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Analysis of cerebrospinal fluid tumor-derived DNA to obviate biopsy of IDH-mutant brainstem glioma in an adult

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ABSTRACT

Adult brainstem gliomas are rare and present unique diagnostic and therapeutic challenges due to their critical location and limited biopsy feasibility. Molecular profiling of tumor-derived DNA (t-DNA) isolated from cerebrospinal fluid (CSF) is emerging as a minimally invasive alternative for characterizing these tumors and guiding targeted therapy. A 34-year-old woman with brainstem glioma was treated with a standard course of radiation and temozolomide (TMZ) and remained stable for several years. After surveillance imaging revealed disease progression and raised suspicion of IDH-mutant disease on MRI spectroscopy, molecular profiling of CSF was ordered. The Belay Summit test, a novel NGS-based liquid biopsy assay for central nervous system (CNS) tumors, identified variants in *IDH1* and *TP53* as well as loss of *CDKN2A/CDKN2B*. Based on these findings, the patient received a short course of radiation and was started on the *IDH* inhibitor vorasidenib. This case demonstrates the use of t-DNA from CSF for molecular profiling of adult brainstem glioma to identify actionable genomic alterations without surgical risk and allow patients to receive targeted therapy without tissue diagnosis.

Short communication

Glioma is the most common brain tumor in adults; however, it is rarely found in the brainstem (1–2 % of adult gliomas) [1]. The low incidence and central, surgically challenging location of brainstem glioma have made it difficult to investigate and characterize. Though further research is warranted, it is understood that adult brainstem glioma is a heterogenous group of tumors that vary in location, radiologic findings, symptoms, and prognoses. Approximately 60 % of cases originate in the pons, but tumors can arise in the midbrain or medulla [2]. About 40 % of adult brainstem gliomas show enhancement on magnetic resonance imaging (MRI), the level of which can vary from minimal to robust [1]. Median survival for adult brainstem glioma is 30–40 months, though this too is subject to variability and prognosticating is limited by biopsy feasibility [3]. When biopsy is an option, adult brainstem gliomas can present with an astrocytic, oligodendroglial-like, or mixed appearance upon histological review.

Efforts to molecularly characterize adult brainstem glioma have identified recurrent mutations in *IDH1* and *H3-3A* (K28M) [4]. These findings are similar to, yet distinct from, what is seen in adult non-brainstem glioma as well as pediatric diffuse intrinsic pontine glioma (DIPG). Further exploration of molecular characteristics among adult brainstem gliomas could expand the treatment selection for this disease, radiation therapy with possible concurrent or adjuvant temozolomide (TMZ) being the current standard. Genomic profiling of tumor derived DNA (t-DNA) in cerebrospinal fluid (CSF) has recently gained recognition as a clinical tool used to identify targets for personalized therapy, particularly in instances when biopsy is out of the question. In the following case description, Belay Summit [5], a novel next-generation sequencing (NGS)-based assay that interrogates oncogenic variants in CSF, was used to inform treatment of brainstem glioma in an adult (Fig. 1).

A 38-year-old female was initially diagnosed with brainstem glioma in 2016 after experiencing headaches, dizziness, and weakness and brain MRI revealed a mass in the pons. The location of the lesion was not amenable to biopsy and a lumbar puncture (LP) was performed for CSF workup. CSF cytology was remarkable only for elevated protein and interpreted as negative for malignancy. The patient was treated with radiation therapy and TMZ for 6 weeks followed by 12 cycles of TMZ. At this time, the patient transferred care to a local oncologist for

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surveillance. The patient was stable from 2017 to 2024 then started showing slow increase in non-enhancing disease in the pons on imaging. She was hospitalized due to difficulty swallowing and found to have frank progression on brain magnetic resonance imaging (MRI) and spectroscopy showed a positive 2-hydroxyglutarate peak, raising concern for IDH-mutant disease.

The Belay Summit test was ordered for genomic analysis of CSF and identified mutations in *IDH1* and *TP53* as well as loss of chr9p21.3, containing *CDKN2A* and *CDKN2B* (Fig. 2) [5]. The IDH1 R132G variant is located in the catalytic site of the IDH1 protein and was detected at a variant allelic frequency (VAF) of 1.7 % in the CSF specimen. This is a well-characterized oncogenic hotspot that confers an enzymatic gain-of-function, allowing IDH1 to convert α-KG to D-2-hydroxyglutarate, an oncometabolite [6,7]. The TP53 Y236H variant, detected at a variant allelic fraction (VAF) of 2.1 %, is located in the DNA-binding domain of the p53 tumor suppressor and has been demonstrated to reduce transcriptional activity in vivo [8,9]. Based on these findings, the patient was treated with a short course of radiation which improved her swallow function and started on IDH inhibitor drug, vorasidenib.

The use of Belay Summit for t-DNA profiling in the present case not only confirmed suspicion of IDH-mutant disease but also provided additional molecular details for a rare tumor type. In a whole-exome sequencing (WES) study of brainstem glioma, 38 % (5 of 13) of adult cases were found to have *IDH1* variants. All of these cases also had an oncogenic variant in *TP53*, concordant with the findings presented in this report [4]. The molecular profiles of the discussed study subset and present case resemble that of cerebral *IDH*-mutant astrocytoma, in which an *IDH1* variant is often accompanied by a variant in *TP53*. Loss of *CDKN2A* and/or *CDKN2B*, identified in this case report, is associated with unfavorable prognosis in non-brainstem *IDH*-mutant astrocytoma and accordingly included in WHO classification. While these findings suggest adult brainstem glioma could be regarded similar to its cerebral counterpart based on parallel molecular profiles, the same WES study identified *H3-3A* in 54 % (7 of 13) of adult brainstem gliomas that were mutually exclusive with *IDH1* variants [4].These cases ultimately bore greater resemblance to pediatric DIPG in which H3-3A K28M is the most common variant.

The presence of *IDH1* or *H3-3A* variants in adult brainstem glioma could have targeted therapeutic potential. Tumors with *H3-3A* variants that are consequently prone to DNA hypomethylation have demonstrated increased sensitivity to TMZ with the use of small-molecular inhibitors targeting H3 demethylases as well as multihistone deacetylase inhibitors [10,11]. Specific to *IDH*-mutant disease, vorasidenib, administered based on Summit results in the present case, is FDA-approved for the treatment of low grade astrocytoma or oligodendroglioma with a susceptible *IDH1* or *IDH2* variant [12]. Current studies, in contrast to *H3-3A* mutant disease, are investigating demethylating agents as well as PARP inhibitors and immunotherapy to treat *IDH*-mutant glioma [13]. In the context of *CDKN2A/CDKN2B* loss, CDK inhibitors are being explored in a phase II clinical trial (NCT03220646) studying the use of abemaciclib in *IDH*-mutant glioma, though a similar trial investigating palbociclib did not demonstrate meaningful tumor response [14].

As growing evidence suggests adult brainstem glioma could fall into one of two molecular subtypes, *IDH* or H3-3A K28M mutant, targeted therapy is becoming a consideration for this patient population. However, personalized treatment ultimately relies on detection of a genomic alteration in the tumor of interest, a particular challenge for brainstem tumors. As demonstrated in this case report, genomic profiling of t-DNA from CSF can be an effective alternative to stereotactic biopsy. In a study that sequenced CSF-derived circulating tumor DNA from 57 patients with primary brainstem tumors, genomic alterations found in tumor tissue were also found in CSF in 97.3 % of cases [15]. Similarly, the Belay Summit assay demonstrated a clinical sensitivity of 90 % across a cohort of 124 primary and metastatic CNS tumors.[5] While survival outcome cannot yet be reported for the present case, the use of an *IDH* inhibitor was decided based on molecular profiling results from CSF, eliminating surgical morbidity and mortality risks associated with brainstem biopsy. For a rare adult tumor type that has historically been exempt from targeted therapy, this case highlights the use of a novel liquid biopsy tool to inform personalized treatment decisions.

CRediT authorship contribution statement

MY – conceptualization, writing – review and editing. AL - Writing – Original draft. VU – writing – review and editing. KFS - writing – review and editing. QN - writing – review and editing. HVR – conceptualization, supervision, Writing – Original draft. All authors approved the final

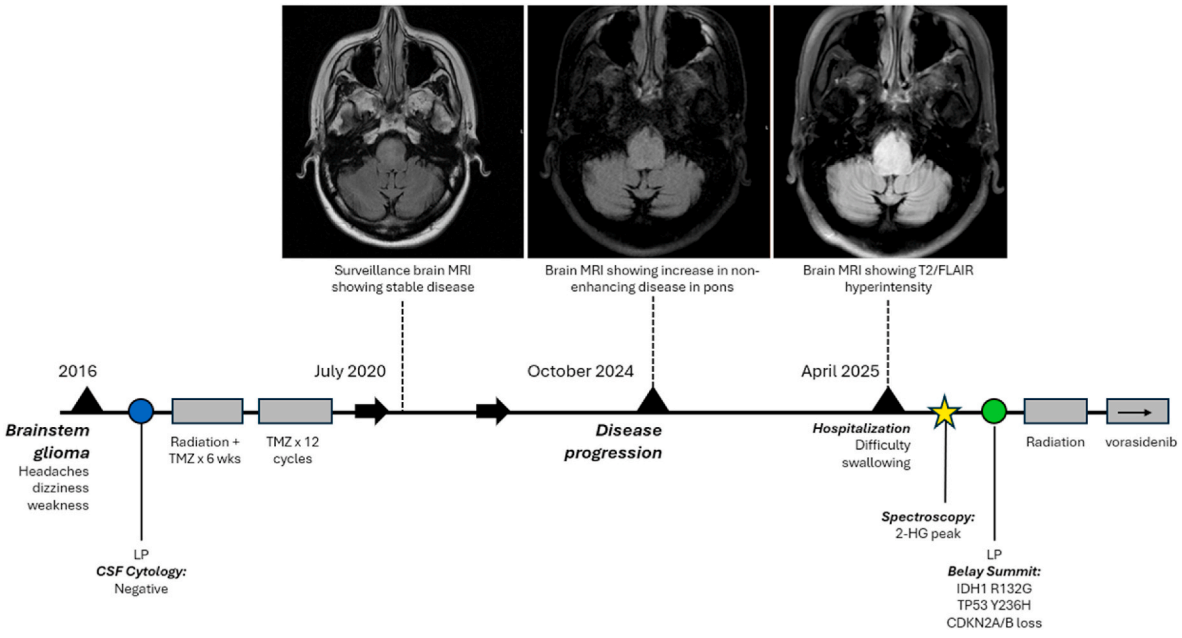

Fig. 1. Clinical history summary. A timeline of key clinical events (black triangles) is presented alongside clinical testing including cerebrospinal fluid (CSF) cytology, spectroscopy, and Summit as well as brain magnetic resonance imaging (MRI) and treatment courses (grey boxes).

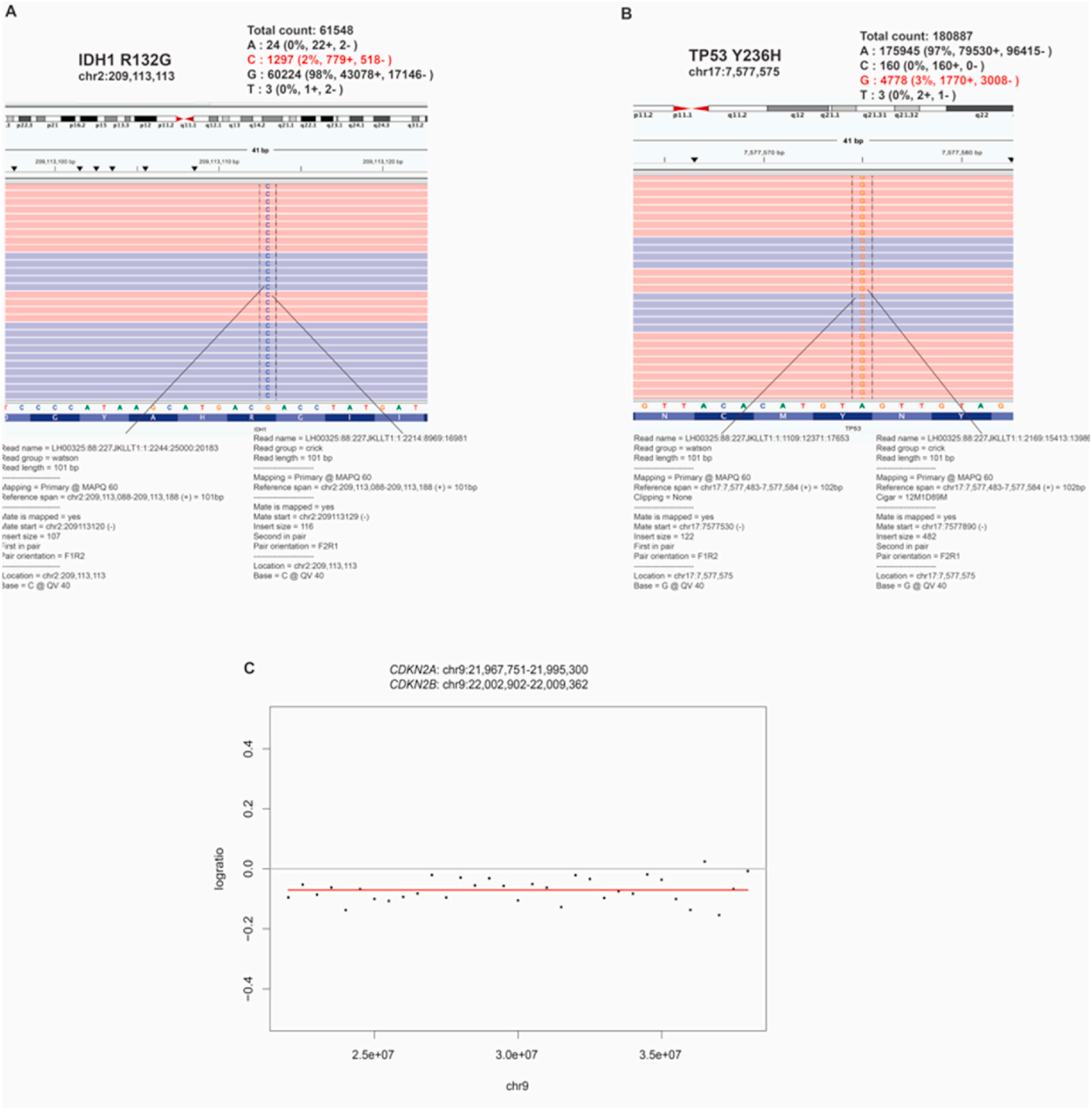

Fig. 2. Genomic alterations detected by Summit. The Belay Summit test uses duplex sequencing technology to detect single nucleotide variants (SNV), multi-nucleotide variants (MNV), and insertions/deletions in a targeted, 32-gene panel as well as low pass whole genome sequencing (WGS) to assess chromosomal arm level alterations in tumor-derived DNA isolated from cerebrospinal fluid (CSF) [5]. In a 38-year-old female with brainstem glioma, Summit identified oncogenic variants IDH1 R132G and TP53 Y236H using duplex sequencing as shown in the IGV displays (A and B). Additionally, loss of chr9p21.3, a region containing notable tumor suppressors *CDKN2A* and *CDKN2B*, was detected through WGS at a $\log_2$ of (−)0.091 as shown in the ichorDNA plot (C).

version manuscript.

Ethics statement

MY has no conflicts to disclose. AL, VU, KFS, QN and HVR are employees of Belay Diagnostics and receive a salary and stock options. Data supporting the findings of this study are available upon reasonable request.

Patient consent/patient permission

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.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors did not use generative AI and AI-assisted technologies in the writing process.

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Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Alexandra Larson, Vindhya Udhane, Kala F Schilter, Qian Nie, Honey V Reddi are employees of Belay Diagnostics and receive a salary and stock options.

References

[1] Salmaggi A, Fariselli L, Milanesi I, Lamperti E, Silvani A, Bizzi A, Maccagnano E, Trevisan E, Laguzzi E, Ruda R, Boiardi A, Soffietti R, Associazione Italiana di N-o. Natural history and management of brainstem gliomas in adults. A retrospective Italian study. J Neurol 2008;255:171–7. https://doi.org/10.1007/s00415-008-0589-0.

[2] Kesari S, Kim RS, Markos V, Drappatz J, Wen PY, Pruitt AA. Prognostic factors in adult brainstem gliomas: a multicenter, retrospective analysis of 101 cases. J Neuro Oncol 2008;88:175–83. https://doi.org/10.1007/s11060-008-9545-1.

[3] Theeler BJ, Ellezam B, Melguizo-Gavilanes I, de Groot JF, Mahajan A, Aldape KD, Bruner JM, Puduvalli VK. Adult brainstem gliomas: Correlation of clinical and molecular features. J Neurol Sci 2015;353:92–7. https://doi.org/10.1016/j.jns.2015.04.014.

[4] Zhang L, Chen LH, Wan H, Yang R, Wang Z, Feng J, Yang S, Jones S, Wang S, Zhou W, Zhu H, Killela PJ, Zhang J, Wu Z, Li G, Hao S, Wang Y, Webb JB, Friedman HS, Friedman AH, McLendon RE, He Y, Reitman ZJ, Bigner DD, Yan H. Exome sequencing identifies somatic gain-of-function PPM1D mutations in brainstem gliomas. Nat Genet 2014;46:726–30. https://doi.org/10.1038/ng.2995.

[5] Nie Q, Schilter KF, Hernandez KAJJR, Acevedo A, Larson A, Domagala BA, Vo SA, Khurana S, Mitchell K, Ellis D, Muhammedov B, Wang Y, Douville C, Coe B, Bettegowda C, Reddi HV. Analytical Validation and Clinical Sensitivity of the Belay Summit™ assay for the detection of DNA variants in cerebrospinal fluid of primary and metastatic CNS cancer. J Mol Diagn 2025.

[6] Dang L, White DW, Gross S, Bennett BD, Bittinger MA, Driggers EM, Fantin VR, Jang HG, Jin S, Keenan MC, Marks KM, Prins RM, Ward PS, Yen KE, Liau LM, Rabinowitz JD, Cantley LC, Thompson CB, Vander Heiden MG, Su SM. Cancer-associated IDH1 mutations produce 2-hydroxyglutarate. Nature 2009;462:739–44. https://doi.org/10.1038/nature08617.

[7] Ward PS, Lu C, Cross JR, Abdel-Wahab O, Levine RL, Schwartz GK, Thompson CB. The potential for isocitrate dehydrogenase mutations to produce 2-hydroxyglutarate depends on allele specificity and subcellular compartmentalization. J Biol Chem 2013;288:3804–15. https://doi.org/10.1074/jbc.M112.435495.

[8] Bouaoun L, Sonkin D, Ardin M, Hollstein M, Byrnes G, Zavadil J, Olivier M. TP53 variations in human cancers: new lessons from the IARC TP53 database and genomics data. Hum Mutat 2016;37:865–76. https://doi.org/10.1002/humu.23035.

[9] Kato S, Han SY, Liu W, Otsuka K, Shibata H, Kanamaru R, Ishioka C. Understanding the function-structure and function-mutation relationships of p53 tumor suppressor protein by high-resolution missense mutation analysis. Proc Natl Acad Sci U S A 2003;100:8424–9. https://doi.org/10.1073/pnas.1431692100.

[10] Zhang X, Li L, Li Y, Dong C, Shi J, Guo X, Sui A. The role of trimethylation on histone H3 lysine 27 (H3K27me3) in temozolomide resistance of glioma. Brain Res 2025;1846:149252. https://doi.org/10.1016/j.brainres.2024.149252.

[11] Banelli B, Daga A, Forlani A, Allemanni G, Marubbi D, Pistillo MP, Profumo A, Romani M. Small molecules targeting histone demethylase genes (KDMs) inhibit growth of temozolomide-resistant glioblastoma cells. Oncotarget 2017;8:34896–910. https://doi.org/10.18632/oncotarget.16820.

[12] https://www.fda.gov/drugs/resources-information-approved-drugs/fda-approves-vorasidenib-grade-2-astrocytoma-or-oligodendroglioma-susceptible-idh1-or-idh2-mutation.

[13] Sharma N, Mallela AN, Shi DD, Tang LW, Abou-Al-Shaar H, Gersey ZC, Zhang X, McBrayer SK, Abdullah KG. Isocitrate dehydrogenase mutations in gliomas: a review of current understanding and trials. Neurooncol Adv 2023;5:vdad053. https://doi.org/10.1093/noajnl/vdad053.

[14] Sepulveda-Sanchez JM, Gil-Gil M, Alonso-Garcia M, Vaz Salgado MA, Vicente E, Mesia Barroso C, Rodriguez Sanchez A, Duran G, De Las Penas R, Munoz-Langa J, Velasco G, Hernandez-Lain A, Hilario A, Navarro Martin M, Benavides M, Oleaga L, Cantero Montenegro D, Ruano Y, Sanchez-Gomez P, Martin-Soberon MC, Morales-Llombart R, Pachon V, Pineda E. Phase II trial of palbociclib in recurrent retinoblastoma-positive anaplastic oligodendroglioma: a study from the Spanish group for research in neuro-oncology (GEINO). Targeted Oncol 2020;15:613–22. https://doi.org/10.1007/s11523-020-00754-6.

[15] Pan C, Diplas BH, Chen X, Wu Y, Xiao X, Jiang L, Geng Y, Xu C, Sun Y, Zhang P, Wu W, Wang Y, Wu Z, Zhang J, Jiao Y, Yan H, Zhang L. Molecular profiling of tumors of the brainstem by sequencing of CSF-derived circulating tumor DNA. Acta Neuropathol 2019;137:297–306. https://doi.org/10.1007/s00401-018-1936-6.