Personalized therapy guided by longitudinal liquid biopsies for treatment of leptomeningeal disease from lung adenocarcinoma: A case report

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Received December 12, 2023; Accepted March 20, 2024

DOI: 10.3892/ol.2024.14432

Abstract. Molecular-based targeted therapies have significantly benefited certain patients with cancer; however, those with leptomeningeal disease (LMD) persistently exhibit a poor prognosis and are often excluded from clinical trials. Tumor-derived cell-free (cf)DNA, found in the cerebrospinal fluid (CSF) of patients with LMD, can assist in diagnosis and tracking of disease progression. However, the utilization of CSF to direct targeted cancer therapy has yet to be extensively explored. The present study reported the case of a patient with lung adenocarcinoma and LMD who was monitored by performing a series of liquid biopsies of CSF and blood. Targeted sequencing was performed on cfDNA from the CSF and plasma, and the variant allele frequencies (VAFs) of BRAF and NRAS mutations were assessed and analyzed in conjunction with the clinical presentation of the patient. The patient then underwent serial chemotherapy, radiation therapy, immunotherapy and targeted treatment based on the results of the liquid biopsies. Upon the LMD diagnosis, a BRAF p.V600E mutation was detected in plasma cfDNA. Consequently, the patient was treated with vemurafenib and responded favorably to this consolidation treatment for 13 months. After a relapse in July 2018, both BRAF p.V600E and NRAS p.Q61K mutations were detected in CSF supernatant and sediment cell samples, suggesting drug resistance. Therefore, the treatment strategy for the patient changed to cobimetnib plus vemurafenib. Notably, the changes of VAF in the CSF supernatant samples were associated with the clinical status of the patient. The patient survived for 33 months post-LMD diagnosis. The present case report highlights the potential use of liquid biopsy in personalized therapy, as it was instrumental in informing the combinational treatment plan of the patient, which ultimately proved beneficial.

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Key words: leptomeningeal disease, cerebrospinal fluid, circulating tumor DNA, BRAF V600E, NRAS Q61K, drug resistance

Introduction

Leptomeningeal disease (LMD) is a serious complication of non-small cell lung cancer (NSCLC). A total of 3-5% of patients with NSCLC will eventually develop LMD, which has a poor prognosis and life expectancy of 3-6 months after the diagnosis of LMD (1). Clinical manifestations, such as headaches, nausea, vomiting, altered mental status, or focal neurological deficits, magnetic resonance imaging (MRI) and cerebrospinal fluid (CSF) biopsy are key factors in diagnosing NSCLC with LMD. CSF cytology alone is insufficient and should be combined with liquid biopsy (2).

Although extended survival has been achieved with advances in lung cancer treatment, the longer course of disease has also resulted in an increase in the incidence of LMD, especially for patients with actionable mutations, such as EGFR T790M and ALK rearrangements. Furthermore, there is still no standard treatment for LMD (3). The efficacy of personalized therapies like tyrosine kinase inhibitors (TKIs) in patients with LMD remains unknown as these patients are often excluded from clinical trials (4).

BRAF mutations occur in 1.5-3.5% of patients with NSCLC, and >50% of these mutations were V600E (5). A case reported that vemurafenib demonstrated efficacy in the improvement of neurologic symptoms and disease control for a patient with BRAF V600E and lung adenocarcinoma with LMD (6). However, resistance is inevitable in most patients with BRAF/MEK inhibition (7). The application of radiotherapy, intrathecal therapy, targeted therapy and immunotherapy provides more options for patients with LMD. Each

treatment method has a certain efficacy and can be used alone or in combination for individual patients. However, there is currently no clear consensus on the optimal management of LMD (8).

Previous studies have demonstrated that CSF collected from patients with LMD contains tumor cell-free (cf)DNA, which can be used to detect LMD and monitor disease progression (9-11). The present study reports the results of a case where longitudinal liquid biopsies of blood and CSF were used to inform the selection of a series of targeted drugs to treat a patient with LMD from lung adenocarcinoma.

Case report

In November 2014, a 42-year-old female patient was admitted to Sun Yat-sen University Cancer Center (Guangzhou, China). The patient was diagnosed with poorly differentiated lung adenocarcinoma with lymph node and bone metastases. The full clinical course of the patient is presented in Fig. 1 and representative MRI images are shown in Fig. 2.

Clinical sample processing and bioinformatic analysis pipelines are described in our previous study (12). Clinical samples were obtained through an approved informed consent process by Guangdong Sanjiu Brain Hospital Institutional Review Board (Guangdong, China), and only excess CSF that was not required for pathological diagnosis was utilized in the present study. Briefly, blood and CSF were collected in BCT® tubes (Streck LLC). cfDNA from plasma and CSF was isolated using the QIAamp® Circulating Nucleic Acid Kit (cat. no. 55114; Qiagen GmbH). following the manufacturer's instructions. Extracted DNA was then quantified by Qubit 2.0 (Thermo Fisher Scientific, USA) in accordance with manufacturer's protocols. Sequencing libraries were prepared using the KAPA Hyper Prep Kit (KK8504l; Kapa Biosystems). Targeted enrichment was performed with a 464-gene panel (HaploX Biotechnology). A full gene list is presented in Table SI. Library concentration was assessed by the Qubit dsDNA HS Assay kit. Fragment length was determined on the 4200 Bioanalyzer using the DNA 1000 Kit (Agilent). Then the libraries were sequenced using 150 bp paired-end runs on the Illumina NovaSeq 6000 system (Illumina, Inc.). Raw sequencing reads were filtered by fastp v0.18.0 (13) and aligned to the hg19 genome (GRch37) using the Burrows-Wheeler Aligner v0.7.15-r1140 (14). Duplicated reads were removed using Gencore v0.12.0 (15). SAMtools v0.1.19 was used to generate pileup files of properly paired reads with a mapping quality ≥60 (16). Somatic variants were identified using VarScan2 v2.3.8 (17) with the following parameters: Minimum read depth at a position=20; variant allele frequency (VAF) threshold ≥0.001; strand-filter ≥1; otherwise by default. VarScan 2 requires minimum phred base quality of 20 and a P-value of <0.05. A full mutation list is presented in Table SII.

Genetic testing of the patient's lung biopsy sample identified no mutations in EGFR, anaplastic lymphoma kinase, ROS proto-oncogene 1, receptor tyrosine kinase and c-MET. The patient received two cycles of chemotherapy with on day 1 of each 21-day cycle comprising pemetrexed (500 mg/m^2) and cisplatin (75 mg/m^2) through intravenous infusion and four cycles of chemotherapy with on day 1 of each 21-day cycle comprising pemetrexed (500 mg/m^2) d1, cisplatin (75 mg/m^2) d1 and bevacizumab (15 mg/kg) through intravenous infusion. Serial computed tomography (CT) scans showed stable disease status (Fig. S1).

In May 2017, the patient presented with nausea, vomiting and dizziness and was admitted to our hospital and underwent a lumbar puncture. For the purpose of cytological examination, cells were collected from the patient's cerebrospinal fluid samples. These cells were obtained by centrifuging 0.5 ml of the sample at 140 x g for 5 min at 4˚C. The post-centrifugation samples were collected onto slides. After collection, the samples were air-dried and then fixed in pure ethanol for 5 min at room temperature. Subsequently, these specimens were stained using the May-Grünwald-Giemsa (MGG) method. Briefly, the slide was fully covered with May-Grünwald-phosphate mixture and incubate for 10 min at room temperature. Then, the May Grunwald-phosphate mixture was decanted from the slide and the slide fully covered with Giemsa-phosphate mixture and incubated for 15 min at room temperature. Next, the mixture was decanted and the slide rinsed with slow running tap water. Air dry the slide. The morphological details of the MGG-stained specimens were examined under an optical microscope at 40x and 100x magnification. CSF cytological assessment demonstrated malignant cells (Fig. S2) and LMD was confirmed by MRI (Fig. 2A, I, B). A blood sample collected at this time underwent circulating tumor DNA sequencing and a BRAF p.V600E mutation was identified. The patient was subsequently treated with vemurafenib orally 960 mg twice per day continuously until disease progression, but the symptoms persisted and an external ventricular drain (EVD) was placed 5 days later. The patient then underwent whole brain radiation therapy (WBRT) with a radiation dose of 30 Gy in 10 fractions in 2 weeks, and the EVD was removed after 2 weeks of its placement.

MRI of the spine in February 2018 (Fig. 2J) identified a shrunk lesion in the spinal cord. Surveillance imaging by conventional MRI or arterial spin labeling MRI perfusion (Fig. 2C, F and K) demonstrated visible brain lesions, which was the radiation induced brain injury. Stable disease remained until the patient's symptoms relapsed in July 2018 (Fig. 2D). A head CT was performed which demonstrated new hydrocephalus (Fig. S3). From June 2018, CSF was regularly sampled and sent for clinical and genetic testing. Fig. 3 presents the molecular change over time. CfDNA in CSF was sequenced with the targeted 464-gene panel (HaploX Biotechnology) for 19 months, and BRAF p.V600E and NRAS p.Q61K mutations were found. NRAS p.Q61K has been reported to be resistant to single-agent BRAF inhibitors (5). Subsequently, the patient received cobimetinib orally at a dose of 60 mg once daily for the first 21 days of each 28-day cycle plus vemurafenib orally at a dose of 960 mg twice daily. The patient presented with transient remission of disease symptoms.

In August 2018, a new lesion was found using MRI (Fig. 2G). The patient underwent ventriculoperitoneal shunt (VPS) placement with an adjustable valve (Sophysa Polaris®; Sophysa SA; Tokibo Co., Ltd.). In November 2018, MRI (Fig. 2L) showed evidence of disease progression. In response, treatment was switched from TKIs to intravenous bevacizumab (300 mg) plus pembrolizumab (100 mg) and pemetrexed (700 mg) every 3 weeks for three cycles. Subsequently, though intracranial SD indicated on the CT scan (Fig. 2E), the patient

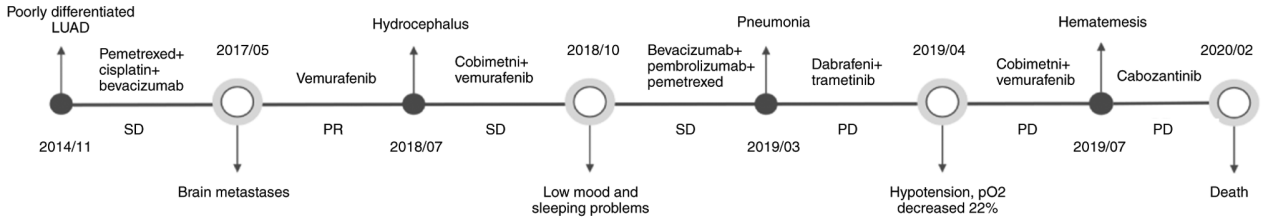

Figure 1. Timeline of the clinical course of the patient, including clinical events, medication and evaluation of treatment efficacy. LUAD, lung adenocarcinoma; SD, stable disease; PR, partial response; PD, progressive disease; pO2, partial pressure of oxygen.

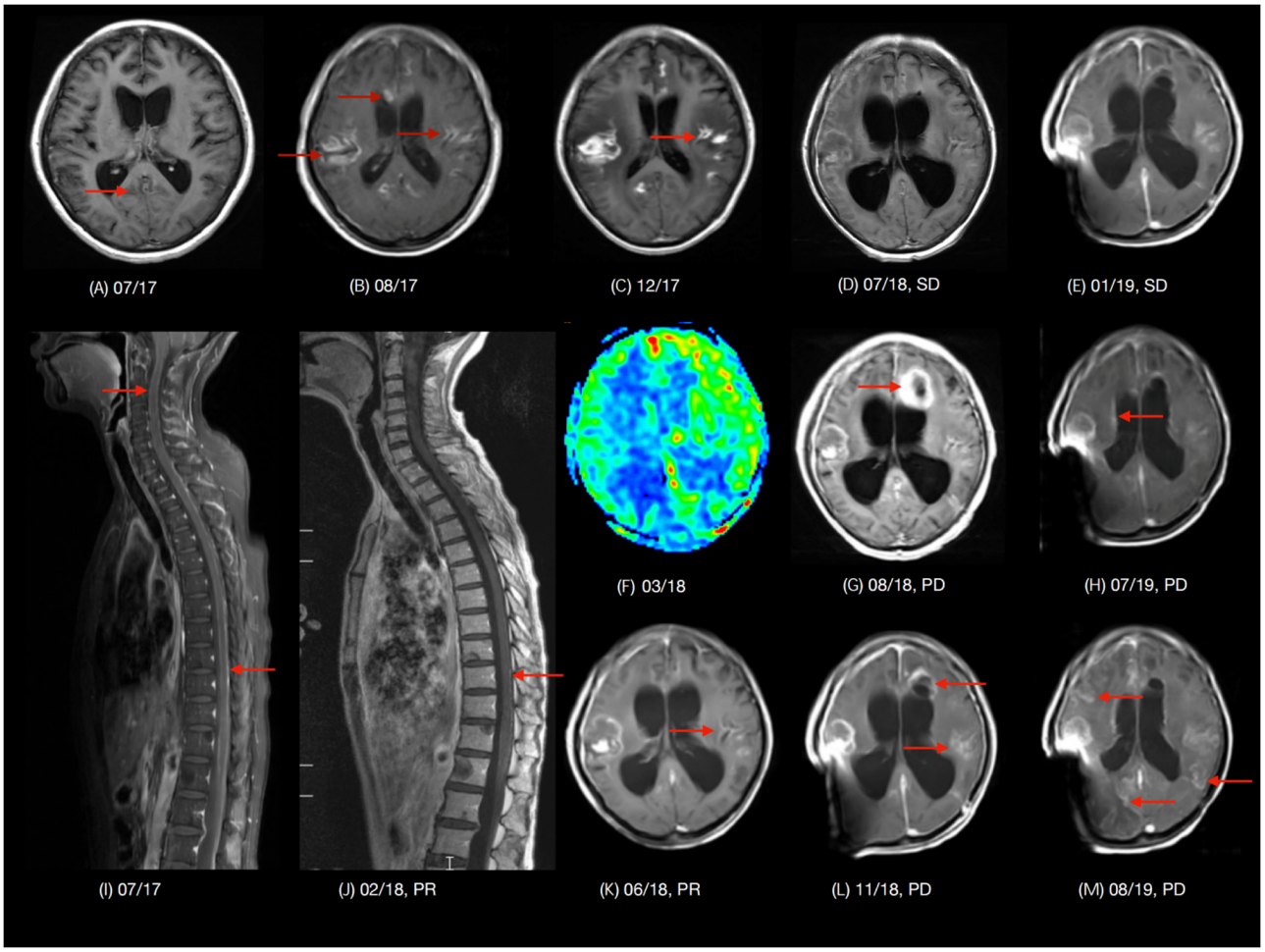

Figure 2. Representative MRI images during the clinical course of the patient. (A) Brain MRI at the time of leptomeningeal disease diagnosis. (B) At 5 days after WBRT, with enlarged region of enhancement due to increased blood-brain barrier permeability by WBRT. (C) Intracranial SD indicated by the highlighted areas on the imaging scans that have not notably changed in size or intensity. (D) SD indicated by the highlighted areas on the imaging scans that have not notably changed in size or intensity. (E) SD indicated by the highlighted areas on the imaging scans that have not notably changed in size or intensity. (F) Radiation-induced brain injury confirmed by arterial spin labeling MRI perfusion. (G) PD indicated by new lesion in left frontal lobe. (H) New lesion found in right lateral ventricle wall. (I) Spinal MRI at the time of leptomeningeal disease diagnosis. (J) Spinal MRI at 6 months after WBRT. (K) Shrunk region of enhancement compared with MRI in Dec 2017. (L) Enlarged enhancement region. (M) New lesions found appearing as bright areas on the MRI scan. Red arrows indicate intracranial lesions. MRI, magnetic resonance imaging; WBRT, whole brain radiation therapy; PR, partial response; SD, stable disease; PD, progressive disease.

began to experience low mood and insomnia after the pemetrexed infusions. Therefore, pemetrexed was removed from the treatment regimen.

In February 2019, the VAF of BRAF p.V600E and NRAS p.Q61K were found to have increased in the CSF. In response, therapy was adjusted to oral dabrafenib 75 mg twice daily and trametinib 2 mg once daily. A month later, the patient presented with severe shortness of breath secondary to pneumonia and was hospitalized for a week. In April 2019, the patient developed hypotension and an increased oxygen requirement. Cobimetnib plus vemurafenib was administered again in clinic. The patient received cobimetinib orally at a dose of 60 mg once daily for the first 21 days of each 28-day cycle plus vemurafenib orally at a dose of 960 mg twice daily. In July 2019, the patient developed hematemesis, and a new lesion was found in right lateral ventricle wall (Fig. 2H). TKIs

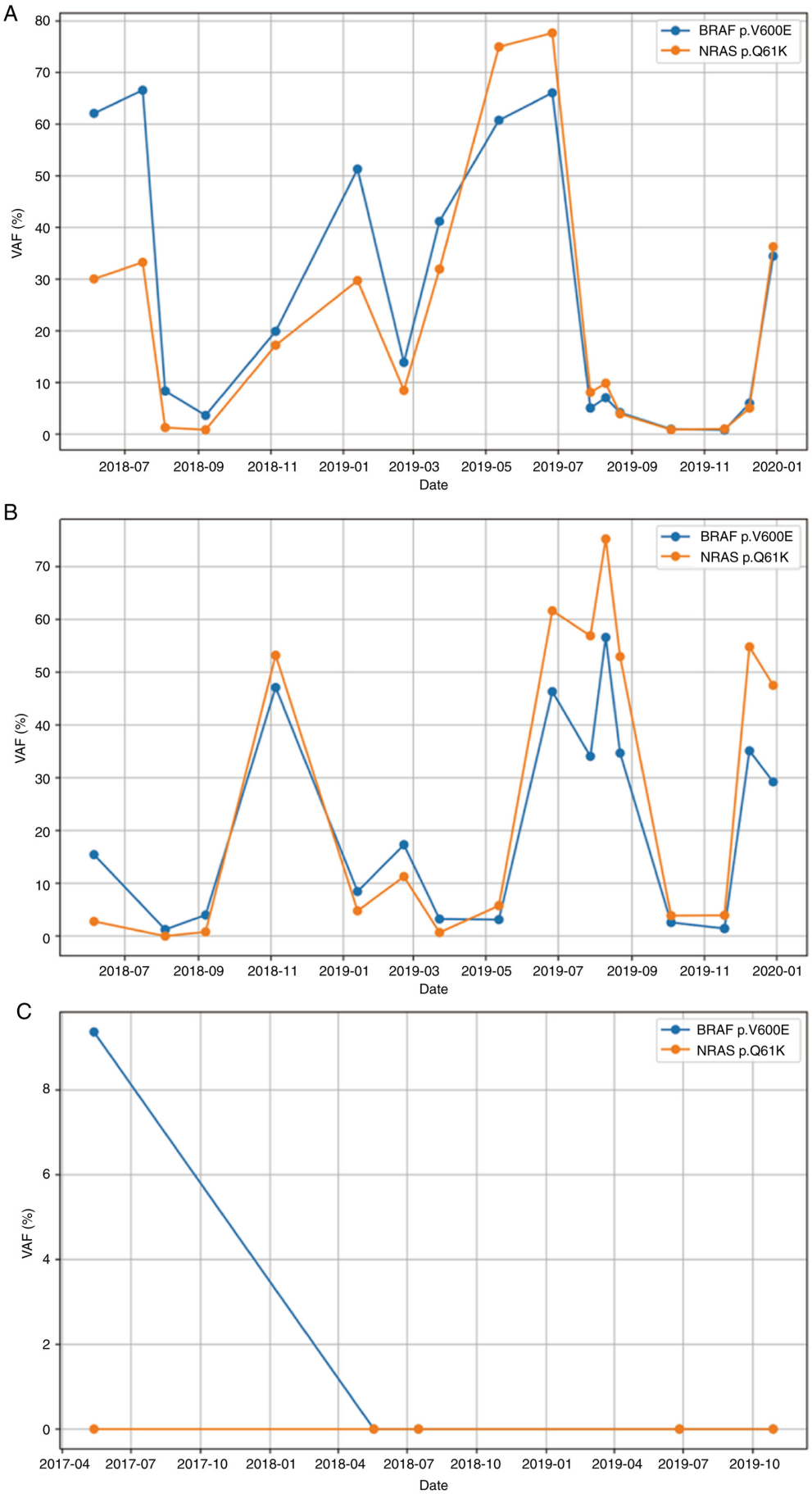

Figure 3. Longitudinal molecular tracking during treatment course. VAF changes of BRAF p.V600E and NRAS p.Q61K driver mutations identified in (A) cell-free DNA in CSF supernatant samples, (B) sediment cell DNA in CSF cell samples and (C) plasma or pleural effusion samples. VAF, variant allele frequency; CSF, cerebrospinal fluid.

were stopped and intravenous pemetrexed at a dose of 700 mg for a cycle of 3 weeks was administered. In August 2019, new lesions were found on the MRI image (Fig. 2M). The patient was started on a new TKI, cabozantinib taken orally at a dose of 20 mg once daily and also temozolomide, an oral active DNA alkylating agent that can cross the blood-brain barrier to treat brain tumors (18), taken at a dose of 100 mg once daily for 5 days. Due to severe constipation, temozolomide was discontinued after 5 days. In September 2019, the patient presented to the hospital with acute respiratory failure and underwent endotracheal intubation. Cabozantinib was increased to a dose of 60 mg once daily. The patient remained hospitalized for 4 months and subsequently died in February 2020.

Discussion

Although CSF cytology can help in the diagnosis of LMD, it does not provide a quantitative assessment of the severity of disease (19). In the present case, CSF was regularly and frequently sampled from the reservoir of the patient's VPS, which allowed the longitudinal tracking of CSF cfDNA and cellular DNA (Fig. 3A and B). A total of 17 time points of CSF supernatant and 16 time points of CSF cell pellets were prepared for targeted sequencing. Unfortunately, CSF from June 2017 (time of diagnosis) to June 2018 was not sent for sequencing due to lack of CSF samples, so genetic information was not obtained in this period. The patient's symptoms were generally consistent with the trend in VAF changes of driver mutations in cfDNA. When the patient presented with severe symptoms or when there was evidence of disease progression, the VAF of driver mutations was high. During periods of time when the patient had evidence of stable disease, the VAF in cfDNA was low. The association of CSF mutation burden in LMD has been reported previously (11), demonstrating its potential as a clinical marker for managing this disease.

In the present case, no actionable mutations were identified in the primary tumor. After presenting with neurologic symptoms in June 2017, the patient was found to have a BRAF p.V600E mutation in blood cfDNA. The patient was started on targeted therapy (vemurafenib), and later also received four rounds of WBRT. Notably, radiation therapy was only applied to the brain, not other sites. Lesions in the spinal cord shrunk after vemurafenib treatment (Fig. 2I and J), indicating the efficacy of this TKI in the central nervous system (CNS). Altogether, the patient had 13 months of progression-free survival from undergoing this regimen in June 2017 to the progression of the disease in July 2018. Subsequent genetic tests using blood samples (May and July 2018) did not identify the BRAF mutation or any other cancer-related mutations (Fig. 3C), indicating that the systemic disease was well controlled after treatment. However, the disease ultimately relapsed in CNS.

As the disease relapsed in June 2018, CSF was regularly sampled and sequenced, and genetic test results were used to inform treatment plans. Notably, sequencing data for CSF pellets from a time point in July 2018 were missing due to inadequate cellular DNA for library preparation. Consolidation of cobimetnib plus vemurafenib was given based on the high mutation frequency of BRAF p.V600E identified in CSF cfDNA sampled in July 2018. After ~8 months following the second disease relapse, the patient's targeted drugs were switched to dabrafenib plus trametinib. The genetic testing of CSF from the previous 7 months identified increasing mutation frequency, indicating possible drug resistance. In August 2019, high mutation frequencies of BRAF p.V600E and NRAS p.Q61K were found in CSF cellular DNA with corresponding lower VAF in cfDNA. This was likely due to a high quantity of malignant cells in CSF. The patient was subsequently started on cabozantinib. Notably, from the longitudinal tracking of CSF DNA over 19 months, the BRAF mutation was initially more abundant than the NRAS mutation both in cellular and cfDNA (Fig. 3A and B). Subsequently, the NRAS mutation became dominant in the cellular DNA, and VAF of these two mutations in cfDNA were similar. We hypothesize that the TKIs placed selection pressure on different tumor clones and resulted in the change of VAF of different mutations.

In summary, the present study reported the case of a patient with an unusual case of LMD from primary lung adenocarcinoma who underwent >20 time points of genetic sampling and testing whilst receiving a combinational treatment of chemotherapy, radiation therapy, immunotherapy and targeted therapy. The patient survived for 33 months after the LMD diagnosis. Although further research is needed to validate the efficacy of targeted therapy in LMD, the present case presents a potential strategy using reliable genetic testing of CSF to help clinical monitoring and treatment of LMD.

Acknowledgements

The authors would like to thank Mr. Siyang Xu and Ms. Manhu Shi (HaploX Biotechnology) for handling the image formats.

Funding

The present study was supported by the Natural Science Foundation of Guangdong Province (grant no. 2019A1515011943) and the Science and Technology Innovation Committee of Shenzhen (grant. no. KQTD20161129103502213).

Availability of data and materials

The data generated in the present study may be found in the Genome Sequence Archive under accession number HRA004612 or at the following URL: https://bigd.big.ac.cn/gsa-human/browse/HRA004612. Access to the data may be requested from the corresponding author.

Authors' contributions

SC, LC, MLa, TM and MLi conceived and designed the work. QH and JL collected clinical sample, patient information and medical records YL and TH contributed to analysis of the patient's data. YL, QH, MLa, TM and MLi drafted the manuscript. All authors have read and approved the final manuscript. SC and LC confirm the authenticity of all the raw data.

Ethics approval and consent to participate

The present research was approved by the Guangdong Sanjiu Brain Hospital Institutional Review (Guangzhou, China; approval no. 2020-010-089). All procedures in the present

study were performed according to the guidelines put forth in the Declaration of Helsinki.

Patient consent for publication

The patient provided written informed consent for the publication of any associated data.

Competing interests

TM, MLi, YL, TH and SC are employed by Shenzhen HaploX Biotechnology, and SC is a stakeholder of HaploX Biotechnology. HaploX Biotechnology performed the sequencing for the study. All other authors declare that they have no competing interests.

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