New Insights into Adjuvant Therapy for Localized Colon Cancer

Nadia Saoudi González, MD, MSC[a],
Francisco Javier Ros Montaña, MD, MSC[a,b],
David García Illescas, MD[a], Iosune Baraibar Argota, MD, PhD[a],
Francesc Salvà Ballabrera, MD, MSC[a],
Ma Elena Élez Fernández, MD, PhD[a,*]

KEYWORDS

• Adjuvant treatment • Colon cancer • liquid biopsy • ctDNA • Immunoscore

KEY POINTS

- Since the MOSAIC trial published in 2004, there have not been any significant advances in new therapies in the adjuvant setting in localized colon cancer. However, in recent years, new tools have been added to support therapeutic management and identify patients likely to benefit from adjuvant therapy.
- The Immunoscore is a novel scoring system accounting for the immune aspect of colon cancer, summarizing the density of CD3+ and CD8+ T cell effectors within the tumor and at the invasive tumor margin. A new TNM classification has been proposed that incorporates the immune component, the TNM-immune classification.
- For patients with stage III colon cancer, noninferiority of 3 months of adjuvant therapy compared with 6 months has not been demonstrated. However, for selected subgroups, the shorter duration of therapy may limit toxic effects without impairing clinical outcomes.
- Circulating tumor DNA (ctDNA) analysis after surgery is a robust prognostic marker in localized colon cancer. Postsurgical ctDNA positive analysis defines a patient subset that remains at high risk of recurrence because of molecular evidence of metastatic disease.

INTRODUCTION

Colorectal cancer (CRC) is the second leading cause of cancer deaths and the third most common cancer diagnosed worldwide.[1] Approximately 80% of newly diagnosed

[a] Department of Medical Oncology, Vall d'Hebron University Hospital, Passeig de la Vall d'Hebron, 119, Barcelona, Catalunya 08035, Spain; [b] Medicine, Università degli Studi della Campania Luigi Vanvitelli, Caserta, Campania, Italy
* Corresponding author.
E-mail address: meelez@vhio.net

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CRC patients present with early-stage disease.[2] For patients with localized disease, defined as stages I, II, and III in the eighth American Joint Committee on Cancer staging system, surgical resection of the primary tumor as well as the regional lymph nodes is the only curative option. Thus, early detection and an accurate adjuvant treatment constitute the 2 main opportunities to achieve a cure for this disease.

Approximately half of all patients with localized colon cancer who undergo surgery without adjuvant therapy will ultimately relapse.[3] Consequently, adjuvant systemic chemotherapy plays a key role in reducing the risk of disease relapse by eradicating clinically undetectable micrometastatic disease, also known as minimal residual disease (MRD). Currently, clinicopathologic characteristics of tumors guide the selection of patients suitable for adjuvant therapy, based on the rationale that patients with a higher risk of recurrence can obtain greater benefit from adjuvant chemotherapy. Therefore, adjuvant chemotherapy is recommended for all patients with stage III colon cancer (with lymph node involvement). The role of adjuvant treatment in stage II disease remains a subject of debate because it is not recommended for all patients. The decision is made on an individual basis, considering all tumor risk factors.[4]

Paradoxically, clinical outcomes can be very different among patients within the same stage. This has driven the development of new pathologic classifications in CRC for accurate tumor staging integrating immune system dynamics. In patients with CRC, a correlation between time to recurrence and overall survival (OS) has been described with regard to the strength of the adaptive immune reaction.[5,6] The Immunoscore is a scoring system that considers the density of CD3+ and CD8+ T cell effectors within the tumor and at the invasive margin using digital pathology in paraffin sections.[7] This novel score estimates the risk of recurrence among patients with localized colon cancer. Patients with a high Immunoscore have a lower risk of relapse compared with patients with low Immunoscore (HR 0.31 95% CI 0.23–0.41; $P < 0.0001$). This tool has the potential to reliably identify patients who will relapse. The strength of Immunoscore is not only its capacity to predict tumor recurrence but also that it is a reproducible and objective platform. Based on the reviewed data, a new TNM classification has been suggested, including the immune component, the TNM-immune classification.[8] The accurate prognostic status provided by Immunoscore can help to identify those patients with more aggressive disease and therefore to identify candidates for standard chemotherapy.

In 2008, Diehl and colleagues demonstrated that circulating tumor DNA (ctDNA) reflects tumor response and progression after surgery and could be used as a marker for MRD and also as a prognostic factor,[9] suggesting that ctDNA could be a promising surrogate marker of MRD. ctDNA could be a predictive biomarker to help identify patients who will benefit the most from adjuvant therapy, thereby avoiding under or overtreating a substantial number of patients. Since then, several clinical trials have been initiated exploring the potential role of ctDNA in stratifying patients with the potential to benefit from adjuvant treatment. In this review, we discuss the current adjuvant treatment paradigm of localized colon cancer considering ctDNA and the optimal duration of adjuvant treatment.

Current Decision-Making in Localized Colon Cancer

The current standard of care for adjuvant therapy in stage III colon cancer (with lymph nodes involvement) is a combination of fluoropyrimidine and oxaliplatin based on 3 large-scale clinical trials: MOSAIC, NSABP C-07, and XELOXA.[10–12] In general, it has been established that adjuvant systemic therapy with fluoropyrimidines alone decreases the risk of death by an absolute 10% to 15% in stage III disease, with a further 4% to 5% improvement for oxaliplatin-containing combinations.[10–12] These benefits

were calculated based on historical clinical trial data before modern-day surgical techniques. The absolute risk of recurrence is now lower, and this, along with the nonnegligible risk of long-term and untreatable peripheral neuropathy associated with oxaliplatin, motivated efforts to examine deescalated chemotherapy approaches. The International Duration Evaluation of Adjuvant Therapy (IDEA) collaboration evaluated a shorter duration of adjuvant chemotherapy and will be discussed later in the review.

For stage II CRC patients with poor prognosis factors, such as pT4 stage or less than 12 lymph nodes assessed during the surgery, the National Comprehensive Cancer Network and European Society of Medical Oncology guidelines recommend adjuvant chemotherapy based on a combination of fluoropyrimidine and oxaliplatin.[4,13] The presence of only one of several additional minor prognostic factors (high-grade tumor, vascular invasion, lymphatic invasion, perineural invasion, tumor presentation with obstruction or perforation, and high preoperative CEA levels) is less significantly associated with risk of relapse, thus treatment with fluoropyrimidines as monotherapy is indicated in microsatellite stable tumors[4] because patients with stage II colon cancer with microsatellite instability or mismatch repair deficiency have an excellent prognosis and a potential resistance to 5-fluorouracil monotherapy.[14] Based on expert panel recommendations, patients with an accumulation of several minor prognostic risk factors might also be considered for the addition of oxaliplatin therapy based on a trend to an increased benefit without statistical significance in a stage II high-risk subgroup analysis of the MOSAIC trial,[12] although this has not been conclusively shown to improve OS.[15,16] In patients with high-risk stage II disease, neither 5-year disease-free survival (DFS) nor OS at 6 years was significantly increased by the addition of oxaliplatin (from 74.6% to 82.3%, and from 83.3% to 85%, respectively).

Regarding the significance of timing and early discontinuation of adjuvant treatment, it has been described that failure to complete planned therapy was significantly associated with adverse prognosis in terms of 5-year disease free-survival.[17] Furthermore, a meta-analysis that included 14 studies showed that adjuvant chemotherapy should be started within 8 weeks after surgery, and that delaying the initiation of adjuvant chemotherapy beyond 8 weeks after surgery significantly decreased OS.[18]

A major challenge in the adjuvant setting is the lack of progress in drug development during the past few decades, with no treatment demonstrated to be effective in this setting after treatment with oxaliplatin and fluoropyrimidine. Treatments that are active overall in the metastatic setting such as irinotecan and biological treatments such as bevacizumab and cetuximab have failed to demonstrate significant survival advantages compared with fluoropyrimidines and oxaliplatin in randomized trials.[19–23]

The International Duration Evaluation of Adjuvant Therapy Collaboration

Driven by the aim of reducing or avoiding the neurotoxicity associated with oxaliplatin, the IDEA collaborators investigated the role of chemotherapy duration, specifically 3 versus 6 months of capecitabine with oxaliplatin (CAPOX) or 5-fluorouracil, leucovorin, and oxaliplatin (FOLFOX).[24] The goal and primary endpoint was to demonstrate the noninferiority of 3 months treatment versus the standard of care of 6 months in stage III colon cancer, in terms of 3-year DFS. The IDEA collaboration is a prospective, pooled data analysis from 6 randomized phase 3 clinical trials of adjuvant chemotherapy that enrolled mostly patients with stage III colon cancer across 12 countries.[25–30]

Of the total 12,834 patients analyzed in the IDEA collaboration, about 60% received FOLFOX and 40% CAPOX. The distribution of T4 and N2 stage tumors, the choice of chemotherapy regimen, and the follow-up duration were heterogeneous between the

trials. CAPOX versus FOLFOX assignment was not randomized for any of the evaluated trials.

Noninferiority of 3 months versus 6 months was not confirmed in the overall population (HR, 1.07; 95% confidence interval [CI], 1.00–1.15; $P = 0.11$); at 3-year, DFS was 74.6% versus 75.5% (95% CI) in the 3 and 6-month therapy groups, respectively. Neurotoxicity grade of 2 or greater and other toxicities were more frequent in the group with longer duration. In an analysis by subgroups of treatment type (5-fluorouracil or capecitabine), FOLFOX for 6 months was superior to FOLFOX for 3 months (HR = 1.16; 95% CI, 1.06–1.26), whereas CAPOX for 3 months demonstrated noninferiority compared with CAPOX for 6 months (HR = 0.95; 95% CI, 0.85–1.06) in the overall population.

An unplanned classification of patients (post-hoc analysis) was done according to risk associated with "T" and "N." Approximately 60% of patients had low-risk disease (T1-3/N1) compared with 40% with high-risk disease (T4/N2). Among low-risk patients, CAPOX for 3 months was noninferior to CAPOX for 6 months, but FOLFOX for 3 months did not meet conditions to prove noninferiority versus 6 months in this group. For high-risk patients, FOLFOX for 3 months was inferior to FOLFOX for 6 months, and CAPOX for 3 months did not demonstrate noninferiority compared with CAPOX for 6 months. An updated analysis of DFS, after a median follow-up of 72.3 months, confirmed the previous findings.[31]

Circulating Tumor DNA and Minimal Residual Disease

Tumor sample collection is an invasive procedure, and furthermore this measure does not fully capture tumor heterogeneity because this technique is static in time and space. In this context, the advantages are clear with the analysis of ctDNA as a noninvasive method that can evaluate broad heterogenous tumor profiles and track the evolution of genomic changes over time. ctDNA represents only a small fraction of the total circulating free DNA (cfDNA) because most cfDNA consists of DNA released from normal cells.[32] ctDNA constitutes from as little as 0.01% or less to greater than 10% of the cfDNA depending on tumor stage, disease burden, biologic shedding, and disease site.[33] DNA fragments derived from cancer cells are typically shorter in length than cfDNA, between 90 and 150 base pairs. Thus, separating these fragments from total circulating DNA improves ctDNA assay sensitivity.[34] The use of ctDNA as a marker in the blood is more reliable than using circulating tumor cells due to it being present at higher concentrations in blood, and its high sensitivity and specificity.[35] Thus, ctDNA could be a useful tool to detect MRD and therefore a good indicator of patients at higher risk of relapse. Nevertheless, the predictive value of ctDNA in advanced colon cancer remains unclear.

Localized CRC has a lower tumor burden than the metastatic setting, making it more challenging to identify ctDNA (approximately 0.01% of total cfDNA).[36] Furthermore, the ctDNA detection rate drops notably following curative tumor resection.[37] Therefore, methods with high sensitivity and consistency are needed to detect these extremely low concentrations of ctDNA in the blood.

Currently, 2 main strategies are used to study ctDNA in this setting, techniques based on the detection of mutations previously found in the primary tissue tumor or techniques based on deep next-generation sequencing (NGS) methods, allowing the detection of mutations not known a priori in the primary tissue, such as whole genome sequencing (WGS) or whole exome sequencing (WES).

Digital polymerase chain reaction (PCR), such as droplet digital PCR (ddPCR),[38] and techniques with beads, emulsion, amplification, and magnetics (BEAMing)[39] have been used by several groups. These techniques allow the detection of known

Table 1
Characteristics and outcomes from retrospective analyses of clinical trial integrating ctDNA analyses in advanced CRC patients

	Tie 2016[37]	Taieb 2019[53]		Reinert 2019[48]			Tie 2019[54]		Tie 2019[55]	Parikh 2019[56]		Tarazona 2019[57]		Khakoo 2020[58]		
Number of Patients	**230**	**805**		**125**			**159**		**96**	**72**		**150**		**47**		
Tumor stage	II	II	III	I 5 (4%)	II 39 (31%)	III 81 (65%)	II 35 (22%)	III 124 (78%)	III	II	III	II	III	I	II	III
Adjuvant chemo-therapy																
Yes	52 (23%)	805 (100%)		77 (33%)			57 (36%)		96 (100%)	30 (41%)		56 (37%)		43 (91%)		
No	178 (77%)	0		153 (67%)			102 (64%)		0	42 (59%)		94 (63%)		4 (9%)		
ctDNA analysis platform	Safe-SeqS	ddPCR		Signatera			Safe-SeqS		Safe-Seq	Guardant health NGS		ddPCR		ddPCR		
+ctDNA post IQ	14 (8%)[a]	109 (14%)		10 (8%)[b]			19 (12%)		20 (21%)	14 (19%)		30 (20%)		6 (13%)		
Risk of recurrence of +ctDNA postsurg	HR 18 (95% CI 7.9–40) $P < 0.001$	HR 1.75 (95% CI 1.25–2.45) $P < 0.001$		HR 7.2 (95% CI 2.7–19) $P < 0.001$			HR 13 (95% CI 5.5–31) $P < 0.001$		HR3.8 (95% CI 2.4–21) $P < 0.001$	7/8 ctDNA + recurred 7/34 ctDNA – recurred		HR 17.56 $P = 0.0014$		3/3 ctDNA + recurred 0/20 ctDNA – recurred $P < 0.001$		
Risk of Recurrence of +ctDNA post-ADJ	HR 11 (95% CI, 1.8–68) $P = 0.001$	NR		HR 17.5 (95% CI 5.4–56.5) $P < 0.001$			NR		HR 6.8 (95% CI 11–157) $P < 0.001$	HR 11.3 $P < 0.0001$		HR 10 (95% CI 9–307) $P < 0.0001$		HR 7.1 (95% CI 2.4–21.5) $P < 0.001$[c]		

Abbreviations: CI, confidence interval; ctDNA, circulating tumor DNA; ddPCR, droplet digital PCR; IQ, surgery; HR, hazard ratio; NGS, next-generation sequencing; NR, not reported; PostADJ, postadjuvant therapy; Postsurg, postoperative.

[a] Only patients not receiving adjuvant treatment.

[b] Plasma collected at day 30 post-IQ was available for 94 patients.

[c] After neoadjuvant chemo-radiation.

Table 2
Ongoing prospective clinical trials including patients with low-risk stage II colon cancer

Trial Name	Dynamic	NRG GI-005 (Cobra)	MEDOCC-CrEATE	Circulate AIO-KRK-0217	Circulate Prodige 70
Identifier	(ACTRN-12615000381583)	NCT04068103	NL6281/NTR6455)	NCT04089631	NCT04120701
Region	Australia	Canada and USA	Netherlands	Germany	France
Population	Stage II	Stage II	Stage II	Stage II Only ctDNA + pts	Stage II Only ctDNA + pts
ctDNA analysis	Safe-SeqS	Guardant LUNAR-1™	PGDx elio™	-	ddPCR **
Timing of liquid biopsy	POp 4–7 wk	Immediate POp	POp 0.5–3 wk	Immediate POp	POp 2–8 wk
Control arm (SoC)	Surveillance or ACT based on clinico-pathological risk	Surveillance	Surveillance	Surveillance	Surveillance
Experimental arm (ctDNA-guided) (+ or –)	+ ACT – surveillance	+ CAPOX/FOLFOX for 6 mo – Surveillance	+ CAPOX 6 mo – Surveillance	ACT (capecitabine or CAPOX)	FOLFOX
Hypothesis/primary objective	POp negative ctDNA reduces using of ACT without impact RFS	6-mo RFS is better in ctDNA + experimental arm	Pts with POp ctDNA + will be more convinced of receiving ACT	ACT achieves better DFS than surveillance	FOLFOX achieves ≥17.5% benefit in 3-y DFS

Table 3
Ongoing prospective clinical trials including patients with high-risk stage II and stage III colon cancer

Trial Name	DYNAMIC-III	TRACC	VEGA	ACT-3	IMPROVE-IT2	PEGASUS[c]
Identifier	(ACTRN-12615000381583)	NCT04050345	UMIN000039205	NCT04259944	NCT04084249	NCT04259944
Region	Australia and New Zealand	United Kingdom	Japan	USA	Denmark	Spain and Italy
Population	Stage III	High-risk stage II and stage III colorectal	High-risk stage II and low-risk stage III Only ctDNA – pts	Stage III Only ctDNA + pts after completion of ACT	High-risk stage II and stage III	High-risk stage II and stage III
ctDNA Analysis	Safe-SeqS	NGS (22 gene panel)	Signatera™	Guardant LUNAR-1™	ddPCR	Guardant LUNAR-1™
Timing of liquid biopsy	POp 5–6 wk	POp <8 wk	POp 4 wk	3–6 wk after ACT	Every 16 wk until 2 y	POp 2–4 wk and after ACT
Control arm (SoC)	ACT based on clinical risk	Capecitabine 6 mo or CAPOX 3 mo	CAPOX 3 mo	Surveillance	Surveillance: CT at 1 and at 3 y	POp ctDNA: + CAPOX 3 mo – Cape 6 mo[c]
Experimental arm (ctDNA-guided) (+ or –)	+ ACT escalation[a] – ACT deescalation[a]	+ standard ACT – ACT deescalation (reescalation if ctDNA + at 3 mo)	Surveillance[b]	MSI: nivolumab BRAF mutated: CEB[d] MSS/BRAF-wt: FOLFIRI	Surveillance by ctDNA: if ctDNA+, 3 monthly PET	After ACT: +/+ ACT escalation –/+ ACT deescalation +/– ACT deescalation –/– deescalation
Hypothesis	Escalation ACT is superior and de-escalation ACT is noninferior to SoC in terms of RFS	ctDNA-guided ACT is noninferior to SoC in 3-y DFS	Surveillance is noninferior to CAPOX	FOLFIRI is superior to surveillance	ctDNA combined to imaging can detect recurrence earlier	De/escalation strategy by double ctDNA assessment is reliable for molecular adjuvant treatment

Abbreviations: ACT, Adjuvant chemotherapy; CAPOX, capecitabine plus oxaliplatin; ddPCR, droplet digital PCR; DFS, disease-free survival; N, number; POp, post-operative; Pts, patients; RFS, recurrence-free survival; SoC, standard of care.

[a] Escalation from preplanned treatment by increasing the number of agents used or the duration of the adjuvant treatment. Deescalation from preplanned treatment by decreasing the number of agents used or the duration of the adjuvant treatment.

[b] If ctDNA + at 3 mo, patients will be randomized to surveillance versus trifluridine/tipiracil (ALTAIR clinical trial, UMIN000039205).

[c] PEGASUS study: Standard of care and experimental are not applicable in this study.

[d] CEB: cetuximab, encorafenib, binimetinib.

mutations of interest, with a high degree of sensitivity that allows identification of the scarcest variant allele with a frequency of 0.01% or lower.[40] Other techniques that allow detection of mutations previously found in the primary tissue tumor are quantitative PCR-based methods, such as amplification refractory mutation system or coamplification at lower denaturation PCR.[41] However, these techniques are unable to detect mutations not known a priori in the primary tissue, thus limiting the assessment of intratumor heterogeneity and emergent mutations that could confer resistance to treatments. Assays based on NGS such WGS or WES, allow for the detection of multiple genetic alterations in one sample. Nevertheless, NGS techniques have potential limitations, such as a requirement for higher amounts of cfDNA to decrease the presence of false-negative results,[42] and the detection of mutations in clonal hematopoietic cells that could be incorrectly interpreted as the presence of ctDNA carrying somatic mutations.[43] New strategies, such as the detection of methylation patterns that could reveal the origin of circulating DNA, or the study of the nucleosome position to identify the tissue source of origin of cfDNA, are being studied to improve sensitivity in the evaluation of ctDNA.[44,45]

The first study evaluating ctDNA as a potential marker of MRD in CRC was conducted in 2008 and included 18 patients with resected colorectal liver metastases.[9] The study demonstrated that ctDNA levels (detected using a BEAMing assay) declined after metastasectomy. At the first follow-up, all patients but one with detectable ctDNA experienced recurrence. In contrast, none of the patients with undetectable ctDNA at first follow-up experienced recurrence. This observation inspired several studies to validate ctDNA as a marker of MRD. All completed ctDNA studies published to date are noninterventional studies, and all affirm the observation that patients with detectable ctDNA after surgery have a high risk of recurrence and predict the appearance of both clinical and radiological recurrence, whereas patients who are completely negative for ctDNA after surgery have a lower probability of recurrence.

The role of ctDNA as a predictive marker has been analyzed retrospectively across several clinical studies. **Table 1** summarizes outcomes from published clinical trials that included the use of liquid biopsy for ctDNA analyses. Most trials that are currently ongoing are prospectively evaluating the role of ctDNA for escalating or deescalating adjuvant chemotherapy to prevent unnecessary toxicity and to identify patients who are cured after radical surgery, distinct from patients with molecular metastatic disease defined as detectable ctDNA.[46] A large number of prospective clinical trials include an evaluation of the potential role of ctDNA for patients with advanced colon cancer in adjuvant setting. **Tables 2** and **3** summarize ongoing randomized interventional adjuvant trials in colon cancer including ctDNA analyses.

ctDNA analysis will offer more precise information for risk stratification, and have the potential to improve decisions over the intensity and the duration of adjuvant therapy, whereas serial ctDNA analysis may also provide an early real-time means of identifying patients with the efficacy of adjuvant treatment.

SUMMARY

Following the landmark of the MOSAIC trial, demonstrating that the addition adjuvant treatment with FOLFOX increases DFS, there has been a lack of any significant advances regarding adjuvant treatment in localized colon cancer.[47] During recent years, this has changed and significant developments have been made, including the development of the Immunoscore classification and liquid biopsies, both demonstrated to be accurate and reliable tools to identify patients who will relapse. Complementing these technical advances are the results from the IDEA trial, which suggest that the

duration of adjuvant chemotherapy could be reduced, thereby reducing toxicity, without deleterious effects on outcomes in specific subgroups.

Currently, significant updates in the adjuvant setting involve better classifications tools rather than novel drugs. With a drug armamentarium that has remained unchanged for many years, clinical efforts have focused on limiting chemotherapy-related toxicity. The importance of immune infiltration within the tumor and at the tumoral edge has emerged as an area of interest to guide the use of adjuvant chemotherapy. Pagès and colleagues demonstrated that the Immunoscore splits patients into 3 different subgroups regarding relapse-free survival. Patients with high Immunoscore have better clinical outcomes compared with those patients with low immune infiltration.[7] Despite the accurate prognostic description that Immunoscore provides, after radical surgery, we cannot know whether or not the patient is cured. However, in recent years the role of liquid biopsies to identify MRD after radical surgery has been demonstrated. Thus, the prognostic role of liquid biopsy—confirming the presence of molecular metastatic disease—in this setting is now well established. Liquid biopsies have allowed physicians to identify those patients at high risk of relapse after surgery, with demonstrations that among 10% of the patients with a positive liquid biopsy after surgery, 90% of them will relapse.[37,48] Despite this high sensitivity, not all patients with positive liquid biopsy will experience recurrence. Moreover, it is of critical importance to note that among patients with a negative liquid biopsy, 10% will relapse, highlighting the need to develop more sensitive techniques. Liquid biopsy using multiple ddPCR or methylation raises the sensitivity of standard techniques. Furthermore, longitudinal tracking of liquid biopsy has been demonstrated to significantly increase the sensitivity of the technique, identifying patients who will relapse, and crucially including patients with a negative liquid biopsy immediately after the surgery.

Some differences in the key studies in localized colon cancer using liquid biopsy are worth highlighting, and their potential repercussions. First, the Danish group included stage I–III tumors and used the Signatera platform,[48] whereas the Australian group focused on stage II tumors and used a different platform, the Safe-Seq assay.[37] Moreover, the 2 groups used different definitions of a positive liquid biopsy: the Danish group defined a positive biopsy as samples with at least 3 detected genomic alterations, whereas the Australian group considered samples with only one identified mutation to be positive, and positivity was defined by a permutation test. Despite these differences, the overall results were similar. This raises some important points to consider. For example, despite the analytical sensitivity of ctDNA assays, false negatives have been described in the metastatic setting due to biological factors. In addition, there is a relationship between the number and the size of the lesions and the amount of ctDNA.[49] Furthermore, some tumor sites play a significant role in the shedding of ctDNA, such as in patients with liver metastases who more frequently have positive liquid biopsies, compared with patients with nodal or lung spread.[50] Similarly, specific histologies (eg, mucinous) have also been related to a high rate of negative liquid biopsy.[33]

Another challenge is to determine the optimal timing for blood sample collection, both postoperatively and over time, to detect progression as soon as possible. Recent studies described that after major trauma—such as after surgery—there is a release of cfDNA that could hamper ctDNA detection until 4 weeks after the surgery because of the longer half-life of cfDNA compared with ctDNA.[51] The optimal timing seems to be approximately 4 weeks postsurgery. However, longitudinal ctDNA monitoring demonstrated the strongest prognostic power overall. Updated results of the aforementioned Danish cohort were recently reported for 260 patients (4 stage I, 90 stage II, and 166 stage III). When a single liquid biopsy was performed, relapses among ctDNA positive

and ctDNA negative groups were reported in 80% and 20% of patients, respectively. However, when longitudinal tracking was done, relapses among ctDNA positive patients increased to 89.3%, and relapses among ctDNA negative patients declined to 10.7%.[52]

Interestingly, the current definition of a positive liquid biopsy has also been redefined beyond a simple positive or negative status. A recent trial with 485 patients including stage II and III colon tumors and locally advanced rectal tumors. Plasma samples were collected 4 to 10 weeks after surgery and mutations in ctDNA were assayed using Safe-SeqS. ctDNA was detected after surgery in 59 (12%) patients overall. Recurrence risk increased exponentially with increasing ctDNA mutant allele fraction (MAF; HR, 1.2, 2.5 and 5.8 for MAF of 0.1%, 0.5% and 1%, respectively). Interestingly, using ctDNA MAF, a cut-off of 0.046% predicted 3-year recurrence-free survival (9% among patients with MAF >0.046%, and 33% among patients with MAF $\leq$ 0.046%).[51]

The prognostic value of ctDNA in the localized scenario is well established; however, its predictive role remains to be clarified. Using a window of opportunity provided by better stratification factors, new clinical trials have been designed aiming to escalate or deescalate adjuvant therapy based on the results of the liquid biopsy. A key driver is the hypothesis that patients with a positive liquid biopsy postsurgery or during the adjuvant treatment could obtain clinical benefit if treatment is escalated, whereas those patients with postsurgical negative liquid biopsy or those patients whose liquid biopsy switched from positive to negative could receive a less toxic treatment. Serial ctDNA analysis may also provide an early real-time read-out of adjuvant treatment efficacy, improving the efficiency of adjuvant trials and novel drug development. In the next few years, the results of these clinical trials will clarify the role of liquid biopsies as a driver of adjuvant treatment. This real-time assessment of treatment benefits could be used as a surrogate endpoint for adjuvant novel drug development.

CLINICS CARE POINTS

- Noninferiority of 3 months vs 6 months was not confrimed in the IDEA trial. Nevertheless, treatment with CAPOX for 3 months could be used among low-risk patients.
- ctDNA is a prognostic biomarker in localized CRC, and may be able to be used to guide treatment decisions and risk stratify patients who have CRC.
- Patients with high Immunoscore have better clinical outcomes compared with those patients with low immune infiltration.

DISCLOSURE

The authors have no COI in relation to this review.

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