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Epidermal Growth Factor Receptor and its Oncogenic *EGFRvIII* Variant in Benign and Malignant Brain Tumors

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Abstract. *Background/Aim: Tumorigenesis and cancer progression might be driven by abnormal activation of growth factor receptors. Importantly, molecular changes in EGFR-dependent signaling is one of the most common characteristics of brain tumors. Patients and Methods: HER1 and EGFRvIII variants in meningiomas and glioblastomas were evaluated at the RNA level. Results: EGFRvIII was found in 18.6% of glioblastomas (GBM), whereas 25% of EGFRvIII positive tumors express wild-type EGFR as well. HER1 was over-expressed in benign meningiomas compared to glioblastomas, whereas HER1 expression in meningiomas differed significantly between sub-types of meningiomas. EGFRvIII and HER1 where positively correlated in glioblastomas. Yet, the patient overall survival did not differ between high- and low-HER1 expressing glioblastomas or between EGFRvIII positive and negative GBMs. Conclusion: HER1 may be considered as an independent factor for classification of benign meningiomas. The mRNA levels of HER1 or EGFRvIII should not be used as independent prognostic factors for patients with gliomas.*

Despite the fact that brain tumors account only for about 2% of all cancers in adult patients, they significantly impact the number of cancer-related deaths (1, 2). The five-year survival rates for brain tumors are among the lowest for all human cancers (3), regardless of the improvement in surgical treatments and the widespread introduction of adjuvant radio and chemotherapy (4). This treatment resistance results from tumor heterogeneity and a high propensity for malignant progression (1, 5). Among the broadly heterogeneous primary brain tumors, meningiomas and gliomas are the two most common types of malignancies.

Meningiomas present with the highest frequency among intracranial tumors in adults, accounting for approximately 37% of all primary brain tumors. These lesions are located along the external surface of the brain or within the ventricular system, and are mostly described as slowly growing. Meningiomas have been classified by WHO as less malignant grade I, grade II which is atypical, and grade III that is characterized as anaplastic. Grade I, grade II and grade III meningeal tumors account for 80%, 15-20%, and 1-3% of all meningiomas, respectively (6). Approximately 86% of patients diagnosed with meningioma (20-44 years) survive for five years or longer. The more aggressive grade II and III meningiomas show rates of recurrence at 5 years approximating 50% and 90%, respectively. The mean 5-year survival for patients diagnosed with atypical meningioma (grade II) is 58% whereas for those diagnosed with grade III malignant meningioma is 8.2-12% (7, 8). Despite their mostly benign histological feature, the recurrence and progression of meningiomas is of crucial importance.

Infiltrating gliomas, histologically and clinically different in comparison to benign brain lesions, are the most often diagnosed primary malignant tumors of the central nervous system among adult patients. The tumors of glial origin are graded as WHO II-IV, and the most malignant glioblastoma cases comprise 45% of all glial tumors and 7% of all brain tumors. Low grade glioma is a poor prognosis disease with a survival averaging approximately 7 years (9); only a few patients diagnosed with glioblastoma survive 2.5 years,

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whereas not more than 5% of patients are still alive 5 years from diagnosis (10). Glioblastoma with its aggressive and highly diffuse characteristic, is the deadliest primary brain malignancy due to high recurrence and resistance to therapy.

A plethora of laboratory and clinical research efforts had been engaged in better understanding these brain tumors in the past few decades. These studies have defined most of the malignancies according to their phenotypical features, including self-sufficiency in growth factors and apoptotic response (11-13). The molecular mechanisms that define these phenotypical features are widely diverse among different types of brain tumors, what was shown in glioblastomas (11, 14-16). Therefore, the development of effective therapeutic strategies should be based on fully understanding these molecular events that determine such variabilities in tumor physiologies (2). Of particular importance is the fact that the intrinsic cellular machinery contributing to their distinct biological characteristics remains not fully elucidated.

The intracellular signal transduction cascades determine the majority of the cell physiological features (17), including also tumor initiation and development. This has been also shown as critical in case of brain malignancies, where the role of receptor tyrosine kinase (RTK) signaling is constantly discussed (18). One of the most often found RTK abnormalities in glioblastomas is related to epidermal growth factor receptor (EGFR) (18, 19). Upregulation of EGFR signaling results in increased proliferation, migration and survival of cancer cells. The emerging role of EGFR-related signaling in glioblastoma has been deeply explored with the use of distinct research approaches, including functional experiments and large-scale studies, on patient tissue samples and clinical data. These research strategies confirmed the importance of the EGFR signaling in glioblastoma biology, showing that the different EGFR network components may determine the aberrant behavior of glioblastomas (2, 11, 20, 21). On the other hand, only a few reports have focused on other types of glial tumors such as oligodendrogliomas, diffuse astrocytomas, malignant lymphomas and last, but not least, meningiomas. In this work, we evaluated the expression of EGFR and its most commonly mutated variant III (EGFRvIII) in various types of central nervous system tumors along with survival analysis for patients who underwent surgery as part of their treatment.

Patients and Methods

Patients and tissue samples collection. The samples were collected upon surgical treatment of patients with brain tumors with the formal agreement of the local Ethics Committee no. KE-0254/139/2012. Each resected tumor tissue was divided into two parts; one part was immediately frozen in liquid nitrogen and the other was histologically assessed by a pathologist. Patients diagnosed from 2013 to 2016 were included in the study. The histological types of the analyzed tumors are shown in Table I. We collected and analyzed tumor samples from 25 males and 18 females with GBM, whose median age was 57 years (minimal 27, maximal 80). The meningiomas were taken from 7 males and 19 females with a median age 56 years (minimal 35, maximal 86).

Table I. *Analyzed tumors and their histopathological classification.*

Type	Number of samples	Subtype	N
Meningioma	26	Fibrous meningioma GI	7
		Meningothelial meningioma GI	9
		Lymphoplasmacytic-rich GI	1
		Transitional meningioma GI	2
		Anaplastic meningioma GIII	1
		Angiomatous meningioma GI	1
		Atypical meningioma GII	3
		Metaplastic meningioma GI	1
		Microcystic meningioma GI	1
Glioblastoma	43	Glioblastoma multiforme GIV	36
		Giant cell glioblastoma GIV	3
		Gliosarcoma GIV	4

N: Number of samples; SEM: standard error of mean.

Table II. *Relative quantity of HER1 expression in glioblastomas (GBMs), meningiomas and in reference material (HB-RNA).*

	N	Mean±SEM RQ	Median RQ	Min-Max RQ
GBM	43	3.63±1.21	0.84	0.01-41.45
Meningiomas	26	3.14±0.87	2.16	0.002-22.42
HB-RNA	4	0.31±0.05	0.28	0.24-0.43

Reference material. As the reference normal human brain material (HB-RNA), we used total RNA from Human Frontal Cortex purchased from Agilent (Agilent Technologies, Mulgrave, Australia).

RNA isolation and cDNA synthesis. The tumor samples were stored in –80˚C for up to 6 months. Total RNA was isolated from brain tumor samples grinded in liquid nitrogen with the use of RNEasy Lipid Tissue Mini KIT purchased from Qiagen (Hilden, Germany) according to the manufacturer's manual. We spectrophotometrically tested the purity and quantity of the RNA using the UV-VIS Genesys 10S instrument (Thermo Fisher Scientific, Madison, WI, USA). The RNA samples with 260/280 nm optical density ratio lower than 1.8 were excluded from further analysis. The RNA quality was also assessed by agarose electrophoresis. An aliquot of 3 μg of total RNA was subsequently reverse transcribed using Transcriptor High Fidelity cDNA Synthesis Kit (Roche Diagnostics, Mannheim, Germany). The reaction was conducted for 30 min at 50˚C in the presence of oligo(dT) primers, and finally the enzyme was inactivated at 85˚C for 5 min, according to the manufacturer's instructions.

Table III. *HER1 expression in different subtypes of meningiomas. N: Number of samples; SEM: standard error of mean.*

	N	Mean±SEM RQ	Median RQ	Min-Max RQ
Fibrous meningiomas	7	3.49±0.65	3.15	2.01-7.07
Meningothelial meningiomas	9	2.09±0.53	1.47	1.10-6.15
Atypical meningiomas GII	3	1.35±1.02	0.70	0.002-3.36
Other meningiomas GI	6	2.05±0.61	2.22	0.33-4.29
HB-RNA	4	0.31±0.05	0.28	0.24-0.43

N: Number of samples; SEM: standard error of mean.

Nested-polymerase chain reaction (nested-PCR). Nested PCR was used for EGFRvIII variant detection according to a modified protocol described by Silva *et al*. (22). The sets of the primers were as follows: 1st stage (For 5'- GTATTGATCGGGAGAGCCG-3', Rev 5'-GTGGAGATCGCCACTGATG-3'); 2nd stage (For 5'-GCGATGCGACCCTCCGGG-3', Rev 5'-TCCGTTACACACTT TGCG-3'); the volume of each reaction was 25 µl, and the reaction mixture contained a cDNA amount corresponding to 25 ng of the RNA, 1 x DreamTaq PCR Master Mix (Thermo Scientific) and 0.4 µM primers for 1st stage reaction. Following 2 min of denaturation at 95˚C, the cDNA was amplified in 40 cycles consisting of 45 s denaturation at 95˚C, 30 s annealing at 60˚C and 90 s elongation at 72˚C. The products of the amplification were then diluted 1:50 in water and 5 µl of diluents were used for amplification using the 2nd stage primers at the same conditions as described above. The products of the 2nd stage reaction were finally visualized on 1.5% agarose gels.

Quantitative polymerase chain reaction (qPCR). The qPCR amplification was performed using a LightCycler® 480 II machine (Roche). The analysis was performed with the use of Universal ProbeLibrary (UPL, Roche) fluorescently labeled oligonucleotide probes in a duplex reaction, which where specific for the HER1 and the reference glyceraldehyde-3-phosphate dehydrogenase (*GAPDH)* amplicons. HER1 probe was labeled with FAM, whereas *GAPDH* probe was labeled with Yellow 555 fluorophore. The primers and probe sets were as following: HER1 (Forward: 5'-CCAT CCTGGAGAAAGGAGAA-3', Reverse 5'-CATCCAGCACTT GACCATGA-3', Probe #83 5'-GGTGGCTG-3'); GAPD: (Forward 5'-CTCTGCTCCT CCTGTTCGAC-3', Reverse 5'-GCCCA ATACGACCAAATCC-3', Probe 5'-CTTTTGCGTCGC-3'). Amplification was performed in 10 µl reaction mixture containing a cDNA amount corresponding to 12.5 ng of total RNA, 1 • LightCycler® 480 Probes Master (Roche) and appropriate set of 0.4 µM primers and 0.2 µM UPL probes for each target and reference duplex. Following 10 min of initial denaturation/incubation (95˚C), the cDNA was amplified in 45 cycles: 10 s denaturation at 95˚C, 30 s annealing at 60˚C and 10 s elongation at 72˚C. The reads were analyzed using a Relative Quantification (RQ) method that includes efficiency correction. Our method allowed to detect the most common HER1 variants including also EGFRvIII, which are listed as: ENST00000455089, ENST00000395504, ENST00000454757, ENST00000275493, ENST00000533450 and NM_201283. The sample showing near average HER1 level was used as the calibrator (RQ=1).

Statistics. The variables deviated from Gaussian distribution, which was initially evaluated with following normality tests: Shapiro-Wilk, D'Agostino and Pearson, and Kolmogorov–Smirnov. The comparisons between two groups were subsequently estimated using the Mann–Whitney test, whereas the comparisons among multiple groups were calculated using the Kruskal–Wallis test. The correlation coefficients were estimated using Spearman's rank test. The data on the graphs were expressed as the mean with the margin of standard error of the mean (SEM) and considered as significant when $*p<0.05$, $**p<0.01$ and $***p<0.001$. The overall survival (OS) was estimated with the use of Kaplan–Meier plots and Cox regression model. Cox–Mantel log-rank test was used for comparing the survival curves.

Results

Expression of HER1 in brain tumors. All assessed samples expressed the *HER1* gene. Table II presents *HER1* expression in GBM, benign tumors, and HB-RNA reference material. Median HER1 expression was approximately 2.5 times higher in meningiomas when compared with GBM ($p=0.0102$; Mann–Whitney test). Moreover, *HER1* expression was significantly lower in reference material (HB-RNA) in comparison to other analyzed groups (HB-RNA *vs*. GBM; $p=0.0190$ and HB-RNA *vs*. Meningiomas; $p=0.0049$; Mann–Whitney test).

For further analysis all meningiomas were subdivided into four subtypes based on their histopathological features: fibrous GI, meningothelial GI, atypical GII and other meningiomas GI. Table III shows *HER1* expression in different subtypes of meningiomas. Importantly, median *HER1* expression was at least two times higher in fibrous meningiomas in comparison to meningothelial meningiomas ($p=0.0360$, Mann–Whitney test). On the other hand, *HER1* expression was significantly lower in HB-RNA when compared to all meningiomas of the GI subtype (fibrous $p=0.0106$, meningothelial $p=0.0028$, other meningiomas GI $p=0.0190$, Mann–Whitney test). The difference in *HER1* expression between HB-RNA and atypical meningiomas was not significant ($p>0.05$, Mann–Whitney test).

Expression of HER1 variants in tumors. Expression of *EGFRvIII* was detected neither in meningiomas nor in HB-RNA reference material while the longest *HER1* variant (*wtEGFR*) was expressed in 95.3% (41/43) of GBM tumors.

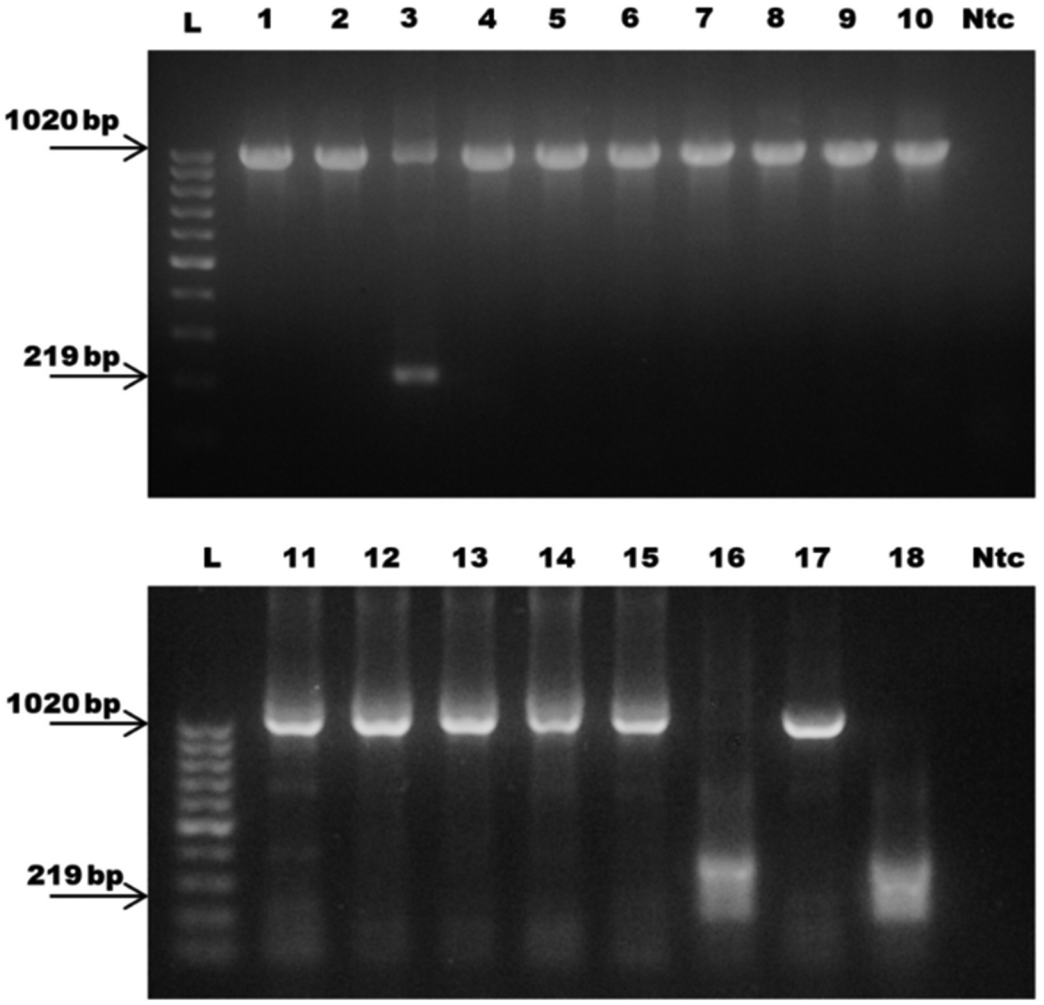

Figure 1. *HER1 variants in brain tumors. L, DNA ladder shows the fragments from 100 bp up to 1,000 bp; 1-18, PCR products from the brain tumors; Ntc, no template control of PCR reaction. Two representative electropherograms of PCR products are shown. The primers used here cover exons 2-7, which are deleted in oncogenic the EGFRvIII variant. The band of 1020 indicates the presence of the longest wtEGFR transcript, whereas the amplicon of 219 bp shows EGFRvIII expression. Tumor sample 3 shows both wtEGFR and EGFRvIII expression, whereas samples 16 and 18 show EGFRvIII only.*

EGFRvIII variant was expressed in 18.6% (8/43) of GBM samples, whilst 25% (2/8) of these tumors showed simultaneous expression of both, *wtEGFR* and oncogenic *EGFRvIII* variants. The presence of *wtEGFR* and *EGFRvIII* was found in 4.65% (2/43) of all GBM tissues. Representative results of the presence of *HER1* variants in the assessed tumors are shown in Figure 1.

Correlation between HER1 levels and EGFRvIII variants in malignant gliomas. The presence of *EGFRvIII* transcripts positively correlated with *HER1* expression in GBM patients (R=0.56; $p>0.00001$; N=43; Spearman test). This finding was confirmed by the comparison of *HER1* RQ values between *EGFRvIII* positive and *EGFRvIII* negative GBM, where median *HER1* expression was about 10 times higher in *EGFRvIII* positive GBM than in negative ones ($p=0.0003$, Mann–Whitney test; Table IV). We did not observe any significant correlations between *HER1* expression and the gender or age of the patients.

Overall survival of patients with GBM. The patients with GBM were followed up from the date of diagnosis until death from any cause (OS) till December 2016 (mean OS 6.5 months, min. 2 days – max. 57 months). Only 4 (4/43, 9.3%) patients were still alive at the end of the follow-up period. All GBM patients were divided into groups according to the

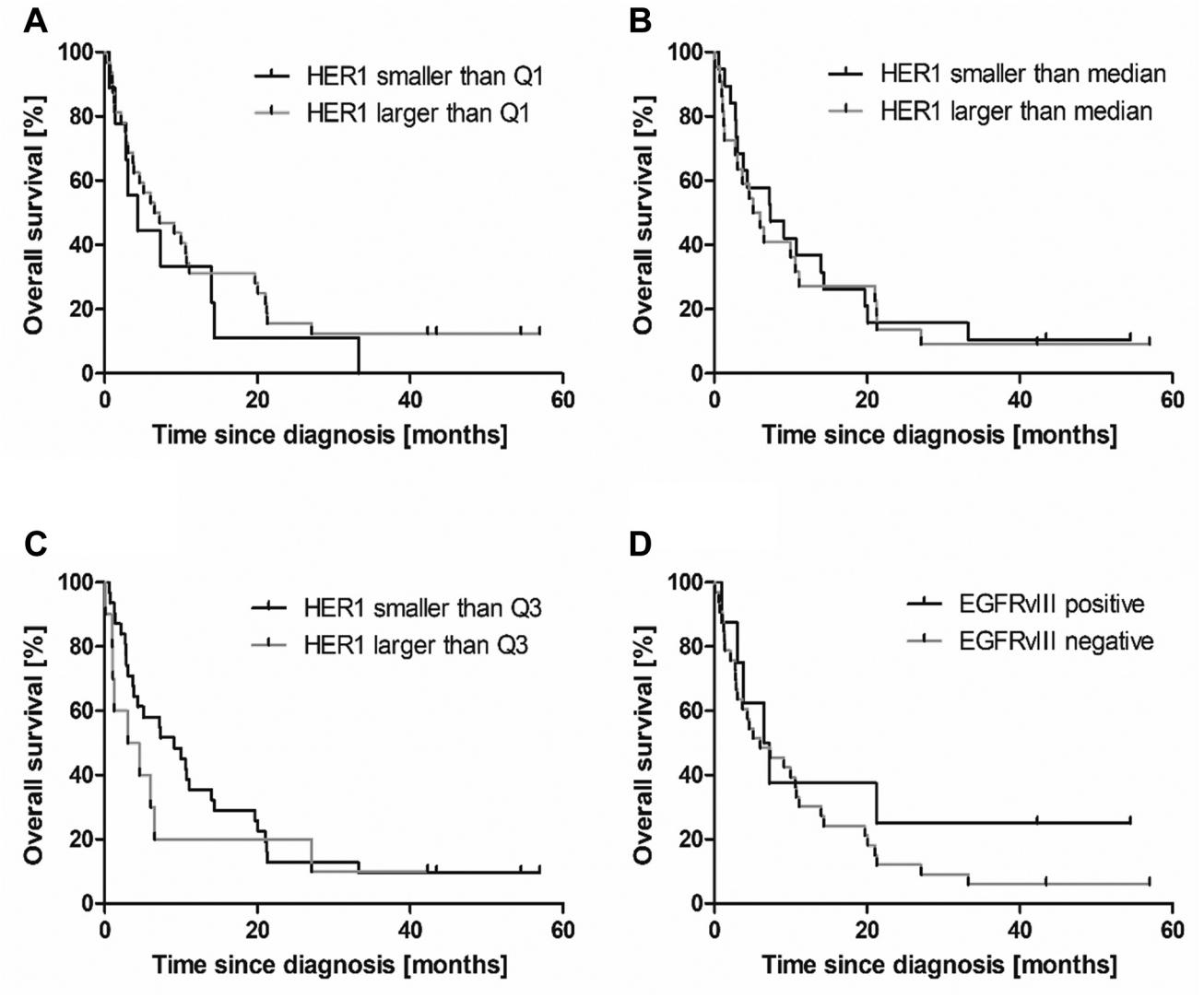

Figure 2. *Overall survival of GBM patients and expression of HER1 (A-C) and EGFRvIII (D).*

cut-off point based on the median *HER1* expression, as well as according to *EGFRvIII* status. The survival of patients in these groups is shown in Table V. Kaplan–Meier survival curves are shown in Figure 2. *HER1* expression or *EGFRvIII* status was not related to OS in the analyzed groups of patients with GBM. Other clinical data such as gender or age of patients were not identified as prognostic factors for patients' survival (data not shown).

Table IV. *HER1 expression in GBM positive and negative for EGFRvIII.*

EGFRvIII	N	Mean±SEM RQ	Median RQ	Min-Max RQ
Negative	35	1.57±0.54	0.74	0.01-15.07
Positive	8	12.66±5.17	6.98	1.14-41.45

N: Number of samples; SEM: standard error of mean.

Discussion

EGFR has been shown to be over-expressed in many cancers (23), including benign and malignant brain tumors (24-27). In the present study, we observed higher expression of *HER1* mRNA in meningiomas compared to GBM tumors and normal brain tissue, suggesting the potential role of EGFR in meningioma development and progression (28). Within the grade I subgroup of meningioma patients, we demonstrated higher mRNA *HER1* expression in fibrous meningiomas than in any other type. Very high mRNA *EGFR* expression in low

Table V. *The overall survival (OS) analysis of glioblastoma patients. Median survival of each analyzed group of the patients and the results of Log-rank cMantel-Cox tests are shown.*

	Smaller than *Q1*	Larger than *Q1*	Smaller than median	Larger than median	Smaller than *Q3*	Larger than *Q3*	EGFRvIII positive	EGFRvIII negative
Censored	0	4	2	2	3	1	2	2
Deaths	9	28	17	20	28	9	6	31
Median survival in months	4.33	6.85	7.3	5.55	9.13	3.77	6.85	6

grade meningioma has been reported previously and postulated that expression of *EGFR* could reflect the histological subtype of the meningioma (29). However, analyses of *EGFR* mRNA expression in meningiomas are rare in the literature, therefore, no definite conclusions have been drawn so far. Moreover, the majority of the studies are based on immunohistochemistry (IHC) and histology assessments, which gives often conflicting results regarding the expression levels of EGFR across malignancy grades (26, 30-37), mainly due to the use of different antibodies (34), and the possible tumor's heterogeneity (28). Other methods of *EGFR* mRNA level assessment (RT-PCR, RNAseq) and *HER1* gene copy number (whole exome sequencing, WES), seem to be superior than protein expression (IHC) in that respect, especially for GBM analysis (38), whereas EGFR IHC might serve as a possible screening tool for evaluation of *EGFR* gene amplification in clinical neuropathology (39). Moreover, a particular regulatory mechanism of EGFR signaling in meningioma has been postulated, supported by the observation that patients diagnosed with tumors expressing high levels of *EGFR* mRNA presented a better progression free survival (PFS) (34), which is in contrast to other brain tumors, including GBM (40). Our study showed that all the types of meningiomas displayed much higher *EGFR* mRNA levels than normal human brain, suggesting that indeed EGFR signaling might play a significant role in meningioma growth or development. Moreover, according to recent immunohistochemical studies, EGFR in meningiomas is present in an activated state, as judged by assessing the phosphorylated forms of the receptor, which are absent in normal meninges (28). Similar data demonstrating higher *EGFR* mRNA levels in meningioma tumors than in normal meninges have been also presented by others (41). We did not detect the mRNA of the *EGFRvIII* variant in any of the analyzed meningioma tumors, showing that this variant is probably not involved in meningioma oncogenesis and progression, which is in agreement with other reports (34). Especially for recurrent meningioma treatment, despite the fact that these tumors are rare, EGFR and its signaling might be used to identify effective therapeutic options or considered as promising candidates for targeted therapy (42, 43).

In contrast, we demonstrated the presence of *EGFRvIII* variant in GBM tumors. Other studies have shown similar or higher frequency of *EGFRvIII* in GMB (21.3-27%) (44-46), which might be explained by the relatively small number of EGFRvIII harboring tissues in our study. Moreover, we did not observe *EGFRvIII* expression in normal brain tissue, which supports a notion that it is a good candidate for targeted therapy in EGFRvIII harboring tumors (40, 47). Despite these promising results, studies that implemented antibodies generated against EGFR, as well as the first generation of EGFR tyrosine kinase inhibitors (TKIs), showed limited activity in GBM patients. The 2nd and 3rd generation of these TKIs seem to be more promising, as analyzed in pre-clinical models. However, the highly heterogeneous nature of GBM and the presence of a blood brain barrier that influences the neuropharmacokinetics of the drugs represent real obstacles to the effective use of these treatments (47). So far, vaccines (including Rindopepimut, CDX110) have been reported, following the phase I clinical trial, to significantly prolong patients' survival when co-administrated with temozolomide (TMZ) (47-49), however, unfortunately, they failed phase III trial (50). Moreover, antibody-drug conjugate Depatuxizumab mafodotin (ABT-414), designed to use EGFR as an entry point to deliver a toxic payload into tumor cells, has been used in GBM combination therapy with TMS and enhanced TMS antitumor activity in the phase clinical trial (51). Targeting of both, EGFRvIII and EGFR by a bispecific T-cell engager (BiTE) has been demonstrated as an advanced promising approach of Chimeric Antigen Receptor (CAR) T-cell therapy in an animal GBM model (52). It is thought, that the lack of a ligand-binding domain and constitutively active EGFRvIII (53, 54) could explain the failure of the TKI targeted therapy treatment. EGFRvIII variant is also linked with the final outcome of patients that suffer from different cancer types, confirming that it could be a prognostic factor. EGFRvIII positive GBM patients surviving at least one year have significantly shorter survival rates in comparison to those having EGFRvIII negative tumors (55). Moreover, EGFRvIII is considered a poor prognostic factor in *EGFR* amplified glioblastomas (47, 56), similar to GBM patients

with *EGFR* gene amplification (57). In our present study, EGFRvIII-positive patients had also almost ten times higher *HER1* expression than *EGFRvIII*-negative patients, but it did not correlate with patients' survival. Our data are supported by studies in larger cohorts indicating that the lack of EGFRvIII is not associated with different progression-free or overall survival (40). Interestingly, we also identified a subset of patients with simultaneous expression of *wtEGFR* and *EGFRvIII*. The presence of both, *wtEGFR* and *EGFRvIII* in the same cell results in altered downstream signaling, that has been proven to be crucial for tumorigenesis and tumor growth promotion (47, 58). However, the mechanism of EGFR and EGFRvIII cooperation remains largely unknown (47) and requires further studies. Since *EGFR* amplification or expression of *EGFR* gene variants seems to be a hallmark of brain tumors, molecular and genomic profiling of these neoplasms should be performed. Although it does not have prognostic value, determination of the *EGFRvIII* status is recommended for patients with glioblastoma in order to consider administering EGFRvIII-targeting agents.

Conflicts of Interest

No potential conflicts of interest in relation to this study were reported by the Authors.

Authors' Contributions

Conceptualization: AS, RR, TT; Methodology and Validation: MK, WJ, MC, ASB; Formal Analysis and Investigation: MK; Statistics: MK, MC; Resources: AS, RR, TT; Writing–Original Draft Preparation, MK, AS, RR; Writing–Review and Editing: MK, DS, PA, JSK, KP, TT; Visualization: MK, DS; Supervision: AS; Funding Acquisition: MK, AS, RR.

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References

1 Black PM and Loeffler JS: Cancer of the nervous system. Lippincott Williams & Wilkins, 2005.

2 Tan AC, Ashley DM, López GY, Malinzak M, Friedman HS and Khasraw M: Management of glioblastoma: State of the art and future directions. Wiley, 2020. PMID: 32478924. DOI: 10.3322/caac.21613

3 Salcman M, Scholtz H, Kaplan RS and Kulik S: Long-Term Survival in Patients with Malignant Astrocytoma. Neurosurgery *34*: 213-220, 1994. PMID: 8177380. DOI: 10.1227/00006123-199402000-00002

4 Avgeropoulos NG and Batchelor TT: New treatment strategies for malignant gliomas. Oncologist *4*: 209-224, 1999. PMID: 10394589.

5 Kim HJ and Kim DY: Present and future of anti-glioblastoma therapies: A deep look into molecular dependencies/features. Molecules *25*, 2020. PMID: 33053763. DOI: 10.3390/molecules25204641

6 Ostrom QT, Gittleman H, Xu J, Kromer C, Wolinsky Y, Kruchko C and Barnholtz-Sloan JS: CBTRUS Statistical Report: Primary brain and other central nervous system tumors diagnosed in the United States in 2009-2013. Neuro Oncol *18*: v1-v75, 2016. PMID: 28475809. DOI: 10.1093/neuonc/now207

7 Violaris K, Katsarides V, Karakyriou M and Sakellariou P: Surgical outcome of treating grades II and III meningiomas: A report of 32 cases. Neurosci J *2013*: 1-4, 2013. PMID: 26317097. DOI: 10.1155/2013/706481

8 Shan B, Zhang J, Song Y and Xu J: Prognostic factors for patients with World Health Organization grade III meningiomas treated at a single center. Medicine *96(26)*: e7385, 2017. PMID: 28658170. DOI: 10.1097/MD.0000000000007385

9 Claus EB, Walsh KM, Wiencke JK, Molinaro AM, Wiemels JL, Schildkraut JM, Bondy ML, Berger M, Jenkins R and Wrensch M: Survival and low-grade glioma: The emergence of genetic information. Neurosurg Focus *38*: E6, 2015. PMID: 25552286. DOI: 10.3171/2014.10.FOCUS12367

10 Tamimi AF and Juweid M: Epidemiology and Outcome of Glioblastoma. *In*: Glioblastoma. Codon Publications, pp. 143-153, 2017. PMID: 29251870. DOI: 10.15586/codon.glioblastoma.2017.ch8

11 Couturier CP, Ayyadhury S, Le PU, Nadaf J, Monlong J, Riva G, Allache R, Baig S, Yan X, Bourgey M, Lee C, Wang YCD, Wee Yong V, Guiot MC, Najafabadi H, Misic B, Antel J, Bourque G, Ragoussis J and Petrecca K: Single-cell RNA-seq reveals that glioblastoma recapitulates a normal neurodevelopmental hierarchy. Nat Commun *11*, 2020. PMID: 32641768. DOI: 10.1038/s41467-020-17186-5

12 Dirkse A, Golebiewska A, Buder T, Nazarov P V., Muller A, Poovathingal S, Brons NHC, Leite S, Sauvageot N, Sarkisjan D, Seyfrid M, Fritah S, Stieber D, Michelucci A, Hertel F, Herold-Mende C, Azuaje F, Skupin A, Bjerkvig R, Deutsch A, Voss-Böhme A and Niclou SP: Stem cell-associated heterogeneity in Glioblastoma results from intrinsic tumor plasticity shaped by the microenvironment. Nat Commun *10*, 2019. PMID: 30992437. DOI: 10.1038/s41467-019-09853-z

13 Ng K, Kim R, Kesari S, Carter B and Chen CC: Genomic profiling of glioblastoma: convergence of fundamental biologic tenets and novel insights. J Neurooncol *107*: 1-12, 2012. PMID: 22002595. DOI: 10.1007/s11060-011-0714-2

14 Verhaak RGW, Hoadley KA, Purdom E, Wang V, Qi Y, Wilkerson MD, Miller CR, Ding L, Golub T, Mesirov JP, Alexe G, Lawrence M, O'Kelly M, Tamayo P, Weir BA, Gabriel S, Winckler W, Gupta S, Jakkula L, Feiler HS, Hodgson JG, James CD, Sarkaria JN, Brennan C, Kahn A, Spellman PT, Wilson RK, Speed TP, Gray JW, Meyerson M, Getz G, Perou CM, Hayes DN and Cancer Genome Atlas Research Network: Integrated genomic analysis identifies clinically relevant subtypes of glioblastoma characterized by abnormalities in PDGFRA, IDH1, EGFR, and NF1. Cancer Cell *17*: 98-110, 2010. PMID: 20129251. DOI: 10.1016/j.ccr.2009.12.020

15 Lopes MB and Vinga S: Tracking intratumoral heterogeneity in glioblastoma via regularized classification of single-cell RNA-Seq data. BMC Bioinformatics *21*, 2020. PMID: 32070274. DOI: 10.1186/s12859-020-3390-4

16 Cheng Q, Li J, Fan F, Cao H, Dai Z-Y, Wang Z-Y and Feng S-S: Identification and analysis of glioblastoma biomarkers based on single cell sequencing. Front Bioeng Biotechnol *8*: 167, 2020. PMID: 32195242. DOI: 10.3389/fbioe.2020.00167

17 Bianco R, Melisi D, Ciardiello F and Tortora G: Key cancer cell signal transduction pathways as therapeutic targets. Eur J Cancer *42*: 290-294, 2006. PMID: 16376541. DOI: 10.1016/j.ejca.2005.07.034

18 Furnari FB, Fenton T, Bachoo RM, Mukasa A, Stommel JM, Stegh A, Hahn WC, Ligon KL, Louis DN, Brennan C, Chin L, DePinho RA and Cavenee WK: Malignant astrocytic glioma: genetics, biology, and paths to treatment. Genes Dev *21*: 2683-2710, 2007. PMID: 17974913. DOI: 10.1101/gad.1596707

19 Pan PC and Magge RS: Mechanisms of egfr resistance in glioblastoma. Int J Mol Sci *21*: 1-21, 2020. PMID: 33187135. DOI: 10.3390/ijms21228471

20 Johannessen CM, Johnson LA, Piccioni F, Townes A, Frederick DT, Donahue MK, Narayan R, Flaherty KT, Wargo JA, Root DE and Garraway LA: A melanocyte lineage program confers resistance to MAP kinase pathway inhibition. Nature *504*: 138-142, 2013. PMID: 24185007. DOI: 10.1038/nature12688

21 Areeb Z, Stuart SF, West AJ, Gomez J, Nguyen HPT, Paradiso L, Zulkifli A, Jones J, Kaye AH, Morokoff AP and Luwor RB: Reduced EGFR and increased miR-221 is associated with increased resistance to temozolomide and radiotherapy in glioblastoma. Sci Rep *10*: 1-12, 2020. PMID: 33082482. DOI: 10.1038/s41598-020-74746-x

22 Silva HAC, Abraúl E, Raimundo D, Dias MF, Marques C, Guerra C, de Oliveira CF and Regateiro FJ: Molecular detection of EGFRvIII-positive cells in the peripheral blood of breast cancer patients. Eur J Cancer *42*: 2617-2622, 2006. PMID: 16956761. DOI: 10.1016/j.ejca.2006.03.033

23 Sasaki T, Hiroki K and Yamashita Y: The role of epidermal growth factor receptor in cancer metastasis and microenvironment. Biomed Res Int *2013*: 546318, 2013. PMID: 23986907. DOI: 10.1155/2013/546318

24 Tse GMK, Lui PCW, Vong JSL, Lau K-M, Putti TC, Karim R, Scolyer RA, Lee C-S, Yu AMC, Ng DCH, Tse AKY and Tan P-H: Increased epidermal growth factor receptor (EGFR) expression in malignant mammary phyllodes tumors. Breast Cancer Res Treat *114*: 441-448, 2009. PMID: 18443904. DOI: 10.1007/s10549-008-0030-5

25 Destro A, Ceresoli GL, Falleni M, Zucali PA, Morenghi E, Bianchi P, Pellegrini C, Cordani N, Vaira V, Alloisio M, Rizzi A, Bosari S and Roncalli M: EGFR overexpression in malignant pleural mesothelioma. Lung Cancer *51*: 207-215, 2006. PMID: 16384623. DOI: 10.1016/j.lungcan.2005.10.016

26 Wernicke AG, Dicker AP, Whiton M, Ivanidze J, Hyslop T, Hammond EH, Perry A, Andrews DW and Kenyon L: Assessment of Epidermal Growth Factor Receptor (EGFR) expression in human meningioma. Radiat Oncol *5*: 46, 2010. PMID: 20509969. DOI: 10.1186/1748-717X-5-46

27 Terry G, Ho L, Londesborough P, Duggan C, Hanby A and Cuzick J: The expression of FHIT, PCNA and EGFR in benign and malignant breast lesions. Br J Cancer *96*: 110-117, 2007. PMID: 17164758. DOI: 10.1038/sj.bjc.6603512

28 Arnli MB, Backer-Grøndahl T, Ytterhus B, Granli US, Lydersen S, Gulati S and Torp SH: Expression and clinical value of EGFR in human meningiomas. PeerJ *5*: e3140, 2017. PMID: 28367377. DOI: 10.7717/peerj.3140

29 Rooprai HK, Martin AJ, King A, Appadu UD, Jones H, Selway RP, Gullan RW and Pilkington GJ: Comparative gene expression profiling of ADAMs, MMPs, TIMPs, EMMPRIN, EGF-R and VEGFA in low grade meningioma. Int J Oncol *49*: 2309-2318, 2016. PMID: 27779709. DOI: 10.3892/ijo.2016.3739

30 Caltabiano R, Barbagallo GM V, Castaing M, Cassenti A, Senetta R, Cassoni P, Albanese V and Lanzafame S: Prognostic value of EGFR expression in de novo and progressed atypical and anaplastic meningiomas: an immunohistochemical and fluorescence in situ hybridization pilot study. J Neurosurg Sci *57*: 139-151, 2013. PMID: 23486338.

31 Diedrich U, Lucius J, Baron E, Behnke J, Pabst B and Zoll B: Distribution of epidermal growth factor receptor gene amplification in brain tumours and correlation to prognosis. J Neurol *242*: 683-688, 1995. PMID: 8568531.

32 Halper J, Jung C, Perry A, Suliman H, Hill MP and Scheithauer B: Expression of TGFα in meningiomas. J Neurooncol *45*: 127-134, 1999. DOI: 10.1023/A:1006365725033

33 Baxter DS, Orrego A, Rosenfeld JV and Mathiesen T: An audit of immunohistochemical marker patterns in meningioma. J Clin Neurosci *21*: 421-426, 2014. PMID: 24231566. DOI: 10.1016/J.JOCN.2013.06.008

34 Guillaudeau A, Durand K, Bessette B, Chaunavel A, Pommepuy I, Projetti F, Robert S, Caire F, Rabinovitch-Chable H and Labrousse F: EGFR soluble isoforms and their transcripts are expressed in meningiomas. PLoS One *7*: e37204, 2012. PMID: 22623992. DOI: 10.1371/journal.pone.0037204

35 Jones NR, Rossi ML, Gregoriou M and Hughes JT: Epidermal growth factor receptor expression in 72 meningiomas. Cancer *66*: 152-155, 1990. DOI: 10.1002/1097-0142(19900701)66:1<152::AID-CNCR2820660127>3.0.CO;2-5

36 Kuratsu J-I, Seto H, Kochi M and Ushio Y: Expression of PDGF, PDGF-Receptor, EGF-Receptor and sex hormone receptors on meningioma. Acta Neurochir (Wien) *131*: 289-293, 1994. PMID: 7754837. DOI: 10.1007/BF01808629

37 Sundaram C, Narla S, Uppin M, Saradhi Mv, Sahu B and Purohit A: Assessment of expression of epidermal growth factor receptor and p53 in meningiomas. Neurol India *62*: 37, 2014. PMID: 24608452. DOI: 10.4103/0028-3886.128276

38 Gan HK, Millward M, Hua Y, Qi C, Sai Y, Su W, Wang J, Zhang L, Frigault MM, Morgan S, Yang L and Lickliter JD: First-in-human phase I study of the selective met inhibitor, savolitinib, in patients with advanced solid tumors: safety, pharmacokinetics, and antitumor activity. Clin Cancer Res, 2019. PMID: 30952639. DOI: 10.1158/1078-0432.CCR-18-1189

39 Lee M, Kang SY and Suh Y-L: Genetic alterations of epidermal growth factor receptor in glioblastoma. Appl Immunohistochem Mol Morphol *27(8)*: 589-598, 2018. PMID: 29912767. DOI: 10.1097/PAI.0000000000000669

40 Felsberg J, Hentschel B, Kaulich K, Gramatzki D, Zacher A, Malzkorn B, Kamp M, Sabel M, Simon M, Westphal M, Schackert G, Tonn JC, Pietsch T, von Deimling A, Loeffler M, Reifenberger G, Weller M and German Glioma Network: Epidermal growth factor receptor variant III (EGFRvIII) positivity in *EGFR*-amplified glioblastomas: prognostic role and comparison between primary and recurrent tumors. Clin Cancer Res *23*: 6846-6855, 2017. PMID: 28855349. DOI: 10.1158/1078-0432.CCR-17-0890

41 Laurendeau I, Ferrer M, Garrido D, D'Haene N, Ciavarelli P, Basso A, Vidaud M, Bieche I, Salmon I and Szijan I: Gene

expression profiling of ErbB receptors and ligands in human meningiomas. Cancer Invest *27*: 691-698, 2009. PMID: 19440932. DOI: 10.1080/07357900802709175

42 Arnli MB, Backer-Grøndahl T, Ytterhus B, Granli US, Lydersen S, Gulati S and Torp SH: Expression and clinical value of EGFR in human meningiomas. PeerJ *5*: e3140, 2017. PMID: 28367377. DOI: 10.7717/peerj.3140

43 Ferluga S, Baiz D, Hilton DA, Adams CL, Ercolano E, Dunn J, Bassiri K, Kurian KM and Hanemann CO: Constitutive activation of the EGFR-STAT1 axis increases proliferation of meningioma tumor cells. Neuro-Oncology Adv *2*, 2020. DOI: 10.1093/NOAJNL/VDAA008

44 Larysz D, Kula D, Kowal M, Rudnik A, Jarząb M, Blamek S, Bierzyńska-Macyszyn G, Kowalska M, Bazowski P and Jarząb B: Epidermal growth factor receptor gene expression in high grade gliomas. Folia Neuropathol *49*: 28-38, 2011. PMID: 21455841.

45 Yoshimoto K, Dang J, Zhu S, Nathanson D, Huang T, Dumont R, Seligson DB, Yong WH, Xiong Z, Rao N, Winther H, Chakravarti A, Bigner DD, Mellinghoff IK, Horvath S, Cavenee WK, Cloughesy TF and Mischel PS: Development of a real-time RT-PCR assay for detecting EGFRvIII in glioblastoma samples. Clin Cancer Res *14*: 488-493, 2008. PMID: 18223223. DOI: 10.1158/1078-0432.CCR-07-1966

46 Viana-Pereira M, Lopes JM, Little S, Milanezi F, Basto D, Pardal F, Jones C and Reis RM: Analysis of EGFR overexpression, EGFR gene amplification and the EGFRvIII mutation in Portuguese high-grade gliomas. Anticancer Res *28*: 913-920, 2008. PMID: 18507036.

47 An Z, Aksoy O, Zheng T, Fan Q-W and Weiss WA: Epidermal growth factor receptor and EGFRvIII in glioblastoma: signaling pathways and targeted therapies. Oncogene *37*: 1561-1575, 2018. PMID: 29321659. DOI: 10.1038/s41388-017-0045-7

48 Colaco RJ, Martin P, Kluger HM, Yu JB and Chiang VL: Does immunotherapy increase the rate of radiation necrosis after radiosurgical treatment of brain metastases? J Neurosurg *125*: 17-23, 2016. PMID: 26544782. DOI: 10.3171/2015.6.JNS142763

49 Swartz AM, Li Q-J and Sampson JH: Rindopepimut: a promising immunotherapeutic for the treatment of glioblastoma multiforme. Immunotherapy *6*: 679-690, 2014. PMID: 25186601. DOI: 10.2217/imt.14.21

50 Weller M, Butowski N, Tran DD, Recht LD, Lim M, Hirte H, Ashby L, Mechtler L, Goldlust SA, Iwamoto F, Drappatz J, O'Rourke DM, Wong M, Hamilton MG, Finocchiaro G, Perry J, Wick W, Green J, He Y, Turner CD, Yellin MJ, Keler T, Davis TA, Stupp R, Sampson JH; ACT IV Trial Investigators: Rindopepimut with temozolomide for patients with newly diagnosed, EGFRvIII-expressing glioblastoma (ACT IV): a randomised, double-blind, international phase 3 trial. Lancet Oncol *18*: 1373-1385, 2017. PMID: 28844499. DOI: 10.1016/S1470-2045(17)30517-X

51 Lassman AB, van den Bent MJ, Gan HK, Reardon DA, Kumthekar P, Butowski N, Lwin Z, Mikkelsen T, Nabors LB, Papadopoulos KP, Penas-Prado M, Simes J, Wheeler H, Walbert T, Scott AM, Gomez E, Lee H-J, Roberts-Rapp L, Xiong H, Ansell PJ, Bain E, Holen KD, Maag D and Merrell R: Safety and efficacy of depatuxizumab mafodotin + temozolomide in patients with EGFR-amplified, recurrent glioblastoma: results from an international phase I multicenter trial. Neuro Oncol *21*: 106-114, 2019. PMID: 29982805. DOI: 10.1093/neuonc/noy091

52 Choi BD, Yu X, Castano AP, Bouffard AA, Schmidts A, Larson RC, Bailey SR, Boroughs AC, Frigault MJ, Leick MB, Scarfò I, Cetrulo CL, Demehri S, Nahed B V., Cahill DP, Wakimoto H, Curry WT, Carter BS and Maus M V.: CAR-T cells secreting BiTEs circumvent antigen escape without detectable toxicity. Nat Biotechnol *37*: 1049-1058, 2019. PMID: 31332324. DOI: 10.1038/s41587-019-0192-1

53 Guo G, Gong K, Wohlfeld B, Hatanpaa KJ, Zhao D and Habib AA: Ligand-Independent EGFR Signaling. Cancer Res *75*: 3436-3441, 2015. PMID: 26282175. DOI: 10.1158/0008-5472.CAN-15-0989

54 Luwor RB, Zhu H-J, Walker F, Vitali AA, Perera RM, Burgess AW, Scott AM and Johns TG: The tumor-specific de2-7 epidermal growth factor receptor (EGFR) promotes cells survival and heterodimerizes with the wild-type EGFR. Oncogene *23*: 6095-6104, 2004. PMID: 15221011. DOI: 10.1038/sj.onc.1207870

55 Heimberger AB, Hlatky R, Suki D, Yang D, Weinberg J, Gilbert M, Sawaya R and Aldape K: Prognostic Effect of Epidermal Growth Factor Receptor and EGFRvIII in Glioblastoma Multiforme Patients. Clin Cancer Res *11*: 1462-1466, 2005. PMID: 15746047. DOI: 10.1158/1078-0432.CCR-04-1737

56 Shinojima N, Tada K, Shiraishi S, Kamiryo T, Kochi M, Nakamura H, Makino K, Saya H, Hirano H, Kuratsu J-I, Oka K, Ishimaru Y and Ushio Y: Prognostic value of epidermal growth factor receptor in patients with glioblastoma multiforme. Cancer Res *63*: 6962-6970, 2003. PMID: 14583498.

57 Hovinga KE, McCrea HJ, Brennan C, Huse J, Zheng J, Esquenazi Y, Panageas KS and Tabar V: EGFR amplification and classical subtype are associated with a poor response to bevacizumab in recurrent glioblastoma. J Neurooncol *142*: 337-345, 2019. PMID: 30680510. DOI: 10.1007/s11060-019-03102-5

58 Fan Q-W, Cheng CK, Gustafson WC, Charron E, Zipper P, Wong RA, Chen J, Lau J, Knobbe-Thomsen C, Weller M, Jura N, Reifenberger G, Shokat KM and Weiss WA: EGFR phosphorylates tumor-derived EGFRvIII driving STAT3/5 and progression in glioblastoma. Cancer Cell *24*: 438-449, 2013. PMID: 24135280. DOI: 10.1016/j.ccr.2013.09.004

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