



Circulating tumour DNA in early stage colorectal cancer: can blood tell all?

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The decision to offer adjuvant chemotherapy after primary resection for non-metastatic colorectal cancer (CRC) is principally guided by histopathologic parameters. However, this approach lacks precision with many patients potentially receiving unnecessary treatment. For example, only about 1 in 20 patients with stage II colon cancer will benefit from adjuvant chemotherapy (1). In large part, this is due to the lack of prognostic biomarkers to precisely stratify patient risk and to guide a personalised approach to treatment. Additionally, for the past 15 years there has been no progress in developing more effective adjuvant therapy beyond oxaliplatin and fluoropyrimidine (2-6).

Tumour derived fragmented DNA, called circulating tumour DNA (ctDNA), is thought to arise from primary and metastatic sites of disease as well as circulating tumour cells (CTCs) (7). ctDNA levels in plasma are typically low, with mutant allele fractions typically less than 10% in metastatic disease and less than 1% in local disease (8). Recent technological advancements, such as digital droplet PCR (ddPCR) and next generation sequencing (NGS) platforms, have enabled the detection of these low frequency tumour DNA fragments in plasma, giving rise to a non-invasive biomarker already demonstrated to align with disease burden and relapse risk (9-11). ctDNA detection is therefore a potentially powerful tool that could lead to a new era of precision oncology in early stage CRC. ctDNA can be used as a marker of minimal residual disease (MRD), defining patients most

likely to benefit from adjuvant therapy, and with serial sampling, also potentially providing an early read-out of adjuvant treatment impact and early detection of metastatic disease.

In their recent publication, Reinert *et al.* demonstrated the potential utility of ctDNA detection in many aspects of early stage CRC care. In their prospective cohort study, they analysed the tumour and plasma of 130 patients with stage I to III CRC and tested for ctDNA at different time points post-surgery (10). They confirmed the prognostic utility of ctDNA, reporting that patients with a positive ctDNA at 30-days post-surgery having higher recurrence rates (70% *vs.* 11.9%; HR 7.2), and also demonstrating the potential utility of serial ctDNA analysis as a monitoring tool during adjuvant treatment and during post-treatment surveillance. This data is all consistent with other early stage CRC studies, demonstrating inferior recurrence free survival (RFS) for post-operative ctDNA-positive patients compared to ctDNA negative patients (11-13).

While the study by Reinert *et al.* adds to the expanding field of ctDNA informed management of early CRC, many important questions remain about how best to incorporate ctDNA analysis into routine clinical practice and how to do this on a stage-by-stage basis. For patients with stage II colon cancers where the benefit of treating unselected patients remains contentious, even for patients with high-risk clinical or pathologic features (14), an obvious next step is to explore adjuvant therapy for patients with detectable

ctDNA. This approach is currently being tested in the DYNAMIC study (ACTRN12615000381583), which recently completed recruitment of just over 450 patients with stage II CRC, randomising patients to a ctDNA informed versus physician's choice adjuvant treatment. Other currently recruiting studies are exploring a similar question, include IMPROVE-IT (NCT03748680), which is enrolling patients with stage I and II disease.

In the management of stage III CRC there is a well-defined role for adjuvant oxaliplatin based treatment, with recent studies examining the optimal duration of therapy (15). While a combined analysis of the studies was unable to demonstrate non-inferiority of 3 versus 6 months of treatment, an *ad hoc* analysis suggested that for low-risk stage III patients, defined as those with T1-3 and N1 cancers, 3 months of CAPOX therapy was non-inferior to 6 months of treatment. For patients with stage III colon cancer ctDNA analysis could allow a risk adjusted approach to treatment. New approaches could be tested in the high-risk ctDNA positive patients, including intensifying adjuvant therapy or offering further treatment to patients who remain with detectable ctDNA at completion of standard therapy. For low risk patients, those in whom ctDNA is not detectable post-surgery, a de-escalation of adjuvant chemotherapy could be considered. Potential options for de-escalation include omitting oxaliplatin or administering treatment for a shorter duration. The currently recruiting DYNAMIC-III trial (ACTRN12617001566325) is seeking to answer such questions related to the use of standard therapy in patients with stage III CRC, escalating or deescalating treatment based on the detection of ctDNA post-surgery.

The chosen methodology of ctDNA detection used by Reinert *et al.* is unique, 16 tumour-derived somatic mutations were identified by whole exome sequencing (WES), creating a personalised multiplex PCR assays with ultradeep NGS to analyse and quantify plasma ctDNA (10). Based on the detection of ctDNA at day 30, this method identified eventual relapse with 41% sensitivity and 96% specificity. The combination of a high number of personalised mutations and deep coverage should theoretically enhance the sensitivity of ctDNA detection. In earlier studies reported by our group, where sample analysis was conducted at John Hopkins by Bert Vogelstein and his team, Safe-Sequencing System (Safe-SeqS), an NGS-based assay using molecular barcoding to enhance the sensitivity of detecting rare mutations, was used to detect only one

patient-specific somatic mutation (11). This method identified relapse post-surgery with 47% sensitivity and 93% specificity for blood samples drawn at 4 to 10 weeks after surgery. The similar reported sensitivity and specificity between these two tumour-informed ctDNA assays would suggest that either provides strong prognostic information, with additional studies required to further refine these methods and to define the optimal approach. As ctDNA moves closer to the clinic, the relative cost-effectiveness of the assay will also need to be considered.

Alternative approaches are also being explored for MRD detection. The methods mentioned previously are tumour-informed, using tumour-specific mutations identified from the primary tumour to inform what mutation(s) to detect in the plasma, and other tumour-informed approach are being developed including CAPP-Seq (CANcer Personalized Profiling by deep Sequencing) as well as digital-PCR platforms with droplet digital PCR and BEAMing (10-11,16-18). An alternative approach involves a tumour-agnostic assay where only the plasma samples are analysed for genetic or epigenetic changes without first analysing the tumour tissue, targeting frequently mutated cancer genes. Examples of this include the Guardant LUNAR assay and methylation assays (19-21). The clear advantage to a tumour-agnostic approach is the saving in time and cost from not needing to undertake tumour sequencing but as with tumour informed assays, performance needs to be demonstrated in prospective studies. Beyond technological and financial considerations, logistical questions such as ctDNA result turn-around time, optimal timing of post-operative blood collection, and frequency of testing will also need to be addressed.

Serial ctDNA analysis may also be able to dynamically monitor response to ACT at a microscopic level, providing a real-time indicator of treatment benefit. Reinert *et al.* identified 10 patients with positive ctDNA post-surgery who received ACT (10). Eight of these patients had longitudinal ctDNA surveillance and of the 4 patients with negative ctDNA after ACT, 2 relapsed compared to all 4 patients who remained persistently positive. Hypothesising that a positive ctDNA post-ACT was a poor prognostic marker, they further analysed a total of 58 patients post-ACT. In total, 7 of 7 patients with positive ctDNA relapsed compared to 7 of 51 patients without ctDNA detection, indicating that ctDNA positivity post-ACT is a clear predictor of relapse. The presence of ctDNA post-chemotherapy would suggest a failure to clear all micro-

metastatic disease. Continuing chemotherapy or switching to an alternative chemotherapy may be considered but large prospective studies designed to explore the treatment of patients with positive ctDNA post-ACT is needed to answer this important question.

Serial ctDNA analysis may also have a role during post-treatment surveillance. In Reinert and colleague's cohort, the detection of ctDNA during surveillance predicted relapse with 88% sensitivity and 98% specificity (10). ctDNA positivity also preceded the emergence of radiographically evident recurrence in 14 patients with a mean lead-time of 8.7 months. Of note, serological CEA did not demonstrate any lead-time over radiological imaging. Reported in the same issue of *JAMA Oncology*, Wang *et al.* found the recurrence rate among patients with positive ctDNA levels during surveillance was 77%, with ctDNA detection preceding radiologic and clinical evidence of recurrence by a median of 3 months (22). This difference in lead-time may be due to difference in surveillance imaging frequency employed in the two studies. It is unknown whether initiating treatment at the time of ctDNA detection during surveillance, prior to when disease is detectable on imaging, will provide a clinically meaningful benefit. When this approach has been explored in ovarian cancer no clinical benefit was seen. The MRC05 trial demonstrated that in asymptomatic women with ovarian cancer, initiating chemotherapy on the basis of Ca125 elevation alone did not improve survival and on average these women started chemotherapy 5 months earlier (23). Therefore, it is unclear when initiation of treatment should begin. We must also consider that patients may suffer psychological distress by being informed of a positive ctDNA result without clear guidance of how to act on such a result. Further randomised prospective studies are therefore needed to determine how ctDNA detection during surveillance should guide treatment decision making.

Reinert *et al.* reported that pre-operative ctDNA was detected in 88.5% of patients, in comparison serological CEA was detected in only 43.3% (10). However, the prognostic and clinical value of pre-operative ctDNA was not addressed. We have shown previously in patients with locally advanced rectal cancer that pre-treatment ctDNA detection was not prognostic, reflecting the current thinking that in the pre-operative setting, the majority of ctDNA is likely released from the in-situ primary tumour which is about to be removed and is therefore not a true assessment

of MRD. So currently, there is uncertainty as to the value of pre-operative DNA analysis.

Intriguingly, Reinert and colleagues also demonstrated the utility of ctDNA in identifying clinically actionable mutations such as RAS and BRAF, selecting patients that could potentially benefit from targeted EGFR therapy such as cetuximab or BRAF and MEK inhibitors such as encorafenib and binimetinib. As a proof-of-concept analysis, 11 patients with known actionable mutations identified by WES of the primary tumour had their plasma from longitudinal surveillance analysed by multiplex PCR. Of these 11 patients, 7 had actionable mutations detected in the first ctDNA-positive sample (10). When all subsequent ctDNA positive samples were tested, a further 2 patients had actionable mutations detected. This result is consistent with other studies in colorectal and lung cancer where it has been demonstrated that ctDNA can be used to determine the presence of actionable mutations. Bachet *et al.* demonstrated that in patients with CRC and positive ctDNA, RAS status can be determined from plasma with 92.9% sensitivity and 87.7% specificity (24). In paired tumour and plasma analysis there was excellent concordance between tumour and plasma (κ coefficient 0.89). There is a clear benefit in using ctDNA where tumour tissue cannot be easily retrieved but this approach may also be advantageous as biopsy from a single site may not represent intra- and inter-tumour heterogeneity.

The potential utility of ctDNA as a powerful prognostic marker and real time marker of treatment efficacy has now been demonstrated in multiple studies. Patients with detectable ctDNA after primary surgery are clearly at very high-risk of relapse. Persistently detectable ctDNA during adjuvant therapy also predicts poor outcomes. However, before ctDNA can become integrated into routine clinical practice, prospective studies demonstrating a clinical benefit are required. In parallel, efforts to improve the technology and to define the optimal testing methods, which may vary for different patients and circumstances, are required.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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