In patients with resected colorectal cancer, minimal or molecular residual disease (MRD) assays are now available either commercially or through expanded access programs/clinical trials. They can be broadly divided into two types of liquid biopsies. First came the tumor-informed circulating tumor DNA (ctDNA)-based platforms in 2019 in the United States (Signatera). Recently, in 2021, plasma-only--based tumor-agnostic MRD assays are also commercially available for patients with colorectal cancer (Guardant Reveal). Here, we present a unique case of a patient with resected stage III colon cancer who underwent testing using both platforms with discordant results secondary to multiple other primary malignancies that were diagnosed later. The case here outlines the need to keep the clinical context and pathologic factors in mind, which may, in turn, influence the performance of any of these assays.

Case Presentation

Our patient is a 66-year-old otherwise healthy gentleman who was diagnosed with colon cancer in 2019 in the setting of melena and iron deficiency anemia. Colonoscopy and imaging revealed a circumferential 5-cm mass in the rectosigmoid area. Biopsy was consistent with a mismatch repair-proficient adenocarcinoma. The patient underwent laparoscopic sigmoid colectomy with final pathology revealing pT3N1a (metastases to one of 30 lymph nodes resected). Three months (four cycles) of systemic chemotherapy with CAPEOX (capecitabine and oxaliplatin) was delivered. The patient was concurrently enrolled in the expanded access program for the tumor-informed ctDNA assay. He underwent surveillance follow-up and imaging alongside the MRD testing.

In his 18th-month follow-up, concerns were raised regarding a new portal vein thrombus on a computed tomography (CT) scan. Follow-up with magnetic resonance imaging revealed that potentially, the thrombosis seen was a tumor thrombus and that there were surrounding areas of heterogenous enhancement in the caudate lobe alongside segments 5, 7, and 8, but no discrete mass that was amenable to a tissue biopsy. The tumor-informed MRD testing continued to be negative. Multidisciplinary tumor board discussion was concerning for possible recurrence of the colorectal cancer. However, with no safe/feasible way to get tissue to prove this, the plasma-only MRD assay was ordered. This was noted to be positive. A positron emission tomography (PET/CT) scan was also subsequently performed. On the PET/CT, although the liver area did not show significant uptake, highly avid hypermetabolic mass was noted in the left palatine region consistent with a primary oropharyngeal malignancy. In addition, left parapharyngeal space and left level 2 cervical adenopathy consistent with metastases were also noted.

Core needle biopsy of the left-sided adenopathy in the neck was consistent with metastatic squamous cell carcinoma, p16-positive. An ultrasound-guided biopsy of the suspicious area of the liver as noted on the magnetic resonance imaging was also attempted and came back as hepatocellular carcinoma, moderately differentiated. The patient has two advanced but not metastatic malignancies (no distant spread). He is starting palliative radiation for the head and neck squamous cell cancer with plans for potentially yttrium-90 radioembolization (Y90-RE) for the advanced unresectable hepatocellular carcinoma. Repeat plasma-only assay 3 months apart continues to be positive on the methylation/epigenomic component but negative on the ctDNA component.

Discussion

This case is very intriguing. With increasing life expectancy and advanced age at diagnosis for a lot of our patients, it is not uncommon to have one or even more secondary distinct malignancies. In this case, the patient had a stage III colon cancer for which curative-intent surgery and adjuvant therapy have been completed with no evidence of disease recurrence. However, in the context of plasma-only liquid biopsy assay results being positive, although further imaging did not reveal colorectal cancer recurrence, we serendipitously stumbled upon two other advanced malignancies, ie, hepatocellular cancer and oropharyngeal squamous cell cancer.
As noted, the plasma-only assay is a composite of two methods of testing.\(^5\) It combines panel-based mutational testing for ctDNA alongside methylation/epigenomic testing. The addition of the latter is what increases the sensitivity of the assay as noted in the recent publication.\(^5\) However, it is likely that methylation/epigenomic markers as in this case are not mutually exclusive to colorectal cancer. It is possible that there is likely an overlap with other malignancies sharing similar pathways, leading to the false-positive signal in the context of colorectal cancer MRD detection. In this patient's case, it was the methylation/epigenomic component that was persistently positive on two occasions; whereas the ctDNA component on both the platforms continues to be negative to date. Although this is truly a hypothesis, on the basis of the understanding and development of other methylation markers in this early detection/MRD space, the odds are that it is the hepatocellular cancer more than the oropharyngeal cancer that is causing the false-positive signal in this patient's case (Figure 1). Several other methylation markers being developed for hepatocellular cancer have been shown to exhibit cross-reactivity to colorectal adenocarcinomas.\(^8,9\)

These are indeed exciting times for patients with colorectal cancer.\(^1\) From panel-based next-generation sequencing
platforms for advanced/metastatic disease that also report on microsatellite instability status to now the availability of several MRD platforms (tumor-based or plasma-only), it is of value to understand the clinical and pathologic variables that may affect testing results.6,7,10 Both the platforms have their pros and cons.11 The plasma-only assay results, since it does not require tissue, are being reported within 7-10 days. The turnaround time here is key since adjuvant therapy-based decisions are being made. The tumor-informed platforms have an initial first sample turnaround that can take 4-6 weeks since a piece of cancer tissue needs to be sent for mutational profiling to develop the custom-built assay.4 However, in the context of situations like this where a patient may have more than one primary malignancy, it is of value as demonstrated here to confirm the pathology before acting up on the result. This is particularly relevant where, for example, the presentation of the recurrence as the tumor thrombus in the liver is not classic for colorectal cancer and more so aligned with hepatocellular cancer.

Our report has several limitations. First, it is an n = 1 case report. In addition, it is possible that the patient could still have recurrent colon cancer that is not being picked up on the mutational panel testing. However, this is less likely given multiple subsequent negative timepoints and no imaging findings at several follow-up timepoints. Furthermore, given the final pathology of pT3N1a and 3 months of CAPEOXY, the estimated 5-year disease-free survival using recently validated nomograms is approximately 80%, again, making it less likely.12 The strength of our report is the unique situation whereby we have both tumor-informed and plasma-only colorectal cancer MRD assay in the same patient with multiple follow-up timepoints. It is also worth mentioning that commercially available next-generation sequencing platforms meant for metastatic disease are different and less sensitive than the ones that are meant for MRD detection. As these have quickly become commercially available, it is important to distinguish the tests on the basis of the indication.

From a future directions’ perspective, besides the application of various MRD platforms for colorectal cancer, it is of value to note that multiple methylation/epigenomic-based assays are being developed for both diagnosis and screening for different malignancies. Future studies need to include positive controls of other malignancies to demonstrate that the performance of the assays is diseasespecific. Finally, active enrollment in ongoing clinical trials that are incorporating the plasma-only or the tumor-informed assays is going to be key in understanding the implications of a positive test result and the role of ctDNA as a predictive marker.13

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AUTHOR’S DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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