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Editorial

Circulating tumor DNA as a biomarker to guide therapy in post-operative locally advanced rectal cancer: the best option?

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1.0 Introduction

Approximately 30% of colorectal cancers arise within the rectum, where neoadjuvant chemo-radiation (CRT) and then surgery is the standard treatment for patients with locally advanced rectal cancer (LARC - cT3/T4 and/or N+, M0). While multiple advances have been made in managing rectal cancer, patient selection for adjuvant chemotherapy after surgery, and the agent(s) of choice remain major clinical dilemmas. Better prognostic markers to identify those patients who actually require adjuvant chemotherapy are needed.

1.1 Circulating tumor DNA (ctDNA) as a Biomarker

Sequencing of DNA from colorectal cancers has identified several genes that are recurrently somatically mutated. These tumor-specific DNA mutations can be detected in the cell-free component of peripheral blood as circulating tumor DNA (ctDNA) in the majority of patients with metastatic disease, allowing for non-invasive molecular characterisation of tumors (1). Additionally, the short half-life of ctDNA (~ 2 hours) makes ctDNA a useful dynamic marker of tumor bulk, with early decreases in ctDNA amounts in patients with metastatic disease reflecting treatment responses that are later confirmed by conventional imaging (2). The possibility that ctDNA could be used to detect micro-metastatic disease in patients who have undergone surgery with curative intent was suggested in an initial series of 18 patients with advanced CRC undergoing metastasectomy (3). Later small series of other solid malignancies such as breast, lung and pancreatic cancers have consistently suggested the utility of ctDNA as a marker of recurrence risk (4-6).

Given the low fraction of tumor-derived DNA amongst the many thousands genome equivalent of non-tumor derived DNA fragments in the circulation of patients with early stage disease, having a sensitive ctDNA assay is critical to the detection of MRD. Several modified targeted sequencing approaches (e.g. Safe-SeqS, CAPP-Seq, TAm-Seq), have been developed to significantly improve the accuracy of commercially available massively parallel sequencing instruments (currently limited by the high sequencing error rate of 0.05 to 1%) in detecting relatively rare mutations. Using unique identifiers (molecular barcodes), the Safe-SeqS assay is able to reduce the background error rate of sequencing and detect, on average, 1 mutant allele in 10,000 DNA template molecules (sensitivity 0.01%) (7). The sensitivity of ctDNA detection could be further enhanced by using patient-specific panels through initially genotyping individual patient's tumor tissue.

1.2 Adjuvant chemotherapy Benefit in Locally Advanced Rectal Cancer

There have been substantial changes in the treatment paradigm for patients with LARC. Currently, standard practice as endorsed by guidelines (NCCN, ESMO) is to administer 4 months of chemotherapy to all patients after surgery. This recommendation is largely based on early studies, predating the modern era, where all chemotherapy was given post-operatively and an overall survival benefit for adjuvant chemotherapy was seen (8). In more recent studies, where patients were treated with the modern standard of neoadjuvant CRT followed by TME surgery, all individual studies and meta-analyses have failed to demonstrate any survival benefit for adjuvant chemotherapy (9, 10). However, the significance of these more recent study results continues to be debated due to poor compliance with adjuvant therapy across all studies and many studies being underpowered.

Most recently, the value of adding oxaliplatin to the standard fluoropyrimidine (5FU or capecitabine) has been explored in multiple trials. A small randomised phase II study (the ADORE trial) (11) and the German AIO study (12), found improved disease-free survival, but other studies have found no benefit from adding oxaliplatin to 5FU or capecitabine (13). The ADORE trial compared adjuvant FOLFOX and single agent 5FU in patients with post-operative pathological stage II (ypT3-4N0) or III (ypTanyN1-2) rectal cancer after pre-operative fluoropyrimidine-based chemo-radiotherapy and surgery. This study demonstrated an 8.7% improvement in 3-year disease-free survival in patients treated with adjuvant FOLFOX (HR 0.657, p = 0.047). However, subgroup analysis suggested that the benefit was limited to patients with pathologic stage III (ypN+) disease. The German AIO study investigated the benefit of adding oxaliplatin to fluorouracil-based pre-operative chemo-radiation and post-operative chemotherapy in patients with clinical T3-4 or node-positive rectal cancer. This study found a 4.7% 3-year disease-free survival benefit in the oxaliplatin arm (HR 0.79, p = 0.03). Although these two studies are relatively early in follow-up, no overall survival advantage is yet apparent.

1.3 Existing Markers of Recurrence Risk After Rectal Cancer Surgery

The response (also known as down-staging) achieved after CRT (the yp or pathologic stage) is the best available marker of recurrence risk in LARC. The best outcomes are seen in the 15–27% of patients that achieve a pathologic complete response (pCR), defined as no residual cancer found in the resection specimen. In a meta-analysis, 5-year disease-free survival of the 484 patients who achieved a pCR was 83%, compared to 66% patients who did not achieve a pCR (P < 0.0001) (14). The worst outcomes were seen in those with persistently involved lymph nodes (ypN+). Many clinicians are now routinely using the pathologic stage to guide adjuvant therapy decisions. Given the excellent prognosis associated with a pCR, oncologists are becoming more comfortable with not offering any adjuvant treatment

to these patients, particularly for older or frail patients. Where initial CRT was poorly tolerated or where there is minimal residual cancer, patients may also be offered no treatment or a fluoropyrimidine alone. Where lymph nodes are still involved after CRT (ypN+), combination oxaliplatin-based treatment is typically given based on the disease-free survival benefit observed in the ADORE and German AIO studies.

2.0 Assessing Minimal Residual Disease in Locally Advanced Rectal Cancer

The presence of circulating DNA molecules that contain somatic mutations matching those found in an individual's tumor promises to be a direct indication that occult tumor cells (variously described as micro-metastatic disease or minimal residual disease - MRD) remain after curative intent surgery. ctDNA measurements should therefore be considered not as a conventional biomarker of recurrence risk, but more like a staging test such as a CT or PET scan.

We have previously demonstrated the ability of post-operative ctDNA analysis using the Safe-SeqS assay to detect minimal residual disease and to predict recurrence in patients with stage II CRC (15). This study demonstrated that stage II colon cancer patients who had detectable ctDNA post-operatively are at an extremely high risk of radiologic recurrence (estimated 3-year RFS of 0%). Conversely, the stage II patients with undetectable ctDNA post-operatively were at a very low risk of radiologic recurrence (3-year RFS of 90%).

2.1 ctDNA analysis in locally advanced rectal cancer (LARC)

In a separate prospective multi-centre study, we enrolled 200 patients with LARC planned for CRT and curative resection (16). Serial plasma samples were collected pre-CRT, 4 to 6 weeks after completion of CRT (post-CRT), and 4-10 weeks after surgery and before adjuvant chemotherapy (post-op). Adjuvant chemotherapy use was at clinician’s discretion, blinded to the ctDNA results. Somatic mutations in individual patients’ tumor were identified via sequencing of 15 genes commonly mutated in CRC. For ctDNA analysis, plasma samples were then examined using personalized Safe-SeqS assays for the matching mutation found in the individual patient’s tumor. The final evaluable population included 159 eligible patients with pre-CRT and post-op ctDNA samples available for analysis.

ctDNA was detectable at diagnosis in a high proportion (77%) of patients with LARC, but the detection of ctDNA at diagnosis was not predictive of recurrence. ctDNA was detected after surgery in 12% of cases and was strongly associated with recurrence (HR 11; $p < 0.001$), irrespective of the use of adjuvant chemotherapy. Combining ctDNA results and pathology results further stratified patients. Thirty-four patients (21%) achieved a pCR, 43 (27%) had pN+ disease. pCR (vs non-pCR) was associated with a trend for lower recurrence risk (HR 0.32, $p = 0.10$) and pN+ (vs pN0) with a higher recurrence risk (HR 4.3, $p < 0.001$). ctDNA analysis was able to

further stratify patients into groups at very high and low risk of recurrence even among patients with pathological lower risk (pCR: HR 15; p <0.01) and higher risk (ypN+: HR 11; p <0.001) disease. Additionally, post-op ctDNA remains independently associated with recurrence-free survival after adjusting for known prognostic pathological factors and the use of adjuvant chemotherapy.

In addition to its prognostic role, that is the ability to identify the likelihood of cancer recurrence, ctDNA could potentially be used as a predictive biomarker by providing genomic information on whether individuals are more or less likely to benefit from a particular treatment. Apart from EGFR inhibition in RAS wild-type colorectal cancer, several molecularly targeted therapies have shown promise in the metastatic setting, such as HER-2 inhibition for HER-2 amplified tumors and BRAF/EGFR/MEK inhibition for BRAF mutated tumors. Whilst cytotoxic chemotherapy remains the cornerstone of adjuvant treatment for colorectal cancer, the role of adjuvant targeted treatment in molecular subtypes of colorectal cancer deserves to be explored given the success stories in other tumor types (e.g. HER-2 inhibition in HER-2 amplified breast cancer, and BRAF/MEK inhibition in BRAF mutated melanoma).

3.0 Conclusion

Despite guidelines recommending the routine use of adjuvant chemotherapy in LARC, there is little evidence in the modern era to support the routine use of post-operative chemotherapy in patients who received pre-operative chemo-radiation therapy. Although the use of adjuvant FOLFOX appears promising in node-positive disease (ypN+), this comes with a toxicity price and the impact on overall survival is yet to be proven.

Post-operative ctDNA has been shown to be a direct indicator of minimal residual disease, with the presence of ctDNA predicting recurrence in separate series of stage II colon cancers and locally advanced rectal cancers. The ultimate clinical utility of this test (e.g. reducing the use of unnecessary chemotherapy or improving survival), remains to be proven but randomised clinical trials comparing ctDNA-informed to standard of care management are now underway.

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Declaration of Interest
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