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Serial Testing of Tumor-Derived DNA in Cerebrospinal Fluid to Monitor Leptomeningeal Disease and Assess Therapeutic Response in Lung Cancer Metastasis to the Central Nervous System - A Case Report

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Abstract

Metastatic Leptomeningeal disease (LMD) most often seen in lung cancer, poses unique diagnostic and therapeutic challenges due to limited sensitivity of standard cerebrospinal fluid (CSF) work-up and genetic divergence from the primary tumor associated with resistance to central nervous system (CNS) penetrating treatments. Among CNS complications, LMD is associated with poor outcomes and average survival following diagnosis is three to six months. Routine methods for diagnosing LMD currently fall short, making it difficult to investigate therapeutic options for this type of disease progression. Sequencing of tumor-derived DNA (tDNA) found in CSF is emerging as a useful clinical tool to inform diagnosis and monitoring of CNS malignancies with the potential to guide treatment. The present case of metastatic lung cancer illustrates the use of serial CSF tDNA analysis via Belay Summit™, a novel liquid biopsy assay, to interrogate LMD driver mutations and guide therapeutic decisions alongside tumor profiling.

Keywords: Liquid biopsy, Leptomeningeal disease, CNS metastasis, Genomic testing, CSF

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Introduction

Traditional methods for diagnosing LMD, magnetic resonance imaging (MRI) and CSF cytology have limited sensitivity and specificity, especially for detection of early or minimal residual disease (MRD) [1-4]. Analysis of tDNA from CSF has demonstrated improved sensitivity for CNS involvement in metastatic disease and offers insight into the molecular underpinnings of metastasis [5,6]. Molecular characterization of LMD is becoming increasingly valuable as it is understood that while CNS disease spread is genetically derived from the primary tumor, metastatic cells also undergo a significant genetic divergence, presumably to penetrate the blood brain barrier (BBB) [6,7]. The BBB further complicates liquid biopsy analysis of LMD using plasma, supporting CSF as a more ideal specimen.

Part of the discussion around tDNA analysis is its clinical utility for disease monitoring beyond initial evaluation. Variant allelic frequency (VAF) in particular is being explored as a way to reliably assess therapeutic response [8]. VAF indicates the percentage of DNA fragments carrying a specific mutation, as detected by next-generation sequencing (NGS), relative to the total fragments sequenced at that genomic location. Since tDNA is released into fluids like plasma or CSF by dying tumor cells, the VAF of a mutation ideally reflects the relative abundance of tDNA at the time of specimen collection and therefore may serve as a surrogate for tumor burden [8]. Longitudinal variant tracking in both plasma and CSF is utilized in the present case as a follow-up to initial tumor profiling of intracranial disease and aided the care team in disease characterization as well as assessment of treatment efficacy.

Case Presentation

A 73-year-old female was found to have locally isolated, mixed giant cell and poorly differentiated adenocarcinoma in the upper lobe of the left lung. Upon lobectomy in 04/2022, margins and lymph nodes were negative for malignancy (Figure 1). She was subsequently started on surveillance

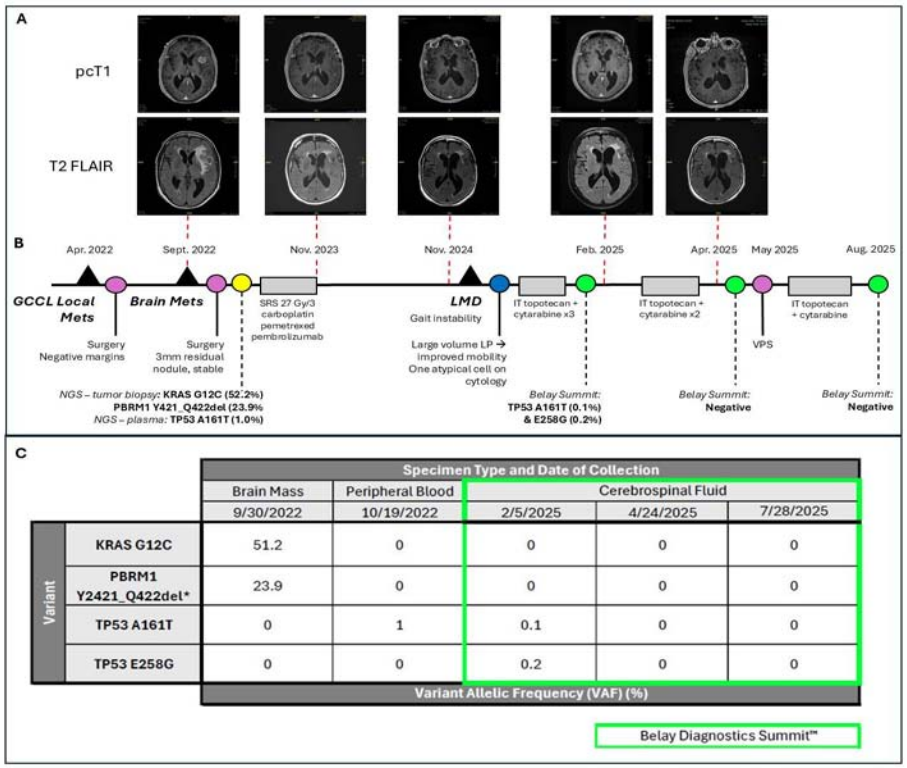

Figure 1: Timeline of Clinical Case Progression (A) Images from brain post-contrast T1-weighted (pcT1) and T2 FLAIR magnetic resonance imaging (MRI) chronologically aligned to clinical history. (B) Graphical timeline summarizing clinical events (black triangles), surgical resection (pink circles), chemotherapy and radiotherapy (grey boxes), large volume lumbar puncture (LP) (blue circle), and tumor/plasma/CSF genomic profiling history (yellow and green circles). Genomic variants are listed "Amino Acid Variant (Variant Allelic Frequency (%))." (C) Comparison of variants detected in different specimens. Giant-cell carcinoma of the lung, GCCL; metastasis, Mets; next-generation sequencing, NGS; stereotactic radiosurgery, SRS; leptomeningeal disease, LMD; intrathecal, IT; ventriculoperitoneal shunt, VPS.

imaging with brain metastasis to the left frontal lobe being detected and resected in 09/2022. Postsurgical cavity margins including a 3 mm residual nodule received stereotactic radiosurgery (SRS) (27 Gray/ 3 fractions). She subsequently received combination, systemic treatment with carboplatin, pemetrexed, and pembrolizumab through 2023, while comprehensive serial imaging restaging and serial blood MRD monitoring during treatment did not show any evidence of disease. Genomic profiling performed on the brain metastatic tumor specimen and matched normal blood specimen detected oncogenic hotspot KRAS G12C at a VAF of 51.2% along with an in-frame, loss-of-function (LOF) deletion in *PBRM1* (Y421_Q422delins*) at a VAF of 23.9%. Both variants are associated with cancer progression, though KRAS G12C is recognized as a common alteration in non-small cell lung carcinoma (NSCLC) and is seen in about 10-13% of cases [9]. Liquid biopsy testing was performed at the same time on blood wherein a missense LOF variant in *TP53* (A161T) was found at a VAF of 1.0%. The 3mm residual enhancing nodule at the anterolateral aspect of the post-surgical cavity remains stable at the time of submission of this manuscript.

The patient experienced worsening gait impairment through 2024, using a walker for mobility assistance in 12/2024. Serial brain MRIs showed slowly worsening, low-grade ventriculomegaly and trans-ependymal absorption. A large volume lumbar puncture (LP) performed in 12/2024 significantly improved patient mobility and restored her gait without the need to use an assistive device for approximately two days. CSF cytology revealed one atypical, degenerated cell, suggestive of potential LMD, though a repeat LP showed no abnormal cells. An Ommaya reservoir was placed on 1/2/2025 to facilitate intrathecal (IT) delivery of topotecan and cytarabine. To assess the presence and extent of LMD following three IT treatments, another LP was performed on 2/5/2025 and ~16 mL of CSF was shipped at room temperature to Belay Diagnostics for genomic profiling using the Summit™ test, a CLIA/CAP-validated CSF liquid biopsy test. The test detects genomic alterations in CSF using duplex sequencing to interrogate single and multi-nucleotide variants in a 32 gene panel and low-pass whole genome sequencing (WGS) to assess chromosomal arm-level aneuploidy [4]. The assay was validated for both primary and metastatic CNS tumors (n=124), demonstrating 100% sensitivity in 7 metastatic lung cancer cases evaluated in the validation cohort [4]. CSF results were reported on 2/14/2025 and identified two variants in *TP53* at low VAF (0.1% and 0.2%), one being TP53 A161T as identified in the blood liquid biopsy performed in 2022 (Figure 1C). The *KRAS* variant found in the brain metastatic mass was not detected and no aneuploidy was observed.

Following two more IT treatments, another CSF specimen was collected on 4/24/2025 for longitudinal tracking of genomic variants using Summit™. No genomic variants were identified in the specimen, suggesting IT treatment was effective. Based on these findings, the care team decided that CSF diversion would be the best next step to address gait impairment. Patient refused adding an in series ventriculoperitoneal shunt (VPS) equipped with an On-Off valve to her Ommaya reservoir and opted instead for revision of her Ommaya reservoir to a VPS. IT treatment continued via LP with topotecan and cytarabine every 6-8 weeks with plans to expand the interval between

IT treatments depending on monitoring of LMD genomic activity using Summit™ for CSF analysis as well as MRD using plasma liquid biopsy tests. Another CSF specimen was collected for Summit™ testing on 7/28/2025. No genomic variants were identified and parallel CSF cytology was negative for malignancy, supporting further therapeutic response and the extension of IT treatment intervals.

Discussion

As cancer relies on genetic maneuvers to bypass cellular growth checkpoints, it is fitting that brain metastatic disease genetically diverges from the primary tumor in NSCLC and further, genetic alterations present in LMD can differ from those in solid brain metastases [1,6,7]. This dynamic evolution, potentially specific to the organ driven tumor environment (lung, brain parenchyma, and CSF), of carcinomatosis demands tissue-specific and accurate genomic profiling for the delivery and overall guidance of targeted therapies. In cases of metastatic NSCLC and other cancers, it is understood that CSF tDNA better represents the genomic profile of the brain tumor relative to plasma tDNA [2,3]. In the present case, parallel profiling of the brain metastatic site and plasma tDNA identified different genomic alterations, high VAF variants in *KRAS* and *PBRM1* verses a low VAF variant in *TP53*, respectively. Interestingly, the same TP53 A161T variant was detected in CSF over two years later following resection and SRS though the driver *KRAS* variant was not found.

Without having further confirmation of the brain tumor genomic landscape post resection and SRS, the Summit™ findings suggest the presence of LMD driven not by KRAS activation but rather inhibition of tumor suppressor p53. A recent meta-analysis of studies across different metastatic cancers with LMD (including NSCLC) highlighted *TP53* as the most commonly altered gene [10]. Another study found that while *KRAS* variants were present in 50% of solid brain metastases from NSCLC, these variants were not identified in cases with LMD[6]. Moreover, growing evidence suggests *TP53* variants are key molecular players in leptomeningeal spread. *TP53*, often referred to as the "guardian of the genome", is a key tumor suppressor that is altered in about half of all human cancers and variants are commonly seen in NSCLC [11]. Inactivation of p53 is associated with poor prognosis as well as potential resistance to SRS of brain metastasis, relevant to the present case, though further investigation is warranted[11]. In addition to the TP53 A161T variant that was identified in plasma during initial work-up, Summit™ also detected TP53 E258G in CSF. The discrepancy in genomic findings between primary tumor, plasma, and CSF exemplifies ongoing clonal evolution and tumor spatial heterogeneity that promote leptomeningeal disease progression [1].

CSF tDNA analysis using Summit™ not only informed metastasis driver mutations in this case but also confirmed clinical suspicion of LMD. Indeterminate CSF cytology results, as seen in this report, are common as the sensitivity of this standard diagnostic method for LMD is about 75% [1]. CSF tDNA analysis is gaining recognition as a means of detecting LMD with one study demonstrating a sensitivity of 93% compared to 72% using cytology in a cohort of 43 LMD-positive samples [5]. In a study specifically investigating LMD from NSCLC, driver variants were detected using CSF tDNA analysis in 100% of cases (n=26, *EGFR*-mutated NSCLC) [12]. This finding was reviewed in comparison to tDNA analysis from plasma wherein driver variants were identified in 73% of cases. Sequencing of tDNA from CSF also yielded unique variants relative to those found in plasma and the primary tumor, similar to findings in the present case. In clinical validation studies, Summit™ achieved similar metrics with a sensitivity and specificity of 90% and 95%, respectively [4].

Taking liquid biopsy a step further, this case exhibits the use of longitudinal variant tracking to assess metastatic disease progression and therapeutic response. Several independent studies in NSCLC report serial measurements of mean or maximum VAF in tDNA that were consistent with patient relapses or response to various therapies including immune checkpoint inhibitors (pembrolizumab-based treatment) [8]. These studies utilized tDNA from plasma, however, and did not follow specific variant presence or VAF. A recent prospective study in 92 NSCLC patients with brain metastases showed serial tDNA concentration measurements (calculated based on mean VAF) in CSF better predicted intracranial tumor response to treatment than paired tDNA plasma analyses [13]. While further research is needed to assess the clinical utility of longitudinal VAF analysis in CSF, this minimally invasive strategy for disease monitoring shows potential in the setting of personalized therapy for metastatic disease.

In the present case, the absence of the initially detected low VAF *TP53* variants on follow-up testing was interpreted as a promising response to IT treatment and aligned with ongoing negative CSF cytology results. Together these findings helped individualize dose and schedule of IT treatment. Regarding the treatment course, topotecan has shown efficacy in metastatic NSCLC, though it is primarily used in cases of small cell lung carcinoma, and cytarabine is recognized as an option for intra-CSF targeting of LMD [1,14]. The care team intends to further investigate targetable genomic variants, particularly the KRAS variant identified in initial tumor profiling since sotorasib is a well-established, FDA-approved therapy for KRAS G12C-mutated NSCLC. While not commonly used against LMD, sotorasib demonstrated promising intracranial activity in a recent metastatic NSCLC case report [15]. Overall, genomic profiling, particularly of the CSF, proved to be critical in the precision medicine approach to this case.

Conclusion

Treatment selection is a multidisciplinary decision that ultimately hinges on continued genomic profiling to determine active driver variants that may differ across intracranial, leptomeningeal, and pleural spaces. In this case, genomic profiling of CSF also allowed treatment dose and schedule adjustment according to LMD therapeutic response. The ongoing efforts of this case highlight the use of innovative molecular strategies to manage LMD, a persistent clinical problem that calls for innovative solutions.

Ethics Statement: The authors confirm that written consent for submission and publication of this case report, including the images and associated text, have been obtained from the patient(s) in line with COPE guidance

Conflict of Interest: GB has no conflicts to disclose. AL, VU, KFS, QN and HVR are employees of Belay Diagnostics and receive a salary and stock options.

Authorship: Writing – Original draft – AL, VU, HVR; Writing review and editing - All authors All authors approved the final version manuscript.

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