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# Using liquid biopsies to guide treatment and monitor response in BRAF V600E positive adenocarcinoma of unknown primary

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## SUMMARY

Liquid biopsies using circulating tumour DNA (ctDNA) have emerged as an alternative to conventional biopsies. They can be used to aid in diagnosing and selecting an agent for treatment and can possibly be used to monitor disease response to treatment. In this report, we present a patient who initially presented with lower abdominal pain. Imaging showed extensive retroperitoneal lymphadenopathy and lymph node biopsy demonstrated poorly differentiated carcinoma. Further workup did not reveal a primary lesion, but his genetic analysis revealed a BRAF V600E mutation and CD274 amplification which was used to guide treatment of the adenocarcinoma as a melanoma of unknown primary. He was initiated on ipilimumab and nivolumab and his ctDNA levels showed rapid improvement. After treatment was stopped due to adverse events, he was monitored via ctDNA, with an increase prompting repeat imaging that demonstrated enlargement of his lesions prompting a resumption of treatment.

## BACKGROUND

Melanomas of unknown primary (MUPs) are a subset of melanoma in which no visible primary lesions can be found and are thought to be due to spontaneous regression of the initial lesions. MUPs comprise roughly 3% of melanomas and are usually found in lymph nodes but, less commonly, can also be found in subcutaneous tissue or visceral organs.[1] The diagnosis is classically made via pathology, though if the diagnosis is unclear, DNA analysis for molecular characterisation can be helpful as specific mutations such as BRAF V600E are common in melanoma.[1 2] Identifying the genetic landscape is not only important for MUPs, but for any melanoma as it can help identify treatment options. For example, if a patient is found to have wild-type BRAF mutation, then the standard of care is immunotherapy with immune checkpoint inhibitors (ICIs) such as ipilimumab and nivolumab which are antibodies that target cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) and programmed death 1 (PD-1), respectively, which are inhibitory immune checkpoint receptors on T cells. The use of ICIs in melanoma is further supported if molecular profiling shows expression of programmed death ligand-1 (PD-L1).[3–5]

Tissue biopsies are the standard for molecular profiling but are often limited because they are invasive and can have long turnaround times.[5] Liquid biopsies have emerged as an alternative to conventional biopsies and are a minimally invasive way to obtain genomic information on a tumour in a timely manner. One method of liquid biopsy is via detecting circulating tumour DNA (ctDNA), which is cell-free DNA (cfDNA) released by tumour cells into the bloodstream via various mechanisms. CtDNA can be detected via next generation sequencing techniques and used to provide insight on the molecular landscape of a tumour.[6–9] This information can then be used to aid in diagnosing and selecting an agent for treatment.[4 5 8 9] The levels of ctDNA can be quantified and there is some data to suggest that these levels can be used to monitor disease response to treatment, progression of disease, and can aid with prognostication of melanoma.[4 10 11] For these reasons, ctDNA has the potential to be a powerful tool in the field of precision oncology.

In this report, we present an interesting case of a man in his fifties who was found to have an adenocarcinoma of unclear primary, where liquid ctDNA next-generation sequencing (NGS) was used to help guide treatment via detection of BRAF V600E mutation and CD274 amplification with rapid turnaround. He started treatment with dual ICIs and had a profound response which was monitored via longitudinal bespoke tumour-informed ctDNA measurements.

## CASE PRESENTATION

A man in his 50s with no significant oncological history initially presented to the urology department with a month-long history of worsening lower abdominal pain that radiated to his pelvis and was associated with testicular pain and a feeling of scrotal fullness during this period along with dysuria. On further questioning, the patient noted he had been experiencing fatigue, chills and excessive night sweats during this period but no weight loss. An initial physical examination, which included a prostate and testicular examination, was unremarkable. He had a family history of various types of cancer, including prostate, testicular, kidney, oesophageal, colon and pancreatic cancer on his paternal side of the family. The patient's last colonoscopy was 4 years ago, and a few benign polyps were removed.

## INVESTIGATIONS

The initial laboratory investigation included a metabolic panel and blood counts that were unremarkable, so he had an initial CT scan of his chest, abdomen and pelvis which showed

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extensive retroperitoneal lymphadenopathy, with the largest pelvic conglomerate measuring 7.2 × 7.2 cm, and hypodense hepatic lesions. A staging MRI of his brain did not show any signs of metastasis. Further labs were obtained including prostate serum antigen 0.77 ng/mL, alpha fetal protein 2.05 ng/mL, beta human chorionic gonadotropin 4 mIU/mL and lactate dehydrogenase 466 U/L. Our patient underwent a left iliac and retroperitoneal lymph node biopsy, which demonstrated poorly differentiated carcinoma, concerning for possible germ cell tumour. He underwent an oesophagogastroduodenoscopy and colonoscopy which did not reveal a primary malignancy, and his scrotal ultrasound did not reveal any masses. His biopsy samples were sent for genetic analysis, but the sample was inadequate. A blood sample via liquid biopsy was also sent for analysis and revealed BRAF V600E mutation and CD274 (PD-L1) amplification and an initial ctDNA level of 4739.81 mean tumour molecules per millilitre (MTM/mL). Pathology from a subsequent biopsy showed poorly differentiated adenocarcinoma positive for CK8/18 potentially suggesting prostatic or germ cell tumour. Immunohistochemical stains for melanoma were negative. An initial whole-body f-fluorodeoxyglucose positron emission tomography (FDG PET) scan showed FDG avidity in his hepatic lesions, multiple paratracheal and abdominal lymph nodes, and an enlarged pelvic conglomerate. He also had a complete skin exam performed by a dermatologist with no lesions identified.

## DIFFERENTIAL DIAGNOSIS

Our patient's pathology did not reveal a primary lesion other than poorly differentiated adenocarcinoma. Based on staining there was some concern for a germ cell tumour though no lesions were seen on examination or imaging. Our patient's molecular profiling showing BRAF V600E and CD274 mutations raised concern for a melanoma even though no primary lesion could be found since BRAF V600E is most commonly found in malignant melanoma.[12] Other malignancies that can also have the BRAF V600E mutation such as colorectal and thyroid tumours[12] were ruled with the above workup. With all this in mind, based on our patient's liquid NGS the decision was made to start treatment of the adenocarcinoma as a MUP.

## TREATMENT, OUTCOME AND FOLLOW-UP

Based on this diagnosis, he was initiated on ipilimumab 3 mg/kg and nivolumab 1 mg/kg infusions with 3 week cycles. His ctDNA levels showed rapid improvement with his levels dropping to 4256 MTM/mL within the first week and down to 154.89 MTM/mL after 1 month (figure 1). He had a repeat CT scan after his second cycle that showed his pelvic mass had significantly decreased in size while his hepatic lesions and lymph nodes had varied responses. His ctDNA level at this time continued to show a robust response to treatment, decreasing to 8.57 MTM/mL and our patient reported improvement in his symptoms. After his third infusion, our patient developed checkpoint inhibitor pneumonitis and hepatitis requiring treatment interruption for 2 months while he was being treated with steroids. After finishing his steroid treatment his ctDNA levels showed complete clearance, and the decision was made to start nivolumab maintenance therapy for improved mortality benefit.[13] Shortly after his first infusion he again developed pneumonitis, so all therapy was stopped, and our patient was started on maintenance steroids. Subsequent PET scan the following month showed a significant decrease in size and FDG avidity of his paratracheal and abdominal lymph nodes and hepatic lesions although not complete resolution. Our patient was started on BRAF/MEK therapy but shortly afterwards developed flu-like symptoms and was deemed intolerant, so treatment was stopped.

After being off all therapy for 2 months his ctDNA increased to 0.29 MTM/mL which prompted a repeat chest CT that showed stable enlargement of his paratracheal and abdominal lymph nodes and liver lesions. As there were still signs of residual disease our patient was started on every 2 week dosing nivolumab with 10 mg of prednisone. This was further supported by his subsequent ctDNA before starting treatment being elevated to 4.29 MTM/mL and repeat PET scan 3 days after initiating treatment showing increased size and FDG avidity in lymph nodes and liver lesions. Our patient tolerated treatment well and his ctDNA levels were 0.04 MTM/mL 1 month after treatment, and since then his subsequent levels have continued to show complete clearance.

## DISCUSSION

ctDNA is a rising diagnostic tool that can monitor various malignancies such as lung cancer and melanoma.[7 10 14] There is preclinical data that suggests it can be used to aid with diagnosing and providing treatment for melanomas. In addition, it is less invasive than tissue biopsies.[4] Serial monitoring of ctDNA can also potentially monitor disease response to treatment and progression.[11] One study showed that in stage III melanomas, baseline and postoperative ctDNA could be used to identify patients with a higher risk of relapse which may be used to guide further treatment.[15] Another study showed that in non-small cell lung cancer patients, ctDNA could be used in combination with CT or PET scans to not only monitor response to treatment but also progression-free survival.[14] Additional cases of patients with carcinomas of unknown primary also used ctDNA, which showed a correlation of ctDNA levels with response to therapy.[16] Overall, ctDNA is a tool that has great potential for guiding treatment, but its exact role will have to be found via clinical trials.

In this case, we further illustrate how liquid biopsy has the potential to serve as a method to assist in diagnosing a patient's

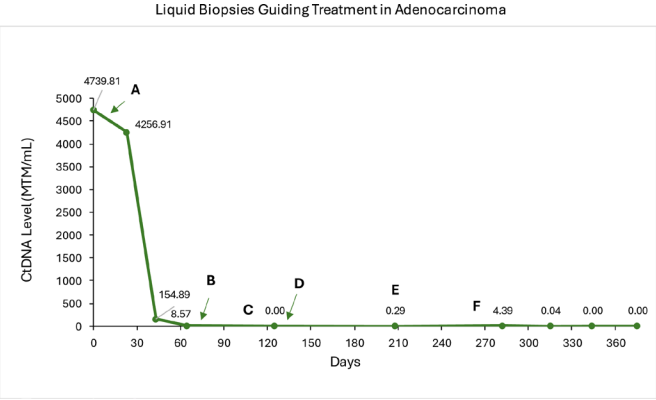


**Figure 1** ctDNA levels in our patient throughout his diagnosis and treatment course. A: Ipilimumab and nivolumab are initiated. B: Our patient developed checkpoint inhibitor-related pneumonitis, treatment is stopped. C: Nivolumab monotherapy is started but stopped after one infusion due to CIP. D: PET scan showed residual FDG avid areas, targeted BRAF therapy started but stopped shortly after due to side effects. E: An increase in ctDNA is found after prior clearance, prompting a repeat PET scan. F: Nivolumab monotherapy is restarted, and infusions are continued every 2 weeks. *This figure is the original work of the authors. ctDNA, circulating tumour DNA; FDG, fluorodeoxyglucose; PET, positron emission tomography.

malignancy and treatment selection expeditiously (7 days) and without procedural risks. Our patient's initial workup did not reveal a primary lesion and pathology was not diagnostic. We used liquid biopsy and NGS techniques for molecular profiling, to reveal BRAF V600E mutation, which is most commonly found in malignant melanoma.[12] This was further supported as other tumours that commonly have this mutation such as colorectal and thyroid tumours were ruled out via workup.[12] Notably, while an eye exam was not performed, ocular melanoma has a different genetic profile with around 90% of ocular lesions having a GNAQ or GNA11 mutation.[17] BRAF mutations, as in our patient, are also rarely seen in ocular melanoma[17] making this diagnosis unlikely. Thus, the presence of BRAF V600E together with the presence of CD274 amplification raised high suspicion for a MUP and he was treated with targeted therapy based on the genomic profile of his malignancy. Our patient was initially started on ICIs over BRAF/MEK inhibitors due to recent literature showing a survival benefit with using dual ICIs as first-line treatment.[18] Notably, some data suggests that in solid tumours CD274 amplification is associated with better response to PD-1 inhibitors.[19] However, the exact role of CD274 as a predictive biomarker for response to ICIs in melanoma is somewhat controversial,[5] and there is data showing survival benefit with ICIs even in patients that are PD-L1 negative.[20] Our patient was eventually switched to BRAF/MEK inhibitors, due to risks of immunotherapy rechallenge in a patient with high-grade ICI-related pneumonitis and hepatitis though was not able to tolerate them due to side effects.[3 18 21] Notably, the decision to use BRAF/MEK inhibitors is further supported by recent literature and FDA approval that supports the use of tissue-agnostic BRAF/MEK inhibitors.[22] Longitudinal liquid biopsies correlated with our patient's response to treatment as his ctDNA showed a rapid decline within weeks of starting treatment and was corroborated by the decreased tumour burden on the PET scan. Once treatment was held due to adverse events, ctDNA was used to monitor for disease recurrence a rise in his levels prompted re-evaluation showing disease progression and reinitiation of therapy. Overall, our case contributes to the literature on the use of liquid ctDNA NGS to obtain a molecular profile of a malignancy which can guide treatment, and highlights ctDNA's potential to serve as a quick and easily obtained method to monitor disease.

## Learning points

- Melanomas of unknown primary (MUPs) are a rare type of melanoma but are something to consider in a patient with poorly differentiated carcinoma if no primary site is able to be found.
- Next-generation sequencing (NGS) via liquid biopsy can serve as a timely method to obtain genetic information on a patient's malignancy which can be used to guide treatment.
- Circulating tumour DNA (ctDNA) has the potential to serve as a fast and easily obtainable method to monitor a patient's response to treatment.

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**Contributors** PNP was the major contributor in writing the report, sourcing the references and making the figure used in the report. CA assisted in structuring and drafting the report. AT was the main clinician caring for the patient in the time period described and oversaw the project and communication with the patient. AT also served as the guarantor. MJ reviewed the report and is the clinician currently caring for the patient.

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