Complete response to immunotherapy in a patient with high-risk stage III colorectal cancer after ctDNA-guided detection of early adjuvant treatment failure

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ABSTRACT
The standard of care for stage III colorectal cancer (CRC) is curative resection with adjuvant chemotherapy (ACT). There is a high risk of recurrence particularly for high-risk patients with stage III disease, making close disease monitoring vital. Circulating tumor DNA (ctDNA) is now established as an effective method of early detection of disease relapse as well as postoperative risk stratification. However there remains a lack of established protocol for using ctDNA to assess response to ACT and in using that data to alter therapy in real time. A case is described of a patient with high-risk stage III CRC in whom failure of ACT was detected early and therapy was quickly changed based on rising ctDNA levels. The described patient had complete radiologic and clinical response to checkpoint inhibitor immunotherapy and remains free of disease after 18 months. This case demonstrates a promising example of how ctDNA can be used to both assess effectiveness of ongoing therapy and drive real-time change in treatment while sparing unnecessary chemotherapy toxicities.

INTRODUCTION
An estimated 25%–30% of stage III colorectal cancers (CRCs) recur after definitive therapy, making postsurgical disease surveillance vitally important. Early detection and intervention of relapse is associated with improved overall survival (OS) and clinical outcomes. Among stage III CRC, the risk factors most associated with shortened time to recurrence include high T (T4) and N (N2) stages. Although the efficacy of immunotherapy is established in stage IV microsatellite instability high (MSI-H) disease, ongoing studies such as Alliance A021502 (NCT-02912559) are evaluating the role of checkpoint inhibitor immunotherapy in stage III MSI-H tumors. Patients with locally aggressive, MSI-H CRC are at high risk of treatment failure, therefore close monitoring during therapy is critical.

Current NCCN guidelines recommend monitoring stage II and stage III CRC postsurgery with serum carcinoembryonic antigen (CEA) levels and CT. However, CT imaging is unable to detect micrometastases and CEA suffers from lack of sensitivity and specificity. Circulating tumor DNA (ctDNA) is an emerging technology, using capture-based next-generation sequencing to detect acellular tumor DNA in the peripheral blood as a marker of molecular residual disease (MRD). There is growing evidence supporting the superiority of tumor-informed ctDNA to conventional disease surveillance. In a prospective study by Reinert et al, ctDNA detected relapse 8.7 months ahead of conventional surveillance methods. Its role in detecting disease recurrence is now well established, as numerous studies show that postoperative ctDNA levels are highly prognostic for disease-free survival (DFS) and OS in resectable CRC independent of other risk factors. Given the increased risk of recurrence in high-risk stage III disease and superior ctDNA sensitivity to alternative surveillance, there is an opportunity to incorporate ctDNA testing for quicker identification of treatment failure. Among patients with alternative treatment options, particularly the MSI-H population, early detection would minimize toxicity from ineffective therapy and potentially improve outcomes.

We describe a case of a patient with high-risk, MSI-H stage III CRC in whom failure of ACT was detected early by rising ctDNA levels. Following rapid initiation of checkpoint inhibitor immunotherapy, the patient achieved a complete and sustained response to programmed cell death protein 1 (anti-PD-1) therapy.

CASE DESCRIPTION
A women in her mid-70s presented to a community hospital with several days of nausea and vomiting. The patient had a past medical history of moderate aortic regurgitation and coloidal thyroid nodules. CT
imaging of the abdomen and pelvis showed an obstructing cecal mass with invasion into the transverse colon and terminal ileum. No gross metastatic disease was observed. Given her symptoms of malignant bowel obstruction, she was transferred to a tertiary care center and taken urgently to surgery. Intraoperatively a friable cecal mass was found, measuring 10 × 8.5 × 6.0 cm³ with invasion into the cecal serosa and the adjacent small bowel lumen. Extended right hemicolectomy with small bowel resection was performed. Histopathology of the cecal mass showed undifferentiated carcinoma grading, with 7 out of 21 abdominal lymph nodes involved, the largest deposit measuring 1.2 cm with extracapsular extension. Based on clinical tumor-node-metastasis staging, the cancer was classified as stage IIIIC (pT4bN2bM0) by the American Joint Committee on Cancer eighth edition. All margins were free of tumor. Perineural invasion was identified, without evidence of lymphovascular invasion or macroscopic perforation. Immunohistochemical staining performed on tissue from the primary cecal mass showed loss of expression of MLH1 and PMS2 consistent with high microsatellite instability. Hypermethylation of MLH1 was negative, and mutational analysis showed wild type BRAF.

High-risk features of her cancer included undifferentiated grade, tumor (T4b) and nodal (N2) staging, and perineural invasion. Baseline postoperative CT imaging was performed which demonstrated no residual or new disease. Due to significant baseline neuropathy and age-related elevated risk-to-benefit ratio, she was deemed not a candidate for oxaliplatin, and thus not a candidate for the aforementioned A021502 Trial. Given her risk, tumor-informed Signatera (Natera) MRD ctDNA testing was performed postoperatively and during adjuvant treatment.

Adjuvant capecitabine monotherapy was initiated 6 weeks postoperatively with an intent to complete eight cycles (6 months) of treatment, followed by surveillance with re-staging at 6-month intervals. After her second cycle, repeat ctDNA levels increased, prompting early re-staging. Positron emission CT scan showing recurrent and metastatic disease, including a soft tissue lesion at anastomosis in the right upper quadrant measuring 2.4 × 2.0 cm² and enlarging gastrohepatic and mesenteric soft tissue foci (Figure 1). She was immediately given pembrolizumab for metastatic MSI-H CRC per Keynote 177. After two cycles of pembrolizumab at 200 mg every 3 weeks her ctDNA Signatera assay levels were undetectable. Re-staging after 3 months showed complete resolution of her tumor burden. Her presurgical and postsurgical CEA levels remained negative at <5.0 ng/mL (Figure 1).

She maintains a complete response to therapy at 18 months and will continue pembrolizumab for at least 2 years, with ctDNA monitoring every 3 months during that time.

Figure 1  Radiologic disease correlating with circulating tumor DNA (ctDNA) and CEA values over time. (A) Postoperative CT (July 09, 2021) following right hemicolectomy, serving as patient’s baseline scan. (B) PET-CT (September 22, 2021) obtained after ctDNA interval increase to 59.7 MTM/mL, which showed new hypermetabolic 2.4 × 2.0 cm² hypermetabolic soft tissue lesion at anastomosis and hypermetabolic gastrohepatic and mesenteric soft tissue foci, both consistent with metastatic disease. (C) CT A/P with contrast (December 09, 2021) following two cycles of pembrolizumab, showing complete radiologic response. (D) Graphical trend of plasma ctDNA and CEA levels over time, with time points that adjuvant capecitabine (July 21, 2021) and pembrolizumab (September 22, 2021) were initiated. *MTM = mean tumor molecules; PET = positron emission tomography.
DISCUSSION

Tumor-informed ctDNA predicts risk for disease recurrence after curative intent surgery or ACT, underscoring its ability to detect MRD. Increasingly, ctDNA is also used to assess response to ACT, particularly in patients at high risk of relapse. However, most studies are observational and assess ctDNA levels after the ACT course is completed. While helpful, given the ability of ctDNA to detect relapse prior to conventional surveillance, earlier testing could spare toxicities from chemotherapy and allow earlier transition to alternative therapy in the setting of treatment failure. This is particularly true in the MSI-H CRC population where immunotherapy is an appealing treatment choice. For our patient with stable, normal-range CEA values and lack of clinical symptoms, without ctDNA results she would have received up to 4 months additional ineffective therapy. Early detection of rising ctDNA during adjuvant therapy prompted cross-sectional imaging which confirmed disease recurrence, allowing transition to front-line immunotherapy. Six weeks after initiating pembrolizumab, ctDNA level was undetectable, signaling she was already without signs of MRD. This is meaningful given data showing positive correlation between ctDNA clearance and progression-free survival. Kotani et al showed that of 182 patients with MRD positivity at 4 weeks postop, patients who did not achieve clearance demonstrated inferior DFS (adjusted HR 11, 95% CI 5.2 to 23.0, p<0.0001). The patient’s postoperative CEA values remained normal in contrast to positive ctDNA levels, corroborating data earlier described showing the superior sensitivity and specificity of ctDNA.

Although there is no established protocol for using tumor-informed ctDNA to provide real-time feedback on effectiveness of adjuvant therapy, there is evidence supporting the use of postoperative ctDNA values to guide ACT (table 1). Tie et al randomized patients 2:1 to ctDNA-informed versus standard (using standard clinicopathologic criteria) arms. Patients in the ctDNA-informed arm who were ctDNA-positive received adjuvant single or doublet chemotherapy, however if negative ctDNA levels at both 4 weeks and 7 weeks postop they were not given ACT. The number of patients in the ctDNA-informed arm who received ACT was 15% compared with 28% in the standard treatment arm, reducing ACT exposure without compromising 2-year relapse-free survival. Kotani et al showed that when randomizing patients with ctDNA positivity at 4 weeks postop to ACT versus observation, 68.48% (65 out of 92) of patients achieved ctDNA clearance versus 12.2% (11 out of 90) in the observation arm.

There are also limited data reinforcing the utility of monitoring ctDNA during ACT course, as shown in this case. A longitudinal biomarker study of patients with

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<th>Author, year</th>
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<td>Kotani et al</td>
<td>Nature Medicine</td>
<td>Stage II–IV (resectable)</td>
<td>1039</td>
<td>Postsurgical ctDNA positivity at 4 weeks was associated with higher recurrence risk (HR: 10.0, p&lt;0.0001) and was highly prognostic for recurrence (HR 10.82, p&lt;0.001). Postsurgical ctDNA positivity identified patients who benefit from ACT (HR 6.59, p&lt;0.0001).</td>
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<td>Reinert et al</td>
<td>JAMA Oncology</td>
<td>Stage I, II, III</td>
<td>125</td>
<td>Longitudinal ctDNA positivity after definitive therapy confers &gt;40 times risk of recurrence versus undetectable ctDNA (HR: 43.5; 95% CI 9.8 to 193.5; p&lt;0.001)</td>
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<td>Taieb et al</td>
<td>Clinical Cancer Research</td>
<td>Stage III</td>
<td>1017</td>
<td>Postoperative or prechemotherapy ctDNA positivity confers worse 3-year DFS of 66.39% vs 76.71% for ctDNA-negativity (p=0.015), (adjusted HR: 1.55, 95% CI 1.13 to 2.12, p=0.006) and OS (HR: 1.65, 95% CI 1.12 to 2.43, p=0.011).</td>
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<td>Tarazona et al</td>
<td>Annals of Oncology</td>
<td>Stage I, II, III</td>
<td>150</td>
<td>Positive postoperative and serial plasma ctDNA confers poorer DFS (HR:17.56; log-rank p=0.0014, and HR: 11.33; log-rank p=0.0001, respectively). ctDNA positivity after ACT was associated with early relapse (HR: 10.02; log-rank p&lt;0.0001).</td>
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<td>Tie et al</td>
<td>Science Translational Medicine</td>
<td>Stage II</td>
<td>230</td>
<td>In patients not treated with ACT 79% (11/14) of those with positive postop ctDNA developed relapse at median follow-up 27 months compared with 9.8% (16/164) with negative ctDNA (HR: 18, 95% CI 7.9 to 40; p&lt;0.001). In patients treated with ACT, positive ctDNA after completion of chemotherapy was associated with inferior RFS (HR: 11, 95% CI 1.8 to 68, p&lt;0.001).</td>
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<td>Tie et al</td>
<td>JAMA Oncology</td>
<td>Stage III</td>
<td>100</td>
<td>Postsurgical ctDNA positivity was associated with inferior RFS (HR: 3.8, 95% CI 2.4 to 21.0; p&lt;0.001) and postchemotherapy ctDNA positivity was associated with worse 3-year RFI of 30% vs 77% when ctDNA was undetectable (HR: 6.8, 95% CI 11.0 to 157.0; p&lt;0.001).</td>
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ACT, adjuvant chemotherapy; CRC, colorectal cancer; ctDNA, circulating tumor DNA; DFS, disease-free survival; OS, overall survival; RFI, recurrence-free interval; RFS, relapse-free survival.
stage I–III CRC by Reinert et al found that of 10 patients with positive postop ctDNA values who received ACT, there were 8 with available serial ctDNA samples during therapy. Of the four patients who retained positive ctDNA during ACT, all relapsed. Tarazona et al studied serial ctDNA levels in patients with stage II–III CRC after surgery and during chemotherapy. They found that in seven patients with positive postop ctDNA and available serial ctDNA values during chemotherapy, three developed rising ctDNA levels during ACT which predicted radiologic relapse. In none of these cases, however, were the results used to modify adjuvant therapy prior to completion.

CONCLUSION

In the described case, disease progression on ACT was detected early in a patient with high-risk stage III CRC using serial tumor-informed ctDNA monitoring. She was transitioned to pembrolizumab immunotherapy and rapidly achieved a complete and sustained remission. This case demonstrates how monitoring serial ctDNA during ACT may critically inform adjuvant therapy decisions in real time. MRD surveillance was particularly salient in this case given her disease was at high risk of relapse and her MSI-H status, which made immunotherapy an attractive option in the setting of disease recurrence. Early detection minimized her exposure to ineffective chemotherapy and enabled therapy change before she developed clinical symptoms of metastatic disease. There are several ongoing randomized controlled clinical trials investigating the ability of ctDNA to guide therapy for CRC during ACT, including NCT-04050345, in which ACT for high-risk patients with stage II–III CRC will be escalated or de-escalated based on postoperative ctDNA positivity. Continued prospective research on using ctDNA to guide adjuvant treatment is needed, particularly in high-risk patients of MSI-H status.

Contributors NL reviewed the background literature and wrote the case report. MJR cared for the patient and formed the structure and premise of the case report, and helped edit and formulate the text and table/figure. JMA provided clinical care for the patient and reviewed and contributed to the submission process.

Funding The authors have not declared a specific grant for this research from any funding agency in the public, commercial or not-for-profit sectors.

Competing interests None declared.

Patient consent for publication Consent obtained directly from patient(s).

Ethics approval This study involves human participants but this patient case was collected not for research purposes but from clinical practice of Dr. Reilley and as a case report is exempted based on institutional guidelines. exempted this study.

Provenance and peer review Not commissioned; externally peer reviewed.

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