

Case Report

# CSF-ctDNA SMSEQ Analysis to Tailor the Treatment of a Patient with Brain Metastases: A Case Report

Wen-Tsung Huang<sup>a</sup> Na-Mi Lu<sup>b</sup> Wen-Yuan Hsu<sup>c</sup> Shih-En Chang<sup>d</sup>  
Alex Atkins<sup>e</sup> Rui Mei<sup>e</sup> Manana Javey<sup>e</sup>

<sup>a</sup>Division of Hematology-Oncology, Department of Internal Medicine, Chi Mei Medical Center, Liouying, Tainan City, Taiwan; <sup>b</sup>Department of Pathology, Chi Mei Medical Center, Liouying, Tainan City, Taiwan; <sup>c</sup>Department of Medical Imaging, Chi Mei Medical Center, Liouying, Tainan City, Taiwan; <sup>d</sup>CellMax Taiwan Co. Ltd, Taipei City, Taiwan; <sup>e</sup>CellMax Inc., Sunnyvale, CA, USA

## Keywords

CellMax SMSEQ liquid biopsy · CSF ctDNA · Circulating tumor DNA · Cancer of unknown primary · Brain metastases · CNS · CSF · Liquid biopsy

## Abstract

Brain metastases are the most common neurological complications of adult cancers, accounting for more than half of brain tumors. The incidence of brain metastases may be increasing due to improved detection of small lesions by advanced imaging technologies. Given the fast evolution of targeted and immunotherapy regimens, it is essential to serially assess brain malignancies during the disease course for disease monitoring and tailoring of the therapeutic management. For such serial and repetitive assessment, cerebrospinal fluid (CSF) could be the biological fluid of choice to supplement cytology examination for the presence or absence of CNS malignancy, as well as provide extensive information on tumor

mutational profile for personalization of treatment. The case described here emphasizes the importance of CSF-ctDNA analysis with the CellMax SMSEQ technology that led to treatment adjustment resulting in clinical remission of the patient.

© 2018 The Author(s)  
Published by S. Karger AG, Basel

## Introduction

Brain metastases are the most common neurological complications of systemic cancer. Among of all the cancer types, lung cancer, breast cancer, and melanoma are the most frequent to metastasize to brain [1]. Brain metastases are most frequently found within the brain parenchyma, cranium, dura, and leptomeninges. In the majority of cases, patients who are diagnosed with brain metastases have an established diagnosis of a primary malignancy; however, up to 15% of all patients have no clearly identified primary site despite intensive clinical, laboratory, and imaging evaluation [2–5]. For an established diagnosis of primary malignancy, the brain metastatic lesions are not always biopsied due to the invasiveness of the procedures. Yet, for the cancer of unknown primary (CUP) cases, the brain lesion biopsies are warranted to establish the diagnosis. Nevertheless, in both scenarios, given the spatial and temporal intratumoral heterogeneity, repeated or serial biopsies are required for an adequate characterization of the somatic genetic alterations for better management during the disease course [6–8]. The tissue biopsy approach for brain lesion sampling has major pitfalls given the limited access to the tumor and the invasiveness of the necessary procedures, along with sampling bias due to tumor heterogeneity. Recently, cell-free circulating tumor DNA (ctDNA) in the peripheral blood has been used to characterize and monitor various types of cancer [8–11]. However, plasma-derived ctDNA analysis of patients with only central nervous system (CNS) lesions revealed either absence or very low levels of tumor DNA in the peripheral circulation [12].

The cerebrospinal fluid (CSF) is in intimate contact with tumor cells of the CNS derived either from primary or metastatic lesions. It has been shown that ctDNA is present in CSF of brain tumor patients [13]. Given the routine, frequent CSF cytology examinations for patients with brain lesions, it could be clinically beneficial to analyze CSF-ctDNA for presence or absence of CNS malignancy and further personalization of the treatment plans. Here we present a case of CUP in a female patient who benefited from having CSF-ctDNA analysis (SMSEQ technology) performed for somatic alterations and treatment adjustment.

## Case Report

A 35-year-old woman with no past history of cancer presented to an outside hospital with severe headache and lower back pain in June 2016. Brain MRI showed hydrocephalus due to leptomeningeal metastases and a focal mass in the spinal cord adjacent to cerebellum. A large tumor was detected at the anterior thecal sac (T5/6 level) along with several enhanced plaques at the posterior (C2–5 level) and terminal sacs (S2 level).

The large spinal tumor causing the cord compression was excised, histopathological examination of which confirmed adenocarcinoma. The tumor was positive for CK7, CDX2,

and PAX-8 and negative for TTF-1, CK20, ER /PR, and ALK rearrangements. In addition, immunohistochemical analysis of HER2 was equivocal (2+) which ultimately reflexed to FISH, demonstrating HER2 positivity on dual probe (HER2/CEP17 ratio 5.08). Furthermore, the tumor was wild type for EGFR and BRAF mutations, and microsatellite stable. Physical examination and series of imaging studies including thoracic and abdominal computed and positron emission tomography (PET/CT), esophagogastroduodenoscopy, and colonoscopy failed to identify the primary site of tumor origin, and a diagnosis of metastatic adenocarcinoma of unknown primary was made. To alleviate the symptoms, a ventriculo-peritoneal shunt was placed, and an Ommaya reservoir was implanted to deliver intrathecal chemotherapy with methotrexate, which ultimately yielded poor response after six cycles. In August 2016, the patient was referred to our hospital, where she was extensively evaluated and given several targeted and immunotherapeutic regimens including gefitinib, trastuzumab, lapatinib, ipilimumab, pembrolizumab, and chemotherapy drugs such as 5-fluorouracil, epirubicin, cyclophosphamide, docetaxel, carboplatin, and capecitabine. Despite myriads of regimens tried, only anti-HER2 therapy resulted in clinical benefit and alleviated lower extremity muscle spasticity and maintained adequate consciousness level. In September 2017, the spasticity in all four extremities worsened, and the patient started to manifest dysarthria and dysphagia. MRI demonstrated multiple enhanced lesions (Fig. 1), and CSF cytology examination confirmed the presence of malignant cells (Fig. 2). A portion (14 mL) of CSF was then spared for ctDNA analysis by CellMax SMSEQ liquid biopsy assay. SMSEQ next generation sequencing (NGS) revealed amplifications of HER2 and MPL, as well as mutations in the PIK3CA, CDKN2A and TP53 genes (Fig. 3; Table 1). Considering the CSF-ctDNA NGS results and recommendations, ado-trastuzumab emtansine was added to the combination regimen of intrathecal trastuzumab and oral lapatinib. Given the CDKN2A mutation, palbociclib was also tried for three weeks, but discontinued due to myelosuppressive side effects. Within two weeks from implementation of a new anti-HER2 regimen combination, the neurological signs of the patient dramatically improved and tumor markers such as CEA and CA19-9 decreased significantly. The following CSF cytology examinations confirmed an absence of malignant cells in three subsequent spinal taps. Currently the patient appears well.

## Discussion

There is an obvious need for sensitive and specific markers to monitor tumor dynamics of both primary as well as metastatic CNS lesions. We decided to take advantage of the CellMax cutting-edge SMSEQ liquid biopsy technology and analyze CSF-derived ctDNA to tailor the cancer management of our patient with CUP. The decision to analyze CSF beyond cytology examination was due to a limited accessibility to the brain lesions and invasiveness of the biopsy procedure. At the same time, the CSF was available as part of standard of care for cytology examination. This patient's spinal tumor was HER2 positive at the time of diagnosis; however, she had undergone anti-HER2 treatment and since it is not unusual for a tumor to change its mutational profile due to treatment, it was necessary to re-assess the HER2 status in addition to confirmation of positive cytology of CSF. In this patient with CUP, CSF-ctDNA analysis helped to characterize the most current tumor mutational profile, corroborate cytology results, and refine the treatment protocol resulting in a clinical remission.

CSF is the biological fluid of choice that is amenable for serial monitoring of CNS tumors, and could be obtained via lumbar puncture. Although the latter is not a noninvasive procedure, it still qualifies as minimally invasive procedure and is currently routinely performed to follow up brain tumor patients with primary or metastatic lesions [14–16]. Cytology examination of CSF provides limited information and is characterized by low sensitivity and specificity [17]. Therefore, further analysis of CSF-ctDNA may be beneficial for all patients with primary or metastatic brain tumors.

CSF-ctDNA analysis may potentially replace cytology examination of CSF for presence or absence of malignancy within CNS; however, it does warrant future comparison studies on a larger scale. For now, we envision CSF-ctDNA to be used in combination with cytology examination and imaging along with clinical parameters. In addition to presence or absence of CNS malignancy, CSF-ctDNA analysis may help provide clinically actionable information that may further personalize the treatment protocols for each individual patient and tailor their disease management plan.

### Statement of Ethics

The authors have no ethical conflicts to disclose.

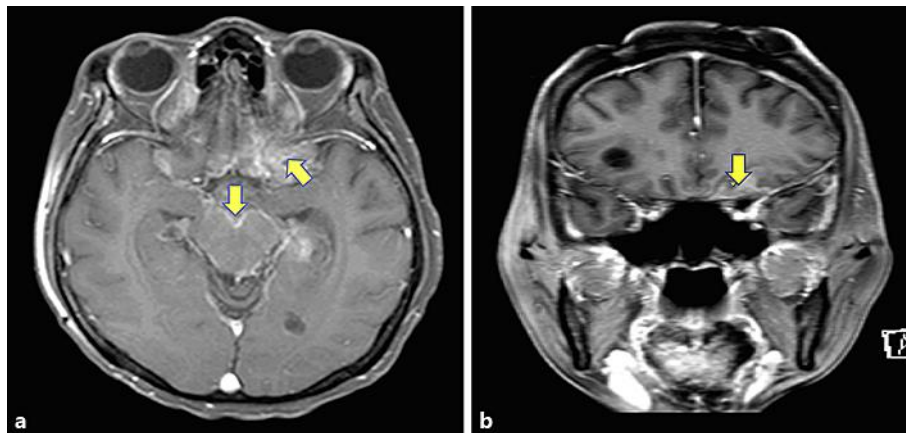
### Disclosure Statement

Wen-Tsung Huang, Na-Mi Lu, and Wen-Yuan Hsu have nothing to disclose and did not receive any gifts from CellMaxLife laboratories. Shih-En Chang, Alex Atkins, Rui Mei, and Manana Javey are employees of CellMaxlife laboratories.

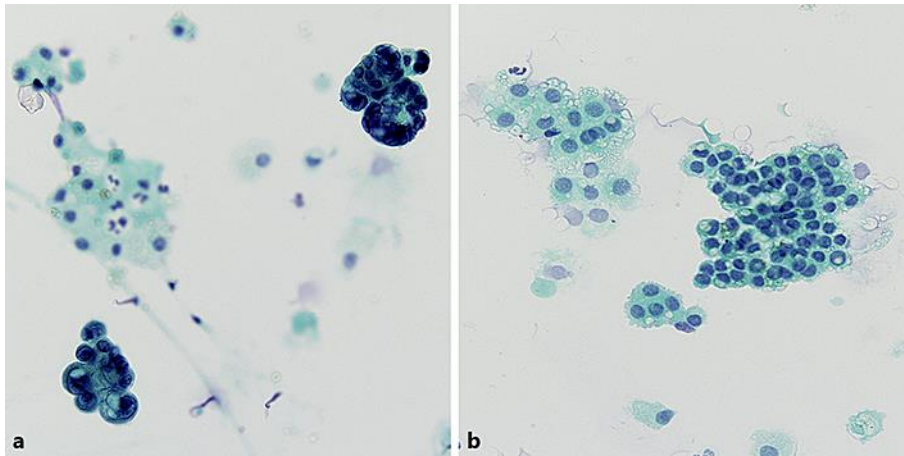
### References

- 1 Gavrilovic IT, Posner JB. Brain metastases: epidemiology and pathophysiology. *J Neurooncol*. 2005 Oct;75(1):5–14.
- 2 Debevec M. Management of patients with brain metastases of unknown origin. *Neoplasma*. 1990;37(5):601–6.
- 3 Nguyen LN, Maor MH, Oswald MJ. Brain metastases as the only manifestation of an undetected primary tumor. *Cancer*. 1998 Nov;83(10):2181–4.
- 4 D’Ambrosio AL, Agazzi S. Prognosis in patients presenting with brain metastasis from an undiagnosed primary tumor. *Neurosurg Focus*. 2007 Mar;22(3):E7.
- 5 Maesawa S, Kondziolka D, Thompson TP, Flickinger JC, Dade L. Brain metastases in patients with no known primary tumor. *Cancer*. 2000 Sep;89(5):1095–101.
- 6 Gerlinger M, Rowan AJ, Horswell S, Math M, Larkin J, Endesfelder D et al. Intratumor heterogeneity and branched evolution revealed by multiregion sequencing. *N Engl J Med*. 2012 Mar;366(10):883–92.
- 7 Sottoriva A, Spiteri I, Piccirillo SG, Touloumis A, Collins VP, Marioni JC et al. Intratumor heterogeneity in human glioblastoma reflects cancer evolutionary dynamics. *Proc Natl Acad Sci USA*. 2013 Mar;110(10):4009–14.
- 8 Tzeng YT, Chang SE, Mei R, Javey M. Liquid Biopsy Prevents Inaccurate Her2 Status Determination by in situ Hybridization in a Patient with Invasive Ductal Adenocarcinoma of the Breast: Case Report. *Case Rep Oncol*. 2017 Sep;10(3):857–62.

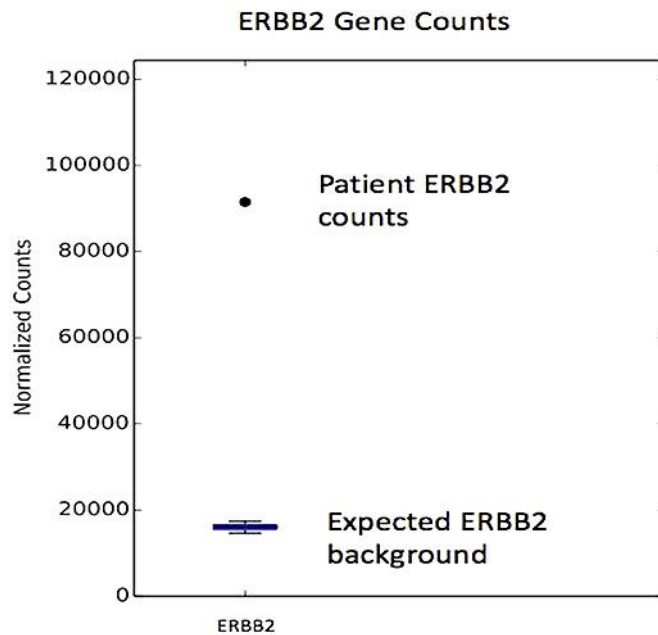
- 9 Murtaza M, Dawson SJ, Tsui DW, Gale D, Forshew T, Piskorz AM et al. Non-invasive analysis of acquired resistance to cancer therapy by sequencing of plasma DNA. *Nature*. 2013 May;497(7447):108–12.
- 10 Dawson SJ, Tsui DW, Murtaza M, Biggs H, Rueda OM, Chin SF et al. Analysis of circulating tumor DNA to monitor metastatic breast cancer. *N Engl J Med*. 2013 Mar;368(13):1199–209.
- 11 De Mattos-Arruda L, Weigelt B, Cortes J, Won HH, Ng CK, Nuciforo P et al. Capturing intra-tumor genetic heterogeneity by de novo mutation profiling of circulating cell-free tumor DNA: a proof-of-principle. *Ann Oncol*. 2014 Sep;25(9):1729–35.
- 12 Bettgowda C, Sausen M, Leary RJ, Kinde I, Wang Y, Agrawal N et al. Detection of circulating tumor DNA in early- and late-stage human malignancies. *Sci Transl Med*. 2014 Feb;6(224):224ra24.
- 13 Pan W, Gu W, Nagpal S, Gephart MH, Quake SR. Brain tumor mutations detected in cerebral spinal fluid. *Clin Chem*. 2015 Mar;61(3):514–22.
- 14 von Hoff K, Rutkowski S. Medulloblastoma. *Curr Treat Options Neurol*. 2012 Aug;14(4):416–26.
- 15 Bartlett F, Kortmann R, Saran F. Medulloblastoma. *Clin Oncol (R Coll Radiol)*. 2013 Jan;25(1):36–45.
- 16 Sasaki S, Yoshioka Y, Ko R, Katsura Y, Namba Y, Shukuya T et al. Diagnostic significance of cerebrospinal fluid EGFR mutation analysis for leptomeningeal metastasis in non-small-cell lung cancer patients harboring an active EGFR mutation following gefitinib therapy failure. *Respir Investig*. 2016 Jan;54(1):14–9.
- 17 Preusser M, Hainfellner JA. CSF and laboratory analysis (tumor markers). *Handb Clin Neurol*. 2012;104:143–8.



**Fig. 1.** Magnetic resonance imaging of the brain after gadolinium injection. **a** FS axial view: enhancement over the midbrain surface and bilateral Sylvian fissure. **b** FS coronal view: nodular enhanced lesion at the left frontal base.



**Fig. 2.** Cytology examination of CSF. **a** Hyperchromatic cell clusters with papillary characteristics. **b** Flat sheet of hyperchromatic cells with noticeable cytoplasmic and nuclear inclusions.



**Fig. 3.** ctDNA SMSEQ analysis of CSF. Blue box, expected ERBB2 (HER2) gene counts; black dot, ERBB2 gene counts (HER2 amplification) in the patient's CSF.

**Table 1.** ctDNA SMSEQ analysis of CSF: somatic variants identified in the patient's CSF

Variant	Map location	Variant allele frequency, %	Coding sequence change
ERBB2 amplification	chr17:37842393-37886915		amplification
PIK3CA-M1043I	chr3:178952074	0.16	c.3129G>C
PIK3CA-E545K	chr3:178936091	0.4	c.1633G>A
MPL amplification	chr1:43802659-43820549		amplification
CDKN2A-R58	chr9:21971186	33.16	c.172C>T
TP53-M246V	Chr17:7577545	22.13	c.736A>G