CtDNA for Colorectal Cancer

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Colorectal cancer (CRC) is one of the leading causes of cancer-related deaths worldwide. While there have been significant developments in the treatments for patients with metastatic CRC in recent years, improving outcomes in the adjuvant setting has been more challenging. Recent technological advances in circulating tumour DNA (ctDNA) assay with the ability to detect minimal residual disease (MRD) after curative intent surgery will fundamentally change how we assess recurrence risk and conduct adjuvant trials. Studies in non-metastatic CRC have now demonstrated the prognostic impact of ctDNA analysis after curative intent surgery over and above current standard of care clinicopathological criteria.

Keywords: ctDNA ; adjuvant chemotherapy ; colorectal cancer

1. Introduction

Colorectal cancer (CRC) is the third most common cancer worldwide and is the second leading cause of cancer-related deaths [1]. The total number of deaths is predicted to rise in rectal and colon cancer by 60% and 71.5% respectively by the year 2035 [2]. Although there have been significant advances in the treatment for patients with metastatic disease with median overall survival now exceeding 24 months [3][4][5], a cure remains elusive for the majority of patients. Early cancer detection and eradication of occult microscopic disease with adjuvant treatment in non-metastatic or early-stage cancer therefore represent the two most substantial opportunities to achieve a cure and improve survival [6][7][8].

The current standard of care for early-stage CRC is surgery, and if indicated, followed by up to six months of adjuvant chemotherapy. While the benefit of adjuvant chemotherapy has been unequivocally established in stage III colon cancer [9][10], the role of adjuvant chemotherapy in stage II colon cancer remains the subject of much debate and is not recommended for all patients [11]. Clinical guidelines currently recommend that adjuvant chemotherapy should be considered in stage II CRC with high-risk clinicopathological features (e.g., T4 extension, lymph node sampling < 12, lymphovascular invasion), following the rationale that patients with a higher risk of recurrence may benefit from adjuvant chemotherapy [12][13]. However, this approach of selectively treating stage II patients with poor prognostic features has not conclusively been shown to improve overall survival [14][15]. To this end, better prognostic and/or predictive biomarkers are needed clinically to help identify the patients who will benefit most from adjuvant therapy.

In stage III CRC, the added absolute benefit of adjuvant chemotherapy is typically quoted to be around 12% with single agent fluoropyrimidine with an additional 6% benefit in combination with oxaliplatin [10][16][17][18]. We have to be mindful that these established benefits were based on historical clinical trial data predating modern-day surgical techniques and pre-operative staging with contrast-enhanced CT scan or positron emission tomography. If the absolute risk of recurrence is lower today due to better surgery and stage migration with better imaging, it is likely that the absolute gain from adjuvant chemotherapy especially oxaliplatin, may be less than they once were. This, along with the risk of long-term peripheral neuropathy associated with oxaliplatin [19][20][21], have motivated an unprecedented international effort to examine a de-escalated treatment approach with a shorter duration (three months) of adjuvant chemotherapy compared to the standard six months of treatment in stage III CRC [22]. The latest overall survival update presented at the 2020 ASCO (American Society of Clinical Oncology) Annual Meeting [23] observed minimal difference (0.4%) between the two groups, setting three months of adjuvant oxaliplatin-based combination treatment as the new standard for stage III CRC, especially those with clinically low-risk disease. Importantly, results from the IDEA (International Duration Evaluation of Adjuvant Therapy) meta-analysis reminds us of the need to continually re-evaluate the risk–benefit ratio of our current treatment recommendation and provides reassurance that less treatment is not necessarily detrimental to patient outcome.

Another challenge beyond the imprecision of patient selection for adjuvant therapy, is the lack of progress with better treatment beyond oxaliplatin and fluoropyrimidine in the past 16 years. Agents that have shown efficacy in the metastatic setting (irinotecan, bevacizumab, and cetuximab) have thus far failed to demonstrate significant survival benefit compared to fluoropyrimidine or oxaliplatin-based combination treatment in eight randomised trials [24][25][26][27][28][29][30]. This

challenge to detect a benefit with new adjuvant therapy may in part be due to the improvement in recurrence risk, hence low event rate, over time with better multidisciplinary medical care as discussed previously. Additionally, the current model of adjuvant clinical trial based on an undifferentiated pathological staging alone (e.g., stage II and III) and an infrastructure which was developed over 50 years ago is highly inefficient, requiring many thousands of patients over a long period of time to capture recurrence and overall survival events. In this regard, biomarkers that could allow prognostic enrichment for high-risk patients and provide early read-out of adjuvant treatment efficacy could expedite novel drug development in the adjuvant setting.

One promising biomarker that has received significant attention in recent years is circulating tumour DNA (ctDNA). ctDNA are DNA fragments that are released by dying cancer cells into the bloodstream and in theory should contain genetic and epigenetic changes identical to the cancer cells they originated from. There are accumulating evidence that ctDNA analysis can be used to evaluate the presence of minimal residual disease (MRD) and predict recurrence in the post-operative setting. For those receiving adjuvant treatment, the non-invasive and dynamic nature of this marker may also reflect adjuvant chemotherapy efficacy in real-time.

2. Circulating Tumour DNA and Minimal Residual Disease

ctDNA is derived from cancer cells and released into the blood stream as a result of tumour cell necrosis [31]. This is distinguished separately from circulating free DNA (cfDNA) which is derived from non-cancer cells, free of molecular pathological alterations (e.g., somatic re-arrangements) and consists of longer base pair lengths compared to ctDNA [32]. ctDNA was first described in 1948 by Mendal and Metais [33] but the relevance to clinical application only became apparent in 1994 when RAS mutations were identified in ctDNA [34]. ctDNA represents only a small fraction of the total cfDNA, but this fraction is highly variable, ranging from less than 0.1% to greater than 10% depending on tumor stage, disease burden, biologic shedding or proliferation, and anatomic factors such as disease site [35][36]. Once in the circulation, ctDNA is cleared rapidly from the bloodstream, with a half-life of approximately 2 h [37], offering a real-time dynamic measure of tumor burden.

ctDNA is now found in both early-stage and metastatic disease across different solid tumour types, but the detection rate varies between tumour types and different stages of the same tumour type [35]. ctDNA has been shown to correlate with disease burden and treatment response in metastatic CRC [38][39]. Clinical use of ctDNA in the metastatic CRC setting includes genomic profiling to guide targeted therapy (e.g., identifying RAS mutations in guiding decision-making for anti-EGFR therapy), tracking resistance mechanisms, and timing of anti-EGFR rechallenge [6][7][8].

Minimal residual disease (MRD) is a term used to describe persistent micro-metastatic disease after definitive treatment (e.g., resection) of primary malignancy and/or completion of adjuvant systemic therapy thereafter. Importantly, this represents an occult state of disease that is not detectable by conventional imaging modalities or blood tests. The prognostic role of ctDNA-based MRD detection is now established in various haematological diseases [40][41][42][43][44][45] where MRD has now been incorporated into standard clinical guidelines [46]. In fact, the expanded U.S. Food and Drug Administration (FDA) approval of blinatumomab in March 2018 to treat adults and children with B-cell precursor acute lymphoblastic leukemia who are in remission, but still have MRD, was the first time the FDA used MRD as a biomarker for a regulatory decision. Despite this success in haematological malignancies, until recently, the clinical validity of ctDNA-based MRD detection in solid tumours was limited by the technical challenge of reliably detecting and quantifying these rare tumour DNA amongst the several thousand genome equivalents of DNA that are present in 1 mL of circulating plasma (typically <0.01% of total cfDNA).

Advances in several molecular techniques allowing high-sensitivity ctDNA analysis has sparked recent interest in pursuing the clinical role of ctDNA for MRD detection across various tumour types [47]. ctDNA detection methodologies will not be the focus of this review but we would like to refer the readers to several excellent papers which have reviewed this topic in detail [48][49][50][51]. Broadly speaking, there are two approaches to ctDNA analysis for MRD detection following curative intent treatment in early-stage cancer: tumor-informed vs. tumor-agnostic approaches. In the tumor-informed approach, somatic mutations are first identified in an individual patient's tumor tissue via targeted sequencing or whole exome sequencing, followed by targeted sequencing of plasma DNA using a personalized assay. Several tumor-informed personalized ctDNA assays have been developed (e.g., SafeSeqS, CAPP-Seq, Tam-Seq, TARDIS, Signatera, ArcherDX PCM, Radar) with limits of detection as low as 0.01% variant allele frequency (VAF) [52][53][54][55][56][57][58]. For the tumor-agnostic approach, ctDNA analysis is performed without prior knowledge of a patient's tumor mutation profile and often includes broad panel-based sequencing or methylation assay (e.g., Guardant Health's 'LUNAR' assay). Beyond NGS-based technique, another sensitive mutation-based ctDNA analysis method includes droplet digital PCR (ddPCR) [59]. However ddPCR assays are limited to specific single mutations or sets of highly related mutations at the same locus [60]. The advantages of the tumor-agnostic approach include its faster turn-around time, lower cost, and ability to detect

emerging resistant mutations. However, the trade-off for not requiring tumor tissue is potentially a lower sensitivity for detecting the low level of ctDNA in the MRD setting. Though more resource-intense, the tumor-informed approach, especially where multiple personalized variants are tracked simultaneously in the plasma, offers the highest analytical sensitivity and is particularly well-suited for MRD detection and recurrence monitoring.

The currently approved FDA ctDNA clinical tests for metastatic disease are: ‘FoundationOne CDx’, ‘Praxis Extended RAS panel’, ‘Cobas KRAS Mutation Test’, ‘therascreen KRAS RGQ PCR Kit’, ‘Dako EGR pharmDx Kit’, and ‘therascreen BRAF V600E RGQ PCR Kit’. For MRD detection, the Signatera assay is approved for colorectal cancer, and the ClonoSeq assay for multiple myeloma, acute lymphoblastic leukemia, and chronic lymphocytic leukemia [61].

3. ctDNA and MRD Detection in Colorectal Cancer

The first evidence of ctDNA’s potential as a marker of MRD came from a study conducted at Johns Hopkins in 2008 involving 18 patients with resected colorectal liver metastases [37]. Using the BEAMing (beads, emulsion, amplification and magnetics) assay, the study demonstrated that ctDNA levels declined precipitously after resection of all visible tumours but remained detectable at first follow-up visit in 12 patients; all but one had experienced recurrence. In contrast, none of the four patients with undetectable ctDNA at first follow-up visit experienced recurrence. This result inspired subsequent clinical validation of ctDNA as a MRD marker in non-metastatic CRC.

Completed ctDNA studies in the non-metastatic setting have thus far been restricted to non-interventional studies (i.e., observation of ctDNA results without active escalation/de-escalation of treatment depending) of which the key studies are summarized in Table 1.

Table 1. Completed observational ctDNA studies in non-metastatic/oligometastatic CRC.

Reference	No. of Patients	Stages Evaluated	Method for ctDNA Analysis	Adjuvant Chemo Given	Key Results	% of Patients ctDNA Positive
Tie et al. 2016 [62]	230	II	Safe-SeqS	23%	In patients not treated with adjuvant treatment, presence of ctDNA after surgery was associated with an inferior recurrence-free survival (HR, 18; p = 0.001) 85% of patients were ctDNA-positive up to or at the time of radiologic recurrence, CEA was only elevated in 41% of patients. The median lead time from ctDNA detection to radiological recurrence was 167 days; range 81–279 days	Post-op: 7.9% Post-Treatment: 11% Surveillance: 11.7%

Reinert et al. 2019 [56]	130	I–III	Signatera	62%	Post-op ctDNA-positive patients were more than 7 times more likely to experience disease recurrence than ctDNA-negative patients (HR, 7.2; $p < 0.001$) Lead time to detect disease recurrence compared with standard surveillance: Mean 8.7 months; range 0.8–16.5 months	Post-op: 10.6% Post-Treatment: 12% Surveillance: 20%
Schøler et al. 2017 [63]	45	I–IV	ddPCR	36.8%	Longitudinal samples from 27 patients revealed ctDNA detection postoperatively in all relapsing patients (n = 14), but not in any of the non-relapsing patients. Of 21 patients treated for localised disease, all 6 ctDNA-positive patients (within 3 months of surgery) relapsed compared with 4 of the remaining patients (HR, 37.7; 95% CI; 4.2–335.5; $p < 0.001$). Time to detect disease recurrence of standard surveillance: Median lead time of 9.4 months, ranging from 0.4 to 14.9 months	Post-op: 28.6% (stages I–III) Post-Treatment: not reported Surveillance: not reported

Taieb et al. 2019 [64]	805	II–III	ddPCR (2 methylation markers)	All patients	2-year DFS was 64% vs. 82% in ctDNA-positive and -negative patients, respectively (HR, 1.75; 95% CI, 1.25–2.45; $p < 0.001$). Post-surgical plasma ctDNA predicted metastatic relapse a median of 10 months before recurrence was visible on radiological scans (HR, 11.33; $p = 0.0001$	Post-op: 13.5% Post-Treatment: not reported Surveillance: not reported
Tie et al. 2019 [65]	159	Locally advanced	Safe-SeqS	35.8% patients	Significantly worse recurrence-free survival was seen if ctDNA was detectable after chemoradiotherapy (HR, 6.6; $p < 0.001$) or after surgery (HR, 13.0; $p < 0.001$). Estimated 3-year recurrence-free survival was 33% for post-operative ctDNA-positive patients and 87% for the postoperative ctDNA-negative patients.	Post-op: 11.9% Post-Treatment: not reported Surveillance: not reported
Tie et al. 2019 [66]	96	III	Safe-SeqS	All patients	Estimated 3-year RFS was 30% when ctDNA was detectable after chemotherapy and 77% when ctDNA was undetectable (HR, 6.8; 95% CI, 11.0–157.0; $p < 0.001$)	Post-op: 21% Post-Treatment: 17% Surveillance: Not tested

					ctDNA detectable at a median of 3 months prior to clinical recurrence.	
Tie et al. 2016 [67]	37	IV (resectable colorectal liver metastases)	Safe-SeqS	70%	Ten of 10 pts (100%) with positive post-treatment (surgery and chemotherapy) ctDNA experienced recurrence vs. 4 of 27 (15%) with negative post-treatment ctDNA (HR, 13.16, $p < 0.0001$)	Post-op: 24.3% Post-Treatment: 27% Surveillance: 32.4%
Khakoo et al. 2020 [68]	47	Locally advanced rectal cancer	ddPCR	91.3%	All 3 patients with detectable ctDNA post-surgery relapsed compared with none of the 20 patients with undetectable ctDNA ($p = 0.001$)	Post-op: 13% Post-Treatment: not reported Surveillance: not reported
Parikh et al. 2019 [69]	72	Stage II–III	Guardant health NGS	41.2%	Patients who were ctDNA-positive after standard therapy completion had a recurrence positive predictive value 93%, negative predictive value 80%, (HR, 11.29; $p < 0.0001$)	Post-op: 19% (surgery arm only) Post-Treatment: 22.2% (chemotherapy arm only) Surveillance: not reported
Overman et al. 2017 [70]	54	IV (resectable liver metastases)	30 kb ctDNA digital sequencing panel (Guardant Health) covering SNVs in 21 genes	Not reported	In 43 patients who underwent successful resection of all visible disease, post-op detection of ctDNA significantly correlated with RFS (HR, 3.1; 95% CI, 1.7–9.1; $p = 0.002$) with 2-year RFS of 0% vs. 47%. ctDNA detected at median of 5.1 months prior to radiographic recurrence.	Post-op: 44% Post-Treatment: Not reported Surveillance: Not reported

Tarazona et al. 2019 [71]	150	Stage II–III	ddPCR	37.2%	Detection of ctDNA after surgery and in serial plasma samples during follow-up were associated with poorer DFS (HR, 17.56; log-rank p = 0.0014 and HR, 11.33; log-rank p = 0.0001, respectively) In patients treated with adjuvant chemotherapy, presence of ctDNA after therapy was associated with early relapse (HR, 10.02; log-rank p < 0.0001)	Post-op: 20.3% Post-Treatment: 28% (patients receiving adjuvant chemotherapy) Surveillance: Not reported

Abbreviations: ctDNA, circulating tumour DNA; CRC, colorectal cancer; HR, hazard ratio; CEA, Carcinoembryonic antigen; ddPCR, droplet digital PCR; CI, confidence interval; DFS, disease-free survival; RFS, recurrence-free survival; NGS, Next-generation sequencing; post-op, post-operative; SNVs, single-nucleotide variant.

A seminal study including 230 patients with stage II colon cancer [62] was among one of the largest and earlier studies which demonstrated the clinical validity of ctDNA (using the tumour-informed Safe-SeqS assay) in the adjuvant setting. In the 178 patients not treated with adjuvant chemotherapy, the study demonstrated that the presence of ctDNA 4 to 10 weeks after surgery predicted a very high risk of recurrence with an estimated 3-year recurrence-free survival (RFS) of 0%, whilst those with undetectable ctDNA after surgery has a 3-year RFS of 90% (hazard ration (HR), 18; p < 0.001). In patients treated with chemotherapy, the presence of ctDNA following completion of chemotherapy was also associated with an inferior recurrence-free survival (HR, 11; p = 0.001). The study also showed superiority of ctDNA over CEA (carcinoembryonic antigen) as a biomarker for detecting radiological recurrence; ctDNA was positive in 85% vs. 41% CEA elevation (p = 0.003) at time of radiological recurrence. A further study using the Signatera assay [56] in 130 patients across stages I–III similarly showed that post-operative ctDNA-positive patients were seven times more likely to relapse than ctDNA-negative patients (HR, 7.2; p < 0.001).

There are a couple of studies which included stage III patients exclusively [64][66]. The first of these is an observation cohort involving 96 patients treated with adjuvant chemotherapy. The post-operative ctDNA detection rate was 21% and ctDNA detection was associated with a significant inferior recurrence-free survival (HR, 3.8; 95% confidence interval (CI), 2.4–21.0; p < 0.001). This prognostic impact was independent of standard clinicopathological criteria. Importantly, ctDNA remains detectable at the end of chemotherapy in 17% of cases with an estimated 3-year RFS of 30% compared with 77% in those whose ctDNA were negative after treatment (HR, 6.8; 95% CI, 11.0–157.0; p < 0.001). To date, the largest reported ctDNA series is a retrospective analysis of 805 patients with stage III colon cancer enrolled in the IDEA-France phase III randomized trial [64] which investigated the outcome of 3 vs. 6 months of adjuvant oxaliplatin-based chemotherapy. Using a tumour-agnostic plasma only methylation assay, post-operative (post-op) ctDNA detection rate was 13.5%. The study has similarly demonstrated that positive post-op ctDNA was an independently prognostic biomarker and perhaps more importantly, also showed that patients with ctDNA-positive disease benefited more from 6 months of adjuvant treatment than those with ctDNA-negative disease.

The ultimate utility of ctDNA is to assess adjuvant treatment efficacy. If clinicians are able to identify which adjuvant therapy is effective during such treatment, as indicated by reduction and subsequent negative ctDNA status, there is a potential to de-escalate toxic therapy. Conversely if adjuvant therapy is not effective at eliminating ctDNA, then switching to alternative therapy including novel drugs may be warranted. Table 1 identifies prospective trials that monitored ctDNA after therapeutic interventions including primary resection (surgery) and during/after adjuvant chemotherapy. It has been

shown across both stage II and III colon cancer that ctDNA positivity after adjuvant treatment completion was associated with poorer RFS [56][62][66]. These data suggest that persistent detection of ctDNA post-treatment reflects presence of micrometastatic disease, which ultimately is the source of clinical recurrence.

Collectively, several trials have now consistently demonstrated the prognostic value of post-op and post-treatment ctDNA assessment in various stages of non-metastatic CRC. Of note, the ctDNA detection rates and prognostic impact vary across studies due to variations in the disease stages included in the studies, ctDNA assays, and pre-analytic variables, such as plasma volume assessed and timing of blood collections.

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