Circulating Tumor DNA Analysis in Colorectal Cancer: From Dream to Reality

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Liquid biopsy is a minimally invasive approach to obtain circulating materials that originate from tumor cells through the sampling of body fluids, mainly peripheral blood. Because of its abilities to detect tumor-derived nucleic acids and proteins and to characterize tumor-specific genomic abnormalities, liquid biopsy has emerged as an approach to orient care of patients with colorectal cancer (CRC). The most advanced approach through which liquid biopsy could be exploited in the CRC field is the analysis of circulating cell-free tumor DNA (ctDNA).

ctDNA is detected in almost all patients with CRC.[1] Available approaches to ctDNA analysis range from the interrogation of a single or a limited number of loci to whole-genome analyses.[2]

Several applications of ctDNA analysis have been hypothesized in the clinical scenario of CRC, including the following: molecular profiling for treatment selection, prognosis assessment and detection of minimal residual disease, monitoring of treatment efficacy and emergence of secondary resistance to ongoing therapies, and identification of candidates for anti–epidermal growth factor receptor (EGFR) rechallenge. However, the role of liquid biopsy as a tool to build a biology-driven care of patients with CRC needs validation to enter clinical guidelines and recommendations.[3,4]

We reviewed available literature about the applications of ctDNA analysis with the aim of disclosing the main gaps that must be filled to push the development of liquid biopsy toward clinical practice.

ctDNA Versus Tumor DNA for *RAS* Profiling

Expectations are high about the possibility of using blood instead of tissue samples to detect *RAS* mutations for anti-EGFR (cetuximab and panitumumab) treatment selection in metastatic CRC (mCRC).

Many retrospective series have described a more than 90% agreement between *RAS* status in matched tumor and ctDNA samples and have highlighted that *RAS* mutations are detectable in ctDNA with high specificity (90% to 100%), but suboptimal sensitivity (89% to 96%; Appendix Table A1).[5-9] Nevertheless, when *RAS* testing on ctDNA was compared with highly sensitive tissue-based techniques,[10,11] concordance rate (78% to 88%), specificity (83% to 91%), and sensitivity (70% to 85%) of plasma-based analyses were less encouraging (Appendix Table A1).

Retrospective data suggested that *RAS* testing on ctDNA results in a similar clinical outcome compared with tissue testing in patients with mCRC who were treated with anti-EGFR–based regimens.[5,10,11] However, some issues hamper *RAS* genotyping on ctDNA as an alternative to tissue analysis for anti-EGFR treatment selection.

First, the standardization of preanalytic variables for ctDNA analysis is still lacking, and this may affect ctDNA quality. Second, the reliability of studies that evaluate the concordance of *RAS* testing between tissue and plasma samples is impaired by the adoption of assays with heterogeneous analytical sensitivity and coverage of genomic regions. There are now several commercially available methods for ctDNA assessment and technology platforms based on digital polymerase chain reaction or next-generation sequencing approaches—each one with specific sensitivity, specificity, throughput, gene coverage, costs, and potential clinical applications.[12] Among them, three test kits are CE-marked in vitro diagnostic devices for detection of *RAS* and *BRAF* mutations on ctDNA in CRC.[13-16]

Third, some clinicopathologic variables are likely to affect the amount of tumor-released ctDNA.[5,6,8,10,11] Whereas liver involvement and tumor burden are positively associated with the *RAS* mutant allele fraction (the proportion of mutant DNA fragments at a given locus), peritoneal, nodal, and lung metastases and mucinous histology are linked to low *RAS* ctDNA detection. Factors that influence ctDNA levels, and the variability in the sensitivity of current technologies, should be considered during interpretation of *RAS* ctDNA results. Although lack of *RAS* mutations detected in ctDNA does not perfectly predict *RAS* wild-type status, the detection of *RAS* mutations is highly reliable for *RAS*-mutant status. Consequently, on the basis of the reported suboptimal sensitivity of ctDNA testing (around 90%), the risk of treating with a null, if not detrimental, effect (ie, with an anti-EGFR-based therapy) false

ASSOCIATED CONTENT

Appendix

Author affiliations and support information (if applicable) appear at the end of this article.

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RAS wild-type cases, as assessed on ctDNA, is not negligible.

The parallel assessment of the mutant allele fractions of other key genomic tumor alterations may help solve challenging cases that have undetectable *RAS* mutations and ctDNA levels at or lower than the analytic sensitivity of adopted assays. To overcome these issues, preanalytic procedures should be standardized, a threshold of detectable mutation rate that confers intrinsic resistance to EGFR inhibition should be set and prospectively validated, and additional investigation to understand when plasma and tissue tests are interchangeable and to improve assay sensitivities is warranted.

Meanwhile, the *RAS* status assessment to address the use of anti-EGFR agents must be performed as the gold standard on tumor specimens.[3,4,17,18] Only when tissue-based testing is technically or logistically unfeasible could it be replaced by ctDNA analysis.[4]

ctDNA to Estimate Prognosis and Detect Minimal Residual Disease

Retrospective studies have focused on the prognostic impact of the quantitative analysis of ctDNA. In the metastatic setting, a correlation between ctDNA concentration and survival has been described, but the independent weight of ctDNA quantification when the relative impact of other well-known prognostic factors is taken into account has not been clarified (Appendix Table A2).

The role of the quantitative assessment of ctDNA in monitoring response during treatment must be defined in the light of its potential added value when compared with easily available and well-established markers, including carcinoembryonic antigen or early radiologic disease reassessment. Recently, ctDNA has been proposed to detect, measure, and monitor residual disease after radical interventions.

A series of proof-of-concept studies reported that liquid biopsy could disclose the persistence of minimal residual disease (MRD) through the detection of ctDNA in patients with CRC who underwent potentially curative surgery (resection of primary tumor in early-stage CRC or radical resection of metastases) even in the absence of clinical or radiologic signs of residual disease (Appendix Table A2). By identifying incomplete eradication of disease after a curative treatment, detectable ctDNA predicts an increased risk of relapse regardless of the exposure to an adjuvant treatment. The development of such a sensitive tool might improve the risk estimation of disease relapse after a curative intervention to properly stratify clinical trials in early-stage CRC and to accordingly drive the therapeutic management.

Theoretically, two applications of the detection of MRD in this setting may be foreseen: to offer chemotherapy to all postoperative ctDNA-positive patients, including those with no histopathologic risk factors to reduce their risk of progression—indeed, ctDNA positivity invariably means residual disease—and to avoid useless adjuvant therapies in postoperative ctDNA-negative patients. Not detecting ctDNA in the postoperative does not invariably mean lack of residual disease, because available assays may have suboptimal sensitivity. As a consequence, postoperative ctDNA cannot yet be regarded as a tool to avoid the recommended treatment in ctDNA-negative patients who are candidates for adjuvant therapy according to clinical practice (ie, those with stage III and high-risk stage II CRC).[19,20] The integration of different sequencing approaches, such as the detection of mutated and methylated ctDNA, could increase the sensitivity of ctDNA detection assays.

Similarly, improvement in the prognostic accuracy for those patients who undergo a radical resection of metastases is clearly needed to establish the best postoperative strategy on an individual basis. If ctDNA is detected immediately after resection, a more intensive approach may be justified.

These applications of ctDNA testing are limited by some open challenges. In early-stage CRC, ctDNA is detected at a lower rate than in the advanced disease.[1] Therefore, highly sensitive techniques are needed to achieve appropriate accuracy to detect MRD.

Most available data have been achieved through a two-step procedure: the identification of specific somatic abnormalities in tissue samples, followed by the search for the same alteration in ctDNA. Currently, a panel including the most frequently and directly detected tumor-specific genomic events in ctDNA has not been validated. Only trials that aim to optimize the treatment of all postoperative ctDNA-positive patients, independent of traditional histopathologic factors, are ethically acceptable.

ctDNA to Track Tumor Response and Resistance to Therapy

Whereas tissue biopsies catch single snapshots of the tumor in a specific spatiotemporal fragment, liquid biopsy may more comprehensively depict the intrinsic and dynamic intratumoral heterogeneity. Serial quantitative and qualitative ctDNA measurements allow longitudinal exploration and tracking of the clonal evolution during and across subsequent systemic treatments.[21-27]

This concept especially fits with the optimization of the use of anti-EGFR agents. Indeed, patients with molecularly selected mCRC who initially benefit from anti-EGFR agents almost invariably experience disease progression.

The molecular landscape of secondary resistance is heterogeneous and partially overlaps with that of primary resistance.[28] Both in vitro and retrospective clinical studies have described the emergence of multiple genomic alterations both inside and outside the EGFR pathway,

including *RAS*, *BRAF*, and *EGFR* ectodomain mutations and *KRAS, HER2,* and *MET* amplifications.[29-33]

Several researchers have used liquid biopsies to monitor treatment effect in patients who receive anti-EGFR–based therapies: the ctDNA analysis of samples collected during treatment has demonstrated the progressive selection of genomic alterations (mainly *RAS* mutant clones) as drivers of secondary resistance to the EGFR blockade even earlier than the radiologic evidence of disease progression. The heterogeneity and dynamism of the tumor clonal evolution under the pressure of targeted treatments are confirmed by the emergence of multiple alterations in the mitogen-activated protein kinase pathway effectors, including *RAS* and *MEK* mutations and *KRAS*, *BRAF*^{V600E}, and *MET* amplification, during the treatment with BRAF/EGFR, BRAF/MEK and BRAF/EGFR/MEK inhibitors in patients with *BRAF*^{V600E}-mutant mCRC.[23-27] Two novel *NTRK1* mutations have been detected as a potential mechanism of acquired resistance to entrectinib, a tyrosine kinase receptor inhibitor, in a patient with *LMNA-NTRK1*–rearranged mCRC.[34] Nevertheless, some steps should be covered to translate liquid biopsy from the investigational setting into clinical practice.

Available data indicate that the frequency of molecular alterations in ctDNA at the time of disease progression is inconsistent among different series, even when the same methods for plasma ctDNA analysis is applied (Table 1). This inconsistency impairs the reliability of adopted techniques and the reproducibility of the findings. Setting and validation of a quantitative threshold to define the clinical relevance of each detectable molecular alteration as clearly associated with a lack of benefit from ongoing therapies may be relevant to biologically guide therapeutic decisions. Currently, no prospective data are available about the usefulness of discontinuing ongoing therapy and initiation of a tailored treatment when signals of acquired resistance emerge in ctDNA before clinical or imaging-based disease progression is noted.

The co-occurrence of multiple and/or subclonal molecular alterations at the time of acquired resistance highlights an increase in tumor heterogeneity, which complicates the definition of clinical value of each identified alteration as therapeutic target for subsequent tailored strategies. In other words, how to therapeutically target the heterogeneous mechanisms of resistance and the subclonal patterns of tumor cell populations that emerge upon drug selection is today still challenging. Most of emerging alterations are not therapeutically actionable, and the most promising approaches (eg, targeting *EGFR* ectodomain mutations by second-generation anti-EGFRs) have failed to prove clinical efficacy.[47]

ctDNA to Select Patients Eligible for Anti-EGFR Rechallenge

In patients who experienced acquired resistance to anti-EGFR agents after an initial clinical benefit, and who are subsequently exposed to at least one other intervening therapy, the reintroduction of an anti-EGFR in later lines has shown promising activity data in a retrospective series.[48] Recent reports disclosed a biologic rationale that supports the reintroduction of the EGFR blockade after an anti-EGFR–free interval. *RAS*-mutated clones emerge at the time of disease progression and then decline with time upon the withdrawal of the anti-EGFR pressure,[7,38,49] which suggests a potential reversibility of the resistant phenotype.

The phase II CRICKET (Phase II Study of Cetuximab Rechallenge in Irinotecan-Pretreated mCRC, *KRAS, NRAS* and *BRAF* Wild-Type Treated in First Line With Anti-EGFR Therapy) trial demonstrated the activity of a cetuximab-based rechallenge strategy in patients with *RAS*/*BRAF* wild-type mCRC whose disease had acquired resistance to first-line cetuximab–containing therapy.[50] This study highlighted the role of liquid biopsy in the selection of optimal candidates for this strategy, because the detection of *RAS* mutations in ctDNA collected before rechallenge is associated with no clinical benefit.

An attempt to strengthen these findings is challenged by the ongoing biomarker-driven CHRONOS (Phase II Trial of Rechallenge With Panitumumab Driven by *RAS* Clonal-Mediated Dynamic of Resistance) study,[51] which adopted ctDNA analysis as an inclusion criterion. In this proof-of-concept study, patients who are candidates for anti-EGFR rechallenge are eligible only if a notable decrease in *RAS* fractional mutational abundance occurs from the time of disease progression after a first-line anti-EGFR–containing therapy to the time of rechallenge.

Liquid biopsy has emerged as a minimally invasive tool to genotype tumors, to assess patient prognosis and detect MRD, to monitor treatment efficacy, and to track the dynamism of clonal evolution over time and therapies. The assessment of ctDNA stands as an intriguing technology to build a personalized and biology-driven continuum of care for patients with CRC.

Awaited results of properly designed ongoing prospective clinical trials, conceived with the purpose of integrating the ctDNA analysis in the therapeutic plan for patients with CRC, could provide acceptable evidence to push liquid biopsy toward clinical practice (Appendix Table A3).

TABLE 1. Summary of Studies That Investigate Genetic Alterations Associated With Secondary Resistance to EGFR Blockade in Metastatic Colorectal Cancer

First Author	No. of Patients	Treatment	Genomic Alterations Investigated in Post-EGFR Therapy Sample	Methods for Plasma ctDNA Analysis	Patients With at Least One Genomic Alteration	Patients With More Than One Genomic Alteration
Bardelli et al[30]	7	Anti-EGFR ± irinotecan	*KRAS* mutations	BEAMing	3 (43)	None
			MET amplification	RT-PCR	3 (43)	
Bettegowda et al[1]	24	Anti-EGFR based	*KRAS* mutations	PCR and Safe-SeqS	22 (92)	15 (63)
			NRAS mutations		9 (38)	
			BRAF mutations		1 (4)	
			EGFR mutations*		2 (8)	
Diaz et al[35]	24	Panitumumab monotherapy	*KRAS* mutations	Ligation and BEAMing	9 (38)	3 (13)
			BRAF mutations		0	
Misale et al[31]	3	Cetuximab monotherapy	*KRAS* mutations	BEAMing	2 (67)	1 (33)
Misale et al[36]	4	Anti-EGFR ± irinotecan	*KRAS* mutations	BEAMing	3 (75)	3 (75)
			NRAS mutations		2 (50)	
			BRAF mutations		0	
Moahn et al[37]	10	Anti-EGFR monotherapy	*KRAS* amplification	Plasma-Seq and ultra-sensitive deep sequencing	4 (40)	1 (10)
			MET amplification		1 (10)	
Morelli et al[38]	62	Anti-EGFR based	*KRAS* mutations	PCR	27 (44)	11 (18)
			EGFR mutation†		5 (8)	
Newhall et al[39]	546	Anti-EGFR monotherapy	*EGFR* mutation†	ddPCR	49 (9)	—
Pietrantonio et al[22]	11	Anti-EGFR ± irinotecan	*KRAS* mutations	ddPCR	4 (36)	2 (18)
			NRAS mutations		0	
			BRAF mutations		1 (9)	
			EGFR mutations‡		1 (9)	
			MET amplification		1 (9)	
			HER2 amplification		1 (9)	
Raghav et al[40]	53	Anti-EGFR based	*MET* amplification	HiSeq	12 (23)	NR
Price et al[41]	164	Panitumumab monotherapy	*RAS* mutations	NGS PlasmaSelect-R	53 (32)	NR
Siena et al[42]	30	Panitumumab + irinotecan	*RAS* mutations	BEAMing	11 (37)	NR
Siravegna et al[7]	16	Anti-EGFR based	*KRAS* mutations	ddPCR and BEAMing	11 (69)	4 (25)
			KRAS amplification		1 (6)	
			EGFR mutations§		2 (13)	
			MET amplification		3 (19)	

(Continued on following page)

TABLE 1. Summary of Studies That Investigate Genetic Alterations Associated With Secondary Resistance to EGFR Blockade in Metastatic Colorectal Cancer (Continued)

First Author	No. of Patients	Treatment	Genomic Alterations Investigated in Post-EGFR Therapy Sample	Methods for Plasma ctDNA Analysis	Patients With at Least One Genomic Alteration	Patients With More Than One Genomic Alteration
Strickler et al[43]	42‖	Anti-EGFR based	*RAS* mutations	Guardant360	28 (67)	—
			BRAF mutations		4 (10)	
			HER2 amplification		5 (12)	
			MET amplification		16 (38)	
			KRAS amplification		10 (24)	
Takegawa et al[44]	18	Cetuximab based	*HER2* amplification	ddPCR	4 (22)	—
Tsuji et al[45]‖	48	Cetuximab based	*RAS* mutations	ddPCR	11/48 (23)	5 (10)
			BRAF mutations		1/46 (2)	
			HER2 amplification		4/42 (10)	
			MET amplification		6/25 (24)	
			EGFR mutations		2/15 (13)	
Vidal et al[5]	18	Anti-EGFR based	*RAS* mutations	OncoBEAM	7 (39)	3 (17)
Xu et al[46]	32	Cetuximab based	*KRAS* mutations	Amplicon deep sequencing	8 (25)	5 (16)
			NRAS mutations		0	
			BRAF mutations		3 (9)	
			EGFR mutations¶		1 (3)	
			PIK3CA mutations		7 (22)	

NOTE. Values represent No. or No. (%). Tsuji et al percentages of genomic alterations were calculated from different total patient numbers, as noted in each cell.

Abbreviations: ctDNA, circulating tumor DNA; ddPCR: digital droplet PCR; EGFR, epidermal growth factor receptor; NGS, next-generation sequencing; NR, not reported; PCR, polymerase chain reaction; RT-PCR: real-time PCR.

*Data refer to analysis of *EGFR* 714 and 749 codons.

†Data refer to detection of *EGFR* p.S492R mutation.

‡Data refer to analysis of *EGFR* exons from 1 to 14.

§Data refer to detection of three *EGFR* point mutations: p.S464L, p.G465R, p.G465E.

‖All patients had extracellular domain *EGFR* mutations on ctDNA.

¶Data refer to analysis of *EGFR* exons 10 and 12.

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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TABLE A1. Overview of Studies That Compare the *RAS* and *BRAF* Molecular Status Tested on Tumor Tissue and Plasma ctDNA

First Author	No. of Patients	Methods of Analysis		*RAS*					*BRAF*				
		Plasma ctDNA	Tumor Tissue	Concordance (%)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Concordance (%)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Thierry et al[a]	95	PCR-based method	SOC	96[b]	92[b]	98[b]	97	95	100	100	100	100	100
Vidal et al[5]	115	OncoBEAM	SOC	93	96	90	90	96	—	—	—	—	—
Normanno et al[11]	92	OncoBEAM	Ion Ampliseq	78	70	83	70	83	—	—	—	—	—
Grasselli et al[10]	146	BEAMing	Real-time PCR and BEAMing	90[c]	89[c]	90[c]	84[c]	93[c]	—	—	—	—	—
				88[d]	85[d]	91[d]	89[d]	88[d]	—	—	—	—	—
Bachet et al[6]	412	NGS	SOC	85	76	98	98	74	—	—	—	—	—
	329	NGS and ddPCR	SOC	95	93	98	98	90	—	—	—	—	—
Thierry et al[e]	—	PCR-based method	SOC	72[f]	85[f]	62[f]	63[f]	84[f]	87[g]	57[g]	89[g]	29[g]	96[g]
				74[h]	83[h]	71[h]	38[h]	95[h]					
				92[i]	67[i]	94[i]	50[i]	97[i]					
Schmiegel et al[8]	90	OncoBEAM	SOC	92	92	93	94	90	—	—	—	—	—
Jones et al[9]	238	OncoBEAM	SOC	93	93	94	94	92	—	—	—	—	—
Siravegna et al[7]	100	ddPCR and BEAMing	SOC	97	95	100	100	94	100	100	100	100	100
Bettegowda et al[1]	206	Safe-SeqS	PCR and Safe-SeqS	95[j]	87[j]	99[j]	98[j]	93[j]	—	—	—	—	—
Diaz et al[35]	28	Ligation and BEAMing	Real-time PCR	96[h]	75[h]	100[h]	100[h]	96[h]	—	—	—	—	—
Toledo et al[k]	25	BEAMing	Ion torrent	100[l]	100[l]	100[l]	100[l]	100[l]	100[m]	100[m]	100[m]	100[m]	100[m]
				100[n]	100[n]	100[n]	100[n]	100[n]					
Spindler et al[o]	221	PCR-based method	PCR and SOC	85[b]	80[b]	96[b]	97[b]	71[b]	—	—	—	—	—
Thierry et al[p]	42	PCR-based method	NGS	71[j]	95[j]	48[j]	64[j]	91[j]	97	100	97	67	100

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TABLE A1. Overview of Studies That Compare the *RAS* and *BRAF* Molecular Status Tested on Tumor Tissue and Plasma ctDNA (Continued)

		Methods of Analysis		*RAS*					*BRAF*				
First Author	No. of Patients	Plasma ctDNA	Tumor Tissue	Concordance (%)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Concordance (%)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Danese et al[q]	85	PCR-based method	PCR-based method	89[j]	85[j]	93[j]	85[j]	93[j]	—	—	—	—	

Abbreviations: ctDNA, circulating tumor DNA; ddPCR, digital droplet PCR; NGS, next-generation sequencing; NPV, negative predictive value; PCR, polymerase chain reaction; PPV, positive predictive value; SOC, standard of care.

[a]Thierry AR, et al: Nat Med 20:430-435, 2014.

[b]Data refer to seven point mutations of *KRAS*.

[c]Data refer to tumor tissue analysis with real-time PCR and plasma analysis with BEAMing, carried out in 146 samples.

[d]Data refer to tumor tissue and plasma analysis with BEAMing, carried out in 130 samples.

[e]Thierry AR, et al: Ann Oncol 28:2149-2159, 2017.

[f]Data refer to *KRAS* exon 2 point mutations, carried out in 121 samples.

[g]Carried out in 97 samples.

[h]Data refer to *KRAS* exons 3 and 4 point mutations, carried out in 34 samples.

[i]Data refer to *NRAS* exons 2 and 3 point mutations, carried out in 34 samples.

[j]Data refer to *KRAS* codons 12 and 13 point mutations.

[k]Toledo RA, et al: Oncotarget 8:35289-35300, 2017.

[l]Data refer to *KRAS* codons 12, 13, 61, and 146 point mutations, carried out in 25 samples.

[m]Data refer to *BRAF* point mutation, carried out in 22 samples.

[n]Data refer to *NRAS* codon 61 point mutations, carried out in 13 samples.

[o]Spindler KL, et al: PLoS One 10:e0108247, 2015.

[p]Thierry AR, et al: Clin Cancer Res 23:4578-4591, 2017.

[q]Danese E, et al: PLoS One 10:e0126417, 2015.

TABLE A2. Overview of Studies That Investigate ctDNA as a Prognostic Tool in Localized and Metastatic Colorectal Cancer

First Author by Disease Stage	No. of Patients	Study Population	Methods for Plasma ctDNA Analysis	Genomic Alterations Investigated on Plasma ctDNA	Key Findings
Early-stage disease					
Diehn et al[a]	144	Curatively resected stage II to III	NGS	One of the single nucleotide variants identified in the tumor tissue	Worse prognosis (2-year RFS, TTR, and OS) for patients with postoperative detectable ctDNA
Schøler et al[b]	21	Curatively resected stage I to III	ddPCR	One of the somatic structural variants and somatic point mutations identified in the tumor tissue	Worse prognosis (RFS and 5-year OS) for patients with postoperative detectable ctDNA
Thomsen et al[c]	294	Curatively resected stage I to III	ddPCR	*RAS* mutation	Worse prognosis (DFS and OS) for patients bearing *RAS* mutation in ctDNA
				BRAF mutation	Worse prognosis (DFS and OS) for patients bearing *BRAF* mutation combined with pMMR assessed on tumor tissue
Tie et al[d]	230	Curatively resected stage II	Safe-SeqS	One of the mutations identified in the tumor tissue	Worse prognosis (3-year RFS) for patients not treated with adjuvant chemotherapy with postoperative detectable ctDNA
					No difference in outcome (RFS) for patients not treated with adjuvant chemotherapy, according to postoperative CEA
					Worse prognosis (RFS) for patients with ctDNA positivity immediately after completion of adjuvant chemotherapy
					Postoperative ctDNA status predicts prognosis status regardless of individual clinicopathologic risk features
					Serial ctDNA measurements appear more sensitive than CEA measurement for predicting radiologic recurrence
Tie et al[e]	159	LARC after curative chemoradiation and surgery	Safe-SeqS	One of the mutations identified in the tumor tissue	Worse prognosis (RFS) for patients with postchemoradiation and postsurgery detectable ctDNA
					Postoperative ctDNA status is an independent predictor of RFS after analysis adjusted for known clinicopathologic risk factors
Tie et al[f]	95	Curatively resected stage II	Safe-SeqS	One of the mutations identified in the tumor tissue	Worse prognosis (RFS) for patients with postoperative detectable ctDNA
					Worse prognosis (RFS) for patients with post-treatment detectable ctDNA
Metastatic disease					
Bedin et al[g]	114	Stages I to IV	ALU-based qPCR	ALU83 and ALU244 fragment dosage (quantification of ctDNA)	Worse prognosis (OS) for patients with baseline elevated ctDNA levels

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TABLE A2. Overview of Studies That Investigate ctDNA as a Prognostic Tool in Localized and Metastatic Colorectal Cancer (Continued)

First Author by Disease Stage	No. of Patients	Study Population	Methods for Plasma ctDNA Analysis	Genomic Alterations Investigated on Plasma ctDNA	Key Findings
Bettegowda et al[10]	206	Stage IV	qPCR	ctDNA mutant fragments	Correlation between ctDNA concentration and survival (OS)
El Messaoudi et al[h]	97	Stage IV	qPCR-based method	*KRAS* and *BRAF* mutations	Worse prognosis (OS) for patients with high ctDNA levels
					Worse prognosis (OS) for patients bearing *BRAF* mutation in ctDNA
Garlan et al[i]	82	Stage IV	ddPCR	mutations of *KRAS*, *BRAF*, *TP53*, or hypermethylation of *WIF1* and *NPY*	Worse prognosis (OS) for patients with high (> 10 ng/mL) *v* low (≤ 0.1 ng/mL) baseline ctDNA levels
					Better outcome (ORR, PFS, and OS) for patients with early change[j] in ctDNA level
Overmann et al[k]	54	Stage IV, with liver metastases (treated with curative intent)	HiSeq	One of the mutations identified in the tumor tissue	Worse prognosis (RFS) for patients with ctDNA positivity after successful resection of liver metastases
Schøler et al[b]	23	Stage IV, with liver metastases (treated with curative intent)	ddPCR	One of the somatic structural variants and somatic point mutations identified in the tumor tissue	Worse prognosis (RFS) for patients with postoperative detectable ctDNA
Spindler et al[l]	229	Stage IV	qPCR-based method	*KRAS* mutations	Worse prognosis (OS) for patients with baseline elevated ctDNA levels.
Tie et al[m]	42	Stage IV	Safe-SeqS	One of the mutations identified in the tumor tissue	Better outcome (early tumor response[n] and PFS) for patients with early change[o] in ctDNA level

Abbreviations: ALU sequence, short stretch of DNA originally characterized by the action of the Arthrobacter luteus (Alu) restriction endonuclease; CEA, carcinoembryonic antigen; ctDNA, circulating tumor DNA; ddPCR, digital droplet PCR; DFS, disease-free survival; LARC, locally advanced rectal cancer; NGS, next-generation sequencing; ORR, objective response rate; OS, overall survival; PCR, polymerase chain reaction; pMMR, proficient mismatch repair; qPCR, quantitative PCR; RFS, relapse-free survival; TTR, time to recurrence.

[a]Diehn M, et al: J Clin Oncol 35, 2017 (suppl; abstr 3591).

[b]Schøler LV, et al: Clin Cancer Res 23:5437-5445, 2017.

[c]Thomsen CEB, et al: Cancer Med 6:928-936, 2017.

[d]Tie J, et al: Sci Transl Med 8:346ra92, 2016.

[e]Tie J, et al: Gut doi: 10.1136/gutjnl-2017-315852 [epub ahead of print on February 2, 2018].

[f]Tie J, et al:. J Clin Oncol 36, 2018 (suppl; abstr 3516).

[g]Bedin C, et al: Int J Cancer 140:1888-1898, 2017.

[h]El Messaoudi S, et al: Clin Cancer Res 22:3067-3077, 2016.

[i]Garlan F, et al: Clin Cancer Res 23:5416-5425, 2017.

[j]Evolution of the ctDNA concentration between baseline (before first cycle) and second and/or third cycle.

[k]Overman MJ, et al: J Clin Oncol 35, 2017 (suppl; abstr 3522).

[l]Spindler KL, et al: PLoS One 10:e0108247, 2015.

[m]Tie J, et al: Ann Oncol 26:1715-1722, 2015.

[n]Defined as 20% or greater reduction in the sum of largest diameters according to RECIST criteria, assessed by computed tomography 8 to 10 weeks after treatment initiation.

[o]Defined as at least 10-fold reduction in ctDNA levels between baseline (before first cycle) and second cycle.

TABLE A3. Ongoing TrialsThat Incorporate ctDNA Analysis As a Criterion for Patient Selection

Trial (trial identifier) by Disease Type	Study Type	Estimated No. of Patients Enrolled	Study Population	Criteria for Patient Selection to ctDNA	Study Intervention	Primary End Point	Study Location
Localized disease							
DYNAMIC (ACTRN12615000381583)[a]	NA	450	Stage II curatively resected	Postoperative detection of ctDNA for arm A	Arm A: ctDNA-positive patients, adjuvant chemotherapy[b]; ctDNA-negative patients, observation *v* Arm B: SOC, at the discretion of the treating clinician	RFS	Australia
DYNAMIC-III (ACTRN12617001566325)[c]	II/III, randomized	1,000	Stage III curatively resected	Postoperative detection of ctDNA for arm B	Arm A: SOC, at the discretion of the treating clinician *v* Arm B: ctDNA-positive patients, escalation adjuvant treatment strategy; ctDNA-negative patients, de-escalation adjuvant treatment strategy	3-year RFR	Australia
DYNAMIC-RECTAL (ACTRN12617001560381)[d]	NA	408	LARC after curative chemoradiation and surgery	Postoperative detection of ctDNA for arm B	Arm A: SOC, at the discretion of the treating clinician *v* Arm B: ctDNA-positive patients, 4 months of adjuvant therapy; ctDNA-negative patients, if pathologic high-risk disease, treatment at the clinician's discretion; if pathologic intermediate- or low-risk disease, observation	No. of patients receiving adjuvant therapy	Australia
IMPROVE (EUDRACT-2018-000070-30)[e]	II, randomized	64	Stage I or II curatively resected[f]	Postoperative detection of ctDNA	Arm A: intensified follow-up *v* Arm B: XELOX for eight cycles and intensified follow-up	3-year DFS	Denmark
Metastatic disease							
PANIRINOX (NCT02980510)[g]	II, randomized	209	Stage IV first-line therapy	*RAS* and *BRAF* wild type	mFOLFOX6 plus panitumumab *v* FOLFIRINOX plus panitumumab	CR rate in FOLFIRINOX plus panitumumab arm	France
CHRONOS (NCT03227926)[h]	II	129	Stage IV third-line therapy[i]	*RAS*-extended mutational load between basal and rechallenge mutation load checkpoints	Rechallenge with panitumumab	ORR	Italy

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TABLE A3. Ongoing TrialsThat Incorporate ctDNA Analysis As a Criterion for Patient Selection (Continued)

Trial (trial identifier) by Disease Type	Study Type	Estimated No. of Patients Enrolled	Study Population	Criteria for Patient Selection to ctDNA	Study Intervention	Primary End Point	Study Location
NCT03087071[j]	II, randomized	84	Stage IV cetuximab-refractory disease	Treatment allocation according to *RAS*, *BRAF*, and *EGFR* mutational status	Panitumumab *v* panitumumab and trametinib	ORR	USA
TRIUMPH (UMIN000027887)[k]	II	36	Stage IV refractory disease	*ERBB2* amplification	Trastuzumab plus pertuzumab	ORR	Japan

Abbreviations: CR, complete response; ctDNA, circulating tumor DNA; DFS, disease-free survival; EGFR, epidermal growth factor receptor; FOLFIRINOX, regimen including 5-fluoruracil, irinotecan, oxaliplatin; LARC, locally advanced rectal cancer; mFOLFOX, modified regimen including 5-fluoruracil and oxaliplatin; NA, not applicable; ORR, objective response rate; RFR, relapse-free rate; RFS, relapse-free survival; SOC, standard of care; XELOX, regimen including capecitabine and oxaliplatin.

[a]Australian New Zealand Clinical Trials Registry: DYNAMIC trial. https://www.anzctr.org.au/Trial/Registration/TrialReview.aspx?id=368173.

[b]Patients treated with chemotherapy receive a single-agent fluorouracil-based regimen (including capecitabine) or fluoropyrimidine plus oxaliplatin.

[c]Australian New Zealand Clinical Trials Registry: DYNAMIC-III trial. https://gicancer.org.au/clinical-trial/dynamic111/.

[d]Australian New Zealand Clinical Trials Registry: DYNAMIC-RECTAL trial. https://gicancer.org.au/clinical-trial/dynamic-rectal/.

[e]EUdract Clinical Trials Registry: IMPROVE trial. https://www.clinicaltrialsregister.eu/ctr-search/trial/2018-000070-30/DK.

[f]Patients without indication for adjuvant chemotherapy according to Dutch Colorectal Cancer Group guidelines.

[g]ClinicalTrials.gov: PANIRINOX trial. https://clinicaltrials.gov/ct2/show/NCT03259009.

[h]ClinicalTrials.gov: CHRONOS trial. https://clinicaltrials.gov/ct2/show/NCT03227926.

[i]Main eligibility criteria: (1) imaging documented complete or partial response (according to RECIST 1.1 criteria) to first-line anti-EGFR–based therapy and progression while on therapy or maintenance regimen, including anti-EGFR agent; (2) planned second-line treatment of any type with the exclusion of additional anti-EGFRs; (3) *RAS*-extended mutational load with more than 3% fractional abundance, measured on plasma ctDNA at baseline mutational load (maximum within 2 weeks of last anti-EGFR administration); (4) a more than 50% decrease in *RAS*-extended mutational load between baseline mutational load and rechallenge mutational load.

[j]ClinicalTrials.gov: Panitumumab in combination with trametinib in cetuximab-refractory stage IV colorectal cancer. https://clinicaltrials.gov/ct2/show/NCT03087071.

[k]UMIN Clinical Trials Registry: TRIUMPH trial. https://upload.umin.ac.jp/cgi-open-bin/ctr_e/ctr_view.cgi?recptno=R000031949