The Future of ctDNA-Defined Minimal Residual Disease: Personalizing Adjuvant Therapy in Colorectal Cancer

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Abstract

Our understanding of the diagnostic and prognostic use of circulating tumor DNA (ctDNA) in colorectal cancer (CRC) has broadly expanded over the past few years. The utilization of ctDNA to detect minimal residual disease is currently being employed across the continuum of cancer care. The lead-time of ctDNA positivity to radiographic recurrence in stage I to III CRC is up to 9 months on average, which provides a therapeutic window for a group of high-risk patients who will ultimately recur. There are several ongoing prospective clinical trials that investigate whether ctDNA can be used as an integral biomarker to risk stratify CRC patients and guide adjuvant treatment decisions. In this review, we summarize the evidence supporting the promise of ctDNA-defined MRD in CRC and highlight the current ctDNA guided adjuvant prospective clinical trials.

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Introduction

Colorectal cancer (CRC) is the third most common cause of cancer-related mortality globally and in the United States. CRC continues to be a major therapeutic challenge with a considerable number of patients experiencing premature death from early disease recurrence. Relapsed CRC may occur in the absence of symptoms, which emphasizes the importance of sensitive screening modalities to optimize outcomes. The current standard of care guidelines for CRC surveillance monitoring relies almost exclusively on the presence of radiographic evidence of disease. This approach, however, is limited as patients with microscopic evidence of disease are not captured via current imaging techniques. Small lymph nodes, for example, may harbor viable tumor cells that are not appreciated on imaging. Furthermore, diagnostic CT chest imaging frequently detects indeterminate lung lesions of unclear significance that may not be amenable to biopsy or FDG-avid on PET.

Abbreviations: CAPOX, Capecitabine+ Oxaliplatin; CRC, Colorectal cancer; CRLM, Colorectal liver metastases; CT, Computed tomography; ctDNA, Circulating tumor DNA; ddPCR, Digital droplet PCR; DFS, Disease free survival; FDG, Flurodeoxyglucose; FOLFIRI, 5-fluorouracil + Irinotecan; FOLFOX, 5-fluorouracil + Oxaliplatin; FOLFOXIRI, 5-fluorouracil + Oxaliplatin + Irinotecan; miRNA, Micro-RNA; MRD, Minimal residual disease; NGS, Next generation sequencing; OS, Overall survival; PET, Positron Emission Tomography; RFS, Recurrence free survival; SOC, Standard of care; TAS-102, Trifluridine and Tipiracil; WGS, Whole genome sequencing.

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Circulating tumor DNA (ctDNA) has emerged as a non-invasive diagnostic and prognostic tool for CRC. The isolation of ctDNA is currently being used across the continuum of cancer care. Multiple studies demonstrate that ctDNA levels can detect minimal residual disease (MRD) and be predictive of recurrence.[1] In patients with stage I to III CRC, detectable ctDNA after completion of adjuvant therapy was associated with an 88% sensitivity to relapse. Importantly, the average lead time of ctDNA detection in plasma to relapse by CT imaging was 8.7 months.[2] The improved lead-time of ctDNA positivity to radiographic recurrence is noteworthy because it provides a therapeutic window for the evaluation of novel therapies in a setting for which there is no current standard of care guidelines. Clearance of ctDNA may even be used as a surrogate end point under the assumption that this is necessary for eventual cure.[3,4] Several ongoing prospective clinical trials are evaluating whether ctDNA can be utilized as an integral biomarker in the adjuvant setting to guide escalation or de-escalation of therapy for stage II and III CRC. Furthermore, accurate MRD detection in CRC can potentially play a role in improving sample size estimates for clinical trials and reducing study costs.

Here, we review the evidence supporting ctDNA as a promising biomarker for MRD detection and discuss ongoing ctDNA based adjuvant clinical trials in CRC.

ctDNA Detection Methods

The concept of ctDNA was first reported in the serum of cancer patients in 1977, however, it took quite some time to garner interest given the obstacle of precisely detecting such low concentra-

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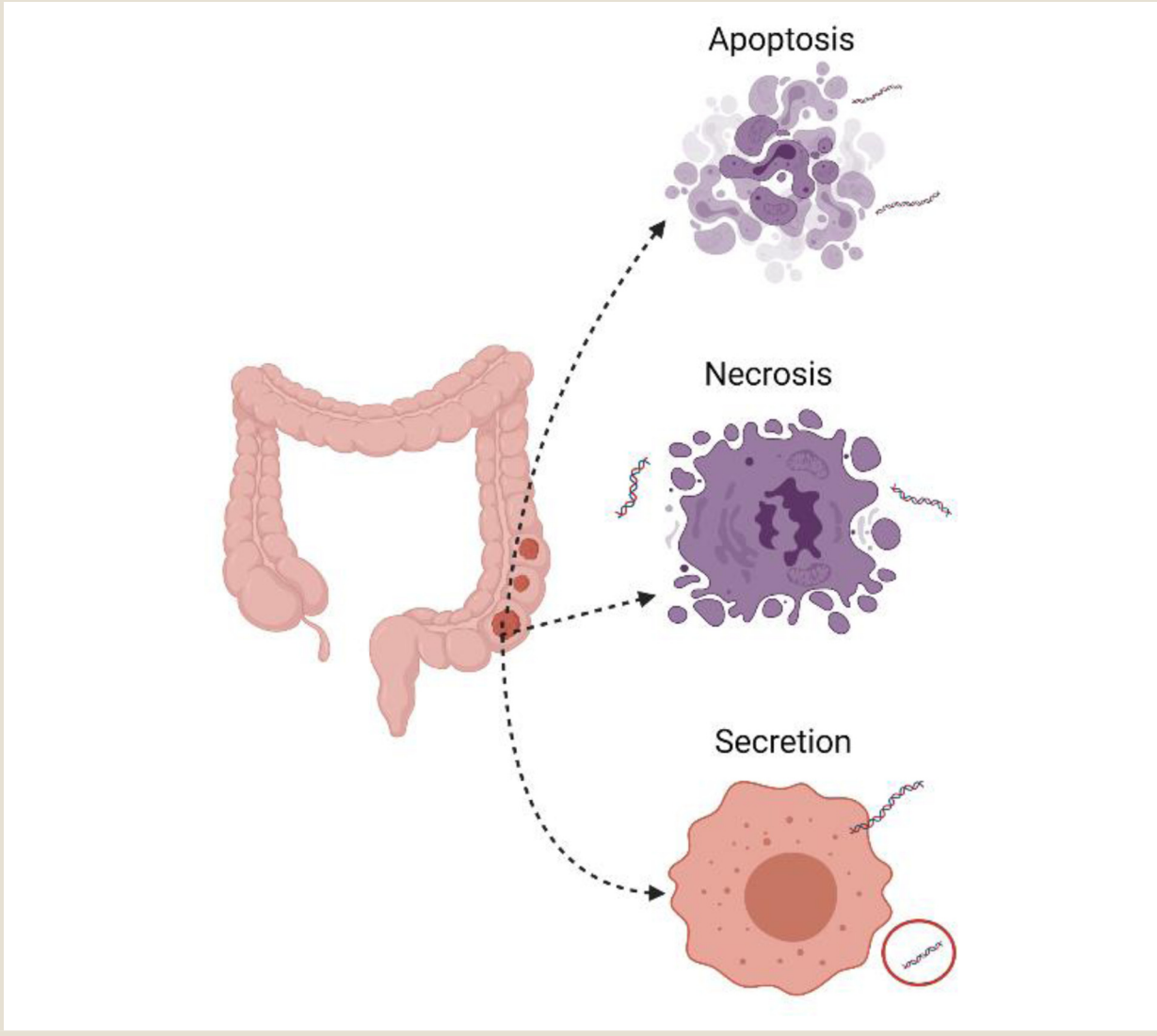
Figure 1 Circulating tumor DNA is composed of short fragments of tumor derived DNA (roughly 130-150 base pairs). Cancer cells release DNA into the circulation via apoptosis, necrosis, or secretion.[38]



tions in the bloodstream.[5] ctDNA is composed of short fragments of tumor derived DNA (roughly 130-150 base pairs) that are released by cancer cells into the circulation via apoptosis, necrosis, or secretion (Figure 1).[6] The proportion of patients in whom ctDNA can be detected depends on the extent of tumor volume and concentrations are typically low in early-stage disease.[7] It is imperative that ctDNA assays provide accurate detection to effectively guide treatment in the absence of radiographic evidence of disease. Highly specific assays are essential for the utilization of ctDNA as an integral biomarker to determine treatment escalation as false positives may subject patients to unnecessary intensive therapy. Successfully identifying which patients may benefit from treatment de-escalation, however, requires exceptional sensitivity to avoid inappropriately selecting high-risk patients to receive less intensive therapy.

Tumor-Informed Assays

Tumor-informed ctDNA detection utilizes mutational signatures that are derived from genomic sequencing of the primary tumor to create patient specific assays. The conventional ctDNA assays can be divided into 2 broad categories including PCR-based and next-generation sequencing (NGS) based assays. Benefits of PCR based methods include high sensitivity for hot spot mutations (<0.001%), monitoring for recurrent resistant mutations, cost effectiveness, and rapid turnaround time without the need for bioinformatic analyses.[8] Digital PCR based methods are limited to known aberrations as they require primers that are specific to a defined mutation or targeted locus, thus these methods are not preferred for ctDNA defined MRD detection.[6,9,10] The development of innovative assay techniques over the years, however, has substantially improved the specificity and sensitivity of detecting tumor products in early-

stage disease. Focused next-generation sequencing (NGS) based approaches have improved sequencing depth and allowed for the detection of low-frequency mutant alleles.[11] Targeted NGS based methods, such as multiplex PCR based NGS and hybrid capture based NGS, have facilitated the evaluation of the genome on a broader scale. Multiplex PCR involves the utilization of primers to amplify multiple unique regions of DNA in a single reaction prior to NGS analysis.[12] Common limitations of multiplex PCR include spurious amplification products, difficulty of reproducing results, and uneven amplification of some target sequences.[12] For hybrid capture based NGS approaches, regions of interest are hybridized to target-specific biotinylated probes and subsequently captured for NGS analysis.[13] Panels can be designed to capture frequently mutated genes from several cancers or personalized for patient specific mutations identified via tumor sequencing.[13] Although these targeted NGS methods don't require prior knowledge of molecular mutations, they warrant comparison with tumor sequencing data to reduce false-positive results.[6] Clinically, focused NGS approaches are useful for evaluating de novo resistance mutations, monitoring clonal evolution in response to therapy, and profiling gene panels. Plasma whole genome sequencing (WGS) allows for the comprehensive exploration of genomic alterations to better understand the landscape of clinically significant mutations.[6,11] Plasma WGS remains an option under further clinical development but is not currently utilized in clinically available assays.

Tumor-Uninformed (Plasma-Only) Assays

The utility of tumor-agnostic (blood-based) ctDNA assays that do not require prior tumor profiling is being explored by several groups. Given the inevitable limitations of a fixed gene panel approach, these assays are oftentimes supplemented with epigenomics including aberrant methylation. Aberrant DNA methylation is common in CRC and seen as an early step for carcinogenesis for most tumors.[14–16] The addition of methyl-groups to the C5 position of cytosines in the DNA can alter the expression of nearby tumor suppressor genes. Methylated DNA provides a unique opportunity to improve sensitivity by detecting CRC beyond testing for somatic mutations. Furthermore, this approach does not require prior tumor sequencing, thus can be performed without prior knowledge of the resected tumor. Parikh and colleagues provide insight into an assay that combines somatic mutations using a focused gene panel and additional methylation signatures to evaluate MRD cells.[17] There are several advantages to tumor-uninformed MRD detection including rapid turn-around time, decreased initial costs, and monitoring in patients with insufficient or unavailable tumor tissue.[18] It is critical, however, to standardize pre-analytical conditions as various extraction methods can considerably affect methylated ctDNA yield and integrity.

Leveraging ctDNA-Defined MRD Detection to Improve CRC Outcomes

Despite meaningful advancements in the treatment of high-risk locoregional CRC, there remains a critical need for further improvement in this setting. Historically, it has been challenging to definitively identify CRC patients at risk of recurrence due to a lack of validated biomarkers, which has complicated the ability to optimize adjuvant drug development. The 5-year survival rate of high-risk stage IIIC CRC patients are only 53% despite standard of care therapies, likely as a result of undetected micrometastatic disease.[19]

The short half-life of ctDNA makes it a sensitive real time marker of tumor burden and its presence likely reflects micrometastases unseen radiographically, otherwise known as MRD.[6,20] Detectable ctDNA can precede radiographic evidence of recurrent disease by several months, thus making it a useful prognostic biomarker[2]. Additionally, serial ctDNA monitoring allows clinicians to capture the evolution of a tumor's molecular profile overtime or in response to cancer therapy. Multiple studies have demonstrated both a high specificity and almost 100% positive predictive value for clinical recurrence in patients with ctDNA-defined MRD.[20,3,21] The detection of ctDNA following completion of curative intent adjuvant therapy is associated with an inferior recurrence free survival and subsequent relapse.[1] In a study evaluating stage I to III CRC patients, detectable ctDNA was associated with an 88% sensitivity to relapse and an average lead time of 8.7 months.[2] The average time from surgery to relapse detection was 19 months for CEA and 13.6 months for CT imaging.[2] Furthermore, all patients experienced increased plasma ctDNA levels over time from initial ctDNA detection to radiographic recurrence and no patients were found to have spontaneous clearance of ctDNA.[2] A more recent study demonstrated an 80% recurrence rate for stage III CRC patients with detectable ctDNA following completion of adjuvant chemotherapy.[22] Of note, only patients who cleared ctDNA permanently following adjuvant therapy did not relapse.[22]

There is no current standard of care guidelines regarding management of CRC patients with ctDNA-defined MRD following adjuvant therapy. The improved detection of ctDNA has provided a unique opportunity to utilize a noninvasive approach to explore therapeutic options and potentially eradicate micrometastatic disease in high-risk patients. Clearance of ctDNA may be used as an end point in early-stage studies to risk stratify patients for more definitive trials and allow for optimization of drug development.

Ongoing ctDNA Defined MRD Clinical Trials in CRC

To Guide Adjuvant Therapy

The goal of adjuvant therapy in CRC is to increase the likelihood of cure while minimizing the risk of toxicity. There are a growing number of clinical trials utilizing ctDNA as a biomarker to risk stratify CRC patients for escalation or de-escalation of adjuvant therapy (Table 1). Ongoing prospective studies are designed to determine whether ctDNA can be successfully used as a surrogate end point in CRC. Clinical trial enrollment based on ctDNA testing in the adjuvant setting can allow for smaller homogenous sample sizes consisting of higher-risk patient populations as opposed to the larger number of patients required to adequately power traditional adjuvant studies due to the heterogenous trial population. Moreover, ctDNA for risk stratification and patient selection can lead to reduced trial length and decreased costs, which may encourage drug development in this space.

There are numerous trials evaluating whether ctDNA-defined MRD detection can be utilized to identify high-risk patients who

Table 1 ctDNA-based MRD Clinical Trials for The Guidance of Adjuvant Therapy

Study Title/ID	Phase	Tumor Type/Stage	ctDNA Analysis	Study Drug	Primary End Point	Study Design	Study Size
IMPROVE-IT NCT03748680	II	Stage I or II colon	ddPCR, NGS	CAPOX or FOLFOX	DFS	ctDNA positive patients 2 wk post resection are randomized to observation vs. CAPOX or FOLFOX x 6 mos	64
COBRA NCT04068103	II/III	Stage II colon (low-risk)	Guardant Reveal	FOLFOX	ctDNA clearance (phase II) RFS (phase III)	Patients s/p resection are randomized to observation vs. adjuvant FOLFOX x 6 mo (if ctDNA positive)	1408
CIRCULATE AIO-KRK-0217 NCT04089631	III	Stage II colon	NGS	Capecitabine or CAPOX	DFS	ctDNA positive patients s/p resection are randomized to observation vs. capecitabine +/- oxaliplatin (investigator's choice) x 6 mo	4812
CIRCULATE-US	III	Stage II or III colon cancer	Signatera	FOLFOX or CAPOX FOLFOXIRI	DFS	ctDNA negative patients post resection will receive SOC chemo vs. observation. ctDNA positive patients will receive either SOC chemo vs. FOLFOXIRI	1912
BESPOKE NCT04264702	II	Stage II or III CRC	Signatera	Treating physician discretion	Percentage of patients who have their adjuvant therapy adjusted based on ctDNA status	ctDNA serially monitored after surgery and adjuvant therapy vs. observation offered based on discretion of treating physician	1000
DYNAMIC II ACTRN12615000381583	III	Stage II colon	Safe-SeqS	5FU, CAPOX, or FOLFOX	RFS	Patients is the study group with detectable ctDNA receive 5FU based adjuvant therapy, while ctDNA-negative patients are observed. Control arm blinded to ctDNA status.	450
DYNAMIC III ACTRN126170015	II/III	Stage III colon		5FU FOLFOX or CAPOX FOLFOXIRI	RFS	ctDNA-positive patients s/p surgery receive an escalation of therapy (FOLFOXIRI if SOC is oxaliplatin based), while ctDNA-negative patients receive a de-escalation of therapy. Control arm is blinded to ctDNA and receive SOC therapy	1000
PEGASUS NCT04259944	II	Stage II (high risk) or III colon	Guardant Reveal	CAPOX Capecitabine FOLFIRI	Number of ctDNA negative cases (post-surgery & adjuvant therapy) later found to be ctDNA positive or have radiographic relapse	Patients with detectable ctDNA post-surgery receive adjuvant CAPOX x 3 mo, while ctDNA negative patients receive capecitabine x 6 mo. ctDNA negative patients retested after 1 cycle of capecitabine and switched to CAPOX if positive. Upon completion of adjuvant therapy, further systemic therapy given x 6 mo if positive (CAPOX if initially ctDNA negative and FOLFIRI if previously positive)	140
GALAXY UMIN000039205	Prospective observational	Stage II-IV colon or relapsed	Signatera	None	DFS	Patients undergo serial ctDNA monitoring post-surgery and based on results may enroll in the phase III VEGA (therapy de-escalation) or the ALTAIL trial (therapy escalation).	2500
VEGA jRCT1031200006	III	Stage II-IV colon or relapsed	Signatera	CAPOX	DFS	Evaluates non-inferiority of adjuvant CAPOX vs. observation for GALAXY patients who are ctDNA negative 1-mo post-surgery	1240

will benefit from early initiation of SOC chemotherapy (Table 1). Notably, majority of these studies have employed the traditional disease-free survival (DFS) end point. Several of these studies (IMPROVE-IT, COBRA,[23] CIRCULATE AIO-KRK-0217) are designed to randomize ctDNA positive patients to either observation or SOC chemotherapy following surgical resection. In the phase III DYNAMIC II study, patients without detectable levels of ctDNA are observed, while ctDNA positive patients receive 5-FU based adjuvant therapy. The BESPOKE study, however, leaves the decision to pursue adjuvant chemotherapy based on ctDNA status up to the treating physician's discretion.

Furthermore, there are several studies evaluating whether ctDNA can be used as a dynamic biomarker to optimize adjuvant therapy via either treatment de-escalation or escalation (Table 1). The CIRCULATE-US study, for example, randomizes ctDNA positive patients to receive SOC adjuvant chemotherapy or FOLFOXIRI. In the DYNAMIC-III study, ctDNA positive patients following resection receive therapy escalation with FOLXFOXIRI, while ctDNA negative patients receive treatment de-escalation. The comparator arm will not undergo ctDNA testing and will receive adjuvant treatment based on standard of care guidelines. The phase II PEGASUS trial is designed to retest patients who were initially ctDNA negative after 1 cycle of capecitabine and escalate to CAPOX if they become ctDNA positive. Additionally, a liquid biopsy will be obtained upon completion of adjuvant therapy to determine whether further systemic treatment is indicated; ctDNA positive patients will receive further systemic therapy for 6 months (CAPOX if originally ctDNA- and FOLFIRI if initially ctDNA+). The GALAXY trial is a prospective observational study for CRC patients following curative surgery who undergo serial ctDNA monitoring using the personalized tumor informed ctDNA assay based on whole exome sequencing of the tumor tissue (Signatera – multiplex-PCR NGS). Based on ctDNA status, patients may enroll in the phase III VEGA (therapy de-escalation) or the ALTAIR trial (therapy escalation). The VEGA trial evaluates the non-inferiority of adjuvant CAPOX vs. observation for patients who are ctDNA negative 1-month post-surgery.

Post-Adjuvant Therapy

Detectable ctDNA after completion of curative therapies can identify high-risk patients with micrometastatic disease and serve as the first prognostic predictive biomarker in CRC. Multiple studies have shown that the positive predictive value (PPV) of ctDNA is roughly 100% by 3 years, which reinforces the notion that CRC patients with ctDNA defined MRD following completion of definitive therapies will inevitably recur radiographically. The lead-time of ctDNA positivity to radiographic recurrence is up to 6 to 9 months, which provides a window for early initiation of systemic therapy to eradicate micrometastatic disease.[2] There are several ongoing clinical trials designed to evaluate whether additional systemic therapy can improve DFS for ctDNA positive patients who relapse despite completion of adjuvant therapy (Table 2).

There are several studies evaluating whether additional chemotherapy can eradicate radiographically undetectable micrometastatic disease. The phase II NCT03803553 study randomizes patients to receive FOLFIRI, encorafenib, bimimetinib, and cetuximab (if BRAF mutation is identified) or nivolumab (if MSI-H) vs. surveillance. Furthermore, there are currently 3 studies evaluating the effects of TAS-102 on MRD. In the phase II NCT04920032 trial, ctDNA positive patients are randomized to TAS-102 in combination with irinotecan vs. investigator's choice. The ALTAIR study investigates the superiority of TAS-102 over placebo for patients who remain ctDNA positive following completion of SOC therapy.[24] Lastly, a single-center phase II trial evaluating 6 months of TAS-102 in CRC patients with ct-DNA defined MRD is pending activation in the near future.

It has been proven that the tumor microenvironment is not well established in MSS patients and subsequently limits an efficient anti-tumor immune response.[25] In addition to immune checkpoints, the tumor microenvironment contains other relevant molecules that play a role in immunosuppression including the cytokine TGFβ.[26] TGFβ has been shown to prevent the activation of T-cells, as well as decrease the function of both T and NK cells.[27,28] The ongoing NCT03436563 study for ctDNA positive MSS patients evaluates whether an anti-PDL1/TGFβ bispecific antibody alone or in combination with a therapeutic cancer vaccine can promote anti-tumor responses.

There has been increased interest in the use of vaccines to explore immunotherapies in CRC patients with ctDNA-defined MRD. ELI-002 is a KRAS targeting vaccine that is being studied in RAS mutated solid tumors with MRD (AMPLIFY-201). Furthermore, the success of the messenger RNA based COVID-19 vaccine has fueled researchers to investigate the use of mRNA-based cancer vaccines to explore individualized treatment options for high-risk patients. RO7198457 is a personalized mRNA-based vaccine that targets specifically expressed tumor associated antigens, subsequently leading to induction of cytotoxic T-lymphocytes and memory T-cell-dependent immune responses against cancer cells. In the phase II NCT04486378 trial, DFS is evaluated in stage II or III ctDNA positive patients randomized to receive either RO7198457 or pursue watchful waiting.

The durable response of immunotherapy due to the high tumor mutational burden in MSI-H CRC patients has been well established in the metastatic and refractory setting. There are several ongoing studies, however, that are evaluating whether early initiation of immunotherapy in MSI-H patients with detectable levels of ctDNA impacts DFS or ctDNA clearance rate (NCT03803553, NCT03832569).

Lifestyle Intervention Studies

Epidemiology data is consistent that diet, vitamin D, aspirin, and exercise are associated with reduced risk of recurrence. Additional studies, however, are necessary to determine whether these changes are causal in nature. There are large clinical trials in Canada and the UK proposed to test these interventions in stage II and III CRC with sample sizes of over 10,000 patients collectively, however, these studies will take several years to read out and can be quite costly. Utilizing ctDNA as a biomarker can provide a more efficient approach to testing these strategies. Furthermore, a lifestyle intervention study is a great option for patients who may not be interested in novel therapeutic agent trials upon completion of SOC therapies. The DAILY study is a phase II study designed to evaluate

Table 2 ctDNA-based MRD Clinical Trials Post-Adjuvant Therapy

Study Title/ID	Phase	Tumor Type/Stage	ctDNA Analysis	Study Drug	Primary End Point	Study Deisgn	Sample Size
NCT03803553	II	Stage III colon	Guardant Reveal	FOLFIRI; Nivolumab; Encorafenib, Bimimetinib, Cetuximab	DFS, ctDNA clearance rate	ctDNA positive patients randomized to FOLFIRI (12 cycles/ or surveillance. If MSI-H - receive nivolumab (12 cycles). If BRAF mutation present, receive encorafenib, bimimetinib, and cetuximab (12 cycles).	500
NCT04486378	II	Stage II colon (high-risk) Stage III colon		RO7198457 (personalized vaccine)	DFS	ctDNA positive patients randomized to RO7198457 (personalized mRNA based vaccine) vs. watchful waiting	15
NCT03832569	I	MSI-High Solid Tumors		Pembrolizumab vs. Placebo	ctDNA clearance rate at 12 mo	ctDNA positive patients randomized to pembrolizumab vs. placebo	10
NCT04920032	II	Stage II or III colorectal cancer	Signatera	TAS-102+Irinotecan	ctDNA clearance rate	ctDNA positive patients randomized to TAS-102 + irinotecan for 6 mo vs. investigator's choice	22
Amplify-201 NCT04853017	I/II	RAS mutated solid tumors		ELI-002	MTD and safety	ctDNA positive (RAS mutated) patients are administered different dose levels of ELI-002 (RAS targeting vaccine)	18
NCT03436563	IB/II	Stage II-IV colorectal cancer	SignateraThis is not a pattern of 'ctgov' external object linking.	M7824 (anti-PDL1/TGFbetaRII fusion protein)	ctDNA clearance rate	ctDNA positive patients s/p resection of all liver mets will receive 6 doses of M7824	74
TAS-102 for ctDNA defined MRD	II	stage II-III colorectal cancer; stage IV CRC s/p R0 resection	Signatera	TAS-102	ctDNA clearance rate	ctDNA positive patients receive TAS-102 × 6 mo	15
DAILY Study NCT05036109	II	stage II or III colorectal cancer; stage IV CRC s/p R0 resection	Signatera	Vitamin D, Aspirin, Plant based diet, exercise	ctDNA clearance rate	ctDNA positive patients receive 3 mo of lifestyle interventions	17
NCT04589468	IA/B	Stage I-III colorectal, breast, and prostate cancer	Signatera	Exercise	Phase II exercise dose	ctDNA positive patients will perform various exercise dose levels for 6 mo	70
ALTAIR NCT04457297	III	Stage II-IV colon or relapsed	Signatera	TAS-102	DFS	Evaluates the superiority of TAS-102 vs. placebo for GALAXY patients who remain ctDNA positive following completion of SOC therapy	240

the effects of vitamin D and aspirin supplementation, a plant-based diet, and physical activity on ctDNA in CRC patients with MRD. The study aims to assess whether such lifestyle changes can eradicate MRD based on clearance of detectable ctDNA. The phase IA/B NCT04589468 trial is a feasibility study evaluating the effects of 6 months of aerobic exercise on stage I to III CRC, breast cancer, and prostate cancer patients with detectable ctDNA. The primary end point is to determine the phase II exercise dose level, which will be further tested to evaluate ctDNA clearance rates.

Future Directions

The inability to completely eradicate MRD following SOC curative therapies is likely a result of the intrinsic disease biology.[3,29] Therapies targeting micrometastatic disease following completion of adjuvant treatment need to better understand the biology and potential vulnerabilities of the cell state to successfully transform cancer care.

Interrogating the Biology of MRD

Metastatic tumors are biologically distinct from their primary counterparts including molecular snapshots and metabolic activity. Adaptive reprogramming and selection pressure produce recurrent and chemoresistant clones that are challenging to detect, target, and treat when radiographically occult.[30] There are distinct multi-gene signatures between primary CRC tumors and CRC liver metastases (CRLM) that can be prognostic of RFS and OS, which may provide insights into the biology of MRD. These changes may not be limited to expressed RNA, as metastasis-specific micro-RNA (miRNA) signatures are also observed with at least 23 known differentially expressed miRNAs in CRLMs vs. primary CRC.[31–33] There is still much to learn about the unique tumor microenvironment

that may differ between MRD and bulky radiographically apparent metastases; for example, in the expression of factors such as matrix metallopeptidases, chemokine receptors, matricellular proteins like osteopontin and periostin among others.[32,34] These factors may reflect maturing interactions of tumor cells with the liver microenvironment, liver specific extracellular matrix, and other liver-specific stromal factors over time and tumor growth. Specifically, microenvironmental changes in TGF- β have been shown in preclinical models to develop as the tumor expands in size, and metastases can be eradicated by genetically and pharmacologically targeting TGF-β.[35] Residual disease after treatment demonstrates significant alterations in protein coding genes related to metabolic and redox processes, as well as immune recognition which may provide therapeutic opportunities for intervention.[36, 37] Emerging evidence suggests that residual and recurrent disease, in the context of CRLM, exhibits cellular functional plasticity which distinguish it biologically from primary CRC.

Conclusions

Overall, serial monitoring of ctDNA in the absence of radiographic evidence of disease may outperform traditional pathological prognostic features for the evaluation of risk of recurrence in CRC. Testing for ctDNA-defined MRD has provided a unique non-invasive opportunity to explore therapeutic options for the eradication of micrometastatic disease in high-risk CRC patients. Findings from the prospective ctDNA based adjuvant trials has the potential to transform clinical practice and significantly improve patient outcomes.

References

1. Luo H, Zhao Qi, Wei Wei, et al. Circulating tumor DNA methylation profiles enable early diagnosis, prognosis prediction, and screening for colorectal cancer. *Sci Transl Med*. 2020;12:1.
2. Reinert T, Henriksen Tenna, Christensen Emil, et al. Analysis of plasma cell-free DNA by ultradeep sequencing in patients with stages I to III colorectal cancer. *JAMA Oncol*. 2019;5:1124–1131.
3. Dasari A, Grothey Axel, Kopetz Scott. Circulating tumor DNA-defined minimal residual disease in solid tumors: opportunities to accelerate the development of adjuvant therapies. *J Clin Oncol*. 2018.
4. Dasari A, Morris Van, Allegra Carmen, et al. ctDNA applications and integration in colorectal cancer: an NCI Colon and Rectal-Anal Task Forces whitepaper. *Nat Rev Clin Oncol*. 2020;17:757–770.
5. Friedrich MJ. Going with the flow: the promise and challenge of liquid biopsies. *JAMA*. 2017;318(12):1095–1097.
6. Wan JCM, Massie Charles, Garcia-Corbacho Javier, et al. Liquid biopsies come of age: towards implementation of circulating tumour DNA. *Nat Rev Cancer*. 2017;17:223–238.
7. Phallen J, Sausen Mark, Adlef Vilmos, et al. Direct detection of early-stage cancers using circulating tumor DNA. *Sci Transl Med*. 2017;9.
8. Perkins G, Lu H, Garlan F, et al. Droplet-based digital PCR: application in cancer research. *Adv Clin Chem*. 2017;79:43–91.
9. Kanagal-Shamanna R. Digital PCR: principles and applications. *Methods Mol Biol*. 2016;1392:43–50.
10. Dressman D, Yan Hai, Traverso Giovanni, et al. Transforming single DNA molecules into fluorescent magnetic particles for detection and enumeration of genetic variations. *Proc Natl Acad Sci U S A*. 2003;100:8817–8822.
11. Chin RI, Chen Kevin, Usmani Abul, et al. Detection of solid tumor molecular residual disease (MRD) using circulating tumor DNA (ctDNA). *Mol Diagn Ther*. 2019;23:311–331.
12. Markoulatos P, Siafakas N, Moncany M, et al. Multiplex polymerase chain reaction: a practical approach. *J Clin Lab Anal*. 2002;16:47–51.
13. Newman AM, Bratman Scott, To Jacqueline, et al. An ultrasensitive method for quantitating circulating tumor DNA with broad patient coverage. *Nat Med*. 2014;20:548–554.
14. Fang JY, Lu Rong, Mikovitz Judy, et al. Regulation of hMSH2 and hMLH1 expression in the human colon cancer cell line SW1116 by DNA methyltransferase 1. *Cancer Lett*. 2006;233:124–130.
15. Laird PW, Jackson-Grusby L, Fazeli A, et al. Suppression of intestinal neoplasia by DNA hypomethylation. *Cell*. 1995;81:197–205.
16. Yi JM, Dhir Mashaal, Guzzetta Angela, et al. DNA methylation biomarker candidates for early detection of colon cancer. *Tumor Biol*. 2012;33:363–372.
17. Parikh AR, Seventer Emily, Siravegna Giulia, et al. Minimal residual disease detection using a plasma-only circulating tumor DNA assay in colorectal cancer patients. *Clin Cancer Res*. 2021;27(20):5586–5594.
18. Bent A, Kopetz S. Going with the flow: the promise of plasma-only circulating tumor DNA assays. *Clin Cancer Res*. 2021;27:5449–5451.
19. Benson AB, Venook Alan, Cederquist Lynette, et al. Colon cancer, version 1.2017, NCCN clinical practice guidelines in oncology. *J Natl Compr Canc Netw*. 2017;15:370–398.
20. Tie J, Cohen Joshua, Wang Yuxuan, et al. Circulating tumor DNA analyses as markers of recurrence risk and benefit of adjuvant therapy for stage III colon cancer. *JAMA Oncol*. 2019;5:1710–1717.
21. Morris V, Dasari Arvind, Kopetz Scott, et al. Can circulating tumor DNA in early-stage colorectal cancer be more than a prognostic biomarker? *JAMA Oncol*. 2019;5:1101–1103.
22. Henriksen TV, Tarazona Noelia, Frydendahl Amanda, et al. Circulating tumor DNA in stage III colorectal cancer, beyond minimal residual disease detection, towards assessment of adjuvant therapy efficacy and clinical behavior of recurrences. *Clin Cancer Res*. 2021.
23. Morris VK, Yothers Greg, Kopetz Scott, et al. *Phase II/III study of circulating tumor DNA as a predictive biomarker in adjuvant chemotherapy in patients with stage II colon cancer: NRG-GI005 (COBRA)*. American Society of Clinical Oncology; 2020.
24. Yukami H, Mishima S, Kotani D, et al. P-120 Prospective observational study monitoring circulating tumor DNA in resectable colorectal cancer patients undergoing radical surgery: GALAXY study in CIRCULATE-Japan (trial in progress). *Ann Oncol*. 2020;31:S128–S129.
25. Lainé A, Labiad Ossama, Hernandez-Vargas Hector, et al. Regulatory T cells promote cancer immune-escape through integrin $\alpha v\beta 8$-mediated TGF-β activation. *Nat Commun*. 2021;12:1–14.
26. Knudson KM, Hicks Kristin, Luo Xiaoling, et al. M7824, a novel bifunctional anti-PD-L1/TGFβ Trap fusion protein, promotes anti-tumor efficacy as monotherapy and in combination with vaccine. *Oncoimmunology*. 2018;7.
27. Lebrun J. The dual role of TGF in human cancer: from tumor suppression to cancer metastasis. *ISRN Mol Biol*. 2012;2012:28.
28. Travis MA, Sheppard D. TGF-β activation and function in immunity. *Ann Rev Immunol*. 2014;32:51–82.
29. Garcia-Murillas I, Schiavon Gaia, Weigelt Britta, et al. Mutation tracking in circulating tumor DNA predicts relapse in early breast cancer. *Sci Transl Med*. 2015;7 302ra133-302ra133.
30. Sharma SV, Lee Diana, Li Bihua, et al. A chromatin-mediated reversible drug-tolerant state in cancer cell subpopulations. *Cell*. 2010;141:69–80.
31. Coebergh Van Den Braak RR, Sieuwerts Anieta, Lalmahomed Zarina, et al. Confirmation of a metastasis-specific microRNA signature in primary colon cancer. *Sci Rep*. 2018;8:1–11.
32. Shiomi A, Kusuhara Masatoshi, Sugino Takashi, et al. Comprehensive genomic analysis contrasting primary colorectal cancer and matched liver metastases. *Oncol Lett*. 2021;21:1–13.
33. Zhang T, Guo Jianrong, Gu Jian, et al. Identifying the key genes and microRNAs in colorectal cancer liver metastasis by bioinformatics analysis and in vitro experiments. *Oncol Rep*. 2019;41:279–291.
34. Bocuk D, Wolff Alexander, Krause Petra, et al. The adaptation of colorectal cancer cells when forming metastases in the liver: expression of associated genes and pathways in a mouse model. *BMC Cancer*. 2017;17(1):1–15.
35. Villalba M, Evans Stephanie, Vidal-Vanaclocha Fernando, et al. Role of TGF-β in metastatic colon cancer: it is finally time for targeted therapy. *Cell Tissue Res*. 2017;370:29–39.
36. Lupo B, Sassi Francesco, Pinnelli Marika, et al. Colorectal cancer residual disease at maximal response to EGFR blockade displays a druggable Paneth cell–like phenotype. *Sci Transl Med*. 2020;12(555).
37. Li Z, Chen Yuling, Ren W, et al. Transcriptome alterations in liver metastases of colorectal cancer after acquired resistance to cetuximab. *Cancer Genom Proteom*. 2019;16:207–219.
38. Schwarzenbach H, Hoon Dave, Pantel Klaus. Cell-free nucleic acids as biomarkers in cancer patients. *Nat Rev Cancer*. 2011;11:426–437.