Acta Neuropathologica Communications

CASE REPORT

Open Access

Management of asynchronous multifocal adult glioblastoma with loss of BRAF^{V600E} -mutant clonality: a case report

Hannah Haile[1,4], Pavan S. Upadhyayula[1], Esma Karlovich[2], Michael B. Sisti[1], Brian J. A. Gill[1,4] and Laura E. Donovan[3,4*]

Abstract

Glioblastoma (GBM) classification involves a combination of histological and molecular signatures including IDH1/2 mutation, TERT promoter mutation, and EGFR amplification. Non-canonical mutations such as BRAF^{V600E}, found in 1–2% of GBMs, activate the MEK-ERK signaling pathway. This mutation can be targeted by small molecule inhibitors, offering therapeutic potential for GBM. In this case report, we describe the management of a 67-year-old male with BRAF^{V600E} -mutant GBM, who experienced both local clonal and distant non-clonal BRAF^{V600E} -mutant recurrences. Initial treatment involved surgical resection followed by radiotherapy and temozolomide (TMZ). Subsequent recurrences were managed with re-resection and dabrafenib/trametinib combination therapy. Notably, a new, non-clonal BRAF^{V600E} -negative tumor developed in a distant location, highlighting the challenge of clonal evolution and resistance in GBM management. The patient's disease ultimately progressed despite multiple lines of therapy, including targeted inhibition. Identifying mechanisms of resistance and tailoring flexible treatment approaches are essential for advancing outcomes in BRAF^{V600E} -mutant GBM. This case emphasizes the value of molecular profiling in personalizing treatment for patients with multifocal disease. The evolving nature of these tumors requires persistent clinical monitoring and treatment adjustments based on tissue diagnostics.

Keywords Glioblastoma, BRAF^{V600E}, BRAF inhibitors, Clonal evolution, Targeted therapy, Glioma, Tumor heterogeneity, Resistance mechanisms, Dabrafenib, Trametinib

Introduction

The current classification of glioblastoma (GBM) relies on a combination of histological and molecular signatures, the latter of which includes an assessment for IDH1/2 mutation, TERT promoter mutation, CDKN2A/B homozygous deletion, and EGFR amplification [1]. GBMs also harbor non-canonical mutations that activate mitogenic signaling pathways. Mutations in V-raf murine viral oncogene homolog B1 (BRAF) - a serine-threonine kinase that activates the MEK-ERK signaling pathway - have been implicated in multiple cancers, including 1–2% of GBMs. The most common BRAF mutation is the substitution of valine for glutamic acid at the 600th amino acid (V600E) [2, 3]. The BRAF^{V600E}

*Correspondence:
Laura E. Donovan
led2137@cumc.columbia.edu
[1]Department of Neurological Surgery, Columbia University Irving Medical Center, New York, NY 10032, USA
[2]Department of Pathology and Cell Biology, Columbia University Irving Medical Center, New York, NY 10032, USA
[3]Department of Neuro-Oncology, Columbia University Irving Medical Center, 710 W. 168th Street, New York, NY 10032, USA
[4]Vagelos College of Physicians & Surgeons, Columbia University, New York, USA

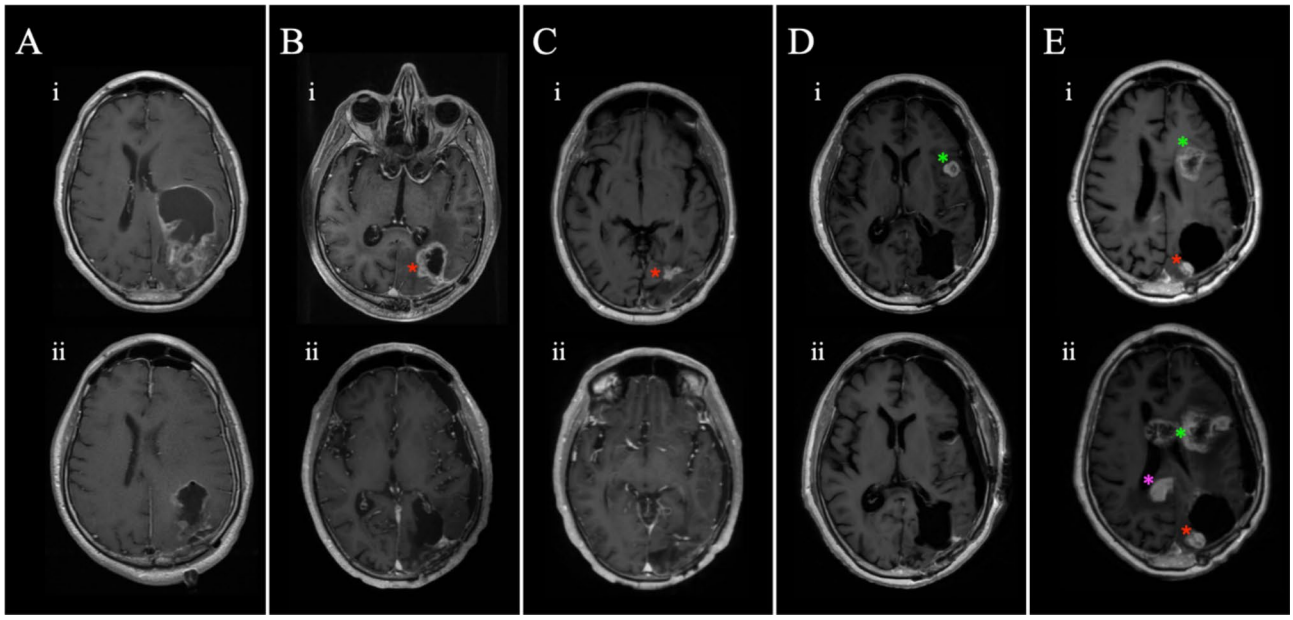

Fig. 1 Pre-operative, Interval, and Post-operative MR Images for Each Surgical Resection

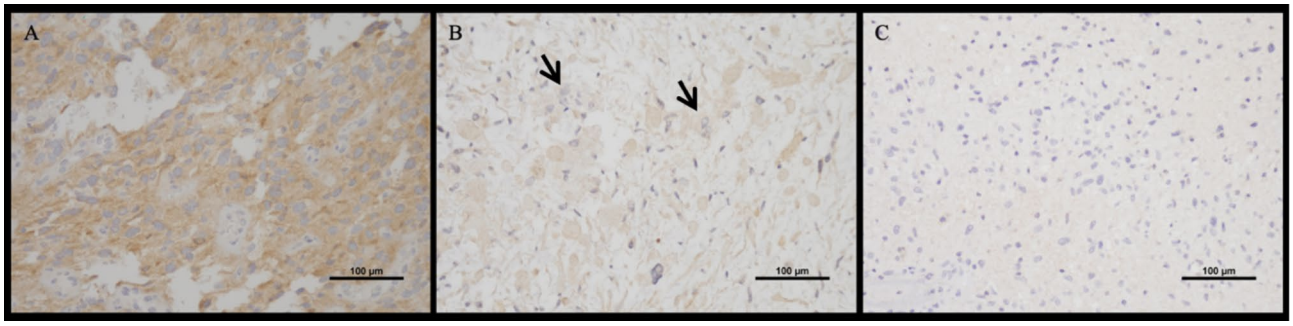

Fig. 2 $BRAF^{V600E}$ immunohistochemical analysis for each surgical resection

mutation is targetable with FDA-approved small molecule inhibitors [4], and when employed, can improve the survival in appropriately selected patients with GBM [5]. In this report, we present and describe the management of a case of $BRAF^{V600E}$ mutant GBM with evidence of local clonal and distant non-clonal recurrences.

Case description

A 67-year-old male presented with progressively worsening headaches, confusion, and left-hand tremulousness over 3 months. Brain magnetic resonance imaging (MRI) revealed a 8.6 × 4.8 × 4.3 cm heterogeneously enhancing left parietal tumor with a large cystic component (Fig. 1A). The patient underwent an uncomplicated left parietal craniotomy for resection of this lesion. His perioperative course was unremarkable. His postoperative MRI demonstrated near-total resection of the tumor (Fig. 1A). The histopathology from the resection was consistent with a GBM. Additional immunohistochemical analysis identified the presence of a $BRAF^{V600E}$ mutation (Fig. 2A; Table 1). A course of concomitant radiotherapy (RT) and temozolomide (TMZ) was initiated.

Three months after the completion of concurrent radiotherapy and TMZ (Fig. 3), a repeat MRI was consistant with disease progression according to RANO 2.0 criteria (Fig. 1B) for which he underwent re-resection [6]. Repeat immunohistochemical analysis demonstrated retention of the $BRAF^{V600E}$ mutation via weakly positive staining in a focal subset of tumor cells (Fig. 2B). In-house genetic sequencing of the tumor confirmed the presence of a $BRAF^{V600E}$ mutation (Table 1). Dabrafenib/Trametinib combination therapy was started and continued for over one year with sustained local response to treatment with near resolution of all contrast enhancement (Fig. 1C) – consistent with a partial response according to RANO 2.0 criteria [6]. 14 months after the initiation of the Dabrafenib/Trametinib therapy and 17 months after his initial chemotherapy and radiation (Fig. 3), an interval brain MRI scan demonstrated the presence of a new a 1.3 × 1.4 × 1.6 cm contrast-enhancing mass in his

Table 1 Genetic alterations in the left parietal primary, left parietal recurrent and left frontal tumors

	Left Parietal Tumor Resection*	Left Parietal Recurrent Tumor Resection		Left Frontal Tumor Resection	
	Surgical Pathology	Surgical Pathology	NGS (Columbia Solid Tumor Panel)	Surgical Pathology	NGS (CARIS)
IDH1/2 mutation	Negative (IHC)	Negative (IHC)	Negative	Negative (IHC)	Negative
BRAFV600E	Positive (IHC)	Positive (IHC)	Positive	Negative (IHC)	Negative
TERT promoter mutation	Positive (Targeted NGS)	Positive (Targeted NGS)	Positive	Positive (Targeted NGS)	Positive
CDKN2A/B deletion	Negative (FISH)	-	-	-	Negative
EGFR Amplification	Negative (FISH)	Negative (FISH)	Negative	Negative (FISH)	Negative
MGMT methylation	Partial	-	-	Positive	Positive
NF1	-	-	Negative	-	Negative
PTEN	-	-	Negative	-	Negative

*NGS was not conducted at the time of initial resection

- : Result not provided

NGS: Next generation DNA Sequencing, IHC: Immunohistochemistry, FISH: Fluorescence in situ hybridization

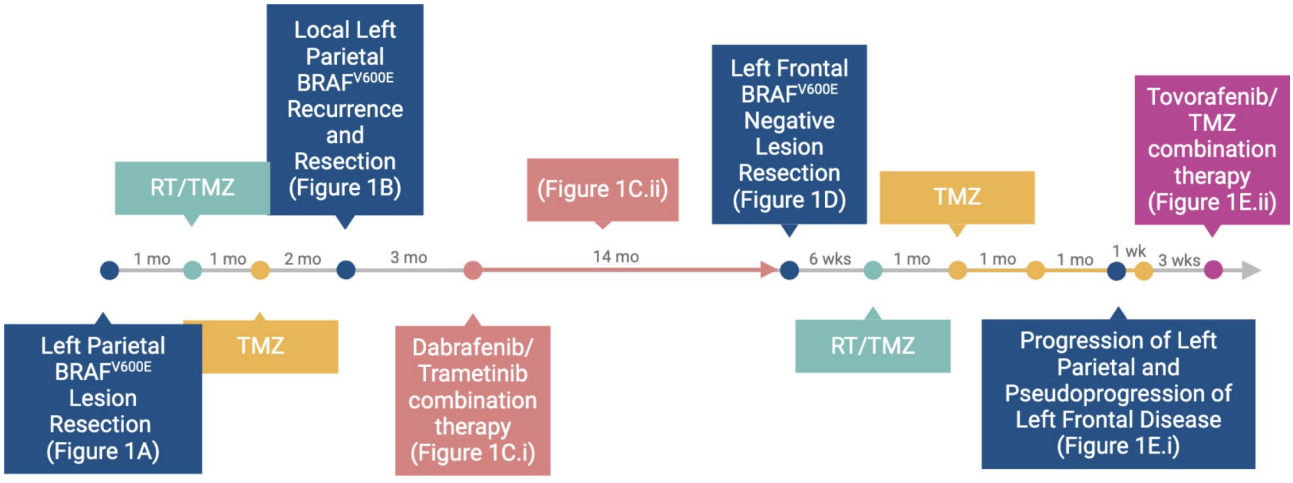

Fig. 3 Timeline

left frontal operculum, distant to the initial site of the tumor (Fig. 1D) [6]. At that time, the left parietal tumor exhibited continued local control on dabrafenib and trametinib therapy (Fig. 1D). An uncomplicated resection of the new left frontal tumor confirmed GBM. Notably, this lesion tested negative for BRAF^{V600E} mutation upon immunohistochemical analysis (Fig. 2C) and next-generation DNA sequencing (Table 1), indicating potential loss of the BRAF^{V600E} clonal population. Consequently, dabrafenib/trametinib was discontinued, and concurrent RT and TMZ were reinstated, followed by 3 cycles of adjuvant TMZ (Fig. 3). Between his 2nd and 3rd cycle of TMZ, there was radiographic evidence of disease progression at the previously controlled left parietal resection site as well as pseudoprogression at the left frontal resection site (Fig. 1E). He was initiated on combined TMZ and tovorafenib therapy to attempt to control the progression at both sites. This was well tolerated; however, neither site of disease responded to this line of treatment as evidence by further disease progression at both sites on his subsequent MRI scan (Fig. 1E).

Preoperative **(A.i)** and postoperative **(A.ii)** axial T1-weighted post-contrast images from the first resection. Preoperative **(B.i)** and postoperative **(B.ii)** axial T1-weighted post-contrast images from the second resection. **Red astersik** denotes tumor progression at parietal resection site. Axial T1-weighted post-contrast images at the initiation of **(C.i)** and 6 months after Dabrafenib/Trametinib therapy **(C.ii)**. Preoperative **(D.i)** and postoperative **(D.ii)** axial T1-weighted post-contrast

MR images from the third resection. **Green asterisk** denotes tumor progression at frontal site. Axial views of T1-weighted post-contrast images obtained after RT and two cycles of TMZ **(E.i)** and after one month of combined TMZ and tovorafenib therapy **(E.ii)**. **Purple asterisk** denotes additional sites of tumor progression.

$BRAF^{V600E}$ immunohistochemistry with 20x magnification is positive in tumor cells from the first resection **(A)**, weakly positive in a focal subset of tumor cells from the second resection **black arrows (B)**, negative in tumor cells from the third resection **(C)**.

Timeline of surgical and adjuvant therapies for the clinical case [7].

Discussion

BRAF mutations are found in up 50% of epithelioid GBMs - a distinct histologic entity associated with worse prognosis compared to traditional GBMs [3]. However, patients with BRAF-mutant GBM often have more durable treatment responses with BRAF inhibition than standard-of-care chemotherapy regimens [8]. Arbour et al. compiled a systematic review of patients treated with BRAF inhibitors for BRAF-mutated gliomas [9]. The progression free survival of the recurrent GBM patients in this review ranged from 3 to 11 months on BRAF inhibitors [9]. While this approach is promising, disease recurrence is still expected and thus it is critical to understand mechanisms for BRAF inhibitor treatment failure in GBM patients.

Kaley et al. studied vemurafenib monotherapy in the VE-BASKET trial, which included recurrent GBM patients who had previously undergone standard of care or other treatment options [10]. The response to vemurafenib monotherapy was variable in the GBM subgroup of six patients, with one patient having a partial radiographic response and two patients achieving stable disease at six months of treatment [10]. The authors hypothesize that this variability is likely due to the significant clonal heterogeneity of these tumors, with monotherapies often selecting for resistant sub-populations. Similarly, the ROAR trial by Wen et al. treated recurrent glioma patients with $BRAF^{V600E}$ mutations using a combination of dabrafenib and trametinib [11]. They reported objective response rates of 38% in their WHO grade III glioma and 32% in their glioblastoma cohorts. However, the median progression free survival was 3.8 months and 2.8 months respectively suggesting that these responses were not durable [11]. Neither study described whether the treatment failures were local.

Resistance to BRAF inhibitors in glioma is thought to be mediated via various mechanisms, which can emerge adaptively during treatment. One such mechanism involves secondary mutations within the BRAF gene itself. For instance, the $BRAF^{L514V}$ mutation, identified after progression in a patient treated with dabrafenib, induces ERK signaling and promotes RAF dimer formation, leading to resistance to the inhibitor [12]. Additionally, upregulation of CRAF has been validated as a resistance mechanism in glioma samples, suggesting that alterations in RAF isoforms contribute to treatment failure [13]. Mechanisms such as loss of PTEN or NF1, although tested for and found negative in this patient's case, are known to confer resistance by maintaining ERK pathway signaling [13]. Furthermore, autophagy has been implicated as an alternative resistance pathway; pharmacologic inhibition of autophagy using chloroquine has shown efficacy in overcoming resistance to BRAF inhibitors, demonstrating the complexity of resistance mechanisms [14].

The case described in this report highlights clonal evolution and selection as potential contributors to disease progression and failure of BRAF-inhibitor therapy in GBM. Similar instances of this have been reported in the literature [10]; however, this report - to the best of our knowledge – may represent the first instance in which this has been identified in two non-contiguous lesions. While our patient did not respond to tovorafenib after progression at the original site of $BRAF^{V600E}$ mutant disease, the recurrent left parietal disease was not biopsied. This could represent a lack of response to second-line BRAF inhibition; however, a loss of BRAF mutation at this site cannot be excluded. Although this report did not include testing for CBL or RAF upregulation, the presence of such undetected mechanisms at the second site could have contributed to the observed resistance. Understanding the mechanisms of resistance and selecting appropriate, adaptive treatment strategies remain critical for improving outcomes in BRAF-mutated GBM. This case underscores these principles and demonstrates the pivotal role of molecular profiling in tailoring targeted therapies for patients with multifocal lesions. Nonetheless, the findings should be interpreted with an understanding of the limitations inherent to this case, including that while molecular testing was performed, not every potential resistance mechanism was evaluated. The dynamic nature of the tumor in these cases necessitates ongoing clinical evaluation, and adaptive treatment strategies guided by updated tissue diagnosis.

Abbreviations

BRAF	V-raf murine viral oncogene homolog B1
FISH	Fluorescence in situ hybridization
GBM	Glioblastoma
IHC	Immunohistochemistry
MRI	Magnetic resonance imaging
NGS	Next generation DNA Sequencing
RT	Radiotherapy
TMZ	Temozolomide
V600E	Substitution of valine for glutamic acid at the 600th amino acid

Acknowledgements
Not applicable.

Author contributions
HH and PSU collected the case information for the report and contributed to the writing of the manuscript. EK performed the histological evaluation of the tumor tissue and provided editorial feedback on the manuscript. BJAG, MBS, and LED were involved in the patient care and provided guidance and editorial feedback on the manuscript. All authors read and approved the final manuscript.

Funding
LD is supported in part by The William Rhodes and Louise Tilzer-Rhodes Center for Glioblastoma at NewYork-Presbyterian Hospital and the NCI-designated Herbert Irving Comprehensive Cancer Center, andthe Matheson Foundation. BG is supported in part by the William Rhodes and Louise Tilzer-Rhodes Center for Glioblastoma at NewYork-Presbyterian Hospital and the NCI-designated Herbert Irving Comprehensive Cancer Center. The Michael Weiner GBM Research Into Treatment Fund.

Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
The patient has consented to the use of their information for the purposes of this case report and a signed consent form is available for review upon request.

Competing interests
The authors declare no competing interests.

Received: 3 October 2024 / Accepted: 18 November 2024
Published online: 28 January 2025

References

1. Louis DN, Perry A, Wesseling P, Brat DJ, Cree IA, Figarella-Branger D, Hawkins C, Ng HK, Pfister SM, Reifenberger G, Soffietti R, von Deimling A, Ellison DW (2021) The 2021 WHO classification of tumors of the Central Nervous System: a summary. Neuro Oncol 23(8):1231–1251. https://doi.org/10.1093/neuonc/noab106
2. Behling F, Barrantes-Freer A, Skardelly M, Nieser M, Christians A, Stockhammer F, Rohde V, Tatagiba M, Hartmann C, Stadelmann C, Schittenhelm J (2016) Frequency of BRAF V600E mutations in 969 central nervous system neoplasms. Diagn Pathol 11(1):55. https://doi.org/10.1186/s13000-016-0506-2
3. Kleinschmidt-DeMasters BK, Aisner DL, Birks DK, Foreman NK (2013) Epithelioid GBMs show a high percentage of BRAF V600E Mutation. Am J Surg Pathol 37(5):685–698. https://doi.org/10.1097/PAS.0b013e31827f9c5e
4. Schreck KC, Grossman SA, Pratilas CA (2019) BRAF mutations and the utility of RAF and MEK inhibitors in primary brain tumors. Cancers (Basel) 11(9). https://doi.org/10.3390/cancers11091262
5. Schreck KC, Langat P, Bhave VM, Li T, Woodward E, Pratilas CA, Eberhart CG, Bi WL (2023) Integrated molecular and clinical analysis of BRAF-mutant glioma in adults. NPJ Precis Oncol 7(1):23. https://doi.org/10.1038/s41698-023-00359-y
6. Wen PY, Bent Mvd, Youssef G, Cloughesy TF, Ellingson BM, Weller M, Galanis E, Barboriak DP, Groot Jd, Gilbert MR, Huang R, Lassman AB, Mehta M, Molinaro AM, Preusser M, Rahman R, Shankar LK, Stupp R, Villanueva-Meyer JE, Chang SM (2023) RANO 2.0: update to the Response Assessment in Neuro-Oncology Criteria for High- and low-Grade gliomas in adults. J Clin Oncol 41(33):5187–5199. https://doi.org/10.1200/jco.23.01059
7. Figure 3. Adapted from (2024) Icon Pack - Timelines (Horizontal), by BioRender.com Retrieved from https://app.biorender.com/biorender-templates. In
8. Lin Z, Xu H, Yang R, Li Z, Zheng H, Zhang Z, Peng J, Zhang X, Qi S, Liu Y, Huang G (2022) Effective treatment of a BRAF V600E-mutant epithelioid glioblastoma patient by vemurafenib: a case report. Anticancer Drugs 33(1):100–104. https://doi.org/10.1097/cad.0000000000001130
9. Arbour G, Ellezam B, Weil AG, Cayrol R, Vanan MI, Coltin H, Larouche V, Erker C, Jabado N, Perreault S (2022) Upfront BRAF/MEK inhibitors for treatment of high-grade glioma: a case report and review of the literature. Neuro-Oncology Adv 4(1). https://doi.org/10.1093/noajnl/vdac174
10. Kaley T, Touat M, Subbiah V, Hollebecque A, Rodon J, Lockhart AC, Keedy V, Bielle F, Hofheinz R-D, Joly F, Blay J-Y, Chau I, Puzanov I, Raje NS, Wolf J, DeAngelis LM, Makrutzki M, Riehl T, Pitcher B, Hyman DM (2018) BRAF Inhibition in BRAFV600-Mutant gliomas: results from the VE-BASKET study. J Clin Oncol 36(35):3477–3484. https://doi.org/10.1200/jco.2018.78.9990
11. Wen PY, Stein A, van den Bent M, De Greve J, Wick A, de Vos F, von Bubnoff N, van Linde ME, Lai A, Prager GW, Campone M, Fasolo A, Lopez-Martin JA, Kim TM, Mason WP, Hofheinz RD, Blay JY, Cho DC, Gazzah A, Subbiah V (2022) Dabrafenib plus Trametinib in patients with BRAF(V600E)-mutant low-grade and high-grade glioma (ROAR): a multicentre, open-label, single-arm, phase 2, basket trial. Lancet Oncol 23(1):53–64. https://doi.org/10.1016/s1470-2045(21)00578-7
12. Wang J, Yao Z, Jonsson P, Allen AN, Qin ACR, Uddin S, Dunkel IJ, Petriccione M, Manova K, Haque S, Rosenblum MK, Pisapia DJ, Rosen N, Taylor BS, Pratilas CA (2018) A secondary mutation in BRAF confers resistance to RAF Inhibition in a BRAFV600E-Mutant brain tumor. Cancer Discov 8(9):1130–1141. https://doi.org/10.1158/2159-8290.CD-17-1263
13. Schreck KC, Morin A, Zhao G, Allen AN, Flannery P, Glantz M, Green AL, Jones C, Jones KL, Kilburn LB, Nazemi KJ, Samuel D, Sanford B, Solomon DA, Wang J, Pratilas CA, Nicolaides T, Levy M, J. M (2021) Deconvoluting mechanisms of Acquired Resistance to RAF inhibitors in BRAFV600E-Mutant human glioma. Clin Cancer Res 27(22):6197–6208. https://doi.org/10.1158/1078-0432.CCR-21-2660
14. Mulcahy Levy JM, Zahedi S, Griesinger AM, Morin A, Davies KD, Aisner DL, Kleinschmidt-DeMasters BK, Fitzwalter BE, Goodall ML, Thorburn J, Amani V, Donson AM, Birks DK, Mirsky DM, Hankinson TC, Handler MH, Green AL, Vibhakar R, Foreman NK, Thorburn A (2017) Autophagy inhibition overcomes multiple mechanisms of resistance to BRAF inhibition in brain tumors. eLife 6:e19671. https://doi.org/10.7554/eLife.19671

Publisher's note