" cfDNA group (n=41) and a "high" cfDNA group (n=40). In the 120-month follow-up period, DFS was significantly longer in the patients in the "low" group than in the "high" group (49.9% vs 21.2%; *P*=.013), but the difference between OS in the 2 groups was not statistically significant (*P*=.164). This study brings up an interesting idea of risk-stratifying patients according to a quantitative analysis of cfDNA; however, the results are mixed, and it is unclear at exactly which time point (pre- or postoperatively) the cfDNA was analyzed. Also, the standardization of what constitutes "low" and "high" groups would require further study and might be a moving target depending on the cohort.

In another mutation-specific study, Hoffmann and colleagues examined a mixed population of patients with ESCC (n=24) and EAC (n=35), specifically looking at *DAPK* and *APC* promoter methylation via ctDNA as a

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Table 2. Summary of Studies of ctDNA MRD in Esophageal Cancer

Author (Y)	Study Design (N)	Summary of Findings
Azad (2020)[41]	Prospective study testing ctDNA for association with recurrence, comparing against standard PET/CT surveillance; mixed EAC and ESCC (45)	- In patients who had localized disease treated with CRT alone, ctDNA detection after CRT was associated with higher risks for recurrence (HR, 18.7; *P*<.0001) and disease-specific death (HR,23.1; *P*<.0001). - Patients who had recurrence after neoadjuvant CRT and resection were more likely to have acquired ctDNA mutations between neoadjuvant CRT and resection (*P*=.027). - ctDNA predicted recurrence with a mean lead time of 114.9 d vs standard PET/CT surveillance (*P*=.0026).
Ococks (2021)[43]	Prospective study of patients undergoing NAC and resection, followed by serial ctDNA monitoring postoperatively; EAC only (63)	- ctDNA positivity at any point was associated with shorter DFS (8.7 vs 26.7 mo; *P*=.001); findings were consistent in multivariate analysis (*P*<.001). - A semilinear increase in ctDNA level was noted qualitatively as recurrence approached; however, finding was not analyzed statistically. - This study specifically used a tumor-agnostic approach.
Liu (2021)[44]	Prospective study of patients undergoing resection +/- adjuvant CRT, checking postop ctDNA to assess for recurrence; ESCC only (23)	- Detectable postop ctDNA was associated with shorter DFS (HR, 27.5; *P*=.005) and shorter OS (HR, 27.6; *P*=.004). - Differences in both DFS and OS remained significant in multivariate analysis. - This study used a relatively novel method for defining ctDNA mutations, specifically filtering for an MAF >5% and excluding mutations seen concurrently on CHIP sequencing.
Hsieh (2016)[45]	Prospective study divided patients into "low" and "high" cfDNA copy number groups on the basis of a comparison with controls, then correlated the groups with risk for recurrence after esophagectomy; ESCC only (81)	- In the 120-mo follow-up period, DFS was significantly longer in patients in the "low" cfDNA copy number group than in patients in the "high" cfDNA copy number group (49.9% vs 21.2%; *P*=.013), but the difference in OS did not reach statistical significance (*P*=.164). - Limitations include a semi-arbitrary cutoff for the 2 groups, as well as lack of clarity on whether cfDNA samples were drawn before or after surgery. - Study provides a novel, quantitative stratification based on cfDNA.
Hoffmann (2009)[46]	Prospective study aimed at detecting residual tumor after definitive treatment by detecting specific *DAPK* and/or *APC* promoter methylation in ctDNA; mixed EAC and ESCC (59)	- "Short" OS and "long" OS were defined arbitrarily as less than and greater than 2.5 y, respectively. - Preop detection of both *DAPK* and *APC* ctDNA methylation predicted "short" survivors with a sensitivity of 99.9% and a specificity of 57.1%. - Postop detection of *APC* methylation was associated with the presence of residual tumor on histopathologic exam of specimen (*P*=.03).

CHIP, clonal hematopoiesis of indeterminate potential; CRT, concurrent chemoradiation therapy; ctDNA, circulating tumor DNA; cfDNA, cell-free DNA; DFS, disease-free survival; EAC, esophageal adenocarcinoma; ESCC, esophageal squamous cell carcinoma; HR, hazard ratio; MAF, mutant allele frequency; mo, month(s); MRD, minimal residual disease; NAC, neoadjuvant chemotherapy; OS, overall survival; PET/CT, positron emission tomography/computed tomography; TNM, tumor, node, metastasis; y, year(s)

means for detecting residual tumor on histopathologic evaluation of surgical specimens.[46] Preoperative and postoperative serum samples were analyzed for these methylation changes. Of the 59 patients treated surgically, 22 had undergone neoadjuvant CRT. The investigators defined "short" vs "long" OS in this study with an arbitrary cutoff time of 2.5 years and found that the preoperative detection of both *APC* and *DAPK* methylation changes predicted short survival with a sensitivity of 99.9% and a specificity of 57.1%. Perhaps more importantly, the postoperative detection of *APC* promoter methylation was associated with the presence of residual tumor on histopathologic examination of the surgical specimen (*P*=.03), a finding that would be direct evidence of MRD, albeit more in a gross sense than at the molecular level to which MRD typically refers.

Overall, ctDNA appears to have a role in detecting MRD and predicting prognosis in both EAC and ESCC, although the body of literature is not as deep and robust as that for CRC. Specifically, ctDNA detection appears to precede the imaging-based diagnosis of recurrence by months, and multiple studies have mentioned potential quantitative and/or specific mutation ctDNA markers that could be studied further to develop ctDNA assays specific to esophageal cancer. As with CRC, there seems to be a role for ctDNA in detecting MRD; however, the data for how these tests can help improve patient outcomes remain scant.

Gastric Carcinoma

Gastric carcinoma (GC) is a relatively common GI malignancy associated with some unique risk factors, such as *Helicobacter pylori* infection. Although GC is often treatable by resection with or without chemotherapy and radiation, recurrence rates are highly variable across multiple large case series, ranging anywhere from 2% in early-stage disease to upwards of 50% in more advanced, node-positive disease.[47,48] With GC the third most common cause of GI cancer–related deaths in the United States in 2021, ctDNA provides an opportunity to decrease recurrence rates and thereby improve survival outcomes. Reviewed below are pertinent studies of ctDNA detection for MRD monitoring in gastric carcinoma (see eTable at www.hematologyandoncology.net).

Yang and colleagues enrolled 46 patients with stages I through III GC who underwent curative resection. The patients were followed with serial ctDNA sampling as well as gastroscopy, measurement of CEA and cancer antigen (CA) 19-9, chest radiography, and abdominal CT.[49] A custom approach in which NGS tumor tissue was matched with ctDNA was used that included CHIP filtering. The use of ACT was left to the discretion of the treating provider; however, the postoperative ctDNA assays used to assess for MRD were drawn before any ACT. ctDNA positivity in this postoperative setting was significantly associated with relapse (100% vs 32%; *P*=.0015) and poorer DFS (HR, 6.56; *P*<.0001), and this remained the case in multivariate analysis with T stage and tumor site (*P*=.005). The sensitivity and specificity of immediate postoperative ctDNA positivity for predicting recurrence were 39% and 100%, respectively. Among the patients who received ACT (n=23), ctDNA positivity was similarly associated with poorer DFS (*P*=.0002) and OS (*P*<.0001). Furthermore, across a median follow-up period of 29.1 months after ACT, the detection of ctDNA at any point was associated with poorer DFS (*P*<.0001) and OS (*P*=.0002). ctDNA detection had a lead time of 179 days in comparison with radiographic recurrence, and interestingly, all the patients with detectable ctDNA but equivocal imaging findings regarding recurrence eventually had recurrence, suggesting a role for ctDNA in interpreting equivocal results of surveillance imaging. This study exemplifies the utility of ctDNA detection in monitoring for MRD in patients with GC treated with curative resection and highlights its multiple roles in this setting, providing a lead time vs imaging and supplementing equivocal findings on surveillance imaging.

Retrospectively examining ctDNA in a subgroup of patients (n=50) from the CRITICS trial (a phase III trial of perioperative treatment for operable GC), Leal and colleagues reported an association between ctDNA detection and recurrence after resection.[50] All patients received 3 cycles of NAC by protocol. The investigators performed targeted sequencing of a prespecified 58-gene panel, and the presence of one of these mutations in cell-free DNA was deemed a positive ctDNA testing result. The preoperative detection of ctDNA was not associated with recurrence when unfiltered, but when CHIP filtering was applied, ctDNA positivity was a significant predictor of recurrence (HR, 3.0; *P*=.012) as well as shorter OS (HR, 2.7; *P*=.03). Similarly, in postoperative ctDNA testing, unmatched ctDNA positivity did not show an association with recurrence or poorer OS, but when filtered via CHIP, ctDNA positivity was associated with a significantly higher risk for recurrence (HR, 21.8; *P*<.001). Although the overall magnitude of the ctDNA effect in this study was small, the study nonetheless underscores the importance of CHIP filtering in ctDNA testing for GC, and likely for GI malignancy as a whole.

A study by Kim and colleagues found utility for ctDNA testing after GC resection, with some caveats, in a study of 25 patients.[51] After surgical resection, tumor tissue was subjected to whole-genome sequencing (WGS); patient-specific tumor mutations were then applied to ongoing ctDNA monitoring, and the mutations were also filtered against healthy controls. Quantitative PCR (qPCR) was used for testing ctDNA in serum, which was drawn the day before surgery and then postoperatively at 1, 3, 6, 9, and 12 months. The authors reported a median lead time of ctDNA positivity vs imaging recurrence of 4.05 months. Interestingly, although any postoperative ctDNA positivity was associated with recurrence within the 12-month postoperative period (*P*=.0294), this was not the case for preoperative ctDNA positivity (*P*=.6372). The investigators also reported that a large proportion of patients (n=8) never had detectable ctDNA by their methods, and that fluctuations in ctDNA positivity were noted within the same patient(s) postoperatively, findings that must be considered potential confounders and limitations for all ctDNA assays.

Ko and colleagues examined specific ctDNA parameters, which were concentrations of long-fragment *LINE1* and methylation proportion of *LINE1* ctDNA,[52] in 49 patients with GC who underwent surgical resection. High

postoperative concentrations (with use of a prespecified cutoff) of long-fragment *LINE1* were associated with significantly shorter RFS (*P*=.009) and OS (*P*=.04), and the differences in both remained significant in multivariate analysis. Postoperative ctDNA methylation status was not associated with recurrence or OS. This study provides yet another specific ctDNA target (in this case, methylation proportion of *LINE1*) that may hold promise in the postoperative setting for predicting recurrence in GC.

Shoda and colleagues provided a small (n=60) but interesting study of *HER2* levels in ctDNA and how this measurement can be related to recurrence after GC resection.[53] They quantified the copy number of *HER2*, a known driver mutation in GC, in peripheral blood samples and expressed this and the copy number of a control gene, *RPPH1*, as a ratio. After resection, 17 patients had recurrence; 13 of these had *HER2*-negative tumors, but increasing *HER2*-to-*RPPH1* ratios were noted in 7 of them. All 4 patients with sufficient follow-up had *HER2*-to-*RPPH1* ratios above the prespecified cutoff at the time of recurrence, and all 4 had steadily increasing *HER2*-to-*RPPH1* ratios as their disease eventually progressed to metastasize widely and cause death. Although limited by the sample size, this study suggests that ctDNA monitoring of *HER2* can predict recurrence, and it raises an interesting question of whether targeted anti-HER2 therapy might have utility in the setting of recurrence with *HER2* positivity on ctDNA despite previously negative results of tumor testing for *HER2* mutation.

Ling and colleagues provided a specific study of *XAF1* methylation in patients with GC.[54] A cohort of surgically treated patients (n=202) underwent tumor-matched reverse transcriptase PCR (RT-PCR) to look for *XAF1* hypermethylation in ctDNA. None of the healthy controls (n=88) had detectable *XAF1* hypermethylation. Of 72 patients with sufficient follow-up, 12 had recurrence, and 10 of the 12 had a negative-to-positive *XAF1* methylation status on serial ctDNA testing. The findings suggest that monitoring of this epigenetic marker might be a noninvasive means of assessing for recurrence. Of note, specific methods of detecting recurrence and comparisons with current methods, such as CEA CA 19-9 measurement, were not specified in this largely proof-of-concept study.

In GC, ctDNA holds promise for detecting MRD and predicting recurrence; however, except for a couple of studies, the data come from very small sample sizes, so that justification for the routine clinical use of ctDNA testing is a challenge at present. In comparison with the other malignancies in this review, the body of literature for GC has a particular focus on individual driver mutations, such as *HER2* and *TP53*, which may be relevant to the targeted treatment landscape, such as trastuzumab. One particularly interesting idea is the acquisition of new mutations after surgical resection, and the question arises of whether these mutations (eg, *HER2*) could be targeted at the time of recurrence despite their absence in tumor tissue at the time of resection. Further study of ctDNA testing for MRD is warranted for GC overall, as well as study of possible targeted salvage therapies based on ctDNA data for acquired mutations.

Limitations

A major consideration in interpreting these studies is the definition of "ctDNA" itself. The heterogeneity among assays leaves ctDNA-based testing open to issues with standardization, which merit discussion. The previously mentioned studies used many different sub-methods to analyze ctDNA—the detection of specific mutations on custom NGS platforms, prespecified gene lists, ddPCR, epigenetic methylation, magnitude of VAF, and more. These can get lumped into a discussion of the presence or absence of ctDNA, each one likely with a different validation standard. Furthermore, several of the studies discuss quantitative cutoffs, some based on data and others appearing arbitrary, and this approach is different from defining the "presence or absence" of ctDNA, which is the approach of most studies. Some of the more frequently used assays are administered by commercial entities, and validation and standardization practices are not always publicly available. Because so many of these varying methods have shown preliminary promise in this setting, another interesting question that is likely to arise is, which method(s) is superior to the others in detecting MRD and predicting prognosis, and head-to-head trials of different assays will be warranted in the future.

Furthermore, most of the studies perform some sort of matching—to a sequencing of the patient's tumor or the patient's own hematopoietic cells (CHIP), or both—and this is a critical step to rule out germline mutations not relevant to malignant processes. Mutation matching or filtering may be key to the validity of the results, and consideration must be given to studies and scenarios in which neither of these mutation-matching efforts is performed.[50,55] Tumor tissue–informed approaches are used in many of the studies in this review and are generally accepted, whereas matching against a patient's own hematopoietic cells (CHIP) is a topic of ongoing study, although it shows significant promise.[43,50]

Perhaps the most important limitation is the paucity of information regarding how these early predictions of recurrence through ctDNA testing can be used to alter treatment. Studies are ongoing regarding offering ACT to patients with ctDNA changes suggestive of recurrence who might not otherwise undergo ACT along with current standard-of-care staging and treatment. More intense ACT regimens, radiotherapy, and/or immunotherapy

could be considered for patients already receiving ACT; however, this approach must be weighed against potential adverse effects, particularly in the absence of randomized data from clinical trials. Finally, the appearance of novel mutations on ctDNA that are not seen on a patient's tumor tissue might present an opportunity for targeted therapy in recurrent disease that was not present at the time of initial therapy; however, this idea is quite novel and comes mostly from the *HER2* study in GC previously noted. The use of ctDNA to guide treatment decisions is an active topic in ongoing studies.[56-58]

Multiple studies are underway to answer some of these outstanding questions, particularly in CRC. Perhaps most pertinent to the questions raised in this review, CIRCULATE-US is a prospective, randomized study that will assign patients with surgically treated CRC to different chemotherapy regimens or surveillance depending on the detection or absence of ctDNA after resection.[59] Specifically, after resection, ctDNA-negative patients will be randomized to either ACT (modified FOLFOX6 [folinic acid, 5-fluorouracil, oxaliplatin] or CAPOX [capecitabine, oxaliplatin]) vs no adjuvant chemotherapy with serial ctDNA testing every 3 months. ctDNA-postive patients, after undergoing resection, will be randomized to less intense (modified FOLFOX6 or CAPOX) vs more intense (FOLFIRINOX [5-fluorouracil, irinotecan, oxaliplatin]) ACT. The results of this study will be pivotal in clarifying the role of ctDNA for risk stratification of ACT vs observation in ctDNA-negative patients, as well as the potential benefit of more intense ACT for ctDNA-positive patients. COBRA is a phase 2/3 study correlating RFS in patients with stage IIA colon cancer with ctDNA detection after resection, with or without ACT.[60] Finally, the MiRDA-C study will evaluate blood-based DNA, RNA, and proteomic profiles to evaluate which assay, or combination of assays, best detects recurrence after definitive treatment for CRC.[61]

Finally, cost-effectiveness must always be considered. Many of the earlier studies developed custom assays to perform these tests that would not be accessible to patients outside large academic centers. As commercial entities have begun to provide more accessible testing, a lack of insurance coverage and therefore out-of-pocket costs could be a significant barrier for patients outside clinical trials. Very little is available in the way of scientific study of the cost-effectiveness of ctDNA testing, and the costs of these assays must be weighed against the costs of current surveillance imaging and serologies, delayed diagnosis of recurrence, and delayed resumption of treatment.

Conclusion

ctDNA provides an intriguing, noninvasive means of evaluating solid organ malignancies—from early diagnosis to personalized treatment decisions to prognosis and risk for recurrence. With regard to the detection of MRD after definitive therapy, in particular surgical resection, by far the most data are behind CRC to suggest that the presence of ctDNA after treatment is an indicator of a poor prognosis, and one that may be present months before recurrence is detected by conventional imaging or CEA monitoring, although similar data of a lower magnitude are certainly available for EAC, ESCC, and GC.

The largest gap in these studies is the lack of prospective, randomized, treatment-based data based on ctDNA profiles. As an example, patients with ctDNA positivity after resection could be randomized to an ACT and/or a radiation arm while those without ctDNA detection after surgery received standard of care. Multiple current randomized trials are ongoing aiming to answer the question of whether treatment intensification or de-intensification based on MRD assays can improve patient outcomes. Applying the results of ctDNA assays that suggest the presence of MRD to change therapy is critical to integrating these tests into meaningful patient care and improving DFS and OS, and this remains a point of ongoing study.

Overall, ctDNA testing shows significant promise in detecting MRD across luminal GI malignancies, in particular CRC. Further study holds promise for improving outcomes, and the potential role of ctDNA testing throughout the course of disease is clearly cause for excitement and optimism.

Disclosures

Drs Ueberroth, Jones, and Bekaii-Saab have no relevant conflicts of interest to declare, and this research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sector.

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Supporting Online Material

eTable. Summary of Studies of ctDNA MRD in Gastric Carcinoma

Author (Y)	Study Design (N)	Summary of Findings
Yang (2020)[49]	Prospective study of patients with stage I-III GC followed after resection with serial ctDNA, gastroscopy, CEA, CA 19-9, and abdominal CT to determine which predicted recurrence (46)	- Postop ctDNA positivity was associated with higher relapse rate (100% vs 32%; *P*=.0015) and shorter DFS (HR, 6.56; *P*<.0001); both differences remained significant in multivariate analysis with T stage and tumor site. - At 30-mo mark, sensitivity and specificity of postop ctDNA positivity at any point for predicting recurrence were 39% and 100%, respectively. - Among patients receiving ACT (n=23), ctDNA positivity was associated with shorter DFS (*P*=.0002) and shorter OS (*P*<.0001). - Of patients with recurrence (n=17), 7 had detectable ctDNA in postop testing, and proportion rose to 16/17 with serial monitoring. - Mean lead time was 179 d for ctDNA positivity vs imaging evidence of recurrence. - All patients with equivocal imaging findings but detectable ctDNA eventually had frank recurrence on imaging.
Leal (2020)[50]	Retrospective examination of patients from CRITICS trial (phase 3 trial of resectable GC with standardized NAC and resection protocol) using prespecified 58-gene ctDNA panel to correlate preop and/or postop ctDNA findings with recurrence risk (50)	- Preop detection of ctDNA was initially not associated with risk for recurrence, but when detection was filtered against concurrent CHIP mutations for each patient, preop ctDNA positivity was a predictor of recurrence (HR, 3.0; *P*=.012) and shorter OS (HR, 2.7; *P*=.03). - Postop ctDNA was not associated with risk for recurrence until CHIP-filtered, after which positivity indicated a higher risk (HR, 21.8; *P*<.001). - Study underscores importance of CHIP filtering in ctDNA testing and possible confounding role of CHIP mutations.
Kim (2019)[51]	Prospective study applying WGS-identified patient-specific tumor mutations to personalized, postop ctDNA monitoring for each patient; qPCR ctDNA testing was done before surgery, then serially after surgery (25)	- ctDNA detection at any time during 12-mo follow-up was associated with recurrence (*P*=.0294). - Preop ctDNA positivity was not associated with postop recurrence (*P*=.6372). - Mean lead time of ctDNA positivity vs recurrence on imaging was 4.05 mo.
Ko (2021)[52]	Prospective study of specific *LINE1* long-fragment concentration and *LINE1* methylation proportion on ctDNA in a subset of patients undergoing definitive resection (49 undergoing definitive resection)	- When prespecified cutoff was used, elevated postop concentration of *LINE1* long fragments was associated with shorter RFS (*P*=.009) and shorter OS (*P*=.04), and differences in both remained significant in multivariate analysis. - Postop *LINE1* methylation by ctDNA was not associated with recurrence risk or OS.
Shoda (2017)[53]	Prospectively examined the ratio of *HER2* copy number to *RPPH1* copy number on ctDNA as a predictor of risk for recurrence (60, but only 4 had sufficient follow-up to fully assess study aims)	- All 4 patients with sufficient data had steadily increasing *HER2*-to-*RPPH1* ratios leading up to progression; also, ratios were higher than prespecified cutoff at time of recurrence. - In overall study group, 7 patients had HER2-negative tumors but went on to exhibit increasing *HER2*-to-*RPPH1* ratios on serial ctDNA monitoring after recurrence, suggesting that this mutation may be acquired en route to recurrence.
Ling (2013)[54]	Prospectively followed *XAF1* methylation status via ctDNA in patients with resected GC to assess if methylation changes predicted recurrence (202)	- Of patients with GC, 69.8% had *XAF1* hypermethylation on ctDNA vs 0/88 healthy controls. - Of 12 patients with recurrence, 10 displayed negative-to-positive *XAF1* methylation status on serial ctDNA testing before or at time of recurrence. - The concordance rate between tumor and ctDNA *XAF1* hypermethylation status was 84.9%.

ACT, adjuvant chemotherapy; CA 19-9, cancer antigen 19-9; CEA, carcinoembryonic antigen; CHIP, clonal hematopoiesis of indeterminate potential; CT, computed tomography; ctDNA, circulating tumor DNA; d, day(s); DFS, disease-free survival; GC, gastric carcinoma; HR, hazard ratio; mo, month(s); MRD, minimal residual disease; NAC, neoadjuvant chemotherapy; OS, overall survival; qPCR, quantitative polymerase chain reaction; RFS, relapse-free survival; WGS, whole-genome sequencing.