Case report

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Glioblastoma with *BRAF*^{V600E} mutation and numerous metastatic foci: a case report

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Abstract

Glioblastoma, the most malignant astrocytic tumour, is associated with limited survival and thus rare metastases. We analysed a particularly interesting case – a 51-year-old male diagnosed within 2 years with primary and recurrent glioblastoma, isocitrate dehydrogenase (IDH)-wild type, as well as with numerous extra-central nervous system (CNS) metastatic foci. Genetic material obtained from primary and recurrent tumours, as well as from pulmonary metastasis was analysed and compared at a molecular level. Next generation sequencing (NGS) analysis revealed BRAFV600E mutation, detected only in 2-5% of glioblastomas, in both the primary tumour and pulmonary metastases. Importantly, this mutation provides a possible therapeutic option as it constitutes a target for clinically approved inhibitors. This case study not only demonstrates a molecular comparison of primary, recurrent and metastatic glioblastoma, but also emphasizes the need for precise molecular diagnostics, which may facilitate treatment choice, especially in tumours currently lacking efficient treatment.

Key words: *glioblastoma, BRAF*^{V600E}*, next generation sequencing.*

Introduction

Glioblastoma (GB), the most malignant astrocytic tumour, constitutes one of the biggest challenges in the oncology field. Its infiltrative nature and location in the brain often forbid complete surgical resection and even with continuous developments in diagnostic and therapeutic approaches, median survival rate of patients still does not exceed one year [18]. Intriguingly, GB metastases are rarely detected and if so, tumours are mostly located within the neuroaxis [5]. Extra-central nervous system (CNS) metastases (only up to 2% of cases) can be usually found in lungs, regional lymph nodes or bones [3,15,17]. Moreover, it was indicated that such tumours are characterized with different vasculature than primary focus [23]. Such rare occurrence of distant metastases may be caused by the fact that the brain is immunologically and anatomically separated by the blood-brain barrier – semipermeable membrane impeding access to the brain. Nevertheless, despite the fact that the exact mechanism of extracerebral metastasis has been poorly understood [22], this is mostly detected in patients with a longer survival

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rate [1] or tending to be associated with disruption of the cellular integrity during surgical procedures [12]. Currently, standard GB treatment involves the maximal feasible surgical resection followed by 60 Gy radiotherapy with concomitant and adjuvant temozolomide-based chemotherapy [26]. In case of glioblastoma patients with no effective therapeutic options available, off-label use of targeted drugs tends to be exceptionally employed, especially since particular targetable molecular alterations are detected in this tumour type. Indeed, *BRAF*^{V600E}, mutation that occurs most commonly in melanomas [2], is detected in pleomorphic xanthoastrocytomas [11] and in low percentage of glioblastomas [9]. Therefore, mutation-specific inhibitors (such as vemurafenib or dabrafenib) can be possibly effective not only in melanoma brain metastases [16], but also glioblastoma tumours with this mutation [6]. Still, administration of targeted therapeutics requires the precise molecular diagnostics of the tumour.

In this paper, a case of glioblastoma patient diagnosed with primary tumour, recurrence and extra-CNS metastases is presented. The aim of the conducted analyses was to define molecular background of this tumour, corresponding with the acquisition of metastasis-prone features, and to molecularly compare the three tumour foci (primary, recurrent and metastatic).

Case presentation

Clinical summary

A 51-year-old non-smoking Caucasian male was admitted to the hospital due to headaches, memory impairment, dropping right mouth corner, psychomotor retardation and confusion. Computed tomography (CT) and magnetic resonance imaging (MRI) revealed a tumour in the right temporal lobe (Fig. 1A,B). The tumour was macroscopically completely resected.

Histopathological analysis shown highly cellular neoplasm with marked cytological atypia, high mitotic activity, focal geographic and pseudopalisading necrosis and extensive microvascular proliferations highlighted by the reticulin stain (Fig. 1C,D). Morphology, immunohistochemical and histochemical stains were consistent with the diagnosis of glioblastoma [GFAP/+/; Ki-67/+/ up to 20% of cells; p53/+/ (Fig. 1E)]. Further sequencing analysis of *IDH1* gene indicated isocitrate dehydrogenase

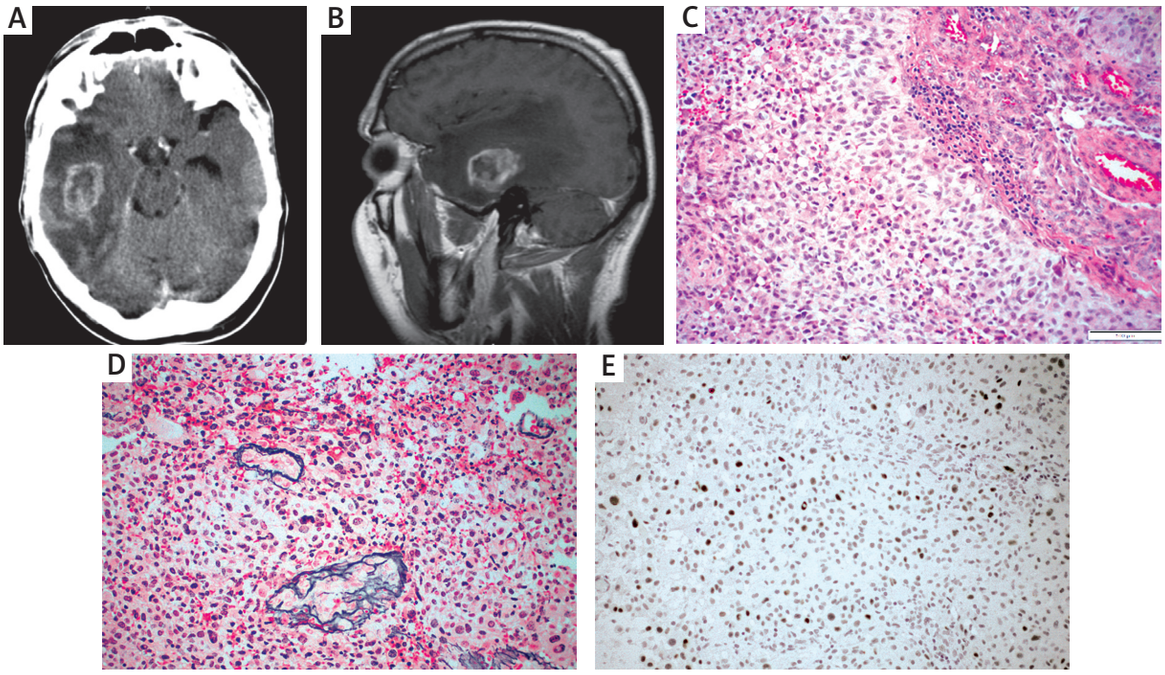

Fig. 1. Primary tumour (35 × 25 × 26 mm) detected in CT (**A**) and MRI (**B**) scans in the right temporal lobe presenting with heterogeneous ring enhancement and massive oedema; H&E (**C**), reticulin (**D**) and TP53 (**E**) stainings of the primary tumour.

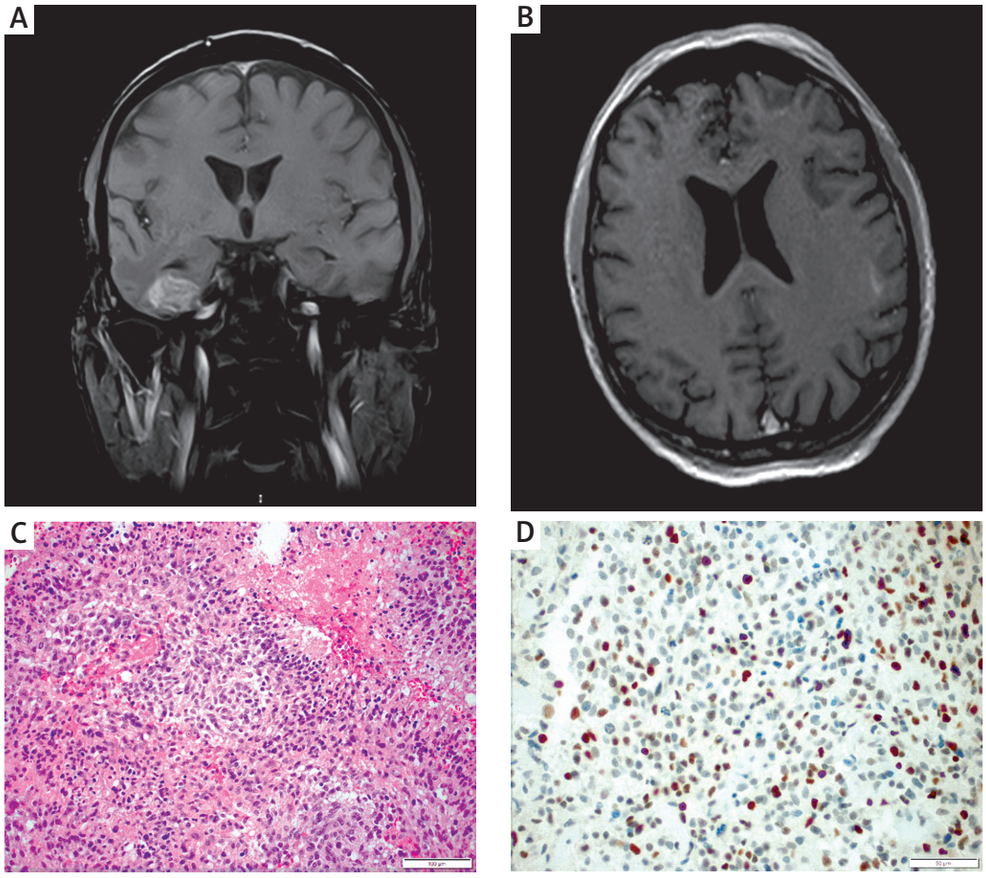

Fig. 2. Recurrent tumour (20 × 35 × 16 mm) detected in MRI in the right temporal lobe (**A**) and possible new focus in the left parietal lobe (**B**); H&E (**C**) and TP53 (**D**) stainings of the recurrent tumour.

(IDH)-wild type glioblastoma, according to the latest WHO classification [14]. The patient was qualified to adjuvant radiotherapy (60 Gy/30 fractions) and temozolomide treatment (150 mg/m^2/day). After 16 months, radiological follow-up revealed recurrent tumour at the base of the right temporal lobe (Fig. 2A) and possible new focus in the left parietal lobe (Fig. 2B). Histochemical and immunohistochemical analyses indicated glioblastoma with TP53 accumulation (Fig. 2C,D). The patient was under strict observation, however, after three months he was admitted to the hospital once again complaining of nonproductive cough, general weakness, night sweats and shivers. Initial diagnosis suggested pulmonary embolism, however, angio-CT revealed diffuse foci in parenchyma of both lungs (Fig. 3A). Histopathological analysis of bronchoscopically collected material confirmed glioblastoma (Fig. 3B,C). Additional tomograms revealed numerous metastatic foci located e.g. in iliacus muscle (Fig. 3D), thoracic vertebrae and soft tissues of lower limbs (data not shown). Material from primary and recurrent tumour samples as well as lung metastatic focus was then delivered to the laboratory in order to isolate DNA and conduct precise molecular analyses, with the emphasis on possible targets of experimental therapy. Unfortunately, the patient died 3.5 months following metastases detection, 90 weeks after the initial diagnosis.

Molecular analyses

All analyses concerning tumour material were approved by the Bioethical Committee of the Medical University of Lodz (Approval No. RNN/234/17/KE). QIAamp DNA Mini Kit (Qiagen) was used for DNA isolation from formalin-fixed and paraffin-embedded tissues prepared for primary tumour, recurrence and lung metastasis. Isolation was preceded by the deparaffinization step with xylene (Sigma). DNA was ana-

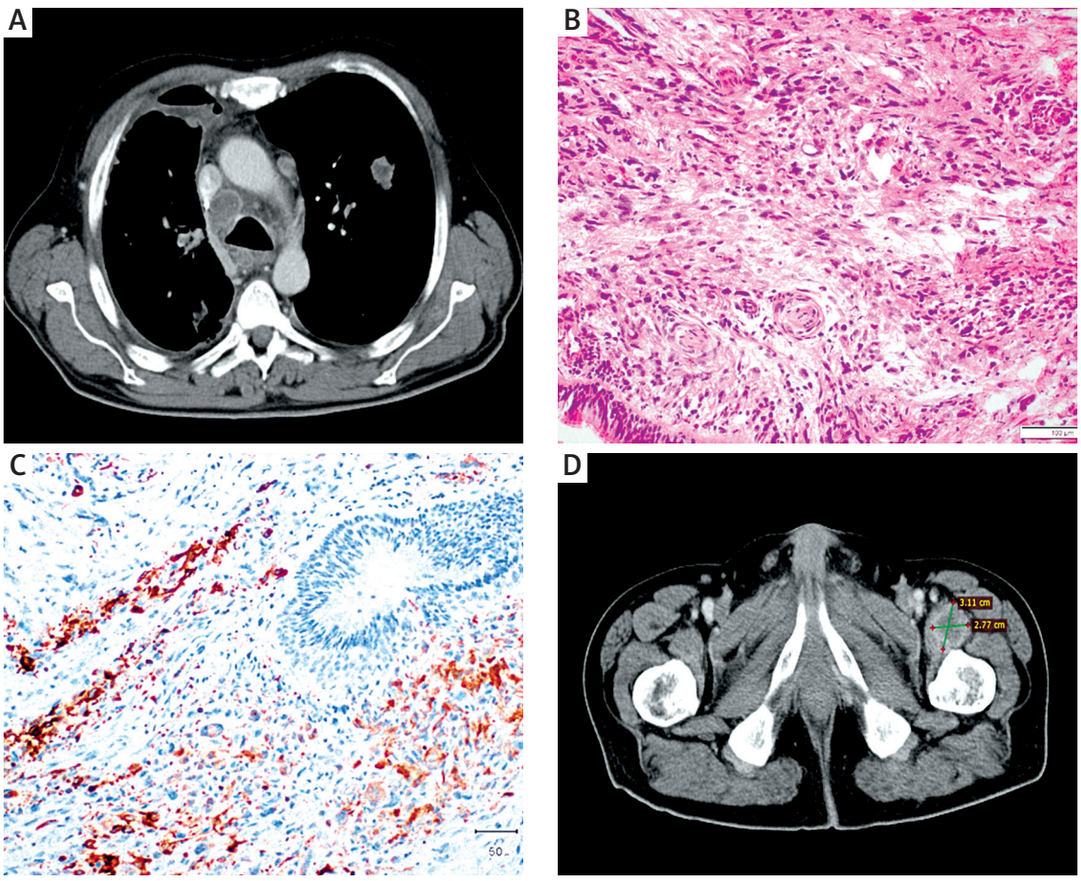

Fig. 3. Angio-CT image of lung metastases (**A**); Histopathology of tumour metastasis to the lung (**B**) revealed nests of malignant cells with strong GFAP positivity (**C**); Another metastatic focus in iliacus muscle (**D**).

lysed using a wide range of molecular techniques, as described previously [24,25], what enabled to compare molecular profiles of these three tumour foci. *BRAF* gene sequencing was conducted with the following primer pair: 5'-AACTCTTCATAATGCTTGCTCTGAT-3' and 5'GTAACTCAGCAGCATCTCAGGG-3'. MLPA analysis using P105 Glioma-2 and P175 Tumor Gain probemixes (MRC Holland) revealed various alterations in the copy number of glioblastoma-associated genes, e.g. *EGFR* amplification or *CDKN2A* and *PTEN* deletion (Fig. 4). Unequivocal identification of the pulmonary mass origin was, however, impossible based on MLPA results only. Therefore, next generation sequencing (NGS) analysis using AmpliSeq Cancer Hotspot Panel v2 (Life Technologies) was performed. This revealed non-hotspot mutations (homozygous in *FLT3* and heterozygous in *IDH1* (synonymous) and *APC*; Table I) in all analysed samples. Other hotspot mutations were detected in *EGFR* (R776C) and *PTPN11* (E69K) in the recurrent tumour and in *EGFR* (W731* – nonsense mutation leading to the formation of stop codon) and *TP53* (M246I) in lung metastasis. Despite the fact that *TP53* mutation was detected neither in primary nor in recurrent tumour in sequencing analyses, IHC analysis for TP53 turned out to be clearly positive. It is consistent with previous report indicating that in case of gliomas, not only *TP53* mutations, but also disturbed TP53 pathway (e.g. by *MDM2* or *MDM4* amplification as well as promoter methylation of *CDKN2A* or *TP53*) may result in abnormal TP53 expression, hence positive IHC staining [28].

Interestingly, a hotspot mutation in *BRAF* gene (V600E) was detected in the primary tumour (20.7%) and lung metastasis (16.2%), which clearly confirmed the origin of metastatic focus. This codon was additionally re-analysed using Sanger sequencing in an attempt to verify the status of *BRAF* gene

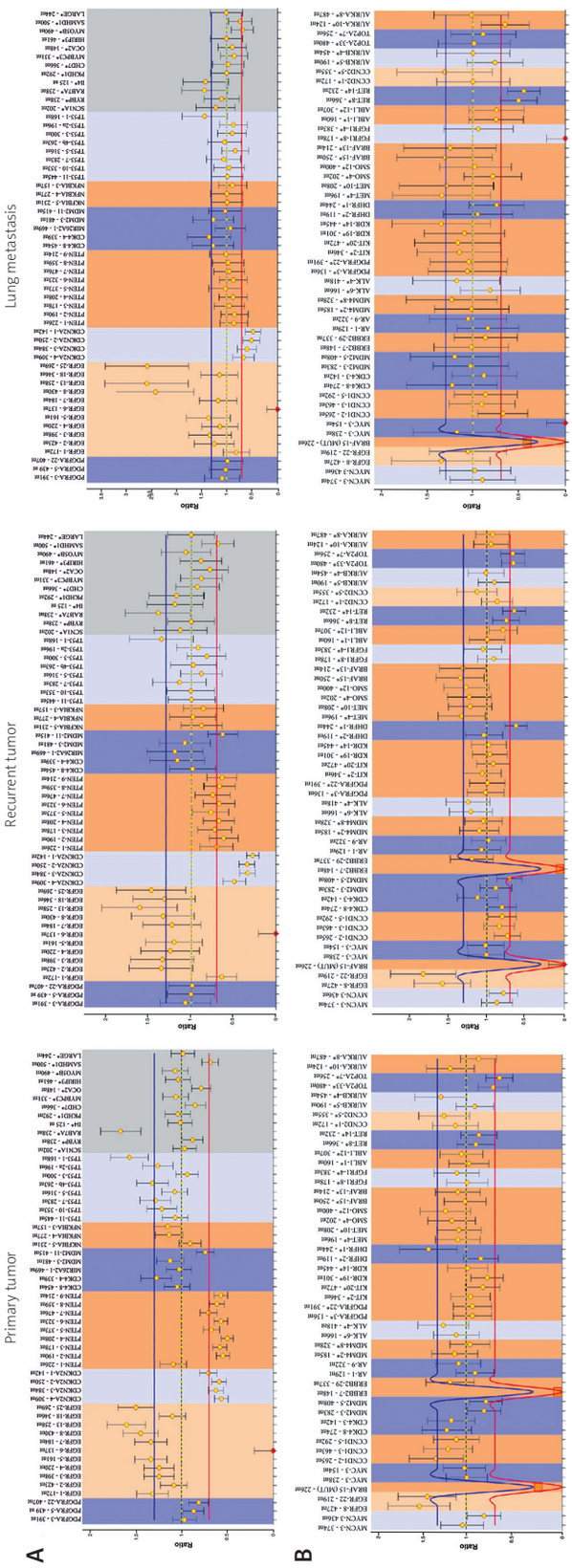

Fig. 4. MLPA analyses of three tumour foci using P105 Glioma-2 (**A**) and P175 Tumor Gain (**B**) probemixes.

in the recurrent tumour with the latter method. For this purpose, DNA isolated from SK-MEL-28 cell line (ATCC), characterized by endogenous homozygous *BRAF*^{V600E} mutation, and from BJ fibroblasts (control wild-type cells; ATCC) was used to obtain dilution series with various percentage of mutated template, in order to verify the detection threshold of Sanger sequencing. Results indicated that in all analysed samples, the percentage of *BRAF*-mutated template was below or near Sanger detection threshold, estimated to be 10-20% of mutated template [7]. Therefore, only NGS results were considered. To be consistent with the latest WHO classification, IDH1 status was also evaluated. Both, NGS and additional Sanger sequencing analysis revealed that all analysed tumour foci were IDH-wild type. Importantly, all the remaining mutations detected by means of NGS in both primary and recurrent tumours or recurrent and metastatic foci were novel (non-hotspot).

Discussion

Glioblastoma, despite its highly aggressive nature, is rarely associated with detectable metastases. Nonetheless, with expected progress in treatment of patients and more precise diagnostic imaging methods in common practice, there will be probably more cases with detected GB metastases. Nevertheless, so far, extra-CNS glioblastoma foci still constitute an interesting research material.

Glioblastoma metastases outside the CNS frequently tend to occur late in the disease course, with patients survival rates reaching approximately two years [1,22]. Therefore, the long survival of the described patient (22.5 months) might have been associated with detection of various metastatic foci. In this case it is not clear whether the tumour location in the frontal lobe close to the frontal corner of the lateral ventricle might have facilitated tumour spread *via* cerebrospinal fluid. However, it may be suggested by the presence of a metastatic focus in the parietal lobe of the opposite hemisphere. Moreover, in case of the analysed patient, extra-CNS metastases, detected e.g. in lungs or kidneys, might have been caused by hematogenous spread *via* sphenoparietal or superior petrosal sinus, etc. [12,21]. Neverthe-

Table I. Selected results of NGS analysis of primary tumour, recurrent tumour and lung metastasis. Results with coverage > 100 were taken into consideration; nd – not detected, reference sequence only; (–) – records excluded from the analysis, not meeting the established technical criteria

CHR.	Position	REF	VAR	Allele Call	Allele source	Allele name	Gene ID	Primary tumour		Recurrent tumour		Metastasis	
								FREQ.	COV.	FREQ.	COV.	FREQ.	COV.
2	209113192	G	A	Hetero	Novel	–	*IDH1*	57.7	234	57.8	526	54.6	1402
2	209113113	G	A	Absent	Hotspot	COSM28747	*IDH1*	nd	573	nd	714	nd	251
2	212812097	T	C	Hetero	Novel	–	*ERBB4*	37.6	149	54.1	486	–	–
5	112175770	G	A	Hetero	Novel	–	*APC*	50.9	169	50.7	369	49.9	959
7	140453136	A	T	Hetero	Hotspot	COSM476	*BRAF*	20.7	116	–	–	16.2	210
7	55249028	C	T	Hetero	Hotspot	COSM6226	*EGFR*	–	–	23.2	164	–	–
7	55242423	G	A	Hetero	Hotspot	COSM13432	*EGFR*	–	–	–	–	3.1	128
10	43613843	G	T	Hetero	Novel	–	*RET*	100.0	195	100.0	417	–	–
12	112888189	G	A	Hetero	Hotspot	COSM13013	*PTPN11*	–	–	12.2	1201	–	–
13	28610183	A	G	Homo	Novel	–	*FLT3*	100.0	169	100.00	401	100.0	800
17	7577543	C	T	Hetero	Hotspot	COSM44310	*TP53*	–	–	–	–	3.5	142

less, when considering all the factors that may have an impact on the pattern of recurrence, including its location (local re-growth *vs.* new focus), surgical resection range, possible ventricular entry, TMZ administration, long progression-free survival or GB recurrence spreading, as in the case of this patient, it still remains difficult for interpretation [10]. Although the profound molecular analysis was not necessary to confirm the same origin of the metastases and primary tumour in the reported case, such molecular comparison not only indicates possible therapeutic options but also enables better understanding of glioblastomas biology.

Molecular analyses of patient's tumour samples revealed a hotspot mutation in the *BRAF* gene (*BRAF*^{V600E}), which is frequently detected in melanoma [2] and constitutes a perfect target for specific small molecule inhibitors, such as vemurafenib or dabrafenib. Interestingly, various alterations in *BRAF* gene are found in gliomas, with *BRAF*^{V600E} mutation present in up to 50% of pleomorphic xanthoastrocytoma [11] and in 2-5% of glioblastomas [9] (with more than half of cases of the epithelioid glioblastoma type). There are several reports of anticancer efficacy of BRAF^{V600E}-targeted inhibitors in patients diagnosed with melanoma metastases to the brain, indicating that these drugs may penetrate CNS tumours [16]. There are also single, successful reports of vemurafenib administration in BRAF^{V600E}-positive anaplastic pleomorphic xanthoastrocytoma [13] and glioblastoma [6,20]. It is not clear whether this mutation may be associated with metastatic potential. Therefore, it may be suggested to put emphasis on analyses of molecular profiles of patients with detected glioblastoma metastases.

So far, there has never been such a large group of GB patients with detected metastases to enable correlation of some molecular markers with metastasis occurrence. There was one report presenting analysis of several genetic alterations in six GB patients, suggesting that *TP53* mutation may have an impact on metastases occurrence [19]. Beyond any doubt one case may not constitute a basis to confirm that BRAF mutation may be associated with longer survival rate and metastasis development. This is, however, in line with literature data indicating that glioblastoma patients harbouring *BRAF*^{V600E} mutation tend to be characterized by prolonged survival, even up to 4 years following diagnosis [27], as well as by younger age [8].

In case of the analysed patient, BRAF^{V600E} inhibitor administration as an adjuvant therapy might have possibly prolong survival. Moreover, hotspot mutations detected only in the recurrent tumour (EGFR R776C and PTPN11 E69K) might have a significant clinical impact. The former hotspot is reported to be associated with reduced sensitivity to particular EGFR TKIs, while the latter constitutes a possible target for SHP2 inhibitors, which are currently under extensive development. Undoubtedly, as early detection of molecular targets as possible is crucial in cancer management. Analyses of circulating tumour DNA,

tumour microRNAs or circulating tumour cells may constitute an interesting option in detection of new, targetable molecular alterations, especially those conferring drug resistance [4].

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Disclosure

The authors report no conflict of interest.

References

1. Anghileri E, Castiglione M, Nunziata R, Boffano C, Nazzi V, Acerbi F, Finocchiaro G, Eoli M. Extraneural metastases in glioblastoma patients: two cases with YKL-40-positive glioblastomas and meta-analysis of the literature. Neurosurg Rev 2016; 39: 37-45.
2. Ascierto PA, Kirkwood JM, Grob JJ, Simeone E, Grimaldi AM, Maio M, Palmieri G, Testori A, Marincola FM, Mozzillo N. The role of BRAF V600 mutation in melanoma. J Transl Med 2012; 10: 85.
3. Awan M, Liu S, Sahgal A, Das S, Chao ST, Chang EL, Knisely JP, Redmond K, Sohn JW, Machtay M, Sloan AE, Mansur DB, Rogers LR, Lo SS. Extra-CNS metastasis from glioblastoma: a rare clinical entity. Expert Rev Anticancer Ther 2015; 15: 545-552.
4. Barciszewska AM. MicroRNAs as efficient biomarkers in high-grade gliomas. Folia Neuropathol 2016; 54: 369-374.
5. Beauchesne P. Extra-neural metastases of malignant gliomas: myth or reality? Cancers (Basel) 2011; 3: 461-477.
6. BebaAbadal K, Walsh MA, Yachnis AT, Tran DD, Ghiaseddin AP. Eleven month progression-free survival on vemurafenib monotherapy in a patient with recurrent and metastatic BRAF V600E-mutated glioblastoma WHO grade 4. JCO Precis Oncol 2017; 1: 1-5.
7. Beck TF, Mullikin JC, NISC Comparative Sequencing Program, Biesecker LG. Systematic evaluation of sanger validation of next-generation sequencing variants. Clin Chem 2016; 62: 647-654.
8. Behling F, Barrantes-Freer A, Skardelly M, Nieser M, Christians A, Stockhammer F, Rohde V, Tatagiba M, Hartmann C, Stadelmann C, Schittenhelm J. Frequency of BRAF V600E mutations in 969 central nervous system neoplasms. Diagn Pathol 2016; 11: 55.
9. Brennan CW, Verhaak RG, McKenna A, Campos B, Noushmehr H, Salama SR, Zheng S, Charkravarty D, Sanborn JZ, Breman SH, Schittenhelm J. The somatic genomic landscape of glioblastoma. Cell 2013; 55: 462-477.
10. Chan DT, Hsieh SY, Kam MK, Cheung TC, Ng SC, Poon WS. Pattern of recurrence and factors associated with cerebrospinal fluid dissemination of glioblastoma in Chinese patients. Surg Neurol Int 2016; 7: 92.
11. Dias-Santagata D, Lam Q, Vernovsky K, Vena N, Lennerz JK, Borger DR, Batchelor TT, Ligon KL, Iafrate AJ, Ligon AH, Louis DN, Santagata S. BRAF V600E mutations are common in pleomorphic xantoastrocytoma: diagnostic and therapeutic implications. PLoS One 2011; 6: e17948.
12. Hamilton JD, Rapp M, Schneiderhan T, Sabel M, Hayman A, Scherer A, Kropil P, Budach W, Gerber P, Kretschmar U, Prabhu S, Ginsberg LE, Bölke E, Matuschek C. Glioblastoma multiforme metastasis outside the CNS: three case reports and possible mechanisms of escape. J Clin Oncol 2014; 32: e80-84.
13. Lee EQ, Ruland S, LeBoeuf NR, Wen PY, Santagata S. Successful treatment of progressive BRAF V600E-mutated anaplastic pleomorphic xanthoastrocytoma with vemurafenib monotherapy. J Clin Oncol 2016; 34: e87-89.
14. Louis DN, Perry A, Reifenberger G, von Deimling A, Figarella-Branger D, Cavenee WK, Ohgaki H, Wiestler OD, Kleihues P, Ellison DW. The 2016 World Health Organization Classification of Tumors of the Central Nervous System: a summary. Acta Neuropathol 2016; 131: 803-820.
15. Lun M, Lok E, Gautam S, Wu E, Wong ET. The natural history of extracranial metastasis from glioblastoma multiforme. J Neurooncol 2011; 105: 261-273.
16. McArthur GA, Maio M, Arance A, Nathan P, Blank C, Avril MF, Garbe C, Hauschild A, Schadendorf D, Hamid O, Fluck M, Thebeau M ,Schachter J, Kefford R, Chamberlain M, Makrutzki M, Robson S, Gonzalez R, Margolin K. Vemurafenib in metastatic melanoma patients with brain metastases: an open-label, single-arm, phase 2, multicenter study. Ann Oncol 2017; 28: 634-641.
17. Mondin V, Ferlito A, Devaney KO, Woolgar JA, Rinaldo A. A survey of metastatic central nervous system tumors to cervical lymph nodes. Eur Arch Otorhinolaryngol 2010; 267: 1657-1666.
18. Ostrom QT, Glittleman H, Xu J, Kromer C, Wolinsky Y, Kruchko C, Barnholtz-Sloan JS. CBTRUS statistical report: primary brain and other central nervous system tumors diagnosed in the United States in 2009-2013. Neuro Oncol 2016; 18: v1-v75.
19. Park CC, Hartmann C, Folkerth R, Loeffler JS, Wen PY, Fine HA, Black PM, Shafman T, Louis DN. Systemic metastasis in glioblastoma may represent the emergence of neoplastic subclones. J Neuropathol Exp Neurol 2000; 59: 1044-1050.
20. Preusser M, Bienkowski M, Birner P. BRAF inhibitors in BRAF-V600E mutated primary neuroepithelial brain tumors. Expert Opin Investig Drugs 2016; 25: 7-14.
21. Schweitzer T, Vince GH, Herbold C, Roosen K, Tonn JC. Extraneural metastases of primary brain tumors. J Neurooncol 2001; 53: 107-114.
22. Seo YJ, Cho WH, Kang DW, Cha SH. Extraneural metastasis of glioblastoma multiforme presenting as an unusual neck mass. J Korean Neurosurg Soc 2012; 51: 147-150.
23. Snopkowska-Wiaderna D, Zielinski KW, Radek M, Papierz W. Extracerebral metastases of glioblastoma have a different vasculature than primary tumour. A case report of glioblastoma extracranial metastases. Folia Neuropathol 2012; 50: 413-416.

24. Stec WJ, Rosiak K, Siejka P, Peciak J, Popeda M, Banaszczyk M, Pawlowska R, Treda C, Hulas-Bigoszewska K, Piaskowski S, Stoczynska-Fidelus E, Rieske P. Cell line with endogenous EGFRvIII expression is a suitable model for research and drug development purposes. Oncotarget 2016; 7: 31907-31925.
25. Stoczynska-Fidelus E, Och W, Rieske P, Bienkowski M, Banaszczyk M, Winiecka-Klimek M, Zieba J, Janik K, Rosiak K, Treda C, Stawski R, Radomiak-Zaluska A, Piaskowski S. Spontaneous in vitro senescence of glioma cells confirmed by an antibody against IDH1R132H. Anticancer Res 2014; 34: 2859-2867.
26. Stupp R, Hegi ME, Mason WP, van der Bent MJ, Taphoorn MJ, Janzer RC, Ludwin SK, Allgeier A, Fisher B, Belanger K, Hau P, Brandes AA, Gijtenbeek J, Marosi C, Vecht CJ, Mokhtari K, Wesseling P, Villa S, Eisenhauer E, Gorlia T, Weller M, Lacombe D, Cairncross JG, Mirimanoff RO; European Organisation for Research and Treatment of Cancer Brain Tumour and Radiation Oncology Groups; National Cancer Institute of Canada Clinical Trials Group. Effects of radiotherapy with concomitant and adjuvant temozolomide versus radiotherapy alone on survival in glioblastoma in a randomized phase III study: 5-year analysis of the EORTC-NCIC trial. Lancet Oncol 2009; 10: 459-466.
27. Takahashi Y, Akahane T, Sawada T, Ikeada H, Tempaku A, Yamauchi S, Nishihara H, Tanaka S, Nitta K, Ide W, Hashimoto I, Kamada H. Adult classical glioblastoma with a BRAF V600E mutation. World J Surg Oncol 2015; 13: 100.
28. Tanboon J, Williams EA, Louis DN. The Diagnostic use of immunohistochemical surrogates for signature molecular genetic alterations in gliomas. J Neuropathol Exp Neurol 2016; 75: 4-18.