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Case Report

Circulating Tumor DNA Testing Overcomes Limitations of Comprehensive Genomic Profiling from Tumor Tissue

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Precision oncology · Circulating tumor DNA · High microsatellite instability · Colorectal cancer

Abstract
"Liquid biopsy" is an established technique for examining circulating tumor DNA (ctDNA) from a routine blood draw and detecting actionable biomarkers. Nonetheless, ctDNA testing is rarely utilized for patients with newly diagnosed metastatic colorectal cancer (CRC). We report a case in which ctDNA testing uncovered an actionable biomarker that was not detected by comprehensive genomic profiling of tumor tissue. An 81-year-old woman with a remote history of non-Hodgkin's lymphoma presented with primary masses in the ascending colon and sigmoid colon. The ascending colon and sigmoid colon tumors were classified as microsatellite stable (MSS) and mismatch repair proficient (pMMR), and both ctDNA and tissue next-generation sequencing (NGS) from the ascending colon mass were ordered. Because tissue NGS results indicated that the ascending colon tumor was MSS, palliative 5-fluorouracil, leucovorin, and oxaliplatin (FOLFOX) chemotherapy was started. However, the ctDNA NGS results that arrived after the start of FOLFOX found high microsatellite instability (MSI-H) and mismatch repair deficiency (dMMR) disease with a serine/threonine-protein kinase B-Raf (*BRAF*^{V600E}) mutation. To treat both her MSS/pMMR ascending colon and sigmoid colon tumors and MSI-H/dMMR metastatic disease, the immunotherapy nivolumab was added to FOLFOX. After 8 months of combined nivolumab and chemotherapy, the patient's metastatic disease had a complete clinical response. This case highlights the complementary role of ctDNA testing for biomarker identification. By performing simultaneous ctDNA testing at the time of diagnosis, an actionable biomarker was discovered that significantly altered this patient's prognosis and treatment options. Orthogonal testing of

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key molecular alterations offers significant advantages for identifying actionable biomarkers and improving management of metastatic CRC.

Introduction

Colorectal cancer (CRC) is one of the most commonly diagnosed cancers in the world and is the second leading cause of cancer-related death in the USA. In 2020, it is estimated that there were approximately 150,000 new cases of CRC, the equivalent of about 400 new cases per day [1]. Based on current treatment algorithms, survival for patients with metastatic CRC is approximately 2–3 years [2–4]. In recent years, the discovery that mismatch repair deficiency (dMMR) and high microsatellite instability (MSI-H) predict sensitivity to immunotherapy therapy has revolutionized the treatment of metastatic CRC. Approximately 3–20% of patients with CRC have tumors that display dMMR/MSI-H with decreased frequency in metastatic tumors as compared with early-stage tumors [5–8]. Traditionally, dMMR is identified in tumor tissue by loss of MMR protein expression as determined by immunohistochemistry (IHC) or by the detection of microsatellite instability through a polymerase chain reaction (PCR)-based test. MSI-H tumors are sensitive to antibodies that target programmed death-1 (PD-1) receptors or its ligands, and both nivolumab and pembrolizumab are approved in the USA and other countries for the treatment of dMMR/MSI-H CRC [9–12]. Additionally, data from the KEYNOTE-177 trial demonstrated that pembrolizumab is superior to cytotoxic chemotherapy in the first-line treatment of dMMR/MSI-H metastatic CRC, resulting in a new standard of care for these patients [13]. Unfortunately, aside from a small subpopulation of patients that may receive benefit from PD-1 blockade, most patients have microsatellite stable (MSS) or mismatch repair proficient (pMMR) tumors, who receive no clinical benefit from PD-1 blockade [14]. Given the importance of MMR/MSI status in the first-line management of patients with metastatic CRC, rapid and reliable biomarker testing is critical.

Current National Comprehensive Care Network (NCCN) guidelines recommend tissue biopsy for biomarker testing and associated first-line management of metastatic CRC [15]. Detection of circulating tumor DNA (ctDNA) from a routine blood draw ("liquid biopsy") is an established method for biomarker testing that is a rapid and noninvasive alternative to tissue biopsy. Already, blood-based next-generation sequencing (NGS) profiling has shown the potential to detect malignancy in asymptomatic patient populations, identify minimal residual disease in patients following surgical resection, identify actionable biomarkers in patients with metastatic disease, predict treatment response, and identify genomic drivers of treatment resistance [16–20]. Its clinical utility and validity have been established for certain cancer types, primarily non-small cell lung cancer, but there is currently no defined role for ctDNA in NCCN CRC guidelines [21].

Here, we report a case in which ctDNA testing identified a highly actionable molecular alteration – MSI-H – that was not discovered from initial tissue testing. Written informed consent was obtained from the patient for publication of this case report. The discovery of the MSI-H biomarker substantially changed the patient's treatment course and long-term outcomes. This case highlights the complementary role of ctDNA to traditional tissue testing and suggests that ctDNA may guide treatment decisions for patients with newly diagnosed metastatic CRC. The CARE Checklist has been completed by the authors for this case report, attached as online supplementary material (for all online suppl. material, see www.karger.com/doi/10.1159/000529813).

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Case Report

An 81-year-old woman with a remote history of non-Hodgkin's lymphoma treated by autologous stem cell transplant presented to the emergency department with 2 days of abdominal pain and diarrhea. Computed tomography of the chest, abdomen, and pelvis revealed a soft tissue mass in the ascending colon, resulting in partial obstruction of the small bowel and small adjacent mesenteric lymph nodes. Colonoscopy demonstrated a large, completely obstructing mass in the ascending colon/hepatic flexure as well as an ulcerated, medium-sized, non-obstructing mass in the sigmoid colon. Endoscopic biopsy samples confirmed infiltrating, moderately differentiated adenocarcinomas in the ascending colon and sigmoid colon. Both tumors were classified as MSS by PCR and pMMR by IHC, and these findings were later confirmed on review by a second pathologist.

She was referred to surgery for possible right colectomy and sigmoid resection. During intraoperative evaluation, she was noted to have bulky ileocolonic nodal disease and carcinomatosis at this site. The primary tumors were deemed unresectable. Palliative diverting ileostomy was not feasible due to the extent of her bulky peritoneal and ileocolonic disease. Biopsies of carcinomatosis were obtained intraoperatively.

The patient was referred to medical oncology after surgery. At the time of initial consultation, she had progressively worsening abdominal pain concerning for intermittent and partial bowel obstruction. A colonic stent was placed to palliate bowel obstruction symptoms. Given the urgency of biomarker testing, tissue NGS (FoundationOne CDx, Foundation Medicine, Cambridge, MA, USA) of the ascending colon tumor biopsy as well as ctDNA NGS (Guardant360, GuardantHealth, Redwood City, CA, USA) were ordered simultaneously. The patient was started immediately on a palliative 5-fluorouracil, leucovorin, and oxaliplatin (FOLFOX) chemotherapy regimen. Bevacizumab was held due to concern for imminent bowel obstruction and colonic stent.

Tissue NGS results from the ascending colon cancer revealed an MSS and TMB-low tumor (shown in Table 1). However, the ctDNA NGS which arrived after FOLFOX treatment had already begun, detected the presence of MSI-H disease with a $BRAF^{V600E}$ mutation. Tissue NGS and MSI testing by PCR were then performed on the peritoneal lesion obtained intraoperatively. This confirmed an MSI-H and $BRAF^{V600E}$-mutated metastasis consistent with the ctDNA profile. Further IHC of the peritoneal metastatic lesion revealed loss of MutL homolog 1 (MLH1), MutS homolog 6 (MSH6), and PMS1 homolog 2 (PMS2) expression. Thus, the patient had two separate colonic primaries, both MSS by PCR and NGS, and a third metastatic adenocarcinoma that was MSI-H/dMMR and $BRAF^{V600E}$ mutated (shown in Fig. 1).

To treat both her MSI-H/dMMR and MSS/pMMR diseases simultaneously, the anti-PD-1 immunotherapy nivolumab was added to FOLFOX. The combination of FOLFOX and nivolumab demonstrated significant anti-cancer activity. Follow-up computed tomography of the chest, abdomen, and pelvis after 2 months showed significantly decreased size of the infiltrative ascending pericolonic mass. After 4 months of FOLFOX, the patient was transitioned to fluorouracil plus nivolumab as maintenance therapy.

After 8 months of disease control, the patient was referred to surgery for removal of the ascending colon and sigmoid colon tumors. Intraoperative evaluation revealed complete clinical response to her metastatic disease. She underwent right colon resection and sigmoidectomy. Pathology revealed complete response in the ascending colon primary and its associated ileocolonic mesentery with previously biopsy-proven metastatic disease. The sigmoid resection confirmed T3N0 adenocarcinoma of the sigmoid colon which was retested and demonstrated to be MSS and pMMR. Following surgery, she has received nivolumab without cytotoxic chemotherapy and has ongoing complete response to treatment (shown in Fig. 2). She is tolerating treatment well with no clinically significant treatment-related side effects.

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Table 1. Genomic alterations, genomic signatures, and additional biomarkers found by tissue NGS of ascending colon mass, tissue NGS of peritoneal nodule, and ctDNA liquid biopsy

Diagnostic endoscopic biopsy of ascending colon mass Commercial 324 gene NGS panel	Liquid biopsy (~2 months post diagnosis) Commercial 83 gene NGS panel	Biopsy of peritoneal nodule Hotspot 50 gene NGS panel
Genomic alterations	Genomic alterations	Genomic alterations
MAP2K1(NM_002755)c.167A>C(p.Q56P)	BRAF(NM_004333)c.1799T>A(p.V600E)	BRAF(NM_004333)c.1799T>A(p.V600E)
TP53(NM_000546)c.428T>C(p.V143A)	MSH6(NM_000179)c.3261delC(p.F1088fs)	
SMARCA4(NM_003072)c.-5_13delTGAAGATGTCCACTCCAG(p.M1?)	BRCA1(NM_007294)c.3538dupA(p.S1180fs)	
CDK8 amplification	ARID1A(NM_006015)c.4582C>T(p.R1528)	
FLT3 amplification	ARID1A(NM_006015)c.3826C>T(p.R1276)	
GNAS amplification	CCND1(NM_053056)c.859C>T(p.P287S)	
	TP53(NM_000546)c.818G>A(p.R273H)	
	TP53(NM_000546) c.526_534delTGCCCCCAC(p.C176_H178del)	
Genomic signatures	Genomic signatures	Genomic signatures
Tissue tumor mutational burden = 7.6 Muts/Mb	Blood tumor mutational burden = 141.1 Muts/Mb	N/A
MSS	MSI-H	
Additional biomarker testing	Additional biomarker testing	Additional biomarker testing
Normal expression of MLH1, MSH2, MSH6, and PMS2 by IHC	N/A	Loss of MLH1, MSH6, and PMS2 expression by IHC
MSS by PCR		MSI-H by PCR
		MLH1 promoter methylation

MSI-H, high microsatellite instability; MSS, microsatellite stable.

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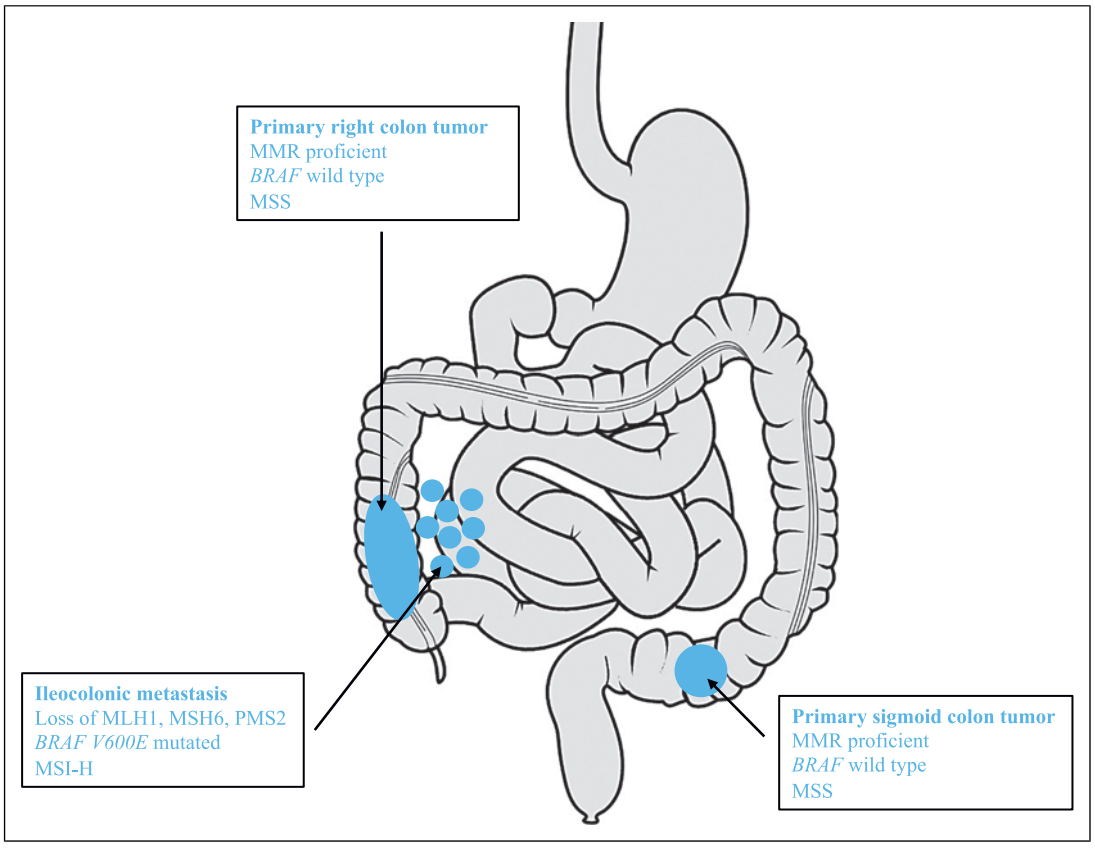

Fig. 1. Spatial arrangement of ascending colon tumor, sigmoid colon tumor, and carcinomatosis with MSS/pMMR and MSI-H/dMMR biomarkers, respectively. Remesz O. Intestinesall.svg. Wikimedia Commons, 2009.

Discussion

This case is an example in which following the standard of care for CRC biomarker testing by molecular profiling of tumor tissue alone would have missed a critically important and actionable therapeutic target. Here, tissue NGS results pointed to a treatment algorithm that relies on cytotoxic chemotherapy and offers modest survival benefit. However, in this case, the ctDNA NGS test identified MSI-H and $BRAF^{V600E}$-mutated metastatic disease which could be treated with anti-PD-1 immunotherapy. In this case, FOLFOX and nivolumab demonstrated significant anti-tumor activity and offered this patient substantially better outcome.

This case highlights how liquid biopsy could be particularly valuable when multiple primary tumors are present. Because blood-based NGS is a more complete representation of all tumors in the body shedding ctDNA, the actionable biomarkers derived from this patient's metastatic disease were recognized.

There are other scenarios in which liquid biopsy may offer advantages over tissue testing. When a tissue biopsy is not feasible due to tumor's location, a liquid biopsy may offer lower risk of complications [22]. Additionally, turnaround time and time to trial enrollment are quicker with ctDNA NGS than tissue NGS. Nakamura-Yoshino et al. reported that the interval between receiving a sample and reporting its results was 19 days for tissue NGS and 7 days for ctDNA NGS and that patient accrual to interventional clinical trials increased from 4.1 patients per month to 8.1 patients per month when ctDNA NGS was added to their testing protocol [23]. Importantly, MMR IHC testing of the metastatic tissue sample would have had the

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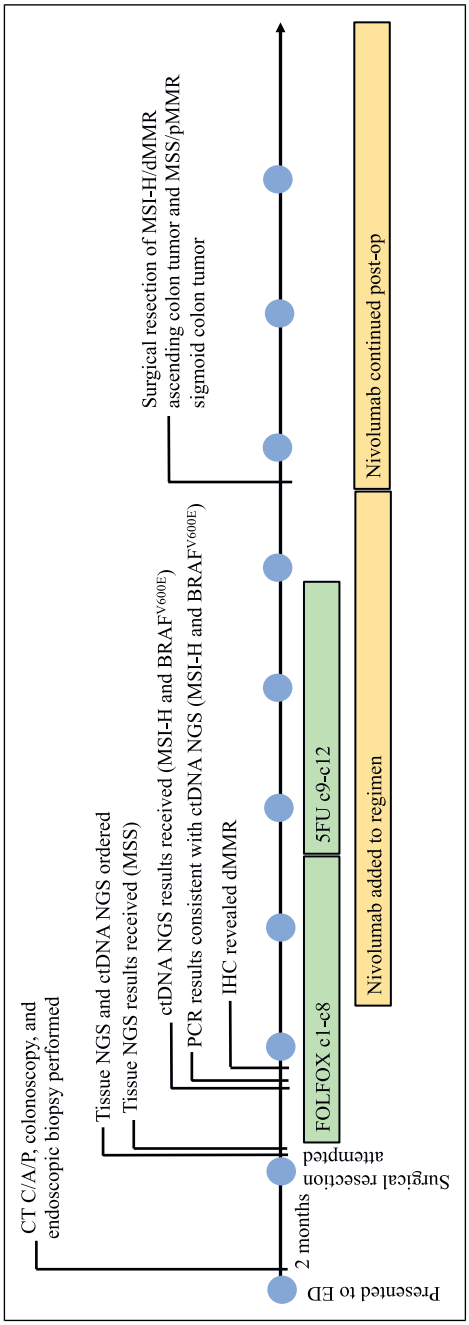

Fig. 2. Timeline of tissue NGS and ctDNA NGS testing and therapeutic regimens of chemotherapy, immunotherapy, and surgical resection for patients with CRC.

quickest relative turnaround time, but because the tissue itself may not always be accessible, liquid biopsy provides significant value. Serial ctDNA monitoring may also provide early assessment of response to treatment [24]. As such, the liquid biopsy shows potential clinical utility where multiple primary tumors are present, tumors express heterogeneity and evolve, tissue cannot be physically reached, time is critical, or when noninvasive approaches are preferred.

When highly actionable molecular alterations are present, it is essential that these biomarkers be detected. Orthogonal testing of molecular alterations increases the probability that actionable biomarkers are detected. This study is limited by being a single case report and because it is rare for a patient to have multiple simultaneous primary tumors. While the rarity of multiple primary tumors limits prospective validation, this case report highlights an example of how actionable driver alterations may be detected from blood when tissue is negative. This case suggests that in patients with newly diagnosed metastatic CRC, there is clinical benefit in testing ctDNA simultaneously with a tissue biopsy test to ensure that actionable molecular targets are identified.

Statement of Ethics

Ethical approval is not required for this study in accordance with local or national guidelines. Written informed consent was obtained from the patient for publication of the details of the medical case.

Conflict of Interest Statement

J.H.S.: consultant for AbbVie, Takeda, AstraZeneca, Bayer, Eli Lilly, GSK, Natera, Pfizer, Seagen, Silverback Therapeutics, and Viatris. Research (institutional) for Amgen, Bayer, Erasca, Eli Lilly, Seagen, Daiichi Sankyo, Gossamer Bio, Astar D3, Sanofi, Roche/Genentech, Curegenix, Nektar, AbbVie, and Silverback Therapeutics.

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Author Contributions

Anivarya Kumar wrote the primary draft of the manuscript. Michelle Green, Julie Thacker, William Jeck, and John H. Strickler critically revised, commented upon, and approved the manuscript before its final submission.

Data Availability Statement

All data generated or analyzed during this study are included in this article and its online supplementary material files. Further inquiries can be directed to the corresponding author.

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