Review Article

Ongoing Clinical Trials and Future Research Scenarios of Circulating Tumor DNA for the Treatment of Metastatic Colorectal Cancer

Laura Roazzi,[1,2,#] Giorgio Patelli,[1,2,3,#] Katia Bruna Bencardino,[2] Alessio Amatu,[2] Erica Bonazzina,[2] Federica Tosi,[2] Brunella Amoruso,[4,5] Anna Bombelli,[2] Sara Mariano,[2] Stefano Stabile,[2] Camillo Porta,[4,5] Salvatore Siena,[1,2] Andrea Sartore-Bianchi[1,2,6]

Abstract

Liquid biopsy using circulating tumor DNA (ctDNA) has emerged as a minimally invasive, timely approach to provide molecular diagnosis and monitor tumor evolution in patients with cancer. Since the molecular landscape of metastatic colorectal cancer (mCRC) is substantially heterogeneous and dynamic over space and time, ctDNA holds significant advantages as a biomarker for this disease. Numerous studies have demonstrated that ctDNA broadly recapitulates the molecular profile of the primary tumor and metastases, and have mainly focused on the genotyping of *RAS* and *BRAF*, that is propaedeutic for anti-EGFR treatment selection. However, ctDNA soon broadened its scope towards the assessment of early tumor response, as well as the identification of drug resistance biomarkers to drive potential molecular actionability. In this review article, we provide an overview of the current state-of-the-art of this methodology and its applications, focusing on ongoing clinical trials that employ ctDNA to prospectively guide treatment in patients with mCRC.

Clinical Colorectal Cancer, Vol. 000, No.xxx, 1–14
Keywords: anti-EGFR, Colorectal cancer, ctDNA, RAS, Resistance dynamics

Introduction

The concept of liquid biopsy, that is the detection and monitoring of tumor evolution in patients' body fluids, gained prominence in the last decade thanks to its advantages over tissue biopsies, such as minimal invasiveness, molecular comprehensiveness and faster turnaround times.[1] Various cancer biomarkers, notably circulating tumor DNA (ctDNA), can be isolated from liquid biopsy samples, blood first, and inform about the presence of a tumor and its characteristics.[1-3] Since colorectal cancer (CRC) is a significant ctDNA shedder among solid tumors,[4-7] the development of liquid biopsy towards clinical practice has been prolific in this disease.

Around 50% of CRC patients are diagnosed with or will eventually develop metastatic disease (mCRC).[8] Precision oncology plays a pivotal role in this context, as the identification of drug targets and resistance alterations refines patients' selection towards more effective therapies and improved outcomes, while avoiding unnecessary toxicity.[6,9] In this clinical scenario, ctDNA offers an opportunity to timely pick the optimal treatment by providing a broader view of tumor molecular features when compared to tissue biopsy, through the identification of gene alterations that are the consequence of spatial and temporal heterogeneity.[1,3] At the same time, ctDNA still faces challenges that have somehow precluded extensive adoption in everyday clinical practice. Cost of testing, technical and biological limitation accounting for the issue of false negative and positive results, and lack of actionability of acquired mutations, all contribute to mitigate full application of liquid biopsy in the clinical setting.[6]

Several reviews are available concerning the role of ctDNA in mCRC.[10-12] Here, we focus on ongoing studies and how they fit into different scenarios of potential application for leveraging the use of ctDNA in this tumor type.

[1]Department of Oncology and Hemato-Oncology, Università degli Studi di Milano, Milan, Italy
[2]Department of Hematology, Oncology, and Molecular Medicine, Grande Ospedale Metropolitano Niguarda, Milan, Italy
[3]IFOM ETS – The AIRC Institute of Molecular Oncology, Milan, Italy
[4]Division of Medical Oncology, A.O.U. Consorziale Policlinico di Bari, Bari, Italy
[5]Interdisciplinary Department of Medicine, University of Bari "Aldo Moro", Bari, Italy
[6]Division of Clinical Research and Innovation, Grande Ospedale Metropolitano Niguarda, Milan, Italy

Submitted: Sep 14, 2023; Revised: Jan 4, 2024; Accepted: Feb 11, 2024; Epub: xxx

Address for correspondence: Salvatore Siena and Andrea Sartore-Bianchi, Department of Oncology and Hemato-Oncology, Università degli Studi di Milano, ASST Grande Ospedale Metropolitano Niguarda, Piazza Ospedale Maggiore, 3, 20162 Milan, Italy
E-mail contact: salvatore.siena@unimi.it, andrea.sartorebianchi@unimi.it

[#] Equally contributed as first author.

1533-0028/$ - see front matter
https://doi.org/10.1016/j.clcc.2024.02.001

Please cite this article as: Laura Roazzi et al, Ongoing Clinical Trials and Future Research Scenarios of Circulating Tumor DNA for the Treatment of Metastatic Colorectal Cancer, Clinical Colorectal Cancer, https://doi.org/10.1016/j.clcc.2024.02.001

ctDNA-Based Molecular Characterization of mCRC: Current Scenario

Several studies have demonstrated ctDNA capability to recapitulate tumor molecular findings with high concordance to tissue analysis, while providing greater sensitivity for low-allele frequency alterations and shorter turnaround times.[9,13-16] As a matter of concern, the concordance of molecular profiles between ctDNA and tissue analyses may be influenced by metastatic sites and disease characteristics, since liver-only metastatic disease and elevated tumor burden were reported to be correlated with a higher *RAS* status concordance.[17]

The first application of ctDNA in mCRC was accordingly the refinement of the mutational status of the *RAS* and *BRAF* genes, that must be confirmed wild type for patient selection towards anti-EGFR treatment together with chemotherapy in first line.[18-24] Besides, ctDNA was employed to assess early tumor response during treatment, as a decrease in overall ctDNA can predict tumor response already after 2 weeks of treatment, well ahead of conventional imaging and CEA standard biomarker.[25-31]

The role of ctDNA, however, goes far beyond *RAS* and *BRAF* mutations, with retrospective studies focusing on additional resistance biomarkers to panitumumab and cetuximab. Several alterations were demonstrated to be implicated in tumor response optimization, such as gene mutations of *ERBB2, EGFR* ectodomain (ECD), *FGFR1, PDGFRA, PIK3CA, PTEN, AKT1* and *MAP2K1*, amplifications of *KRAS, ERBB2, MET*, and fusions of *ALK, ROS1, NTRK1-3* and *RET*.[32-38] This was proved to be relevant both before treatment administration for primary resistance mechanisms, and during treatment for the emergence of acquired mutations as a mechanism of secondary resistance.[39,40] These findings were indeed confirmed and extended further by the preliminary results of a retrospective biomarker analysis (NCT02394834) from the prospective PARADIGM phase 3 trial (NCT02394795). This study showed a correlation between the absence of ctDNA alterations in most of the aforementioned primary resistance genes and improved benefit by anti-EGFR therapy. Importantly, when comparing chemotherapy with panitumumab or bevacizumab, negative ultra-selection through ctDNA analysis led to a longer OS with panitumumab over bevacizumab, independently from primary tumor sidedness.[41]

Similarly, ctDNA is applicable for the detection of drug targets beyond anti-EGFR treatment in mCRC and their related resistance mechanisms, enabling further tailored therapeutic strategies in the *continuum of care*.[42,43] This is the case of $BRAF^{V600E}$ mutations for the combination of encorafenib and cetuximab, *ERBB2* amplifications for dual blockade therapies such as trastuzumab with pertuzumab or lapatinib or tucatinib, $KRAS^{G12C}$ mutations that are sensitive to sotorasib or adagrasib with panitumumab/cetuximab, microsatellite instability for treatment with immune checkpoint inhibitors, but also rarer *NTRK1-3, RET, FGFR2-3, ALK*, and *ROS1* fusions.[29,44-52]

Novel applications of ctDNA in precision oncology lay in the selection of patients for anti-EGFR rechallenge and "enhanced" rechallenge for mCRC. Rechallenge therapy refers to the retreatment with anti-EGFR agents in chemorefractory *RAS* wild-type patients with mCRC, that previously achieved benefit and subsequently progressed to panitumumab or cetuximab-based therapies.[53] In this situation, resistant clones are selected upon the exposure to EGFR blockade, but then tend to decline upon the withdrawal of such agents, allowing for renewed sensitivity to rechallenge strategies.[9,53-55]

Studies have shown that patients who had multiple anti-EGFR lines experience fluctuating levels of ctDNA *RAS* mutations, which provides a molecular basis for the efficacy of rechallenge. This was confirmed by several trials, which revealed that having *RAS* wild-type ctDNA at the time of rechallenge was a necessary precondition for response.[56-61] The screening of candidates for rechallenge was indeed improved by ctDNA triage: the CHRONOS phase II trial adopted for the first time a prospective ctDNA assessment for *RAS, BRAF* and *EGFR* ECD mutations to guide panitumumab rechallenge in patients with mCRC. The trial demonstrated encouraging outcomes with a 30% overall response rate (ORR), favorably comparing with standard third-line treatments.[62]

Finally, with the term "enhanced anti-EGFR rechallenge" we refer to the simultaneous targeting, together with anti-EGFR, of another actionable biomarker (or broader contexts of susceptibility), that is choosen based on ctDNA analysis.[10] This approach, which involves combinations of drugs to overcome resistance, is currently under investigation in several ongoing trials, although some initial reports, such as with the use of combined EGFR and MEK inhibitors, were negative.[63]

A comprehensive report of the completed studies that provide original data supporting ctDNA advancements in mCRC is listed in Table 1 . Table 2 summarizes an update of ongoing studies that still address ctDNA retrospectively in this disease.

Future Scenarios: Ongoing Trials and the Promise of ctDNA-Guided Strategies in mCRC

ctDNA-guided strategies refer to those approaches that employ ctDNA as a molecular biomarker to guide treatment decisions and monitor disease progression in patients with mCRC. These strategies involve the detection, analysis, and interpretation of ctDNA at baseline to inform personalized treatment choices. As previously discussed, the CHRONOS trial (NCT03227926) was the first published ctDNA-guided study that successfully employed liquid biopsy to triage patients with mCRC towards anti-EGFR rechallenge.[62] In parallel, the REMARRY-PURSUIT phase II trial (UMIN000036424; jRCTs031190096) similarly addressed ctDNA *RAS* dynamics to drive anti-EGFR rechallenge, but revealed a lower-than-expected ORR (14%) potentially because *BRAF* and *EGFR* mutations were not included in the molecular exclusion criteria for rechallenge (differently from CHRONOS).[64] Apart from anti-EGFR agents, ctDNA-guided selection for targeted therapies is limited to anti-HER2 agents in mCRC. Indeed, the TRIUMPH phase II trial (UMIN000027887) adopted ctDNA-guided treatment with pertuzumab plus trastuzumab for *ERBB2*-amplified mCRC, stratifying patients according to efficacy with similar accuracy to tissue genotyping (ORR 28%).[29,44] These three ctDNA-triaged trials represent the only published examples of such kind and are presented along with their results in Table 3. Other ctDNA-guided studies are currently in progress and could provide further insights; in the following sections we present ongoing ctDNA-

Please cite this article as: Laura Roazzi et al, Ongoing Clinical Trials and Future Research Scenarios of Circulating Tumor DNA for the Treatment of Metastatic Colorectal Cancer, Clinical Colorectal Cancer, https://doi.org/10.1016/j.clcc.2024.02.001

Table 1 Completed/Retrospective ctDNA Studies Adopting ctDNA in mCRC

Trial/Author	N Pts	Phase/Design	Targeted Agents	Line of Therapy
EGFR-targeted therapy				
PARADIGM/Watanabe J, JAMA. 2023	733	III/randomized	Panitumumab + chemotherapy	First line
FIRE-4/AIO KRK-0114/Stintzing S, JCO, 2023	673	III/randomized	Cetuximab + chemotherapy	First line
MODUL/NCT02291289	609	II/randomized	Any targeted therapy + chemotherapy maintenance	First line
WJOG8916G/Izawa N, Target Oncol. 2023	56	II	Cetuximab + trifluridine-tipiracil	Refractory (rechallenge)
VELO/Napolitano S, JAMA Oncol. 2023	49	II/randomized	Panitumumab + trifluridine-tipiracil	Refractory (rechallenge)
CONSORT/Topham JT, J Clin Oncol. 2023	169	Observational	Cetuximab or panitumumab	Third line
PANIB/Jassens K, Clin Cancer Res. 2023	40	II/randomized	Panitumumab + chemotherapy	First line
PLATFORM-B/Vidal J, Clin Cancer Res. 2023	100	Observational	Cetuximab + chemotherapy	First line
CALGB/SWOG-80405/Raghav K, J Clin Oncol. 2023	61	III/randomized	Cetuximab + chemotherapy	First line
PRJCA008093/Yang W, Front Oncol. 2022	22	Observational	Cetuximab + chemotherapy	Any
Mariani S, Front Oncol. 2022	26	Observational	Panitumumab or cetuximab-based therapy	Any
Sato S, Anticancer Res. 2022	129	Observational	Panitumumab or cetuximab-based therapy	First line
PERSEIDA/Valladares-Ayerbes M, Cancers (Basel). 2022	119	Observational	Panitumumab + chemotherapy	First line
NCT04831528/Yang W, Front Oncol. 2022	22	Observational	Cetuximab-based therapy	Any
NCT04228614/Wang F, Gut. 2022	171	Observational	Any	First line
CAVE/Martinelli E, Jama Oncol. 2021	67	II	Cetuximab + avelumab	Refractory (rechallenge)
Lim Y, Sci Rep. 2021	93	Observational	Cetuximab-based therapy	First line
PARERE/Moretto R, Clin Colorectal Cancer. 2021	214	II/randomized	Panitumumab monotherapy	Refractory
JACCRO CC-08 and CC-09/Sunakawa J, JCO Precis Oncol. 2020	16	Observational	Panitumumab or cetuximab-based therapy	Refractory
Knebel FH, Cancers (Basels). 2020	10	Observational	Panitumumab or cetuximab-based therapy	Any
CRICKET/Cremolini C, Jama Oncol. 2019	25	II	Cetuximab + chemotherapy	Refractory (rechallenge)
IMPACT-CRC/van Helden E, Mol Oncol. 2019	34	I-II	Cetuximab monotherapy	Refractory
Maurel J, JCO Precis Oncol. 2019	178	II	Panitumumab or cetuximab-based therapy	First line
ASPECCT/Peeters M, Clin Cancer Res. 2019	261	III/randomized	Panitumumab monotherapy	Refractory
Siena S, Ann Oncol. 2018	39	II	Panitumumab + chemotherapy	Refractory
Montagut C, JAMA Oncol. 2018	193	II/randomized	Futuximab and modotuximab	Refractory
CAPRI-GOIM/Normanno N, Ann Oncol. 2018	92	II	Cetuximab + chemotherapy	First line
Strickler JH, Cancer Discov. 2018	24	Observational	Panitumumab or cetuximab-based therapy	Any
Grasselli J, Ann Oncol. 2017	146	II	Panitumumab or cetuximab-based therapy	Any
Toledo RA, Oncotarget. 2017	23	Observational	Cetuximab + chemotherapy	First line
Pietrantonio F, Clin Cancer Res. 2017	22	Observational	Panitumumab or cetuximab-based therapy	Any
Vidal J, Ann Oncol. 2017	115	Observational	Panitumumab or cetuximab-based therapy	Any
PLACOL/Garlan F, Clin Cancer Res. 2017	82	Observational	Any	First or second line
Siravegna G, Nat Med. 2015	100	Observational	Panitumumab or cetuximab-based therapy	Any
Misale S, Nature. 2012	3	Observational	Panitumumab or cetuximab-based therapy	Any
Diaz L, Nature. 2012	28	II	Panitumumab monotherapy	Refractory
Spindler K-L G, Clin Cancer Res. 2012	108	Observational	Cetuximab + chemotherapy	Refractory
Montagut C, Nat Med. 2012	10	Observational	Cetuximab-based therapy	Any
HER2-targeted therapy				
Strickler JH, J Clin Oncol. 2023	71	II/randomized	Trastuzumab + tucatinib	Refractory
DESTINY-CRC01/Siena S, J Clin Oncol. 2021	53	II	Trastuzumab-deruxtecan	Refractory
HERACLES A/Siravegna G, Clin Cancer Res. 2019	29	II	Trastuzumab + lapatinib	Refractory

(*continued on next page*)

Please cite this article as: Laura Roazzi et al, Ongoing Clinical Trials and Future Research Scenarios of Circulating Tumor DNA for the Treatment of Metastatic Colorectal Cancer, Clinical Colorectal Cancer, https://doi.org/10.1016/j.clcc.2024.02.001

Table 1 (*continued*)

Trial/Author	N Pts	Phase/Design	Targeted Agents	Line of Therapy
BRAF-targeted therapy				
URBAN/Bergamo F, J Clin Oncol. 2023	40	Observational	BRAFi + anti-EGFR +/- MEK inhibitors	Second/Third line
BEACON/Koptez S, J Clin Oncol. 2022	544	III/randomized	Encorafenib + cetuximab +/- binimetinib	Second/Third line
NCT04790448/Fang-Ye L, Drug Resist Updat. 2022	36	Observational	Vemurafenib + cetuximab + chemotherapy	Refractory
KRAS G12C-targeted therapy				
NCT04585035/Ruan D, J Clin Oncol. 2023	24	I-II	D-1553	Refractory

Abbreviations: N = number; pts = patients.

guided trials according to the relevant clinical question they are addressing for mCRC management (Table 4).

1. In First Line: can ctDNA inform the decision for the best combination (anti-EGFR vs anti-VEGF) with backbone chemotherapy?

Robust scientific evidence that derives from decades of clinical research has established the standard first-line therapy for microsatellite stable (MSS) mCRC. This consists of a combination of cytotoxic chemotherapy with 5-fluoruracil, oxaliplatin, and/or irinotecan, along with a monoclonal antibody targeting either EGFR (cetuximab or panitumumab) or VEGF (bevacizumab). Anti-EGFR agents are considered the primary treatment option for *RAS*/*BRAF* wild-type tumors, while mutations in *RAS* (*KRAS, NRAS*) and *BRAF* render anti-EGFR agents ineffective, allowing for the utilization of bevacizumab.[16,18,65-71]

Additionally, in right-sided mCRC, it is recommended to avoid anti-EGFR therapy due to limited clinical benefit observed in this subset of patients.[18] This recommendation is based on the understanding that right-sided tumors commonly exhibit distinct molecular characteristics that confer resistance to EGFR inhibition, including activation of multiple downstream signaling effectors.[72-75] While the mutational status of mCRC is typically determined through tissue sample analysis, which may involve archive specimens, it is important to acknowledge that a single spatially circumscribed test may not fully capture the full molecular characteristics of a tumor. Given the elevated intratumoral heterogeneity commonly observed in mCRC, efforts are being made to overcome this limitation.[1,3] These include exploring the use of ctDNA for comparison with tissue analysis and incorporating ctDNA-guided strategies in ongoing trials to guide more tailored treatment approaches.

In this respect, Stintzing et al. presented baseline liquid biopsy data from FIRE-4 trial (NCT02934529), focusing on patients with *RAS* wild-type based on tumor tissue samples, detecting an additional 13% of patients having a *RAS* mutation at baseline. Remarkably, these patients showed outcome characteristics expected for *RAS* mutant patients when treated with first-line FOLFIRI-cetuximab. On the other hand, authors showed that emerging *RAS* mutation during anti-EGFR therapy did not correlate with shorter overall survival, but rather with prolonged exposure to anti-EGFR treatment.[76]

The LIBImAb study (NCT04776655) is also addressing optimization of outcomes to anti-EGFR in first line through molecular refinement by ctDNA. This ongoing phase III study aims to evaluate ctDNA-guided decisions regarding the first-line treatment choice between bevacizumab or cetuximab in combination with FOLFIRI in patients with mCRC harboring *RAS*/*BRAF* wild-type tumors, initially determined by tissue analysis. In the event of a detectable *RAS* mutation in ctDNA at baseline, patients are randomly assigned to 1 of these 2 options, with the primary objective of assessing whether bevacizumab demonstrates superiority over cetuximab in terms of progression-free survival (PFS) for this specific subset of patients. Furthermore, even for patients with baseline *RAS* wild-type ctDNA, a subsequent liquid biopsy is adopted after 8 cycles of FOLFIRI and cetuximab to detect potential acquired resistance and reintroduce the possibility of randomization at this point.

A different design is adopted by another ongoing, first-line, phase II study (PANIRINOX, NCT02980510), comparing panitumumab with FOLFIRINOX or mFOLFOX6 in patients that are selected solely by the absence of *RAS*/*BRAF* mutations according to ctDNA. Although the primary endpoint of the study is to investigate the complete response rate of the triplet chemotherapy plus panitumumab, this trial stands as one of the first examples of a clinical approach to molecularly triage tumors by relying on ctDNA. According to preliminary results, the intensification of upfront chemotherapy in combination with anti-EGFR agents did not confer additional benefits for patients with ctDNA-selected *RAS*/*BRAF* V600E wild-type mCRC.[77]

Finally, some ongoing studies are testing broader gene panels, including also *ERBB2*, and adopting therapeutic strategies to prevent acquired resistance, such as in the phase Ib/II OrigAMI-1 trial (NCT05379595) that employs a bispecific EGFR/MET-targeted antibody (amivantamab) in anti-EGFR naïve or pretreated patients with negative ctDNA for *RAS*/*BRAF*/*ERBB2*/*EGFR* ectodomain mutations.

2. Feasibility and timeliness: can ctDNA-guided therapeutic switching be anticipated before radiologic progression?

Patients with mCRC who are not eligible for definitive treatments face significant challenges in terms of therapeutic options, as available treatments for the chemorefractory disease have shown limited efficacy in providing substantial additional benefits. In this scenario, delayed assessment of progression could lead to early clinical deterioration or unnecessary treatment-related toxicities in patients whose tumors progress early during the course of treatment. The use of

Please cite this article as: Laura Roazzi et al, Ongoing Clinical Trials and Future Research Scenarios of Circulating Tumor DNA for the Treatment of Metastatic Colorectal Cancer, Clinical Colorectal Cancer, https://doi.org/10.1016/j.clcc.2024.02.001

Table 2 Ongoing/Retrospective ctDNA Studies Adopting ctDNA for mCRC

Trial	Phase/Design	Targeted Agents	Line of Therapy
NCT05051592	Observational, prospective	Cetuximab/panitumumab + chemotherapy	First line
RASINTRO/NCT03259009	Observational, prospective	Cetuximab or panitumumab rechallenge	Refractory
FOLICOLOR/NCT04735900	Observational, prospective	Panitumumab + chemotherapy	First line
COCA/MACS/NCT02872779	Observational, prospective	Any targeted therapy + chemotherapy	First line
COBRAF/NCT05639413	Observational, prospective	Anti-BRAF/anti-EGFR agents	First to third line
NCT05694936	II/randomized	Cetuximab/panitumumab + sodium valproate maintenance	First-line
NCT05775900	Observational, prospective	Cetuximab q3w + capecitabine maintenance	First-line
REPROGRAM-02/NCT05462613	II-III/randomized	Regorafenib + metronomic chemotherapy + low-dose aspirin	Second line
NCT04169347	II	Panitumumab + chemotherapy	First line
NCT03446157	II/randomized	Cetuximab + palbociclib	Refractory
BESPOKE/NCT04761783	Observational, prospective	Immune checkpoint inhibitors	Any
NIPIRESCUE/NCT05310643	II	Nivolumab + ipilimumab	Refractory
NCT02600949	I	Synthetic tumor-associated peptide vaccine therapy	Refractory
NCT03526835	I-II	MCLA-158 (anti-EGFR/LGR5)	Refractory
AMPLIFY-7P/NCT05726864	I-II/randomized	ELI-002 7P immunotherapy	Refractory
NCT05397171	I/II	AZD8853 (anti-GDF15)	Refractory
NCT05708599	Observational, prospective	Any	Any
NCT03401957	Observational, prospective	Cetuximab-based infusional 5-FU regimen	First line
BEYOND/NCT03751176	II	Cetuximab + chemotherapy	Second line
SUNRISE-CRC/NCT03909724	II/randomized	Sunitinib vs trifluridine/tipiracil	Refractory
COLOMATE/NCT03765736	Observational, prospective	Any targeted therapy	Any
METLIVER/NCT05398380	Observational, prospective	Anti-angiogenic therapy (bevacizumab or aflibercept)	Refractory
PREDICTION/NCT05806151	Observational, prospective	Any	Any
NCT03829410	Observational, prospective	Onvansertib + bevacizumab + chemotherapy	Second line
AIO-KRK-0114/NCT02934529	Observational, prospective	Cetuximab or bevacizumab + chemotherapy	First line
AIO-KRK-0116/NCT04034459	Observational, prospective	Cetuximab or bevacizumab + chemotherapy	First line
KISIMA-01/NCT04046445	Observational, prospective	ATP128 +/- BI 754091; ATP128 + VSV-GP128 + BI 754091	Any
EmutRAS/NCT03908788	Observational, prospective	Cetuximab or panitumumab	First line
COCA-MACS/NCT02872779	Observational, prospective	Targeted therapy +/- chemotherapy	First line
NCT03946917	I-II	Regorafenib + JS001	Refractory
NCT04607421	III/randomized	Encorafenib + chemotherapy	First line
NCT03668431	II	Dabrafenib + trametinib + PDR001	Any
NCT04689347	Ib	Bevacizumab + chemotherapy	First line
NCT04166604	II	Trifluridine/tipiracil	Refractory
NCT02997228	III/randomized	Chemotherapy + bevacizumab + atezolizumab vs atezolizumab	First line
NCT03947385	I-II	IDE196 + binimetinib + crizotinib	Any
NCT03594448	Observational, prospective	Any	Any
NCT01983098	Observational, prospective	Any	Any
IMPROVE/NCT04425239	II/randomized	Panitumumab + chemotherapy	First line
NCT04555369	Observational, prospective	Any	Any
RX-CROME	Observational, prospective	Regorafenib + XmAb20717	Refractory
NCT05141721	II-III/randomized	GRT-C901/GRT-R902 + immunotherapy + bevacizumab + chemotherapy vs chemotherapy + bevacizumab	Any
NCT04587128	II	Cetuximab or panitumumab + chemotherapy	Any
NCT02948985	Observational, prospective	Chemotherapy ± cetuximab	Any
NCT01189903	II	Regorafenib	Refractory

Please cite this article as: Laura Roazzi et al, Ongoing Clinical Trials and Future Research Scenarios of Circulating Tumor DNA for the Treatment of Metastatic Colorectal Cancer, Clinical Colorectal Cancer, https://doi.org/10.1016/j.clcc.2024.02.001

Please cite this article as: Laura Roazzi et al, Ongoing Clinical Trials and Future Research Scenarios of Circulating Tumor DNA for the Treatment of Metastatic Colorectal Cancer, Clinical Colorectal Cancer, https://doi.org/10.1016/j.clcc.2024.02.001

Table 3 Completed ctDNA-Guided Studies in mCRC Treatment

Trial/Author	N Pts	Design	Targeted Agents	Line of Therapy	Significance	ORR (%)	DCR (%)	PFS (months)	OS (months)
EGFR-targeted therapy									
CHRONOS/ Sartore-Bianchi A, Nat Med. 2022	27	II	Panitumumab monotherapy	Refractory (rechallenge)	First phase II study of ctDNA-based screening to guide rechallenge treatment with panitumumab, excluding patients with acquired resistance to anti-EGFR therapy (RAS, BRAF and EGFR ECD mutations) and allowing personalized intervention	30	63	4.0	13.8
REMARRY and PURSUIT/ Kagawa Y, J Clin Oncol. 2022	50	II	Panitumumab monotherapy	Refractory (rechallenge)	ctDNA-based RAS-only screening is insufficient to improve outcomes of rechallenge treatment with panitumumab	14	80	3.6	N/A
HER2-targeted therapy									
TRIUMPH/ Nakamura Y, Nat Med. 2021	30[a]	II	Trastuzumab + pertuzumab	Refractory	Positive predictive value of ERBB2 copy number at baseline for anti-HER2 dual therapy, with comprehensive ctDNA genotyping demonstrating the capacity to predict primary and acquired resistance	30a, 28b	67a, 60b	4.0a, 3.1b	10.1a, 8.8b
KRAS G12C-targeted therapy									
NCT04449874/ Desai J, Nat Med. 2023	29[b]	Ib	Divarasib + cetuximab	Refractory	ctDNA-driven identification of KRASG12C was adopted as an inclusion criterion. A decline in KRASG12C variant allele frequency was associated with tumor response.	63c, 60d	96c, 100d	8.1c, N/Ad	N/A

Abbreviations: DCR = disease control rate; N = number; N/A = not available; ORR = overall response rate; OS = overall survival; PFS = progression-free survival; pts = patients.
[a] ERBB2 amplification as either tissue-positivity or ctDNA-positivity (27 tissue-positive, 25 ctDNA positive). a. In tissue HER2-positive patients with mCRC. b. In ctDNA-positive patients for ERBB2 copy number variation.
[b] KRASG12C mutation identified by either tissue or ctDNA analysis (22 ctDNA positive). c. In 24 patients who had not previously received a KRAS G12C inhibitor before enrollment. d. In 5 patients pretreated with a KRAS G12C inhibitor.

Table 4 Ongoing ctDNA-Guided Studies in mCRC

Trial	Phase/Design	Targeted Agents (+/- Chemotherapy)	Line of Therapy	Role of ctDNA
1. In First Line: Can ctDNA Inform the Decision for Best Combination (anti-EGFR vs anti-VEGF) with Backbone Chemotherapy?				
LIBImAb/NCT04776655	III/randomized	Cetuximab + FOLFIRI vs Bevacizumab + FOLFIRI	First line	ctDNA-guided monoclonal antibody choice; ctDNA-guided therapeutic switch before radiologic progression;
PANIRINOX/NCT02980510	II/randomized	Panitumumab + chemotherapy (FOLFOXIRI o FOLFOX)	Any	ctDNA-guided enrollment of patients
OrigAMI-1 trial (NCT05379595)	Prospective, phase Ib/II	Amivantamab +/- chemotherapy	Any line	ctDNA-guided enrollment of patients
2. Feasibility and Timeliness: Can ctDNA-Guided Therapeutic Switching Be Anticipated Before Radiologic Progression?				
Rapid 1/NCT04786600	II/randomized	Cetuximab/panitumumab vs bevacizumab-based chemotherapy	Refractory	ctDNA-guided therapeutic switch before radiologic progression
NCT03844620	II/randomized	Regorafenib vs trifluridine/tipiracil	Refractory	ctDNA-guided therapeutic switch before radiologic progression
3. Beyond Progression: Can Anti-EGFR Therapy Continue with Only a Chemotherapy Switch if ctDNA Remains Negative for Resistance Mechanisms?				
CAPRI 2 GOIM/NCT05312398	II	Cetuximab-based chemotherapy	First, second and third line	ctDNA-guided anti-EGFR continuation beyond progression with chemotherapy switch only for RAS/BRAF wild-type patients with mCRC in liquid biopsy
4. Rechallenging Current Knowledge: ongoing studies of ctDNA-driven anti-EGFR rechallenge				
MOLIMOR/NCT04775862	II/randomized	Cetuximab or panitumumab-based therapy vs regorafenib or trifluridine-tipiracil	Refractory (rechallenge)	ctDNA-guided anti-EGFR rechallenge
PARERE/NCT04787341	II/randomized	Panitumumab monotherapy vs regorafenib	Refractory (rechallenge)	ctDNA-guided anti-EGFR rechallenge
PULSE study/NCT03992456	II/randomized	Panitumumab monotherapy vs regorafenib or trifluridine/tipiracil	Refractory (rechallenge)	ctDNA-guided anti-EGFR rechallenge
NCT04509635	III/randomized	Cetuximab + chemotherapy vs chemotherapy	Refractory	ctDNA-guided anti-EGFR rechallenge
CAVE-2 GOIM/NCT05291156	II/randomized	Cetuximab + avelumab vs cetuximab monotherapy	Second line	ctDNA-guided anti-EGFR rechallenge
NCT03087071	II	Panitumumab +/- trametinib	Refractory (rechallenge)	ctDNA-guided anti-EGFR rechallenge
C-PRECISE-01/NCT04495621	I/Ib	Cetuximab + MEN1611 (PI3K inhibitor)	Refractory (rechallenge)	ctDNA-guided enrollment of patients and anti-EGFR rechallenge
INTRINSIC/NCT04929223	I/Ib	Inavolisib + cetuximab Inavolisib + bevacizumab Divarasib + cetuximab +/- FOLFOX/FOLFIRI Atezolizumab + bevacizumab +/- tiragolumab Atezolizumab + SY-5609	Refractory	ctDNA-guided enrollment of patients and anti-EGFR rechallenge
CITRIC/2020-000443-3	II/randomized	Cetixumab and irinotecan vs anti-EGFR free regimens	Refractory (rechallenge)	ctDNA-guided anti-EGFR rechallenge
5. Direct ctDNA-driven targeting: Can ctDNA Guide the Selection of Targeted Therapy?				
NCT04831528	II	Any targeted agent	Second line	ctDNA-guided choice of second line targeted therapy after failure of first line
COPE/NCT04258137	II/randomized	Any treatment	First line	ctDNA-guided choice of first-line targeted therapy
6. Re-sensitization to anti-EGFR: What Role Does ctDNA-NeoRAS Play?				
C-PROWESS/s031210565	II	Panitumumab + chemotherapy	Refractory	ctDNA-guided anti-EGFR therapy after demonstration of neoRAS wild-type status in blood despite initial mutant status in archival tissue

Please cite this article as: Laura Roazzi et al, Ongoing Clinical Trials and Future Research Scenarios of Circulating Tumor DNA for the Treatment of Metastatic Colorectal Cancer, Clinical Colorectal Cancer, https://doi.org/10.1016/j.clcc.2024.02.001

ctDNA levels has been proposed as a possible alternative to radioimaging to guide early treatment switch, as ctDNA reflects the burden of disease and was shown to anticipate radiological detection of progression by several months.

The Rapid 1 study (NCT04786600) is an ongoing phase II, randomized clinical trial that is investigating the use of an individualized biweekly ctDNA assay to monitor disease response. The primary objective of the study is to compare overall survival (OS) in subjects receiving ctDNA-guided treatment decisions versus standard 3-monthly CT scans. According to this study, a significant increase in ctDNA will be considered as progressive disease and will guide the therapeutic switch in the intervention arm. The study relies on tumor-informed technology to increase sensitivity for ctDNA analysis, meaning that tumor specimens are first analyzed to extrapolate individual cancer-specific DNA alterations that then direct ctDNA identification in plasma. Eligible patients for this study must have *BRAF* wild-type, MSS mCRC and be at least in the second line of treatment.

A similar randomized phase II study (TACT-D, NCT03844620) is testing the same hypothesis in patients with chemorefractory disease who are candidates to either regorafenib or trifluridine/tipiracil (TFD/TPI). Since those late-line therapies could be toxic and non-beneficial to patients, the aim of this investigation is to evaluate the capability of ctDNA monitoring to spare toxicities. In the investigation arm, patients will continue treatment beyond the first cycle depending on ctDNA results, avoiding prosecution of potentially ineffective treatment regimens. Of note, recent evidence supports TFD/TPI with bevacizumab as the new standard of care for late lines, restraining the need for further studies with regorafenib or TFD/TPI alone in the treatment of mCRC. However, we feel that the potential implications of such a ctDNA-guided study design may overcome this limitation, providing proof-of-principle evidence to support future trials employing ctDNA-based decisions.

Finally, as previously discussed, also the LIBImAb study (NCT04776655) features randomization for RAS wild-type patients with mCRC to the switch from cetuximab to bevacizumab and FOLFIRI (versus the continuation of cetuximab-FOLFIRI in first line) before radiological progression in the case of emergence of RAS mutationa in serial ctDNA analyses.

However, it is important to consider that emerging limitations are associated with this approach, as indicated by the findings of two recent retrospective studies that have highlighted the low prevalence of MAPK-related acquired mutations in the first-line setting.[37,78] Specifically, it has been observed that the development of alterations in the MAPK pathway, which are commonly associated with resistance to anti-EGFR drugs, may be less likely when the agents are combined with chemotherapy.[37,78] Instead, it was suggested that transcriptomic alterations may play a more predominant role in driving resistance in this context.[37,78] As a result, the applicability of switch maintenance strategies that are focused on targeted agents in the first-line setting may be more limited than previously anticipated, leading to slower accrual of ongoing trials.

3. Beyond progression: can anti-EGFR therapy continue with only a chemotherapy switch if ctDNA remains negative for resistance mechanisms?

Another important issue in the management of patients with mCRC is that the tumor genotype might not necessarily change upon treatment exposure, since progression could depend on the emergence of biological mechanisms different from mutations in oncogenes, such as post-transcriptional, epigenetic, metabolic, microenvironmental or immune changes.[37,78] In case of retained *RAS*/*BRAF* wild-type status after progression to first-line treatment, several investigators have been indeed proposing the continuation of anti-EGFR therapy while switching the chemotherapy backbone. Indeed, in this situation, a switch from FOLFOX to FOLFIRI (or *vice versa*) may help to regain disease control, while maintaining anti-EGFR effects. In this regard, longitudinal analysis by ctDNA is the most suitable tool to address the dynamics of tumor molecular alterations.

The CAPRI 2 GOIM study (NCT05312398) is a phase II clinical trial that is currently testing the aforementioned hypothesis. In case of progression, only the chemotherapeutic regimen is switched, while anti-EGFR therapy is continued if ctDNA analysis does not identify the emergence of *RAS*/*BRAF* mutations. This concept will be applied after progression to both first line and second line in this single arm study that has ORR as its primary endpoint. While this approach differs from a pulsatile anti-EGFR/VEGF therapeutic switch (as in the LIBImAb trial), ctDNA analysis acts as a bridge between these two therapeutic strategies, that become in fact part of a spectrum when the therapeutic decision is informed by liquid biopsy. According to this paradigm, in case acquired resistance mechanisms are documented, pulsatile early-switching could have a rationale to prevent the further consolidation of anti-EGFR resistant clones; conversely, when MAPK susceptibility to anti-EGFR agents is still intact, anti-EGFR therapy continuation beyond-progression may be effective in principle.

4. Rechallenging current knowledge: ongoing studies of ctDNA-driven anti-EGFR rechallenge

Rechallenge with anti-EGFR drugs in the case of *RAS* wild-type ctDNA for chemorefractory patients with mCRC showed activity in the CHRONOS and other anti-EGFR rechallenge studies.[56-62,79] However, given the heterogeneity of results (possibly due to different study designs), further confirmation by ongoing trials would be desirable to corroborate this strategy.[64] Also, the results of randomized interventions will more robustly assess the best treatment sequence for this subset of patients, also considering the evolution of later-line therapeutic options now including the combination of TFD/TPI and bevacizumab.[80]

In the phase II, randomized PARERE study (NCT04787341), panitumumab rechallenge before or after regorafenib will be tested in chemorefractory patients with mCRC not bearing *RAS*/*BRAF* wild-type ctDNA and who had achieved previous benefit from first-line anti-EGFR-based treatment. The objective of this study is to understand the best treatment sequence (regorafenib after panitumumab or *vice versa*) by comparing OS between the 2 groups. The REVERCE study, a phase II randomized Japanese trial, previously reported a higher OS rate in *RAS* wild-type patients with mCRC treated with regorafenib followed by cetuximab with or without irinotecan, as compared to the reverse sequence.[81] However, the study had limitations that prevent drawing definitive conclu-

Please cite this article as: Laura Roazzi et al, Ongoing Clinical Trials and Future Research Scenarios of Circulating Tumor DNA for the Treatment of Metastatic Colorectal Cancer, Clinical Colorectal Cancer, https://doi.org/10.1016/j.clcc.2024.02.001

sions, including the lack of ctDNA guidance to support anti-EGFR therapy and premature conclusion due to poor accrual.[81] Similarly, the PULSE randomized phase 2 trial (NCT03992456) is investigating the efficacy of rechallenge with panitumumab in *RAS* wild-type patients with mCRC, compared to investigators' choice standard treatment (regorafenib or TFD/TPI).

In addition, the landscape of clinical practice has changed, as anti-EGFR agents are now routinely administered in earlier lines of therapy for eligible patients.[18] However, the PARERE has the limitation of adopting regorafenib as comparator, when TFD/TIP + bevacizumab might represent a more appropriate comparison considering the results of the SUNLIGHT trial (NCT04737187).[80] This is also the case of the ongoing phase III study NCT04509635 that is comparing cetuximab plus chemotherapy *versus* chemotherapy alone in third line for patients with mCRC with *RAS* wild-type ctDNA, having disease control rates (DCR) as the primary endpoint. The CITRIC study (EudraCT Number: 2020-000443-31) is another ongoing, randomized, phase II trial that aims to compare the ORR of cetuximab plus irinotecan rechallenge in the third-line setting to investigator choices of non-anti-EGFR treatment (potentially including also TFD/TPI and bevacizumab) in patients with *RAS*/*BRAF*/*EGFR* ECD ctDNA wild-type mCRC who previously gained clinical benefit from first-line anti-EGFR therapy.

Finally, several trials are investigating "enhanced rechallenge" strategies, taking advantage of ctDNA to refine a population of patients (*RAS*/*BRAF*/*EGFR* wild type) in which rechallenge is then escalated by adopting potentially synergistic treatments, or directly by targeting molecular biomarkers of acquired anti-EGFR resistance. For example, the CAVE 2 study (NCT05291156) is an ongoing, randomized phase II trial that is comparing cetuximab rechallenge with or without anti-programmed death-ligand 1 (PD-L1) immunotherapy, testing the hypothesis of an immune-stimulating effect of cetuximab on top of the molecular selection by ctDNA. Indeed, evidence from preclinical studies has shown that tumor exposition to anti-EGFR therapy could down-regulate the expression of mismatch repair (MMR) proteins, thus inducing microsatellite instability and increased immunogenicity.[82] Moreover, cetuximab has a potential antibody-mediated cytotoxicity (ADCC) effect that can recall a rich immune-cell tumor infiltration.[83] In this trial, a combination of avelumab and cetuximab will be tested against cetuximab alone in patients who previously benefited from an anti-EGFR-based treatment and were selected for *RAS*/*BRAF* wild-type ctDNA status at baseline. A similar approach based on a different biological rationale is currently employed by combining panitumumab and trametinib (MEK inhibitor) as a reinforced strategy for anti-EGFR rechallenge (NCT03087071). Preliminary results from a study that tested this approach showed poor outcomes both in patients with acquired mutations (*RAS*/*RAF*/*MAP2K1*) on pretreatment liquid biopsy who received upfront panitumumab plus trametinib and in patients who added trametinib after progression during panitumumab monotherapy without acquired mutations.[63]

In the C-PRECISE-01 trial (NCT04495621), on the other hand, selection of patients by ctDNA is required for targeting at rechallenge PI3K together with EGFR only in patients showing acquired *PIK3CA* mutations at the moment of treatment. Similarly, the phase I/Ib INTRINSIC study (NCT04929223) is evaluating the safety and efficacy of biomarker-guided multi-arm treatment in patients with pretreated mCRC, including rechallenge with cetuximab in combination with inavolisib (a highly selective PI3K alpha inhibitor) for patients with *PIK3CA* mutations and *RAS* wild-type status at ctDNA analysis.

5. Direct ctDNA-driven targeting: can ctDNA guide the selection of targeted therapy?

Another interesting opportunity of ctDNA longitudinal analysis is to guide the early assignment of patients to targeted therapies. Primary tumor tissue, when available, may not always offer sufficient information for accurately stratifying patients and selecting the most effective treatment approach. Re-analyzing metastatic lesions through invasive needle biopsy is a potential approach, but it might be hindered by invasiveness-related safety issues and by the inherent issue of heterogeneity observed among individual lesions within the same patient.[84] Liquid biopsy has the potential to serially capture this heterogeneity, enabling sequential monitoring of disease progression and evolution. Furthermore, ctDNA allelic fraction in liquid biopsy could represent an additional parameter to estimate aggressiveness and prognosis in certain mutant subtypes of cancers, as suggested by a study conducted in patients with *BRAF* mutated mCRC.[85]

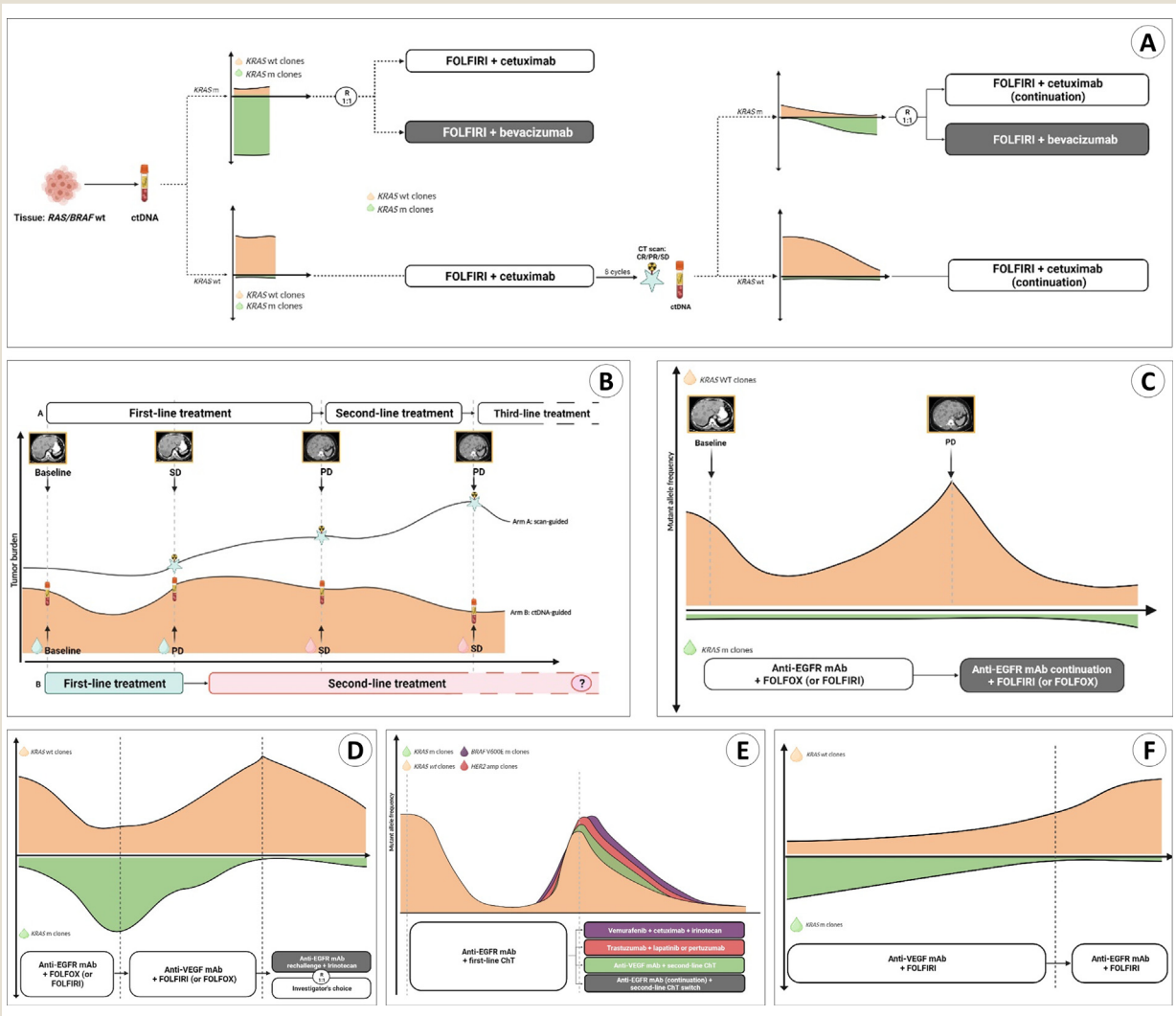
In this context, integration of liquid biopsy analysis and discussion of ctDNA results by a qualified molecular tumor board is promising to guide the best therapeutic opportunity for patients.[1,3,14,38,86-88]

The COPE randomized phase II study (NCT04258137) is evaluating the benefit, in terms of OS, of choosing the most tailored treatment strategy in first line according to ctDNA profiling. All the patients carrying an actionable alteration according to ctDNA will be proposed to receive a matched drug or to enter in a matched clinical trial depending on the possibility of inclusion at the time of the molecular report. A similar strategy is investigated by another study (NCT04831528) in second line, although available therapies are pre-specified and limited to patients with *RAS* and *BRAF* wild-type mCRC who failed first-line treatment containing cetuximab. Treatment choices include cetuximab continuation with second-line chemotherapy (FOLFOX/FOLFIRI/irinotecan monotherapy) for ctDNA *RAS*/*BRAF* wild-type mCRC; switch to bevacizumab and second-line chemotherapy for acquired *RAS* mutations; vemurafenib + cetuximab and irinotecan for $BRAF^{V600E}$ mutations; trastuzumab + lapatinib or trastuzumab + pertuzumab for *ERBB2* amplifications.

Besides, both PI3K and KRAS G12C inhibitors were reported to be in clinical trials for chemorefractory patients, whose inclusion was based on ctDNA molecular criteria. As previously discussed, beside allowing treatment with inavolisib and cetuximab in *PIK3CA* mutant, *RAS* wild-type mCRC patients, the INTRINSIC study (NCT04929223) also encompasses a treatment arm with inavolisib and bevacizumab for *PIK3CA* and *RAS* mutant mCRC patientspatgei. In addition, divarasib (a KRAS G12C inhibitor) was reported in combination with cetuximab for chemorefractory $KRAS^{G12C}$ mutant

Please cite this article as: Laura Roazzi et al, Ongoing Clinical Trials and Future Research Scenarios of Circulating Tumor DNA for the Treatment of Metastatic Colorectal Cancer, Clinical Colorectal Cancer, https://doi.org/10.1016/j.clcc.2024.02.001

Figure 1 Overview of potential developmental scenarios and pivotal ongoing clinical trials for ctDNA-guided intervention in mCRC. Relevant issues in current investigations that are related to clinical unmet needs in the oncology practice for patients with mCRC: (A) Incorporation of ctDNA analysis in the decision-making process for the first-line treatment of mCRC, guiding the choice for the most effective monoclonal antibody to be combined with chemotherapy (anti-EGFR vs anti-VEGF agents). After induction, ctDNA is reassessed for potential emergence of RAS mutations and switch maintenance from anti-EGFR to anti-VEGF agents in combination fluoropyrimidines. Open question regarding this approach is addressed by the randomized trial design of the LIBImAb study; (B) Early detection of disease progression by ctDNA over radio-imaging, and timely initiation of a non-cross resistant treatment line to avoid clinical deterioration and unnecessary toxicity, as addressed in the Rapid-1 trial; (C) Tracking of persistently negative RAS dynamics in plasma as a predictive factor for anti-EGFR continuation beyond progression with chemotherapy switch alone (i.e., CAPRI-2 trial); (D) Clinical utility of ctDNA-guided anti-EGFR rechallenge, addressing direct comparison with other treatment options in randomized trials for chemorefractory patients; (E) Potential role of ctDNA to anticipate patients' selection towards targeted therapies in early treatment lines; (F) The concept of "neoRAS" by longitudinal ctDNA analysis as indicated by patients with RAS mutant mCRC according to tissue analysis but RAS wild type by ctDNA exploration. RAS dynamics may be serially tackled with the aim of testing anti-EGFR agents in such patients. Abbreviations: ctDNA = circulating tumor DNA; LB = liquid biopsy; CT scan = computed tomography scan; PD = progression disease; LoD = limit of detectable; w = weeks, EGFR = epidermal growth factor receptor; VEGF = vascular endothelial growth factor; mAb = monoclonal antibodies, ChT = chemotherapy; KRAS = Kirsten Rat Sarcoma; BRAF = B-raf proto-oncogene.



Please cite this article as: Laura Roazzi et al, Ongoing Clinical Trials and Future Research Scenarios of Circulating Tumor DNA for the Treatment of Metastatic Colorectal Cancer, Clinical Colorectal Cancer, https://doi.org/10.1016/j.clcc.2024.02.001

patients with mCRC in a phase 1b trial, achieving up to 62.5% ORR and 95.8% DCR. This is one of the first clinical study being reported in mCRC leveraging ctDNA as a molecular inclusion criterion for targeted treatment (Table 3).[89] Similarly, divarasib with cetuximab is also being evaluated as part of the INTRINSINC multi-cohort study.

Since most studies previously confined matched targeted agents in later lines of treatment, both these investigations have the potential to generate new hypotheses about pursuing molecular actionability earlier in the course of the disease taking advantage of ctDNA. Indeed, the enthusiasm for precision cancer medicine trials adopting matched targeted therapies still faces setbacks from limited results of several randomized trials in solid tumors, that previously failed in demonstrating improved PFS compared with treatment at physician's choice.[90-92] Since major contributors for the lack of targeted effectiveness could lie in the heavy pretreatment, early ctDNA-based selection is relevant in this context.[90]

6. Re-sensitization to anti-EGFR: what role does ctDNA-NeoRAS play?

Several observational ctDNA studies described the occurrence of "neoRAS" patients with mCRC, referring to *RAS* mutant mCRC that shift towards a repopulation by wild-type clones after treatment as assessed by ctDNA sampling. Although this situation is considered uncommon and lack a clear plausible explanation, these patients could be easily identified by serial ctDNA testing. So far, literature data are still immature to establish the real incidence of this phenomenon.[93-100] A previous study in this population showed a good activity for cetuximab + FOLFIRI in 16 baseline-neoRAS patients with mCRC.[97] Given the limited data, it will be important to expand knowledge and confirm the clinical utility of this approach. An ongoing phase II study (C-PROWESS, s031210565) aims to confirm this suggestion in chemorefractory, acquired neoRAS patients, by assessing the activity (ORR) of panitumumab + irinotecan for *RAS* mutant mCRC that converted to ctDNA *RAS* wild type after progression to previous therapies.

Considering the dynamic fluctuations observed in *RAS* ctDNA levels over time, in particular following the cessation of anti-EGFR therapy, an emerging hypothesis has been introduced regarding the possibility of intermittent/pulsatile administration of cetuximab or panitumumab. This strategy would theoretically preserve the sensitivity of tumor clones by periodically introducing and discontinuing the targeted agent.[53] An application of this concept is being tested in the neoRAS setting. The MoLiMoR study (NCT04554836) is a randomized phase II clinical trial that is proposing to investigate whether the association of intermittent cetuximab, based upon the presence or not of *RAS* mutations in ctDNA, could be superior to FOLFIRI alone in patients with *RAS* mutated mCRC. In the FOLFIRI + cetuximab arm, treatment is shifted to FOLFIRI upon the emergence of *RAS* mutations, while reversion to *RAS* wild-type status would allow the reintroduction of cetuximab. The study evaluates this strategy in the first-line setting and its primary endpoint is PFS. The trial status is currently on hold, maybe limited by the small size of this population.

Discussion and Conclusions

The use of ctDNA-guided strategies in the management of mCRC holds great promise for personalized treatment decisions. In this review, we reported and discussed the study design and the translational questions of ongoing clinical investigation, highlighting the potential of ctDNA analysis in different settings of the *continuum of care*. We identified 6 clinical questions related to topical unmet needs in the oncology practice for patients with mCRC (Figure 1): (A) the incorporation of ctDNA in the decision-making process for the first-line treatment of mCRC, guiding the choice for the most effective monoclonal antibody to be combined with chemotherapy (anti-EGFR vs anti-VEGF agents); (B) the early detection of disease progression by ctDNA over radio-imaging, and the timely initiation of a non-cross resistant treatment line to avoid clinical deterioration and unnecessary toxicity; (C) the tracking of persistently negative *RAS* dynamics in plasma as a predictive factor for anti-EGFR continuation beyond progression with chemotherapy switch alone; (D) the corroboration of the clinical utility of ctDNA-guided anti-EGFR rechallenge, addressing direct comparison with other treatment options for chemorefractory patients and the combination of anti-EGFR with other agents; (E) the potential role of ctDNA to anticipate patients' selection towards targeted therapies in early treatment lines; (F) the collection of further evidence regarding the concept of "neoRAS" by longitudinal ctDNA analysis.

The remarkable number of ongoing, prospective, interventional trials demonstrate the growing importance of ctDNA-guided strategies in mCRC. In future years, liquid biopsy may experience a substantial evolution, also by leveraging the integration of broader multi-omic molecular assays. Indeed, such approach might significantly expand the prowess of analysis of liquid biopsy in multiple settings of the disease, including early detection.[101] Although many interventional studies are now focused on improving efficacy of anti-EGFR treatment by taking advantage of ctDNA refinement of molecular selection, the field is moving forward by shifting to novel approaches such as "enhanced" rechallenge and direct targeting of biomarkers identified through liquid biopsy, paralleling the broader applications now reached in early-stage drug development for many solid tumors.[88,102] Overall, the studies addressed by the present review aim at optimizing molecular-based decisions and tailoring treatments, therefore having the potential to improve outcomes and contribute to reshape clinical practice for mCRC in the era of precision medicine.

Clinical Practice Points

- Liquid biopsy using circulating tumor DNA (ctDNA) is expected to revolutionize the molecular characterization and treatment of metastatic colorectal cancer (mCRC).
- Since CRC is particularly complex at the molecular level, due to its mutability and heterogeneity, ctDNA offers a minimally invasive and potentially comprehensive approach to measure the dynamics of changes during treatment, specifically for RAS mutational status before and during anti-EGFR-based therapy.
- Ongoing clinical trials are shaping ctDNA-guided mCRC treatment, marking a significant step towards precision medicine in this field.

Please cite this article as: Laura Roazzi et al, Ongoing Clinical Trials and Future Research Scenarios of Circulating Tumor DNA for the Treatment of Metastatic Colorectal Cancer, Clinical Colorectal Cancer, https://doi.org/10.1016/j.clcc.2024.02.001

Disclosure

ASB is an advisory board member for Amgen, Bayer, Novartis, Sanofi and Servier. AA is an advisory board member for Roche and Bayer and received honoraria from CheckmAb. SS is an advisory board member for Agenus, AstraZeneca, Bayer, BMS, CheckmAb, Daiichi-Sankyo, Guardant Health, Menarini, Merck, Novartis, Roche-Genentech, and Seagen. The remaining authors declare no competing interests.

CRediT authorship contribution statement

Laura Roazzi: Data curation, Formal analysis, Writing – original draft, Writing – review & editing. **Giorgio Patelli:** Data curation, Formal analysis, Writing – original draft, Writing – review & editing. **Katia Bruna Bencardino:** Methodology, Writing – review & editing. **Alessio Amatu:** Methodology, Supervision, Writing – review & editing. **Erica Bonazzina:** Data curation, Writing – review & editing. **Federica Tosi:** Supervision, Writing – review & editing. **Brunella Amoruso:** Data curation, Writing – original draft. **Anna Bombelli:** Resources, Writing – review & editing. **Sara Mariano:** Resources, Writing – review & editing. **Stefano Stabile:** Methodology, Writing – review & editing. **Camillo Porta:** Methodology, Supervision, Writing – review & editing. **Salvatore Siena:** Conceptualization, Funding acquisition, Methodology, Resources, Supervision, Writing – review & editing. **Andrea Sartore-Bianchi:** Conceptualization, Methodology, Resources, Supervision, Writing – review & editing.

Acknowledgments

Authors are supported by Fondazione Oncologia Niguarda Onlus.

Giorgio Patelli is a PhD student within the European School of Molecular Medicine (SEMM).

References

1. Siravegna G, Marsoni S, Siena S, Bardelli A. Integrating liquid biopsies into the management of cancer. *Nat Rev Clin Oncol*. 2017;14(9):531–548. doi:10.1038/nrclinonc.2017.14.
2. Leon SA, Shapiro B, Sklaroff DM, Yaros MJ. Free DNA in the serum of cancer patients and the effect of therapy. *Cancer Res*. 1977;37(3):646–650.
3. Patelli G, Vaghi C, Tosi F, et al. Liquid biopsy for prognosis and treatment in metastatic colorectal cancer: circulating tumor cells vs circulating tumor DNA. *Target Oncol*. 2021;16(3):309–324. doi:10.1007/s11523-021-00795-5.
4. Bettegowda C, Sausen M, Leary RJ, et al. Detection of circulating tumor DNA in early- and late-stage human malignancies. *Sci Transl Med*. 2014;6(224):224ra24. doi:10.1126/scitranslmed.3007094.
5. Siegel RL, Miller KD, Sauer AG, et al. Colorectal cancer statistics, 2020. *CA Cancer J Clin*. 2020;70(3):145–164. doi:10.3322/caac.21601.
6. Mauri G, Vitiello PP, Sogari A, et al. Liquid biopsies to monitor and direct cancer treatment in colorectal cancer. *Br J Cancer*. 2022;127(3):394–407. doi:10.1038/s41416-022-01769-8.
7. Zhang Y, Yao Y, Xu Y, et al. Pan-cancer circulating tumor DNA detection in over 10,000 Chinese patients. *Nat Commun*. 2021;12(1):11. doi:10.1038/s41467-020-20162-8.
8. Cancer of the Colon and Rectum - Cancer Stat Facts. SEER. 2023. Accessed August 8, 2023. Available at: https://seer.cancer.gov/statfacts/html/colorect.html.
9. Siravegna G, Mussolin B, Buscarino M, et al. Clonal evolution and resistance to EGFR blockade in the blood of colorectal cancer patients. *Nat Med*. 2015;21(7):827. doi:10.1038/nm0715-827b.
10. Patelli G, Mauri G, Tosi F, et al. Circulating tumor DNA to drive treatment in metastatic colorectal cancer. *Clin Cancer Res*. 2023;29(22):4530–4539. doi:10.1158/1078-0432.CCR-23-0079.
11. Loft M, To YH, Gibbs P, Tie J. Clinical application of circulating tumour DNA in colorectal cancer. *Lancet Gastroenterol Hepatol*. 2023;8(9):837–852. doi:10.1016/S2468-1253(23)00146-2.
12. Zhou H, Zhu L, Song J, et al. Liquid biopsy at the frontier of detection, prognosis and progression monitoring in colorectal cancer. *Mol Cancer*. 2022;21(1):86. doi:10.1186/s12943-022-01556-2.
13. Di Nicolantonio F, Vitiello PP, Marsoni S, et al. Precision oncology in metastatic colorectal cancer: from biology to medicine. *Nat Rev Clin Oncol*. 2021;18(8):506–525. doi:10.1038/s41571-021-00495-z.
14. Strickler JH, Loree JM, Ahronian LG, et al. Genomic landscape of cell-free DNA in patients with colorectal cancer. *Cancer Discov*. 2018;8(2):164–173. doi:10.1158/2159-8290.CD-17-1009.
15. Bachet JB, Bouché O, Taieb J, et al. RAS mutation analysis in circulating tumor DNA from patients with metastatic colorectal cancer: the AGEO RASANC prospective multicenter study. *Ann Oncol*. 2018;29(5):1211–1219. doi:10.1093/annonc/mdy061.
16. Kim TW, Peeters M, Thomas A, et al. Impact of emergent circulating tumor DNA RAS mutation in panitumumab-treated chemoresistant metastatic colorectal cancer. *Clin Cancer Res*. 2018;24(22):5602–5609. doi:10.1158/1078-0432.CCR-17-3377.
17. Kagawa Y, Elez E, García-Foncillas J, et al. Combined analysis of concordance between liquid and tumor tissue biopsies for RAS mutations in colorectal cancer with a single metastasis site: the METABEAM study. *Clin Cancer Res*. 2021;27(9):2515–2522. doi:10.1158/1078-0432.CCR-20-3677.
18. Cervantes A, Adam R, Roselló S, et al. Metastatic colorectal cancer: ESMO clinical practice guideline for diagnosis, treatment and follow-up. *Ann Oncol*. 2023;34(1):10–32. doi:10.1016/j.annonc.2022.10.003.
19. Amado RG, Wolf M, Peeters M, et al. Wild-type KRAS is required for panitumumab efficacy in patients with metastatic colorectal cancer. *J Clin Oncol*. 2008;26(10):1626–1634. doi:10.1200/JCO.2007.14.7116.
20. Karapetis CS, Khambata-Ford S, Jonker DJ, et al. K-ras mutations and benefit from cetuximab in advanced colorectal cancer. *N Engl J Med*. 2008;359(17):1757–1765. doi:10.1056/NEJMoa0804385.
21. Di Nicolantonio F, Martini M, Molinari F, et al. Wild-type BRAF is required for response to panitumumab or cetuximab in metastatic colorectal cancer. *J Clin Oncol*. 2008;26(35):5705–5712. doi:10.1200/JCO.2008.18.0786.
22. Douillard JY, Siena S, Cassidy J, et al. Final results from PRIME: randomized phase III study of panitumumab with FOLFOX4 for first-line treatment of metastatic colorectal cancer. *Ann Oncol*. 2014;25(7):1346–1355. doi:10.1093/annonc/mdu141.
23. Van Cutsem E, Köhne CH, Hitre E, et al. Cetuximab and chemotherapy as initial treatment for metastatic colorectal cancer. *N Engl J Med*. 2009;360(14):1408–1417. doi:10.1056/NEJMoa0805019.
24. Normanno N, Esposito Abate R, Lambiase M, et al. RAS testing of liquid biopsy correlates with the outcome of metastatic colorectal cancer patients treated with first-line FOLFIRI plus cetuximab in the CAPRI-GOIM trial. *Ann Oncol*. 2018;29(1):112–118. doi:10.1093/annonc/mdx417.
25. Vidal J, Fernández-Rodríguez MC, Casadevall D, et al. Liquid biopsy detects early molecular response and predicts benefit to first-line chemotherapy plus cetuximab in metastatic colorectal cancer: PLATFORM-B study. *Clin Cancer Res*. 2023;29(2):379–388. doi:10.1158/1078-0432.CCR-22-1696.
26. Tie J, Kinde I, Wang Y, et al. Circulating tumor DNA as an early marker of therapeutic response in patients with metastatic colorectal cancer. *Ann Oncol*. 2015;26(8):1715–1722. doi:10.1093/annonc/mdv177.
27. Garlan F, Laurent-Puig P, Sefrioui D, et al. Early evaluation of circulating tumor DNA as marker of therapeutic efficacy in metastatic colorectal cancer patients (PLACOL study). *Clin Cancer Res*. 2017;23(18):5416–5425. doi:10.1158/1078-0432.CCR-16-3155.
28. Parikh AR, Mojtahed A, Schneider JL, et al. Serial ctDNA monitoring to predict response to systemic therapy in metastatic gastrointestinal cancers. *Clin Cancer Res*. 2020;26(8):1877–1885. doi:10.1158/1078-0432.CCR-19-3467.
29. Nakamura Y, Okamoto W, Kato T, et al. Circulating tumor DNA-guided treatment with pertuzumab plus trastuzumab for HER2-amplified metastatic colorectal cancer: a phase 2 trial. *Nat Med*. 2021;27(11):1899–1903. doi:10.1038/s41591-021-01553-w.
30. Tan L, Tran B, Tie J. A phase Ib/II trial of combined BRAF and EGFR inhibition in BRAF V600E positive metastatic colorectal cancer and other cancers: the EVICT (erlotinib and vemurafenib in combination trial) study. *Clin Cancer Res*. 2023;29(6):1017–1030. doi:10.1158/1078-0432.CCR-22-3094.
31. Yaeger R, Weiss J, Pelster MS, et al. Adagrasib with or without cetuximab in colorectal cancer with mutated KRAS G12C. *N Engl J Med*. 2023;388(1):44–54. doi:10.1056/NEJMoa2212419.
32. Misale S, Di Nicolantonio F, Sartore-Bianchi A, Siena S, Bardelli A. Resistance to anti-EGFR therapy in colorectal cancer: from heterogeneity to convergent evolution. *Cancer Discov*. 2014;4(11):1269–1280. doi:10.1158/2159-8290.CD-14-0462.
33. Sartore-Bianchi A, Nicolantonio FD, Nichelatti M, et al. Multi-determinants analysis of molecular alterations for predicting clinical benefit to EGFR-targeted monoclonal antibodies in colorectal cancer. *PLoS One*. 2009;4(10):e7287. doi:10.1371/journal.pone.0007287.
34. Randon G, Maddalena G, Germani MM, et al. Negative ultraselection of patients with RAS/BRAF wild-type, microsatellite-stable metastatic colorectal cancer receiving anti-EGFR-based therapy. *JCO Precis Oncol*. 2022;6:e2200037. doi:10.1200/PO.22.00037.

Please cite this article as: Laura Roazzi et al, Ongoing Clinical Trials and Future Research Scenarios of Circulating Tumor DNA for the Treatment of Metastatic Colorectal Cancer, Clinical Colorectal Cancer, https://doi.org/10.1016/j.clcc.2024.02.001

35. Arena S, Bellosillo B, Siravegna G, et al. Emergence of multiple EGFR extracellular mutations during cetuximab treatment in colorectal cancer. *Clin Cancer Res*. 2015;21(9):2157–2166. doi:10.1158/1078-0432.CCR-14-2821.
36. Montagut C, Argilés G, Ciardiello F, et al. Efficacy of Sym004 in patients with metastatic colorectal cancer with acquired resistance to anti-EGFR therapy and molecularly selected by circulating tumor DNA analyses: a phase 2 randomized clinical trial. *JAMA Oncol*. 2018;4(4):e175245. doi:10.1001/jamaoncol.2017.5245.
37. Raghav K, Ou FS, Venook AP, et al. Acquired genomic alterations on first-line chemotherapy with cetuximab in advanced colorectal cancer: circulating tumor DNA analysis of the CALGB/SWOG-80405 trial (alliance). *J Clin Oncol*. 2023;41(3):472–478. doi:10.1200/JCO.22.00365.
38. Topham JT, O'Callaghan CJ, Feilotter H, et al. Circulating tumor DNA identifies diverse landscape of acquired resistance to anti-epidermal growth factor receptor therapy in metastatic colorectal cancer. *J Clin Oncol*. 2023;41(3):485–496. doi:10.1200/JCO.22.00364.
39. Misale S, Yaeger R, Hobor S, et al. Emergence of KRAS mutations and acquired resistance to anti-EGFR therapy in colorectal cancer. *Nature*. 2012;486(7404):532–536. doi:10.1038/nature11156.
40. Diaz LA, Williams RT, Wu J, et al. The molecular evolution of acquired resistance to targeted EGFR blockade in colorectal cancers. *Nature*. 2012;486(7404):537–540. doi:10.1038/nature11219.
41. Shitara K, Muro K, Watanabe J, et al. Negative hyperselection of patients with RAS wild-type metastatic colorectal cancer for panitumumab: a biomarker study of the phase III PARADIGM trial. *JCO*. 2023;41(4_suppl):11. doi:10.1200/JCO.2023.41.4_suppl.11.
42. Schrock AB, Pavlick D, Klempner SJ, et al. Hybrid capture-based genomic profiling of circulating tumor DNA from patients with advanced cancers of the gastrointestinal tract or anus. *Clin Cancer Res*. 2018;24(8):1881–1890. doi:10.1158/1078-0432.CCR-17-3103.
43. Takegawa N, Yonesaka K, Sakai K, et al. HER2 genomic amplification in circulating tumor DNA from patients with cetuximab-resistant colorectal cancer. *Oncotarget*. 2016;7(3):3453–3460. doi:10.18632/oncotarget.6498.
44. Siravegna G, Sartore-Bianchi A, Nagy RJ, et al. Plasma HER2 (ERBB2) copy number predicts response to HER2-targeted therapy in metastatic colorectal cancer. *Clin Cancer Res*. 2019;25(10):3046–3053. doi:10.1158/1078-0432.CCR-18-3389.
45. Kopetz S, Murphy DA, Pu J, et al. Evaluation of baseline BRAF V600E mutation in circulating tumor DNA and efficacy response from the BEACON study. *JCO*. 2022;40(4_suppl):162. doi:10.1200/JCO.2022.40.4_suppl.162.
46. Tan L, Tran B, Tie J, et al. A phase Ib/II trial of combined BRAF and EGFR inhibition in BRAF V600E positive metastatic colorectal cancer and other cancers: the EVICT (erlotinib and vemurafenib in combination trial) study. *Clin Cancer Res*. 2023;29(6):1017–1030. doi:10.1158/1078-0432.CCR-22-3094.
47. Thein KZ, Biter AB, Banks KC, et al. Identification of KRASG12C mutations in circulating tumor DNA in patients with cancer. *JCO Precis Oncol*. 2022;6:e2100547. doi:10.1200/PO.21.00547.
48. Clifton K, Rich TA, Parseghian C, et al. Identification of actionable fusions as an anti-EGFR resistance mechanism using a circulating tumor DNA assay. *JCO Precis Oncol*. 2019;3:1–15. doi:10.1200/PO.19.00141.
49. Cao W, Xu Y, Chang L, et al. Genotyping of circulating tumor DNA reveals the clinically actionable mutation landscape of advanced colorectal cancer. *Mol Cancer Ther*. 2019;18(6):1158–1167. doi:10.1158/1535-7163.MCT-18-1247.
50. Silveira AB, Bidard FC, Kasperek A, et al. High-accuracy determination of microsatellite instability compatible with liquid biopsies. *Clin Chem*. 2020;66(4):606–613. doi:10.1093/clinchem/hvaa013.
51. Willis J, Lefterova MI, Artyomenko A, et al. Validation of microsatellite instability detection using a comprehensive plasma-based genotyping panel. *Clin Cancer Res*. 2019;25(23):7035–7045. doi:10.1158/1078-0432.CCR-19-1324.
52. Deng A, Yang J, Lang J, et al. Monitoring microsatellite instability (MSI) in circulating tumor DNA by next-generation DNA-seq. *JCO*. 2018;36(15_suppl):12025. doi:10.1200/JCO.2018.36.15_suppl.12025.
53. Mauri G, Pizzutilo EG, Amatu A, et al. Retreatment with anti-EGFR monoclonal antibodies in metastatic colorectal cancer: systematic review of different strategies. *Cancer Treat Rev*. 2019;73:41–53. doi:10.1016/j.ctrv.2018.12.006.
54. Parseghian CM, Loree JM, Morris VK, et al. Anti-EGFR-resistant clones decay exponentially after progression: implications for anti-EGFR re-challenge. *Ann Oncol*. 2019;30(2):243–249. doi:10.1093/annonc/mdy509.
55. Khan KH, Cunningham D, Werner B, et al. Longitudinal liquid biopsy and mathematical modeling of clonal evolution forecast time to treatment failure in the PROSPECT-C phase II colorectal cancer clinical trial. *Cancer Discov*. 2018;8(10):1270–1285. doi:10.1158/2159-8290.CD-17-0891.
56. Cremolini C, Rossini D, Dell'Aquila E, et al. Rechallenge for patients with RAS and BRAF wild-type metastatic colorectal cancer with acquired resistance to first-line cetuximab and irinotecan: a phase 2 single-arm clinical trial. *JAMA Oncol*. 2019;5(3):343–350. doi:10.1001/jamaoncol.2018.5080.
57. Sunakawa Y, Nakamura M, Ishizaki M, et al. RAS mutations in circulating tumor DNA and clinical outcomes of rechallenge treatment with anti-EGFR antibodies in patients with metastatic colorectal cancer. *JCO Precis Oncol*. 2020;4:898–911. doi:10.1200/PO.20.00109.
58. Nakamura M. Phase II study of cetuximab rechallenge in patients with RAS wild-type metastatic colorectal cancer: e-rechallenge trial. *Ann Oncol*. 2019;30:vi116. doi:10.1093/annonc/mdz338.112.
59. Martinelli E, Martini G, Famiglietti V, et al. Cetuximab rechallenge plus avelumab in pretreated patients with RAS wild-type metastatic colorectal cancer: the phase 2 single-arm clinical CAVE trial. *JAMA Oncol*. 2021;7(10):1529–1535. doi:10.1001/jamaoncol.2021.2915.
60. Ohhara Y, Shinozaki E, Osawa H, et al. Liquid biopsy for optimizing the rechallenge of cetuximab in metastatic colorectal cancer: additional study of e-rechallenge trial. *JCO*. 2019;37(4_suppl):585. doi:10.1200/JCO.2019.37.4_suppl.585.
61. Karani A, Felismino TC, Diniz L, Macedo MP, Silva VSE, Mello CA. Is there a role for rechallenge and reintroduction of anti-EGFR plus chemotherapy in later lines of therapy for metastatic colorectal carcinoma? A retrospective analysis. *Ecancermedicalscience*. 2020;14:1069. doi:10.3332/ecancer.2020.1069.
62. Sartore-Bianchi A, Pietrantonio F, Lonardi S, et al. Circulating tumor DNA to guide rechallenge with panitumumab in metastatic colorectal cancer: the phase 2 CHRONOS trial. *Nat Med*. 2022;28(8):1612–1618. doi:10.1038/s41591-022-01886-0.
63. Parseghian CM, Vilar Sanchez E, Sun R, et al. Phase 2 study of anti-EGFR rechallenge therapy with panitumumab with or without trametinib in advanced colorectal cancer. *JCO*. 2022;40(16_suppl):3520. doi:10.1200/JCO.2022.40.16_suppl.3520.
64. Kagawa Y, Kotani D, Bando H, et al. Plasma RAS dynamics and anti-EGFR rechallenge efficacy in patients with RAS/BRAF wild-type metastatic colorectal cancer: REMARRY and PURSUIT trials. *JCO*. 2022;40(16_suppl):3518. doi:10.1200/JCO.2022.40.16_suppl.3518.
65. Van Cutsem E, Lenz HJ, Köhne CH, et al. Fluorouracil, leucovorin, and irinotecan plus cetuximab treatment and RAS mutations in colorectal cancer. *J Clin Oncol*. 2015;33(7):692–700. doi:10.1200/JCO.2014.59.4812.
66. Douillard JY, Oliner KS, Siena S, et al. Panitumumab-FOLFOX4 treatment and RAS mutations in colorectal cancer. *N Engl J Med*. 2013;369(11):1023–1034. doi:10.1056/NEJMoa1305275.
67. Bokemeyer C, Köhne CH, Ciardiello F, et al. FOLFOX4 plus cetuximab treatment and RAS mutations in colorectal cancer. *Eur J Cancer*. 2015;51(10):1243–1252. doi:10.1016/j.ejca.2015.04.007.
68. Maughan TS, Adams RA, Smith CG, et al. Addition of cetuximab to oxaliplatin-based first-line combination chemotherapy for treatment of advanced colorectal cancer: results of the randomised phase 3 MRC COIN trial. *Lancet*. 2011;377(9783):2103–2114. doi:10.1016/S0140-6736(11)60613-2.
69. Hurwitz H, Fehrenbacher L, Novotny W, et al. Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. *N Engl J Med*. 2004;350(23):2335–2342. doi:10.1056/NEJMoa032691.
70. Saltz LB, Clarke S, Díaz-Rubio E, et al. Bevacizumab in combination with oxaliplatin-based chemotherapy as first-line therapy in metastatic colorectal cancer: a randomized phase III study. *J Clin Oncol*. 2008;26(12):2013–2019. doi:10.1200/JCO.2007.14.9930.
71. Peeters M, Price T, Boedigheimer M, et al. Evaluation of emergent mutations in circulating cell-free DNA and clinical outcomes in patients with metastatic colorectal cancer treated with panitumumab in the ASPECCT study. *Clin Cancer Res*. 2019;25(4):1216–1225. doi:10.1158/1078-0432.CCR-18-2072.
72. Arnold D, Lueza B, Douillard JY, et al. Prognostic and predictive value of primary tumour side in patients with RAS wild-type metastatic colorectal cancer treated with chemotherapy and EGFR directed antibodies in six randomized trials. *Ann Oncol*. 2017;28(8):1713–1729. doi:10.1093/annonc/mdx175.
73. Stintzing S, Modest DP, Rossius L, et al. FOLFIRI plus cetuximab versus FOLFIRI plus bevacizumab for metastatic colorectal cancer (FIRE-3): a post-hoc analysis of tumour dynamics in the final RAS wild-type subgroup of this randomised open-label phase 3 trial. *Lancet Oncol*. 2016;17(10):1426–1434. doi:10.1016/S1470-2045(16)30269-8.
74. Tejpar S, Stintzing S, Ciardiello F, et al. Prognostic and predictive relevance of primary tumor location in patients with RAS wild-type metastatic colorectal cancer: retrospective analyses of the CRYSTAL and FIRE-3 trials. *JAMA Oncol*. 2017;3(2):194–201. doi:10.1001/jamaoncol.2016.3797.
75. Venook AP, Niedzwiecki D, Innocenti F, et al. Impact of primary (1°) tumor location on overall survival (OS) and progression-free survival (PFS) in patients (pts) with metastatic colorectal cancer (mCRC): analysis of CALGB/SWOG 80405 (alliance). *JCO*. 2016;34(15_suppl):3504. doi:10.1200/JCO.2016.34.15_suppl.3504.
76. Stintzing S, Heinemann V, Fischer von Weikersthal L, et al. Phase III FIRE-4 study (AIO KRK-0114): Influence of baseline liquid biopsy results in first-line treatment efficacy of FOLFIRI/cetuximab in patients with tissue RAS-WT mCRC. *JCO*. 2023;41(16_suppl):3507. doi:10.1200/JCO.2023.41.16_suppl.3507.
77. Mazard T, Ghiringhelli F, Winter A, et al. LBA30 panitumumab (P) + FOLFIRINOX or mFOLFOX6 in unresectable metastatic colorectal cancer (mCRC) patients (pts) with RAS/BRAF wild-type (WT) tumor status from circulating DNA (cirDNA): first results of the randomised phase II PANIRINOX-UCGI28 study. *Ann Oncol*. 2023;34:S1270. doi:10.1016/j.annonc.2023.10.022.
78. Parseghian CM, Sun R, Woods M, et al. Resistance mechanisms to anti-epidermal growth factor receptor therapy in RAS/RAF wild-type colorectal cancer vary by regimen and line of therapy. *J Clin Oncol*. 2023;41(3):460–471. doi:10.1200/JCO.22.01423.
79. D'Onofrio R, Caputo F, Prampolini F, et al. CtDNA-guided rechallenge with anti-EGFR therapy in RASwt metastatic colorectal cancer: Evidence from clinical practice. *Tumori*. 2023;109(4):387–393. doi:10.1177/03008916221122554.

Please cite this article as: Laura Roazzi et al, Ongoing Clinical Trials and Future Research Scenarios of Circulating Tumor DNA for the Treatment of Metastatic Colorectal Cancer, Clinical Colorectal Cancer, https://doi.org/10.1016/j.clcc.2024.02.001

80. Prager GW, Taieb J, Fakih M, et al. Trifluridine–tipiracil and bevacizumab in refractory metastatic colorectal cancer. *N Engl J Med*. 2023;388(18):1657–1667. doi:10.1056/NEJMoa2214963.
81. Shitara K, Yamanaka T, Denda T, et al. REVERCE: a randomized phase II study of regorafenib followed by cetuximab versus the reverse sequence for previously treated metastatic colorectal cancer patients. *Ann Oncol*. 2019;30(2):259–265. doi:10.1093/annonc/mdy526.
82. Russo M, Crisafulli G, Sogari A, et al. Adaptive mutability of colorectal cancers in response to targeted therapies. *Science*. 2019;366(6472):1473–1480. doi:10.1126/science.aav4474.
83. Kurai J, Chikumi H, Hashimoto K, et al. Antibody-dependent cellular cytotoxicity mediated by cetuximab against lung cancer cell lines. *Clin Cancer Res*. 2007;13(5):1552–1561. doi:10.1158/1078-0432.CCR-06-1726.
84. Siravegna G, Lazzari L, Crisafulli G, et al. Radiologic and genomic evolution of individual metastases during HER2 blockade in colorectal cancer. *Cancer Cell*. 2018;34(1):148–162 e7. doi:10.1016/j.ccell.2018.06.004.
85. Ros J, Matito J, Villacampa G, et al. Plasmatic BRAF-V600E allele fraction as a prognostic factor in metastatic colorectal cancer treated with BRAF combinatorial treatments. *Ann Oncol*. 2023;34(6):543–552. doi:10.1016/j.annonc.2023.02.016.
86. Nakamura Y, Taniguchi H, Ikeda M, et al. Clinical utility of circulating tumor DNA sequencing in advanced gastrointestinal cancer: SCRUM-Japan GI-SCREEN and GOZILA studies. *Nat Med*. 2020;26(12):1859–1864. doi:10.1038/s41591-020-1063-5.
87. Van Emburgh BO, Arena S, Siravegna G, et al. Acquired RAS or EGFR mutations and duration of response to EGFR blockade in colorectal cancer. *Nat Commun*. 2016;7:13665. doi:10.1038/ncomms13665.
88. Pal A, Shinde R, Miralles MS, Workman P, de Bono J. Applications of liquid biopsy in the pharmacological audit trail for anticancer drug development. *Nat Rev Clin Oncol*. 2021;18(7):454–467. doi:10.1038/s41571-021-00489-x.
89. Desai J, Alonso G, Kim SH, et al. Divarasib plus cetuximab in KRAS G12C-positive colorectal cancer: a phase 1b trial |. *Nat Med*. 2024;30(1):271–278. doi:10.1038/s41591-023-02696-8.
90. Le Tourneau C, Delord JP, Gonçalves A, et al. Molecularly targeted therapy based on tumour molecular profiling versus conventional therapy for advanced cancer (SHIVA): a multicentre, open-label, proof-of-concept, randomised, controlled phase 2 trial. *Lancet Oncol*. 2015;16(13):1324–1334. doi:10.1016/S1470-2045(15)00188-6.
91. Massard C, Michiels S, Ferté C, et al. High-throughput genomics and clinical outcome in hard-to-treat advanced cancers: results of the MOSCATO 01 trial. *Cancer Discov*. 2017;7(6):586–595. doi:10.1158/2159-8290.CD-16-1396.
92. Trédan O, Wang Q, Pissaloux D, et al. Molecular screening program to select molecular-based recommended therapies for metastatic cancer patients: analysis from the ProfiLER trial. *Ann Oncol*. 2019;30(5):757–765. doi:10.1093/annonc/mdz080.
93. Nicolazzo C, Barault L, Caponnetto S, et al. True conversions from RAS mutant to RAS wild-type in circulating tumor DNA from metastatic colorectal cancer patients as assessed by methylation and mutational signature. *Cancer Lett*. 2021;507:89–96. doi:10.1016/j.canlet.2021.03.014.
94. Klein-Scory S, Wahner I, Maslova M, et al. Evolution of RAS mutational status in liquid biopsies during first-line chemotherapy for metastatic colorectal cancer. *Front Oncol*. 2020;10:1115. doi:10.3389/fonc.2020.01115.
95. Henry J, Willis J, Parseghian CM, et al. NeoRAS: incidence of RAS reversion from RAS mutated to RAS wild type. *JCO*. 2020;38(4_suppl):180. doi:10.1200/JCO.2020.38.4_suppl.180.
96. Montes AF, Lago NM, Gómez JD, La C, et al. FOLFIRI plus panitumumab as second-line treatment in mutated RAS metastatic colorectal cancer patients who converted to wild type RAS after receiving first-line FOLFOX/CAPOX plus bevacizumab-based treatment: phase II CONVERTIX trial. *Ann Oncol*. 2019;30:iv23–iv24. doi:10.1093/annonc/mdz155.088.
97. Bouchahda M, Saffroy R, Karaboué A, et al. Undetectable RAS-mutant clones in plasma: possible implication for anti-EGFR therapy and prognosis in patients with RAS-mutant metastatic colorectal cancer. JCO Precision *Oncol*. 2020(4):1070–1079. doi:10.1200/PO.19.00400.
98. Raimondi C, Nicolazzo C, Belardinilli F, et al. Transient disappearance of RAS mutant clones in plasma: a counterintuitive clinical use of EGFR inhibitors in RAS mutant metastatic colorectal cancer. *Cancers (Basel)*. 2019;11(1):42. doi:10.3390/cancers11010042.
99. Nicolazzo C, Barault L, Caponnetto S, et al. Circulating methylated DNA to monitor the dynamics of RAS mutation clearance in plasma from metastatic colorectal cancer patients. *Cancers (Basel)*. 2020;12(12):3633. doi:10.3390/cancers12123633.
100. Moati E, Blons H, Taly V, et al. Plasma clearance of RAS mutation under therapeutic pressure is a rare event in metastatic colorectal cancer. *Int J Cancer*. 2020;147(4):1185–1189. doi:10.1002/ijc.32657.
101. Bessa X, Vidal J, Balboa JC, et al. High accuracy of a blood ctDNA-based multimodal test to detect colorectal cancer. *Ann Oncol*. 2023;34(12):1187–1193. doi:10.1016/j.annonc.2023.09.3113.
102. Use of circulating tumor deoxyribonucleic acid for early-stage solid tumor drug development; draft guidance for industry; 2022. Accessed August 22, 2023. Available at: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/use-circulating-tumor-deoxyribonucleic-acid-early-stage-solid-tumor-drug-development-draft-guidance.

Please cite this article as: Laura Roazzi et al, Ongoing Clinical Trials and Future Research Scenarios of Circulating Tumor DNA for the Treatment of Metastatic Colorectal Cancer, Clinical Colorectal Cancer, https://doi.org/10.1016/j.clcc.2024.02.001