Editorial Commentary

Assaying circulating-tumor DNA to predict recurrence of localized colon cancer

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Colorectal cancer (CRC) is a genetic disease in which cells have accumulated multiple changes in the genome to advantage excessive uncontrolled proliferation for morphological growth and capability for metastases (1,2). The genetic and epigenetic changes commence in affected stem cells to generate precursor adenomas, with additional changes accruing that advance the histology and malignant potential to an early stage cancer, and further progression to advanced stage cancer. Directly assaying human CRCs demonstrate hypermutated tumors in ~15% of cases that are driven most commonly by epigenetic silencing of the DNA mismatch repair gene *MLH1* causing coding exon frameshift mutations, and non-hypermutated tumors in ~85% of cases that commonly show mutations of *APC*, *KRAS*, and *TP53* genes as principal drivers (2-4). These two major genetic pathways for CRC development and progression show differences in histology, immune infiltration, location within the colon, patient outcome, and response and approach to treatment (5-10). The march towards more accurate precision medicine for patients with CRC is deemed worthy as the genetics of CRC are further characterized, and despite the fact that tumors can be heterogeneous while at the same time be genetically unique for each patient (2,11).

Screening is the principal approach in preventing CRC (12,13); once CRC develops, surgical resection is the primary method for attempted cure of the patient (for patients with low rectal tumors, they first receive neoadjuvant chemoradiotherapy) (7,14). However, among those patients who undergo curative surgery and adjuvant treatment, as many as 40% may recur or relapse metachronously (15,16). The mechanism for recurrence is presumed through the occurrence of microscopic metastases that are not apparent by currently-utilized diagnostic techniques at the time of initial staging. Methods that can detect the presence of residual disease after attemptive curative surgery will change surveillance and management of patients, and can categorize patients for enhanced surveillance and additional treatments from those who don't possess micrometastases. This will require the use of accurate and highly predictable biomarkers as compared to the current use of radiographic imaging and current use of the serum marker carcinoembryonic antigen (CEA) to diagnose recurrence, as both are not sensitive enough for the purpose of detecting micrometastases (*Table 1*). In addition, neither radiographic imaging or CEA can fully quantitate patient tumor burden nor specify changes in the genetic and epigenetic profile of the patient's tumor that might have resulted from initial treatments towards curing the tumor. This last point had been only previously possible with direct assay of the primary and/or metastatic tissue, and could not be re-performed after tumor resection or significant regression of tumor size. Thus, more ideal biomarkers that can detect residual disease after curative resection and genetically profile the remaining disease would offer precise care for the patient with potential tailored therapy specific to the genetic makeup of the residual tumor to offer the best chance for continuance of

Table 1 Diagnostic and prognostic biomarkers, and treatment approaches for CRC

Clinical parameter	Adenoma	Early stage CRC	Advanced stage CRC	Recurrent CRC
Diagnosis	• Screening	• Screening	• Screening • Symptom evaluation • Radiographic imaging	• Surveillance • Radiographic imaging
Current diagnostic biomarkers	• Fecal hemoglobin • Fecal DNA	• Fecal hemoglobin • Fecal DNA	• Fecal hemoglobin • Fecal DNA	• Radiographic imaging
Current prognosis biomarkers		• Histologic characteristics • Tissue MSI and tumor mutational burden	• Histologic characteristics • Tissue MSI and tumor mutational burden	• CEA (serum) • Tissue MSI and tumor mutational burden
Current actionable biomarkers		• Tissue MSI and tumor mutational burden • Tissue WGS	• Tissue MSI and tumor mutational burden • Tissue WGS	• CEA (serum) • Tissue MSI and tumor mutational burden • Tissue WGS
Promising biomarkers for detection or prognosis	• Fecal DNA improvements	• Tissue immunotyping • ctDNA • RNA transcriptome, including fusions • Proteomics	• Tissue immunotyping • ctDNA • RNA transcriptome, including fusions • Proteomics	• ctDNA • RNA transcriptome, including fusions • Proteomics
Treatment	• Endoscopic polypectomy	• Endoscopic removal (selected cases) and/or Surgical removal • Adjuvant chemotherapy (non-MSI) or neo-adjuvant chemotherapy (rectal only) in selected cases	• Surgical removal • Chemotherapy (5-fluorouracil, leucovorin, oxaliplatin, irinotecan, bevacizumab, anti-EGF, immune checkpoint inhibitors (MSI) • Radiation (rectal only)	• Surgical removal • Chemotherapy (5-fluorouracil, leucovorin, oxaliplatin, irinotecan, bevacizumab, anti-EGF, immune checkpoint inhibitors (MSI) • Radiation (rectal only)

CRC, colorectal cancer.

patient survival.

Colorectal tumors shed cells and DNA into the fecal stream due to its direct exposure to the colonic lumen, and can be utilized for CRC screening purposes (17). Tumor DNA is also shed and can also be detected in the bloodstream when a CRC is present, presumably due to its direct access to the patient's vascular supply, and comes from apoptotic or necrotic cancer cells (18,19). To detect DNA in either fecal material or blood, sophisticated methods had to be developed to assay the minute amounts of tumor DNA from the much more abundant non-tumor DNA present (17). Cell-free circulating tumor DNA (ctDNA) detected from the blood, plasma, or serum is often termed "liquid biopsy" referencing the blood as a tissue and the ability to extract tumor DNA from it. There are a number of differences between a tissue biopsy and a liquid biopsy;

Table 2 Comparison of tissue and liquid biopsies to detect cancer mutations

Decision input	Tissue marker	Liquid biopsy for ctDNA
Advantages	• Direct measurement of tissue • Many markers can be assayed by local pathology departments	• Can assay multiple times (dynamic and real-time) • Can measure post-therapy for effectiveness of therapy • Can provide genetic profile of tumor at given point in time • May predict presence of residual disease • May provide total and more complete genetic picture of a heterogeneous tumor (avoids sampling error) • May measure remaining tumor burden
Disadvantages	• Tissue heterogeneity • Measured only at pre-therapy	• Need access to sophisticated equipment for assays • Need to differentiate from the larger pool of circulating-free DNA

the main difference is that liquid biopsies are a dynamic reflection of tumor behavior that has "spilled" into the bloodstream, and its presence and can be assayed multiple times even after tumor resection and treatment (*Table 2*). Thus, ctDNA can be used as a monitoring tool for patients with CRC, and may benefit the patient with the use of this diagnostic information.

Extraction of ctDNA from blood is the initial challenge—then appropriate analysis of that tumor DNA needs to follow to obtain diagnostic and/or prognostic information. Analyses can be performed by utilizing single locus or multiplexed PCR assays, targeted DNA sequencing approaches, or genome-wide sequencing (18). Using these and other molecular techniques, a number of correlations between ctDNA and other markers and clinicopathological characteristics have been observed. The strongest of these correlations is with the presence of liver metastasis and with tumor diameter, and intermediate association with the presence of lung metastasis, CEA, CA19-9, and lactate dehydrogenase levels, presence of lymph node metastasis, and the number of metastatic organs (18). Use of ctDNA has been assessed in a number of clinical situations. Several studies show a relationship between ctDNA and advanced cancer stage, larger tumor volumes (with ctDNA changes mirroring tumor volume changes), adequacy of surgical resection (detecting residual disease), selection of treatment based on ctDNA markers, and use to monitor response to systemic therapy (19). Analyses of ctDNA hold great promise as a precision medicine and treatment tool to improve patient outcome.

Relative to patients with localized CRC, knowing if residual disease exists can predict relapse and allow therapeutic interventions prior to observable clinical metastases to prevent clinical advancement. This would abate overtreatment (after cure by surgery) or undertreatment (using stage of disease alone for determination) with chemotherapy for these patients. Tie *et al.* reported on the use of ctDNA to detect the presence of minimal residual disease in stage II CRC patients (20). ctDNA was detected in 14 of 178 patients (7.9%) who had recieved no adjuvant chemotherapy, and radiographic recurrence was observed in 11 of those 14 patients. On the other hand, 164 of 178 patients (92.1%) had no ctDNA detected, and recurrence was observed in 16 (9.8%) patients (20). This study established the feasibility and relative accuracy of the use of postoperative ctDNA in predicting recurrence of colon cancer.

In a recent issue of *Annals of Oncology*, Tarazona *et al.* further examined ctDNA accuracy in predicting minimal residual colon cancer disease in a prospectively-enrolled cohort (21). They enrolled 150 patients with localized staged colon cancer between 2015 to 2017, and collected plasma samples for ctDNA analysis at baseline, 6–8 weeks after surgery, and every 4 months for up to 5 years. Computed tomography scans were done every 6 months. The authors obtained a tissue biopsy prior to surgery and had its DNA sequenced, finding at least one mutation in 132 samples and two or more mutations in 86 samples, with the most common genes mutated being *TP53*, *APC*, *KRAS* and *PIK3CA* (21). To analyze ctDNA, the authors utilized

digital droplet PCR (ddPCR) assays to track mutations identified from tissue biopsies; they also custom-designed a 29-gene next generation sequencing panel to detect molecular alterations with current prognostic relevance or those that could be targeted by future therapies (21). Their primary outcome measure was disease-free survival (DFS).

A total of 94 colon cancer patients completed the plasma ctDNA analysis, with ctDNA ddPCR assays in agreement with mutations identified from tissue sequencing to pursue serial tracking. The authors detected ctDNA in 60 of 94 (63.8%) of colon cancer patients, with concentrations of ctDNA lower in stage I versus stage II-III patients. Fourteen of 69 (20.3%) colon patients had ctDNA detected at 6–8 weeks post-surgery. During the median follow-up of 24.7 months, 18 of 85 (21.2%) patients demonstrated radiological recurrence of their cancer. The authors noted a recurrence rate among patients with postoperative positive ctDNA of 57.1% (8 of 14) whom showed poorer DFS (HR 6.96, P=0.0001). Multivariable adjustment showed that postoperative ctDNA presence was the only significant predictor of DFS (HR 11.64; 95% CI, 3.67–36.88, P<0.001) (21). Additionally, tracking serial plasma samples, the authors show that detection of ctDNA in serial samples associated with poorer DFS (HR 8.03, P=0.006), and was a significant predictor of early relapse using multivariable modeling (HR 11.12; 95% CI, 2.53–55.65, P=0.002). Among patients relapsing during follow-up, 8 of 17 (47.1%) had ctDNA detected in their first plasma sample, and this proportion increased to 14 of 17 (82.4%) through the tracking of the serial samples. Among patients not relapsing during follow-up, only 6 of 53 (11.3%) had ctDNA detected in their first plasma sample, with 4 of these patients having ctDNA undetectable in subsequent serial samples (21). These data ardently demonstrate the use of ctDNA as a powerful biomarker and predictor of minimal disease and patient survival, as outlined in *Table 2*.

These authors also evaluated the impact of adjuvant chemotherapy upon serial ctDNA analysis. Seven of 25 colon patients (28%) with available plasma had ctDNA detected postoperatively and after adjuvant chemotherapy; six of those 7 patients demonstrated recurrence (85.7%) (21). The ctDNA detection preceded radiological recurrence by a median lead time of 11.5 months. The presence of ctDNA after chemotherapy associated with poorer DFS (HR 10.02; 95% CI, 9.202–307.3, P<0.0001). These data show the impact of ctDNA as a predictor for the effectiveness of adjuvant chemotherapy and its ability to remove micrometastases for long-term patient survival.

The manuscript published by Tarazona *et al.* highlights an exciting time for personalized oncology. The use of sophisticated technology to perform liquid biopsies in real time and assay the genetic profile of residual heterogeneous disease that are prognostic and may direct future therapeutics serves as powerful biomarker that more accurately cares for cancer patients than traditional staging. ctDNA analysis predicted tumor burden, the potential for relapse postoperatively and after chemotherapy (typically utilized to neutralize micrometastases), and has the potential to reguide therapy through mutation tracking in serial ctDNA analyses. This last point on mutation tracking requires custom approaches to specific existing and newly-acquired mutations in each individual, as general commercial panels may not account for all mutation permutations. Trazona *et al.* followed the initially-determined the tissue-derived mutations with serial ctDNA analysis using panels custom to the tissue-derived mutations. This approach more accurately tracked residual disease, but could miss novel-acquired mutations without a combined full sequencing approach. The relative non-invasive nature of ctDNA coupled with sensitive cancer genomic analysis of the ctDNA make ctDNA a potent biomarker. As demonstrated in Tarazona *et al.*, ctDNA presence preceded confirmed relapses. There remains a number of issues to optimize use of ctDNA, including the timing of obtaining serial plasma samples, the full ability to detect heterogeneous mutations in wide spread disease, and standardization of analysis of obtained ctDNA as well as thresholds for ctDNA detection and utilization. However, appropriately treating colon cancer patients will improve greatly when ctDNA becomes more mainstream in clinical practice. This will ultimately depend on the availability, feasibility, and cost-effectiveness of use of ctDNA (*Table 2*), but its power in use has been clearly demonstrated in this study.

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