

Implementation of cell-free tumor DNA sequencing from the cerebrospinal fluid to guide treatment in a patient with primary leptomeningeal melanoma: A case report

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Abstract. Primary leptomeningeal melanoma (PLM) is a rare type of cancer that represents a major clinical and molecular diagnostic challenge. A definitive diagnosis requires consistent magnetic resonance imaging findings and cerebrospinal fluid (CSF) cytology. Due to the small number of malignant cells in the CSF, routine testing for mutations in the *BRAF* gene is difficult, which prevents the stratification of these patients to potentially beneficial therapies. We herein present the case of a 62-year old man with CSF cytology indicating PLM, where *BRAF* mutation testing, from cell-free (cf) tumor DNA isolated from the CSF and plasma was implemented to guide clinical decision making. Testing for *BRAFV600E* mutation from the CSF and plasma was technically feasible, yielded concordant results, and guided the treatment for this patient. This case suggests that mutation testing of cfDNA isolated from the CSF is technically feasible and may guide therapy in cases where a tissue diagnosis is not possible for PLM and other malignancies with defined oncogenic driver mutations.

Introduction

Melanoma is the deadliest form of skin cancer and continues to have an increasing incidence in the last decades (1). About ~50% of melanomas harbor a T to A substitution in codon 600 of the *BRAF* gene, resulting in a substitution of Valine to Glutamic Acid (*BRAFV600E*), causing tonic activation of the RAF/MEK/ERK pathway, proliferation, and cell survival (2,3). While a combination of selective RAF/MEK inhibitors results in brisk responses in most patients with *BRAFV600E*, treatment with these drugs in *BRAF* wild-type (WT) tumors leads to paradoxical activation of the pathway with the potential to accelerate tumor growth (4,5). Determining the *BRAF* mutation status is therefore critical prior to initiation of RAF/MEK-inhibitors. In contrast, immune checkpoint inhibitors, which are monoclonal antibodies that target CTLA-4 (i.e., ipilimumab) or the PD-1/PD-L1 axis (e.g., nivolumab or pembrolizumab) exhibit activity irrespective of the *BRAF* mutation status, and are therefore the preferred first-line therapy for patients with *BRAF* WT melanoma (6-8). Testing for the *BRAF* mutation is usually performed on a tissue biopsy, such as a core needle biopsy. However, there are instances, in which potentially significant morbidity prohibits procedures for obtaining tissue. Primary leptomeningeal melanoma (PLM) is a rare and very aggressive type of melanoma with an estimated frequency of 1 in 10 million individuals (9-12). Due to its localization involving the leptomeninges, biopsies cannot be easily performed, which may limit potential therapeutic benefits for these patients. Here, we describe a case of a patient with PLM who underwent *BRAF* mutation testing from cell-free DNA (cfDNA) isolated from cerebrospinal fluid (CSF) to guide therapy choices.

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Case report

A 62-year-old Caucasian man with a past medical history of essential hypertension and obstructive sleep apnea was

Table I. CSF cell count and chemistry from two LPs performed on hospital days 1 and 4.

LP no.	WBC (/μl)	RBC (/μl)	PMN (%)	LYM (%)	MONO (%)	MACRO (%)	OTHER (%)	Glucose (mg/dl)	Protein (mg/dl)
1 (HD1)	28	16,750	42	48	7	1	4	60	516
2 (HD4)	5	7,950	7	57	6	23	6	53	459
Reference range	<5	0	0	0	0	0	0	45-80	15-45

CSF, cerebrospinal fluid; LP, lumbar puncture; HD, hospital day; WBC, white blood cells; RBC, red blood cells; PMN, polymorphonuclear leukocytes; LYM, lymphocytes; MONO, monocytes; MACRO, macrophages.

admitted to Beth Israel Deaconess Medical Center with altered mental status, headaches and gait difficulties in March of 2016. Three months prior to presentation, the patient had noticed lower back pain with radiation to the buttocks. Magnetic resonance imaging (MRI) without contrast of the lumbar spine at that time was unrevealing. Over the following 6 weeks, the patient developed worsening gait difficulties and intermittent confusion and, 3 days prior to presentation, he developed headaches and was persistently confused.

On arrival to our emergency room, the patient was somnolent and only oriented to name. The vital signs were notable for a temperature of 102°F, heart rate 74 beats/min, blood pressure 162/98 mmHg, respiratory rate 20 breaths/min, and oxygen saturation 97% at ambient air. Physical examination revealed somnolence with responses only to noxious stimuli, nuchal rigidity with a positive Brudzinski sign, and bilateral papilledema. An immediate non-contrast head computed tomography (CT) scan showed extensive communicating hydrocephalus and transependymal flow. The patient underwent a large-volume lumbar puncture where the opening pressure was 34 cm H₂O, with subsequent mental status improvement. A complete cell count and chemistry of the CSF is summarized in Table I. The patient was admitted to the neurological intensive care unit for further care.

An MRI scan of the head and spine with and without contrast revealed diffuse leptomeningeal enhancement involving intracranial and spinal meninges on post-contrast T1-weighted imaging, as well as communicating hydrocephalus. There was no parenchymal central nervous system (CNS) involvement (Fig. 1A-C). A CT scan of the chest, abdomen and pelvis did not reveal evidence of visceral metastatic disease. A full skin examination by a dermatologist reported no evidence of a primary cutaneous melanoma, and the ophthalmic examination did not reveal any suspicious lesions. Cytology of the CSF revealed malignant cells with strong staining for human melanoma black-45 (HMB-45), confirming a diagnosis of malignant melanoma (Fig. 1D and E).

Overall, this presentation was consistent with a primary leptomeningeal melanoma (PLM). The patient was treated with dexamethasone and required emergent placement of an external ventricular drain (EVD) due to interval worsening mental status in the setting of hydrocephalus. CSF was collected for isolation of cfDNA and sequencing of the *BRAF* gene. Plasma was collected at the same time for cfDNA sequencing. *BRAF* mutation testing of CSF and plasma was performed using the Biocept Target Selector™ assay.

The patient received five fractions of whole-brain radiation therapy (2,000 cGy) and palliative radiation of the spine from level T12 to S3. He had significant improvement of his neurological symptoms, allowing for removal of the EVD on day 12 of his hospitalization, and was discharged from the hospital on day 20.

Although previous efforts have used targeted next-generation sequencing to evaluate small panels of genes involved in melanoma biology, including *BRAF* and *NRAS* (13), only mutant *BRAF* is a currently actionable target and may help guide the choice of targeted therapy vs. immunotherapy. Sequencing of cfDNA revealed wild-type (WT) *BRAF* gene in both the CSF and plasma. Based on this finding, treatment with either ipilimumab, a PD-1 checkpoint inhibitor, or temozolomide was discussed. Given the patient's good clinical status, treatment with ipilimumab was initiated, with a plan to administer four cycles, potentially followed by PD-1 checkpoint blockade. Although the patient received his first infusion without treatment-related complications, the course was complicated by the development of a pulmonary embolism, which delayed a planned second infusion. Five weeks after the first ipilimumab infusion (~9 weeks after the initial diagnosis), the patient developed rapidly progressing confusion and gait instability with worsening hydrocephalus and succumbed to the disease 3 days later.

Discussion

Primary leptomeningeal melanoma (PLM) is a very rare type of cancer that is considered to arise from melanocytes in the pia and arachnoid (10). Diagnostic criteria for PLM have been suggested (9), including hyperintensity of the meninges on T1-weighted MRI and cytology with positive immunostaining for lineage-specific HMB-45 and S-100 (14-16).

In ~25% of patients, PLM is associated with giant melanocytic nevi, which frequently carry treatment-sensitizing oncogenic driver mutations (17,18) including *BRAFV600E* and *NRASQ61*. Among patients with metastatic cutaneous melanoma, ~50% harbor sensitizing *BRAF* mutations, most commonly *BRAFV600E*. Treatment with RAF or RAF/MEK-inhibitors in this subset of patients has resulted in unprecedented response rates and improvement of progression-free and overall survival (4,19). However, patients with wild-type *BRAF* melanoma are not candidates for RAF/MEK inhibition, as BRAF inhibitors may promote growth of *BRAF*-WT cells and further exacerbate

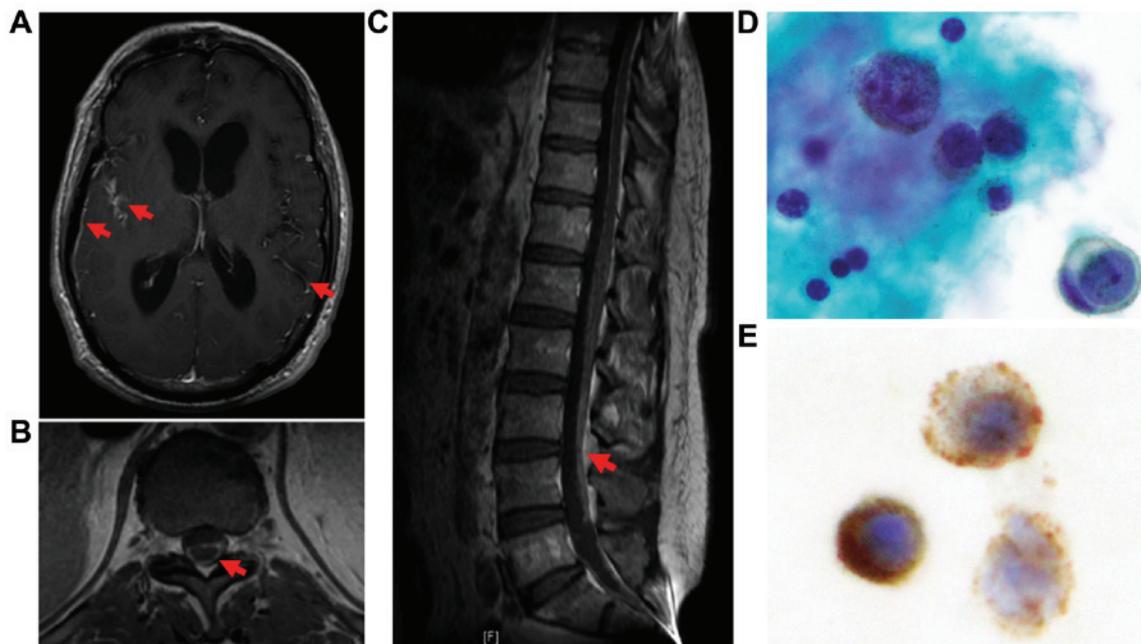


Figure 1. Imaging and CSF pathology revealed PLM with CSF involvement. (A) MRI of the brain with and without contrast: Post-contrast axial T1 sequence. Diffuse leptomeningeal enhancement (arrows) with enlarged third ventricle. (B) MRI of the thoracic spine post-contrast axial T1; arrow indicates leptomeningeal enhancement. (C) MRI of the thoracolumbar spine post-contrast sagittal T1; arrow indicates leptomeningeal enhancement. Cytological analysis of the CSF including (D) ThinPrep and (E) HMB-45 staining. Examination of the CSF revealed dyshesive large atypical cells with high nuclear-to-cytoplasmic ratio, macronucleoli, and fine cytoplasmic pigment. Cannibalistic cells and occasional mitotic figures were present. Immunohistochemical stains performed on cytospin preparations were strongly positive for HMB-45 and negative for cytokeratin, confirming involvement of the CSF by malignant melanoma. CSF, cerebrospinal fluid; PLM, primary leptomeningeal melanoma; MRI, magnetic resonance imaging; HMB-45, human melanoma black-45.

the disease (20), highlighting the importance of targeted *BRAF* testing in this patient. In patients with *BRAF*-WT melanoma, first-line immunotherapies are currently under investigation as an alternative strategy. To this end, the Food and Drug Administration has approved immunotherapies, such as the CTLA-4 inhibitor ipilimumab and PD-1 checkpoint inhibitors, including nivolumab and pembrolizumab, as first-line therapies for patients with metastatic melanoma. Single-agent treatment with any of these compounds or combinations of ipilimumab and PD-1 inhibitors produce deep and long-lasting responses in a subset of patients (6-8,21), including those with leptomeningeal disease (22).

The ideal choice of first-line therapy, RAF/MEK-inhibitors or immunotherapies, in patients with *BRAF*-mutant melanoma remains unclear and is mostly guided by the clinical setting. For example, in patients with rapidly progressing *BRAF*-mutant melanoma, treatment with BRAF/MEK inhibitors may induce faster responses and is the preferred treatment modality. In patients without sensitizing *BRAF* mutations (*BRAF*-WT), as in the present case, immunotherapy is the first-line treatment.

Understanding the molecular profile of these tumors is crucial for employing treatments such as targeted therapies or immune checkpoint inhibitors, which may induce dramatic responses in leptomeningeal melanoma (22,23). However, testing from malignant CSF with as few as 1 malignant cell per μl , as in the case presented here, is challenging with current approaches. We herein report the successful use of targeted *BRAF* sequencing of cfDNA isolated from the CSF as well as the plasma in a patient with PLM. The results from *BRAF* mutation testing were instrumental in selecting the

treatment for this patient, given the potential harm in treating a *BRAF*-WT patient with RAF/MEK inhibitors. Recent reports indicating the feasibility of molecular profiling from CSF (24-27) have mostly focused on primary CNS tumors. With regard to cfDNA sequencing from CSF for melanoma, previous reports have focused only on monitoring treatment response in metastatic lesions for which the genomic status of the primary melanoma lesion was known (28,29). In contrast, our case displays the utility of using cfDNA to guide treatment choice in a primary leptomeningeal melanoma for which no genetic information was available. This study indicates that rapid targeted sequencing of cfDNA from the CSF is clinically feasible and should be considered for guiding treatment in patients in whom a tissue biopsy cannot be obtained, including those with PLM and leptomeningeal metastatic disease.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' contributions

JCM, KH, TJB, SS, JM and BI took clinical care of the patient. JT provided pathology slides. JCM, RT and BI wrote the paper. All authors read, reviewed and approved the manuscript.

Ethics approval and consent to participate

Not applicable.

Consent for publication

The family agreed to publication of the case and material presented here.

Competing interests

VS is an employee of BioCept Inc. The other authors declare that they have no competing interests.

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