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Crossing barriers with CSF-based sequencing for leptomeningeal disease in EGFR mutant NSCLC

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ABSTRACT

This case report highlights the critical role of cerebrospinal fluid (CSF)-based sequencing in precision oncology for a 64-year-old female with metastatic *Epidermal growth factor receptor* (*EGFR*)-mutant non-small-cell lung cancer (NSCLC) with leptomeningeal metastases spread. Isolating cell-free DNA (cfDNA) and capturing true live single circulating tumor cells (sCTCs) from CSF fluid is extremely challenging due to rapid degradation of DNA and extremely low abundance of sCTCs. In progressive disease, we successfully extracted cfDNA and sCTCs in CSF that revealed actionable mutations such as *EGFR* Exon 19 deletion and *ERBB2* amplification. The findings guided personalization of precision therapy, including intrathecal trastuzumab combined with systemic treatments, leading to significant clinical improvement and effective control of leptomeningeal disease.

CSF profiling opens a new diagnostic avenue in identifying resistance mechanism, when blood analysis showed no actionable information. This case demonstrates the transformative potential of CSF-based liquid biopsies to uncover critical mutations, monitor disease progression, and optimize outcomes for central nervous system metastases. Sequential molecular profiling and CSF analysis were instrumental in effective therapeutic strategies for this complex clinical case.

1. Introduction

Non-small-cell lung cancer (NSCLC) accounts for approximately 85 % of lung cancer cases globally presenting a significant clinical challenge [1] and also representing a paradigm of precision oncology when driven by actionable mutations such as *Epidermal growth factor receptor* (*EGFR*) mutations. *EGFR*-mutated NSCLC is characterized by its responsiveness to *EGFR* tyrosine kinase inhibitor (TKIs) frontline therapy, e.g. erlotinib, gefitinib, and osimertinib, which have demonstrated remarkable efficacy in prolonging progression-free survival (PFS) and improving quality of life (QoL) in patients [2,3]. Despite promising initial responses, resistance inevitably develops due to various acquired mechanisms [4,5].

Central nervous system (CNS) metastases like leptomeningeal disease complicate management due to poor prognosis and limited treatment options [6]. Blood-brain barrier (BBB) hampers drug delivery and detection of circulating tumor DNA (ctDNA) in peripheral blood, making both diagnosis and treatment challenging [6].

This case highlights the critical role of Next Generation Sequencing (NGS)-based molecular profiling in guiding treatment decisions where sequential and multi-modal biopsies, including **circulating tumor DNA (ctDNA)** and true live **single circulating tumor cell (sCTC) DNA** from **cerebrospinal fluid (CSF),** allowed clinicians to track tumor evolution and identify actionable alterations, enabling metastasis site-specific therapy [7–10]. Notably, the coexistence of *EGFR* and *Erb-B2 Receptor Tyrosine Kinase 2 (ERBB2)* drivers in CSF and the successful exploitation of both through sequential systemic and intrathecal therapy establish a framework for how CSF-NGS can guide dual-driver targeting in

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oncogene-driven NSCLC. Leptomeningeal metastases pose a major challenge due to poor prognosis and limited therapies [6]. CSF analysis enables detection of sCTC DNA and ctDNA, allowing targeted interventions that improve outcomes in aggressive CNS cases.

2. Case presentation

A 64-year-old diabetic female presented in December 2020 with complaints of acute onset breathlessness for 3–4 days. She was normotensive but required supplemental oxygen and had bilateral lung crepitations with muffled heart sounds.

Computed Tomography (CT) imaging showed a large pericardial effusion, a mass in the left upper lobe with lobulated and spiculated margins, multiple lung and discrete nodules of varying sizes in both lungs (Fig. 1A and B). Electrocardiogram and echocardiogram were consistent with CT findings.

Patient was diagnosed with cardiac tamponade and acute kidney injury, likely due to a malignant left lung mass. Following pigtail pericardial drainage, the patient's breathlessness and renal functions improved. Pericardial fluid analysis confirmed malignant cells, and immunohistochemistry identified adenocarcinoma.

Full staging confirmed metastatic non-small cell adenocarcinoma (NSCLC) of the lung. Magnetic Resonance Imaging (MRI) of the brain was normal. Given her phenotype as an Asian, non-smoker female, molecular testing (Real-time polymerase chain reaction; RT-PCR) was conducted on the cell block, which revealed *EGFR* exon 19 p.E746_A750del.

In December 2020, following FLAURA trial guidelines [3], patient was started on Osimertinib, which she tolerated well and achieved complete metabolic response remaining stable for nearly two years. In September 2022, a follow-up scan showed disease progression in the left lung after PFS of 21 months and given her disease genotype [3], oligo-progression at the primary site was managed with localized Stereotactic Body Radiation Therapy (SBRT), and Osimertinib was continued with good tolerance and no significant radiation pneumonitis.

In June 2023, patient developed clinical signs of disease progression and a repeat Positron emission tomography (PET) imaging revealed frank disease progression. A repeat biopsy in July 2023 confirmed adenocarcinoma, and patient began cytotoxic chemotherapy with a platinum doublet, pending comprehensive genomic profiling (CGP) results. Despite good tolerance, symptoms worsened after first cycle, with **High-Resolution Computed Tomography (HRCT) chest scan** confirming progression (Fig. 1C).

Three weeks later, the CGP results revealed ***EGFR* exon 20 p.C797S**, in addition to ***EGFR* exon 19 p.E746_A750del** and ***Tumor Protein p53 (TP53)* exon 7 p.I232S** mutation (49 %) (Table 1). C797S is classified as Cis or Trans based on its position relative to T790M, often guiding the use of combined first- and third-generation *EGFR* TKIs. When both mutations co-occur on Exon 20, their close 21-basepair proximity allows easier identification of the Cis or Trans status unlike this case where absence of T790M made this distinction unfeasible. ***EGFR* exon 20 p.C797S** mutation confers resistance to third-generation TKIs but remains sensitive to first-generation TKIs [11]. Since the patient was ***EGFR* Exon 19 Deletion+ (83 %)/C797S+/T790M-)** [12], she remained responsive to gefitinib without use of third-generation combination therapy. CT Chest two weeks post gefitinib showed a rapid response to first-generation TKI therapy (Fig. 1D). Patient remained on gefitinib from August 2023 to February 2024 with good tolerance.

3. Development of *ERBB2* amplification

After six months of progression-free survival, February 2024 scans showed disease progression. As the patient declined a repeat tissue biopsy, liquid biopsy was performed revealing ***EGFR* exon 19 p.**

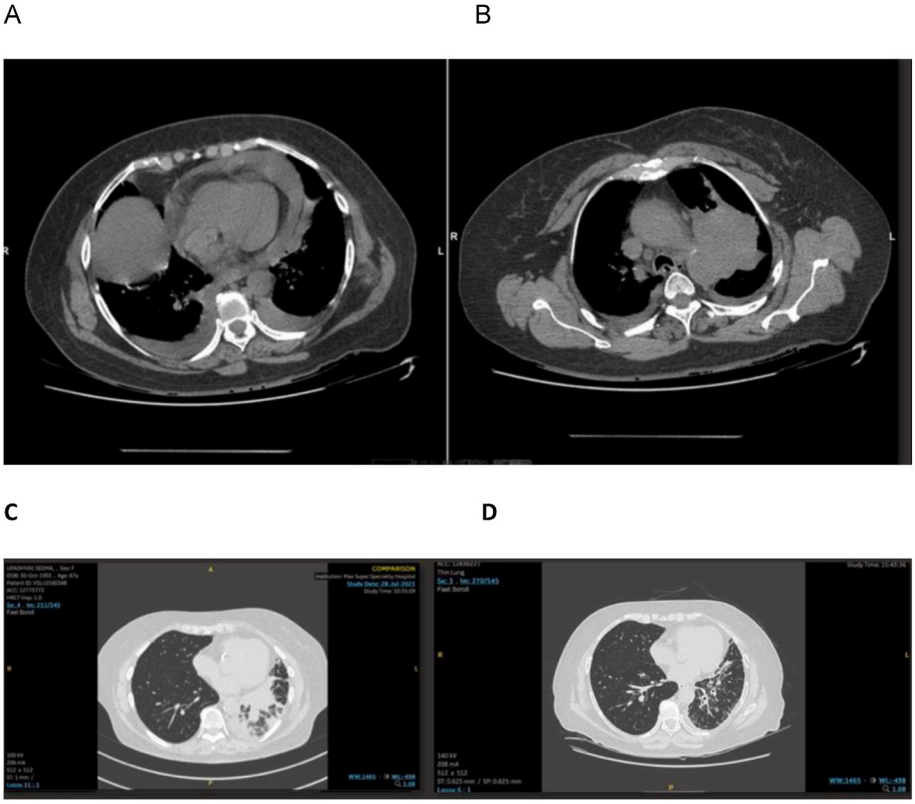

Fig. 1. (A) Computed Tomography (CT) imaging (Dec 2020) showing pericardial effusion. **(B) Computed Tomography (CT)** imaging (Dec 2020) showing left lung mass. **(C) Chest High-Resolution Computed Tomography (HRCT)** (July 2023) demonstrates evidence of disease progression following chemotherapy. **(D)** Follow up **Chest High-Resolution Computed Tomography (HRCT)** (Aug 2023) showing a rapid response to Gefitinib.

Table 1

Longitudinal Comprehensive Genomic Profiling Across Sample Types Unveiling Molecular Tumor Evolution and Advancing Precision Diagnosis through NGS profiling. Post Next Generation Sequencing (NGS), bioinformatics analysis for variant analysis was conducted using the iCare™ software platform. Briefly, adapter and barcode sequences were trimmed from raw **FAST-All Quality** (FastQ) files following quality assessment. The trimmed reads were aligned to the **Genome Reference Consortium human build 38 (**GRCh38) human reference genome, and variant analysis and annotation were performed in accordance with American College of Medical Genetics and Genomics (ACMG)/Association for Molecular Pathology (AMP)/ American Society of Clinical Oncology (ASCO)/College of American Pathologists (CAP) guidelines.

Gene	Alteration	Time point 1 (July 23) Post Osimertininb Progression)		Time point 2 (Feb 24) Post Gefitinib Progression		Time point 3 (May 24) At Onset of LMD		Time point 4 (Oct 24) LMD on treatment	
		Sample Type	%VAF	Sample Type	%VAF	Sample Type	%VAF	Sample Type	%VAF
EGFR	E746_A750del (Exon 19 deletion)	Tissue	83%	Blood	15%	CSF	92%	CSF	Not Detected
		Blood	1%			Blood	Not Detected	Blood	0.20%
EGFR	C797S (Exon Number:20)	Tissue	56%	Blood	Not Detected	CSF	Not Detected	CSF	Not Detected
		Blood	1%			Blood	Not Detected	Blood	
ERBB2	Amplification	Tissue	Not Detected	Blood	Copy Number 6	CSF	Copy Number 21	CSF	Not Detected
		Blood	Not Detected			Blood	Not Detected	Blood	
TP53	I232S	Tissue	49%	Blood	9.30%	CSF	87%	CSF	Not Detected
		Blood	1%			Blood	Not Detected	Blood	
TP53	H193L	Tissue	Not Detected	Blood	0.08%	CSF	Not Detected	CSF	Not Detected
		Blood	Not Detected			Blood	Not Detected	Blood	
CCND1	Amplification	Tissue	Not Detected	Blood	Copy Number 4.6	CSF	Not Detected	CSF	Not Detected
		Blood	Not Detected			Blood	Not Detected	Blood	
TP53	Y163C	Tissue	Not Detected	Blood	Not Detected	CSF	Not Detected	CSF	Not Detected
		Blood	Not Detected			Blood	Not Detected	Blood	0.90%
KIF1A	S252N	Tissue	Not Detected	Blood	Not Detected	CSF	Not Detected	CSF	Not Detected
		Blood	Not Detected			Blood	Not Detected	Blood	5%

E746_A750del), ***ERBB2* amplification** (plasma copy number 6), and the loss of C797S (Table 1) along with two pathogenic ***TP53* circulating variants (*TP53* exon 7 p.I232S)** and **(*TP53* exon 6 p.H193L)** suggesting impaired tumor suppressor function, contributing to poor prognosis.

Patient declined biopsy for Immunohistochemistry (IHC) confirmation. Patient also had an *ERBB2* mutation (*ERBB2* exon 8 p.R340Q), not yet reviewed by **Oncology Knowledge Base** (OncoKB) [13,14]. In view of *ERBB2* amplification and unwillingness for biopsy and chemotherapy, patient was started on investigational trastuzumab deruxtecan (TDxd), which is established for *ERBB2* mutations but not established for amplification [15]. Patient tolerated the therapy well, with only grade 2 fatigue and repeat May 2024 PET-CT scan revealed significant improvement. After two weeks, patient experienced headache, photophobia, and giddiness. Brain MRI (Fig. 2A) revealed leptomeningeal disease, confirmed by CSF examination, suggesting that systemic therapies had not crossed the BBB. Patient declined whole-brain radiation and CSF was sequenced before starting intrathecal methotrexate.

Patient was flown near testing site where CSF was collected in a PAXgene Blood **Circulating cell-free DNA** (ccfDNA) tube (BD Biosciences, New Jersey, USA), transported at 2–8 °C to the lab within 30 min followed by ccfDNA isolation using the QIAamp MinElute kit (Qiagen, Hilden, Germany). True live sCTCs were isolated via the OncoIncytes platform. Illumina-compatible libraries were prepared with the OncoIndx® panel [16] using target hybridization and sequenced on the NextSeq 2000 (Illumina, San Diego, CA, USA).

Variant analysis and annotation (as per American College of Medical Genetics and Genomics (ACMG)/Association for Molecular Pathology (AMP)/American Society of Clinical Oncology (ASCO)/College of American Pathologists (CAP) guidelines) conducted on the iCare™ platform revealed high variant allele fractions (VAFs) for *EGFR* exon 19 p.E746_A750del (92 %) and *ERBB2* amplification (21 copies) (Table 1), as major drivers of brain metastasis. Absence of these drivers in the blood, indicated control of systemic disease and importance of **sampling more proximally to metastatic site**.

NGS of CSF enabled targeted treatment of leptomeningeal disease. *ERBB2*-targeted therapies were considered as a promising treatment option [17,18]. A combined regimen targeting both *ERBB22* amplification and *EGFR* mutation was initiated: osimertinib 80 mg daily, intrathecal trastuzumab (3 mg/kg), and biweekly low-dose paclitaxel/carboplatin. Mild grade 1–2 cytopenias were managed by pausing osimertinib for two days post-infusion. Clinical improvement followed, with July 2024 MRI showing resolution of leptomeningeal enhancement (Fig. 2B).

The patient remained clinically stable, with PET-CT in September 2024 showing stable systemic disease. In October, MRI revealed new asymptomatic parenchymal lesions in the right frontal (Fig. 2C) and occipital lobes (Fig. 2D), while leptomeningeal disease remained controlled. SBRT was planned, with a brief pause in systemic and intrathecal therapy. CGP of CSF and blood showed *EGFR* exon 19 p.E746_A750del (0.2 %) and *TP53* mutations (*TP53* exon 5 p.Y163C, *TP53* exon 5 p.V173M) (Table 1) in blood, but no ctDNA in CSF. Treatment resumed post-SBRT and she continued to do well. Her CSF exam in December 2024, eight months post-leptomeningeal diagnosis, was acellular and negative for malignant cytology. At the time of routine re-evaluton in March 2025, while her CSF was negative for malignant cells and PET CT was suggestive of stable disease, her MRI brain revealed new lesions in the left cerebellum, left occipital and left frontal

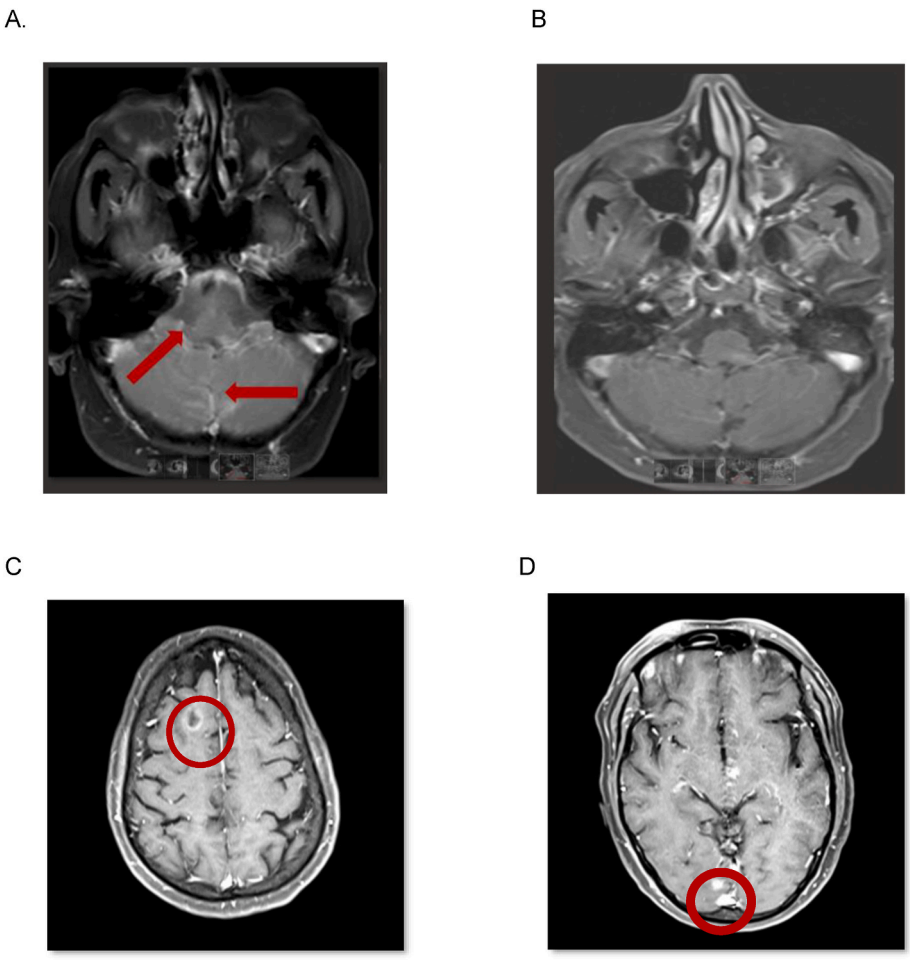

Fig. 2. (A) Magnetic Resonance Imaging (MRI) Brain (May 2024) depicting evidence of leptomeningeal disease. **(B)** MRI brain (July 2024) depicting resolution of the leptomeningeal disease. **(C)** Axial post-contrast T1-weighted MRI showing a **new ring-enhancing lesion** in the **right frontal lobe**. **(D)** Axial post-contrast T1-weighted MRI depicting an **enhancing lesion in the left occipital lobe**.

periventricular regions. She underwent SBRT to these lesions and subsequently her systemic therapy was changed to Amivantamab along with Osimertinib in May 2025 while continuing intrathecal trastuzumab.

At present, almost 1.5-years after onset of leptomeningeal disease and almost 5-years after being diagnosed, she continues to do well. She is mentally agile and is able to take care of herself with some support. Two new small lesions were noticed in the right cerebellum and right precentral gyrus in July 2025 which have remained stable on the current treatment. Her CSF, last checked in September 2025, continues to be negative.

4. Discussion

Treatment of post-osimertinib progression in *EGFR*-mutant NSCLC is complex and often requires individualized sequencing. In this patient, emergence of the *EGFR* exon 20 p.C797S mutation, a P-loop and αC-helix compressing (PACC) alteration that distorts the kinase domain and diminishes third-generation TKI binding—resulted in re-sensitization to gefitinib, consistent with prior reports emphasizing the importance of allelic context in therapeutic decision-making [12,19].

Subsequent disease progression was driven by *ERBB2* amplification, a recognized bypass resistance mechanism observed in 3 % of de novo and up to 15 % of acquired cases after EGFR-TKI therapy [20,21]. It is important to distinguish between ERBB2 amplification and mutation, as they carry distinct clinical significance [21]. Amplification represents a validated bypass resistance mechanism with therapeutic relevance to HER2-targeted therapy, whereas the *ERBB2* exon 8 p.R340Q mutation detected here remains of uncertain pathogenicity and without current clinical actionability. Treating amplification and mutation as equivalent risks overstating therapeutic benefit, and both should be interpreted independently. TDxd has shown efficacy in HER2-mutated and overexpressed NSCLC [15,22] though evidence for *ERBB2*-amplified cases is limited [23]. Unlike in breast cancer, *ERBB2* amplification and overexpression are poorly correlated in NSCLC [24]. While TDxd has shown up to 90 % intracranial disease control in NSCLC [25], patient subsequently developed leptomeningeal disease reflecting the common CNS-predominant progression pattern in oncogene-driven NSCLC despite systemic control.

To guide treatment in this setting, NGS was performed on CSF. CSF genomic analysis is technically challenging due to low ctDNA stability and high background cfDNA, and most published reports have relied on the CellSearch® system [26]. Prior to use of CSF-NGS in NSCLC, *EGFR* mutations were considered as key drivers for progression to leptomeningeal metastases, but additional alterations have also been described. Nie et al. identified non-*EGFR* drivers such as *Anaplastic Lymphoma Kinase (ALK)* rearrangement, *C-ros Oncogene 1, Receptor Tyrosine Kinase (ROS1)* fusion, *RB Transcriptional Corepressor 1 (RB1)* mutation, and *Mesenchymal-Epithelial Transition proto-oncogene, receptor tyrosine kinasee (MET)* amplification [27], Liang et al. reported *MET* copy-number gain as the most frequent non-*EGFR* alteration, associated with improved survival after intrathecal pemetrexed [28] and Li et al. showed that CSF cfDNA not only captured primary *EGFR* drivers with higher allele fractions than plasma but also revealed co-alterations

including *TP53* and *RB1* mutations as well as recurrent copy-number variations such as *MET* amplification [29]. In contrast, our patient's CSF demonstrated a novel dual-driver profile consisting of an *EGFR* exon 19 deletion and high-level *ERBB2* amplification, findings that were entirely absent in plasma. This demonstrates the unique capacity of CSF genotyping to reveal clinically significant alterations that remain undetectable by circulating blood-based assays.

This dual-driver detection informed a combined approach of continued *EGFR* inhibition with osimertinib and intrathecal trastuzumab for *ERBB2* amplification, achieving durable intracranial control. Interestingly, while leptomeningeal disease remained suppressed, new parenchymal metastases emerged. At this stage, ctDNA and CTCs were no longer detected in CSF but appeared in plasma, consistent with prior observations that CSF is the predominant shedding site in leptomeningeal disease, whereas parenchymal lesions more often release tumor DNA into blood [4,21].This highlights the metastasis site–dependent biology of liquid biopsy and the value of sampling proximally to the disease site.

Nevertheless, limitations must be acknowledged. The absence of confirmatory immunohistochemistry (IHC) for *ERBB2* amplification limits orthogonal validation. As a single-patient experience, generalizability remains constrained especially with limited data on frequency of such dual drivers across these patients. Although patient continues to do well, another shortcoming is lack of structured neurological and cognitive evaluation during limited follow-up of 1.5 years.

In summary, this case highlights how NGS on CSF can identifty dual actionable drivers not evident in plasma. It provides a framework to tailor systemic & intrathecal strategies and demonstrates the clinical value of liquid biopsy in managing CNS progression in oncogene-driven NSCLC. More broadly, it illustrates the potential of genomics-driven, individualized treatment to enhance mutation detection, refine therapy, and achieve meaningful disease control even in challenging scenarios such as leptomeningeal metastasis.

Patient consent

The patient has consented to the use of his/her specimen/information derived from the test for research purposes in a non-annotated manner.

Author contributions

Bhuvan Chugh: Conceptualization, Methodology, Investigation, Writing – Original Draft, Writing – Review & Editing. Jayant Khandare: Conceptualization, Writing – Review & Editing. Hrishita Kothavade: Conceptualization. Gowhar Shafi: Conceptualization, Writing – Original Draft, Writing – Review & Editing. Ganesh Khutale: Investigation. Saloni Andhari: Investigation. Atul Bharde: Investigation, Methodology, Writing – Original Draft, Writing – Review & Editing. Sumit Halde: Investigation. Madhura Basavalingegowda: Investigation. Kanchan Hariramani: Investigation. Alain D'Souza: Investigation. Sandhya Iyer: Formal Analysis, Visualization, Writing – Review & Editing. Aarthi Ramesh: Formal Analysis, Visualization, Writing – Review & Editing. Richa Dave: Writing – Original Draft, Writing – Review & Editing. Aravindan Vasudevan: Writing – Review & Editing.

Additional information

None.

Consent

I have no objection to use and share my medical reports and clinical data for publication.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

The data and materials used in the current study are available from the corresponding author upon reasonable request. The raw sequencing data are not publicly available due to informed consent restrictions.

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