Glioblastoma Multiforme and Genetic Mutations: The Issue Is Not Over Yet. An Overview of the Current Literature

Nicola Montemurro[1,2]

[1] Unit of Neurosurgery, "Di Venere" City Hospital, ASL Bari, Bari, Italy
[2] Department of Basic Medical Sciences, Neurosciences and Sensory Organs, University of Bari Aldo Moro, Bari, Italy

Address for correspondence Nicola Montemurro, MD, "Di Venere" City Hospital, ASL Bari - Unit of Neurosurgery, Via Ospedale Di Venere, 1 Bari 70131, Italy (e-mail: montemurronic@hotmail.com).

J Neurol Surg A 2020;81:64–70.

Abstract

Background and Objective Glioblastoma multiforme (GBM) is still a deadly disease with a poor prognosis and high mortality, despite the discovery of new biomarkers and new innovative targeted therapies. The role of genetic mutations in GBM is still not at all clear; however, molecular markers are an integral part of tumor assessment in modern neuro-oncology.

Material and Methods We performed a Medline search for the key words "glioblastoma," "glioblastoma multiforme," and "genetic" or "genetics" from 1990 to the present, finding an exponential increase in the number of published articles, especially in the past 7 years.

Results The understanding of molecular subtypes of gliomas recently led to a revision of the World Health Organization classification criteria for these tumors, introducing the concept of primary and secondary GBMs based on genetic alterations and gene or protein expression profiles. Some of these genetic alterations are currently believed to have clinical significance and are more related to secondary GBMs: TP53 mutations, detectable in the early stages of secondary GBM (found in 65%), isocitrate dehydrogenase 1/2 mutations (50% of secondary GBMs), and also O6-methylguanine-DNA methyltransferase promoter methylation (75% of secondary GBMs).

Conclusion From the introduction of the first standard of care (SOC) established in 2005 in patients with a new diagnosis of GBM, a great number of trials have been conducted to improve the actual SOC, but the real turning point has never been achieved or is yet to come. Surgical gross total resection, with at least one more reoperation, radiation therapy plus concomitant and adjuvant temozolomide chemotherapy currently remains the current SOC for patients with GBM.

Keywords
- glioblastoma multiforme
- prognostic biomarker
- genetics
- isocitrate dehydrogenase 1/2
- O6-methylguanine-DNA methyltransferase (MGMT)

Introduction

Glioblastomas multiforme (GBMs) are the most common malignant tumors of the central nervous system, accounting for 12 to 15% of all intracranial tumors, with an annual incidence of 5 per 100,000 people and ~ 17,000 new cases diagnosed each year.[1] The incidence of GBMs increases with age, peaking between 75 and 84 years. GBMs have a poor prognosis, and the 5-year survival is < 10%. Diffuse gliomas are highly invasive. Thus curative surgical resection can never be achieved, which leads to recurrence. The median overall survival (OS) from diagnosis was 14 months versus 22 months in patients who did not undergo second surgery and those with surgery at recurrence, whereas the median survival from the time of second surgery was ~ 9.7 months.[2–5]

received
June 24, 2018
accepted after revision
February 6, 2019
published online
September 24, 2019

DOI https://doi.org/10.1055/s-0039-1688911.
ISSN 2193-6315.

GBMs can be classified into primary (accoun90–95%ting for of all GBMs), more common among older adults, and secondary GBMs, which evolve from low-grade gliomas (LGGs) or anaplastic astrocytomas (AAs) over the years, which are more common among young people.[6] GBM is still a deadly disease with a poor prognosis and high mortality, despite the discovery of new biomarkers. Some of these need validation to be used in the clinical routine, such as advances in technologies, for example genomics, epigenomics, transcriptomics, and new innovative targeted therapies. The role of genetic mutations in GBMs is still unclear; however, molecular markers are an integral part of tumor assessment in modern neuro-oncology, helping clinicians make therapeutic and clinical decisions for their patients with GBM.[7]

Materials and Methods

We performed a Medline search for the key words "glioblastoma," "glioblastoma multiforme" and "genetic" or "genetics" from 1990 to the present to identify the most recent advances in the genomics of GBMs and summarize genetic review articles, clinical studies in patients with primary or secondary GBM, and validated or not yet validated biomarkers that may affect patients' outcome.

Results

More than 9,500 studies were found using Ovid Medline databases, an exponential increase in the number of published articles, especially in the past 7 years, that discuss potential genetics perspectives in patients with GBMs (►**Fig. 1**). Recent studies were eligible for inclusion if they were not single cases, reported original data on genetic mutations about GBMs, and were targeted therapies and reviews (►**Fig. 2**). Our digital search was supplemented by examining the reference lists of the selected studies, analyzing the most important.

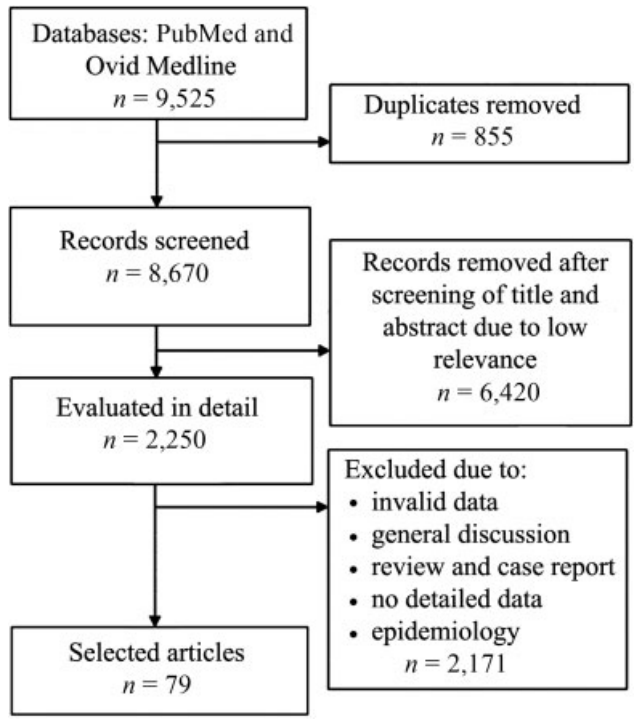

Fig. 2 Flow diagram of study selection of the current literature.

Discussion

GBM was one of the first tumors profiled by The Cancer Genome Atlas Network, using molecular parameters in addition to histology to define many tumor entities.[8] The understanding of molecular subtypes of GBMs and other grades of gliomas recently led to a revision of the World Health Organization (WHO) diagnostic and classification criteria for these tumors.[9] The 2016 WHO classification divides gliomas into LGGs and GBMs per histology. LGGs are further divided into isocitrate dehydrogenase (IDH) 1/2 wild-type or mutant and further classified if there is a

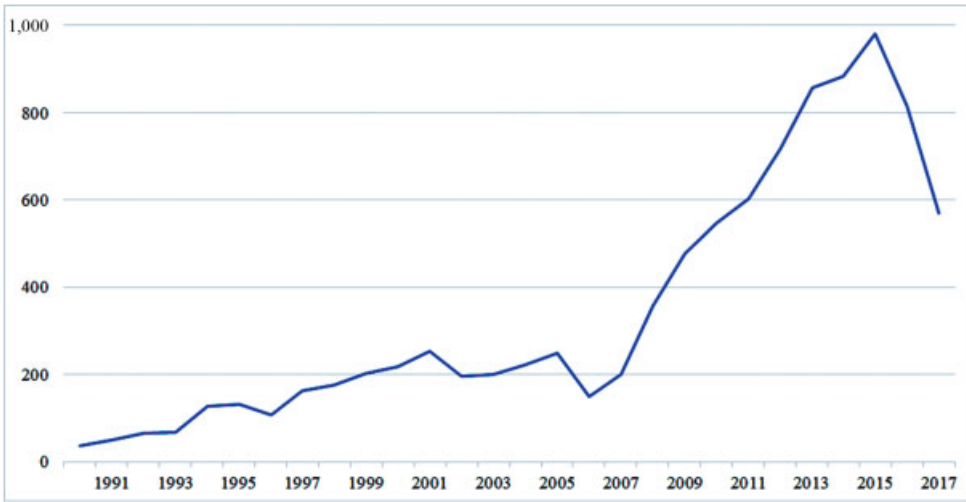

Fig. 1 Diagram showing exponential increase in the number of published articles debating primary and secondary glioblastomas and both genetic alterations and gene expression profiles.

complete deletion of both the short arm of chromosome 1 and the long arm of chromosome 19 (1p/19q codeletion) (oligodendroglioma) or intact 1p/19q loci (diffuse astrocytoma). GBMs are divided into IDH wild-type (primary or de novo GBMs) and IDH mutant (secondary GBMs).[9–11] This new classification merges classical histologic classification, grading, immunohistochemical, and molecular genetic data, allowing a better characterization of gliomas.

Most (~ 90%) of GBMs are primary tumors. They are highly invasive neoplasms, more common in older patients, and arise in the absence of prior disease. Secondary GBMs are much less common, develop from LGGs or AAs, and are associated with a better prognosis. Primary and secondary GBMs are histologically indistinguishable, but they develop from different genetic precursors and show different genetic alterations that permit differentiation. Patients with secondary GBMs were found to be significantly younger than those with primary GBMs.[12] With respect to anatomical localization, Li et al reported secondary GBMs are more commonly located in the frontal lobe (68%), whereas the frontal and temporal lobes were the most typically involved sites in both primary and secondary GBMs.[12]

In primary GBMs, the most frequent genetic alterations observed are loss of heterozygosity (LOH) at 10q (65% of cases), amplification or mutation of epidermal growth factor receptor (EGFR) (22–40%), amplification of platelet-derived growth factor receptor (PDGFR) (7%), tumor protein 53 (TP53) mutation (28–31%), *cyclin-dependent kinase inhibitor 2 A/B* (*CDKN2A/B*) deletion (31%), phosphatase and tensin homolog (PTEN) mutation or deletion (24–30%), IDH1/2 mutation (5%), telomerase reverse transcriptase (TERT) promoter (10%), O6-methylguanine-DNA methyltransferase (MGMT) promoter methylation (36%), loss of expression of the *retinoblastoma gene* (*RB1*) (2%), phosphatidylinositol-4,5-bisphosphate 3-kinase A (PIK3CA) (1%), *murine double minute 2* (*MDM2*) (7–12%), neurofibromatosis type 1 (NF1) deletion or mutations (11%), and glioma-associated *oncogene homolog 1* (*GLI1*) (5–22%).[8,13–21]

In secondary GBMs, the most frequent genetic alteration observed are TP53 mutation (65% of cases), LOH at 22q (70–80%), LOH 19q (40–50%), IDH1/2 mutation (45–50%), MGMT promoter methylation (75%), *CDKN2A/B* deletion, PDGFR gene amplification (7%) 1p/19q codeletion (15–20%), and EGFR (5–7%).[16,17,20,22–26]

Secondary GBMs

Although some genetic aberrations are more frequently expressed in both secondary and primary GBMs, and both subgroups are capable with time of undergoing further malignant degeneration, their role appears to be predominantly related to one of them. Some of them are currently considered clinically significant and are more related to secondary GBMs: TP53 mutations, detectable in the early stages of secondary GBM (found in 65% versus 28% of primary GBMs); IDH1/2 mutations (50% of secondary versus 5% of primary GBMs); and also MGMT promoter methylation (75% of secondary GBMs versus 36% of primary GBMs).[17,27]

TP53 mutation is predominantly related to secondary GBMs, suggesting that TP53 mutation is a specific and stereotyped event in secondary GBM oncology, whereas TP53 mutation in primary GBM is potentially a consequence of widespread genomic instability. In 2008, Parsons et al first reported recurrent mutations in the IDH1/2 genes in patients with gliomas.[28] Subsequent studies identified IDH1 mutations in 45 to 50% of patients with grade II/III gliomas and secondary GBMs.[22,23,25,29] These patients with IDH mutation showed a better prognosis in every grade of glioma and different disease characteristics relative to patients with a glioma with wild-type IDH.[30–32] IDH1/2 mutations were identified by WHO as a critical biomarker in the classification of gliomas and the most reliable marker of secondary GBMs than any other pathologic criterion to differentiate primary from secondary GBMs, whereas an IDH wild type in grade II/III gliomas showed an OS rate more similar to that of GBMs.[9,26,28,30,33]

Nobusawa et al reported a small subgroup of patients with primary GBM and IDH mutation (3.4%) who were younger than other patients and who had TP53 mutations and absence of EGFR amplification (features of the secondary GBMs), suggesting cases of rapidly progressive secondary GBMs, rather than a true primary GBM.[34] For the same reason, a histologic AA with an IDH wild type is now considered a GBM, and a GBM with IDH1 mutation and 1p/19q codeletion is considered an anaplastic oligodendroglioma (AO) in determining patient survival.[35] Studies reported that IDH1 mutation occurs before other mutations, whereas TP53 and α thalassemia/mental retardation syndrome X-linked (ATRX) mutations occur in the setting of development of an AA, and a 1p/19q codeletion occurs in the setting of the development of an AO.[26,29]

ATRX mutation, playing a role in chromatin modulation and maintenance of telomeres, represents a prognostic factor in gliomas. About 50% of MGMT promoter methylation occurs in secondary GBMs, which are more sensitive to chemoradiotherapy, increasing OS when surgery is followed by temozolomide (TMZ) concurrently with radiation therapy.[36–38] Since 2008, several studies have reported an increase in overall survival from 30 to 40 months in patients with GBMs or AAs with IDH1 mutation versus those without this mutation.[16,28,39–41] This survival benefit was also identified in patients with grade II gliomas, showing an increase in OS of 7 years in patients with IDH1 mutation versus patients without this mutation.[39] The best prognosis was found in patients with IDH1/2 mutant and 1p/19q codeleted tumors, whereas patients with IDH1/2 wild-type gliomas and glioblastoma-like genomic alterations (loss on chromosome arm 10q and TERT promoter mutation) tended to have the worst outcome.[15,42] Intermediate survival was seen in patients with IDH1/2 mutant but 1p/19q intact or in patients with IDH1/2 wild-type gliomas with glioblastoma-like genomic alterations.[42] Similarly, ATRX mutation with IDH1/2 wild type is a marker of grade II/III gliomas and secondary GBMs and associated with more favorable outcomes.[43]

Primary GBMs

Genetic aberrations that are more common in primary GBMs are EGFR, *CDKN2A/B* deletion, PTEN mutation or deletion,

PDGFR, TP53 mutation, and TERT promoter.[8,14,16–18,21] EGFR and its most common EGFR-activating mutant variant (EGFRvIII) are tyrosine kinase growth factor receptors and associated with high-grade malignancy, poor prognosis, and shorter OS. The amplification of the *EGFR* gene has high incidence in all gliomas, and it is found in 40 to 50% of primary GBMs.[19] Currently, EGFR status can be used to predict patient response to EGFR-targeted therapies.

CDKN2A/B deletions, found in both primary and secondary GBMs, are more common in primary GBMs.[17,26] After TP53 mutation, *PTEN* is the second most commonly mutated tumor suppressor gene in all tumors. *PTEN,* found in the advanced stages of disease in primary GBM, is central in inhibiting cell proliferation and regulating the ability of cells in migration and invasion.[44,45] *PTEN* mutation rates in primary GBMs and in AAs are 26 to 34% and 18%, respectively. Patients with a *PTEN* mutation tend to have a poor prognosis. The contribution of TERT mutation to tumor aggressiveness is not clear because this mutation, detected in 46% of all GBMs, was found in both primary and secondary tumors and associated with a shorter OS.[46]

The prognostic effect of TERT mutations, according to Labussière et al, may depend on the context because they reported that in LGGs without IDH mutations, TERT mutation is a negative prognostic factor, but in IDH-mutated LGGs, TERT mutation is a positive prognostic factor.[47,48] Eckel-Passow et al reported that gliomas with only TERT mutations are primary GBMs, and patients with GBMs with only TERT mutations have a high prevalence of loss of chromosome 4 and acquired PIK3CA mutations and tend to have the poorest prognosis.[25]

Recent research reported the important role of the expression of vascular endothelial growth factor (VEGF), a common genetic alteration in GBMs, detected in 58.9% of cases, which contributes to the angiogenesis of tumors, as does the X-linked inhibitor of apoptosis (XIAP)-associated factor (XAF) 1, a tumor suppressor that is a precise indicator of IDH1 mutations in grade III gliomas.[49,50] Although mutations in certain cancer genes, such as B-Raf (BRAF) and RAS genes, have rarely been observed in GBMs, mutations in the PIK3CA and PIK3R1 genes, although rare, have been described.[8] BRAF is seen more frequently in pleomorphic xanthoastrocytomas (50–60%), in pilocytic astrocytomas ($<$ 10%), and in gangliogliomas (20–75%).[51,52]

Verhaak et al and other studies analyzing somatic mutations and gene expression identified four transcriptional subtypes of GBMs: classic, proneural, neural, and mesenchymal.[8,21,51,53,54] The classic category showed a greater preponderance of EGFR amplification and CDKN2A alterations, decreased rates of TP53 mutation, loss of chromosome 10, and amplification of chromosome 7 and mitogen-activated protein kinase.[53] The proneural category is associated with PDGFR amplification, IDH1 and TP53 mutations, and activation of the phosphatidylinositol 3-kinase (PI3K). Patients with proneural subtypes GBMs were younger, responded better to therapy, and had a prolonged OS.[53] The neural subtype was associated with a variety of neuron markers and closest to the normal brain, whereas the mesenchymal subtype was found to have a greater degree of NF1 mutations and alterations of PTEN and a greater degree of necrosis histologically.[53]

Although primary GBMs could be of any subtype and mutations, secondary GBMs are more linked with the proneural subtype,[53] predominantly associated with IDH1/2 mutations. This subtype was further subclassified as either CpG island methylator phenotype + (CIMP +) or CIMP− (of which the CIMP+ shows a better prognosis and is associated with longer survival).[21,33] Turcan et al demonstrated that the IDH1 mutation alone is capable of remodeling the genomic methylation profile of the tumor, thus promoting the CIMP+ profile.[55]

Chemotherapy, Immunotherapy, and Targeted Therapy

From the introduction of the first standard of care (SOC) established by Stupp and colleagues in 2005 in patients with new diagnosis of GBM, a great number of trials have been conducted to improve the actual SOC, but the real turning point has never been achieved or, more optimistically, is yet to come.[56–66] MGMT promoter methylation is used as a predictive biomarker of response to TMZ and associated with a better response to it and a better OS in MGMT methylated patients compared with MGMT unmethylated GBM patients.[67,68] Although it is difficult to withhold an approved chemotherapy from MGMT unmethylated patients with primary or secondary GBMs, at recurrence there is no evidence for treating unmethylated patients with TMZ at any dose or schedule.[67]

In a multivariate analysis on 180 patients, Azoulay et al[69] reported that repeat surgery and MGMT promoter methylation were independent prognostic factors that positively affect survival. In contrast, Franceschi et al,[70] in a multivariate analysis of 232 patients, showed that resurgery did not affect survival, whereas MGMT methylation were significantly correlated with OS, reporting that the median time between the first and second surgery was significantly longer in patients with methylated MGMT than in patients with unmethylated MGMT (19.3 versus 13 months). Similarly, mutations in IDH1/2 and TP53 are used as a strong predictive marker for response to chemotherapy in patients with GBMs.[21] However, these last few years have represented a challenge for neuro-oncologists, who try to improve the OS in patients with GBMs, intensifying the dose or prolonging the exposure to TMZ, adding integrin-inhibitor cilengitide or anti-VEGF antibody bevacizumab.[58–60,62,65] In any case, combination therapies require management of toxicities, therapeutic response monitoring, and drug interactions. Tyrosine kinase inhibitors drugs like gefitinib, erlotinib, and cetuximab have been tested for use in GBMs; however, they have not been proven effective for monotherapy.[64,66]

The brain has long been considered an immune-privileged site precluding potent immune responses, but the failure of conventional chemotherapy has led to a consideration of immunotherapy (brain tumor vaccines, immune checkpoint blockade, monoclonal antibodies, radiolabeled antibodies injected directly into the tumor, recombinant interleukin-2 and lymphokine activated killer cells, recombinant immunotoxins specific for EGFR) as a promising strategy.[71] Targeting the mutation EGFRvIII using vaccine alone or in combination with tyrosine kinases inhibitors and TMZ reduced tumor development in xenograft models, but a more recent phase III clinical trial using this approach showed no survival benefit;

nor did other studies using specific targets toward EGFR that also showed disappointing results.[63,71–74]

Immunosuppression is one of the primary reasons for a poor prognosis in GBM. Reduction in T-cell mediated immune response is due to coinhibitory receptors on T cells known as immune checkpoint molecules. Blocking such immune checkpoint molecules, such as cytotoxic T-lymphocyte antigen 4 (CTLA-4) and programmed death 1 (PD-1), tumor regression and promotion of long-term survival can be induced.[75,76] The combination of anti-PD-1 antibodies and radiotherapy doubled median survival and enhanced long-term survival in 15 to 40% of GBM mice.[77] With improvement in diagnostic imaging and methods of delivery, gene therapy may be used in conjunction with surgery, chemotherapy/radiotherapy, or in cases of recurrences following excision as well as chemotherapy-resistant tumors, but at this time no gene therapy for GBM has yet been approved in the United States or Europe.[78] Second surgery, chemotherapy given at recurrence, target therapy, and radiotherapy led to a prolongation of the median OS to 18 to 20 months in the most recent trials.[2,61] Future research should focus on identifying synergistic interactions between chemotherapy, radiotherapy, and immunotherapy to maximize the antitumor potential of individual treatment approaches.

Conclusions

These molecular approaches to GBMs revolutionized the understanding of the pathophysiology of gliomas, pathologic classification of tumors, and therapeutic approaches, even if all these genetic classifications do not routinely influence treatment choices.[79] It is possible that further GBM subclassifications will lead toward individualized treatments for distinct molecular subtypes. GBM patient samples taken during the first tumor resection and after first-line chemotherapy, done during second tumor resection for recurrent GBMs, can be useful to understand the changes occurring in gene and protein expression, to improve pharmacologic research, and to establish the most effective pharmacotherapy for the single patient. At the present time, surgical gross total resection, with at least one more reoperation, radiation therapy plus concomitant and adjuvant TMZ chemotherapy remains the current SOC for patients with GBM. Ultimately, the goal would be an individualized approach to implement a personalized medicine for this deadly disease.

Conflict of Interest
None declared.

References

1 Omuro A, DeAngelis LM. Glioblastoma and other malignant gliomas: a clinical review. JAMA 2013;310(17):1842–1850
2 Wann A, Tully PA, Barnes EH, et al. Outcomes after second surgery for recurrent glioblastoma: a retrospective case-control study. J Neurooncol 2018;137(02):409–415
3 Montemurro N, Perrini P, Blanco MO, Vannozzi R. Second surgery for recurrent glioblastoma: a concise overview of the current literature. Clin Neurol Neurosurg 2016;142:60–64
4 deSouza RM, Shaweis H, Han C, et al. Has the survival of patients with glioblastoma changed over the years? Br J Cancer 2016;114 (02):146–150
5 Ohba S, Hirose Y. Current and future drug treatments for glioblastomas. Curr Med Chem 2016;23(38):4309–4316
6 Soomro SH, Ting LR, Qing YY, Ren M. Molecular biology of glioblastoma: classification and mutational locations. J Pak Med Assoc 2017;67(09):1410–1414
7 Siegal T. Clinical relevance of prognostic and predictive molecular markers in gliomas. Adv Tech Stand Neurosurg 2016;(43):91–108
8 Cancer Genome Atlas Research Network. Comprehensive genomic characterization defines human glioblastoma genes and core pathways. Nature 2008;455(7216(:1061–1068
9 Louis DN, Perry A, Reifenberger G, et al. The 2016 World Health Organization Classification of Tumors of the Central Nervous System: a summary. Acta Neuropathol 2016;131(06):803–820
10 Malzkorn B, Reifenberger G. Practical implications of integrated glioma classification according to the World Health Organization classification of tumors of the central nervous system 2016. Curr Opin Oncol 2016;28(06):494–501
11 Sturm D, Orr BA, Toprak UH, et al. New brain tumor entities emerge from molecular classification of CNS-PNETs. Cell 2016; 164(05):1060–1072
12 Li R, Li H, Yan W, et al. Genetic and clinical characteristics of primary and secondary glioblastoma is associated with differential molecular subtype distribution. Oncotarget 2015;6(09):7318–7324
13 Crisman TJ, Zelaya I, Laks DR, et al. Identification of an efficient gene expression panel for glioblastoma classification. PLoS One 2016;11(11):e0164649
14 Karsy M, Guan J, Cohen AL, Jensen RL, Colman H. New molecular considerations for glioma: IDH, ATRX, BRAF, TERT, H3 K27M. Curr Neurol Neurosci Rep 2017;17(02):19
15 Karsy M, Neil JA, Guan J, Mahan MA, Colman H, Jensen RL. A practical review of prognostic correlations of molecular biomarkers in glioblastoma. Neurosurg Focus 2015;38(03):E4
16 Liu A, Hou C, Chen H, Zong X, Zong P. Genetics and epigenetics of glioblastoma: applications and overall incidence of IDH1 mutation. Front Oncol 2016;6:16
17 Lombardi MY, Assem M. Glioblastoma genomics: a very complicated story. In: De Vleeschouwer S, ed. Glioblastoma. Brisbane, Australia: Codon Publications; 2017:3–25
18 Rao SK, Edwards J, Joshi AD, Siu IM, Riggins GJ. A survey of glioblastoma genomic amplifications and deletions. J Neurooncol 2010;96(02):169–179
19 Roth P, Weller M. Challenges to targeting epidermal growth factor receptor in glioblastoma: escape mechanisms and combinatorial treatment strategies. Neuro Oncol 2014;16(Suppl 8): viii14-9
20 Wee CW, Kim E, Kim N, et al. Novel recursive partitioning analysis classification for newly diagnosed glioblastoma: A multi-institutional study highlighting the MGMT promoter methylation and IDH1 gene mutation status. Radiother Oncol 2017;123(01):106–111
21 Brennan CW, Verhaak RG, McKenna A, et al; TCGA Research Network. The somatic genomic landscape of glioblastoma. Cell 2013;155(02):462–477
22 Brat DJ, Verhaak RG, Aldape KD, et al; Cancer Genome Atlas Research Network. Comprehensive, integrative genomic analysis of diffuse lower-grade gliomas. N Engl J Med 2015;372(26): 2481–2498
23 Ceccarelli M, Barthel FP, Malta TM, et al; TCGA Research Network. Molecular profiling reveals biologically discrete subsets and pathways of progression in diffuse glioma. Cell 2016;164(03):550–563
24 Colman H. Toward more informative biomarker-based clinical trials in glioblastoma. Neuro Oncol 2017;19(07):880–881
25 Eckel-Passow JE, Lachance DH, Molinaro AM, et al. Glioma groups based on 1p/19q, IDH, and TERT promoter mutations in tumors. N Engl J Med 2015;372(26):2499–2508

26 Jiao Y, Killela PJ, Reitman ZJ, et al. Frequent ATRX, CIC, FUBP1 and IDH1 mutations refine the classification of malignant gliomas. Oncotarget 2012;3(07):709–722
27 Nakamura M, Watanabe T, Yonekawa Y, Kleihues P, Ohgaki H. Promoter methylation of the DNA repair gene MGMT in astrocytomas is frequently associated with G:C–> A:T mutations of the TP53 tumor suppressor gene. Carcinogenesis 2001;22(10):1715–1719
28 Parsons DW, Jones S, Zhang X, et al. An integrated genomic analysis of human glioblastoma multiforme. Science 2008;321 (5897):1807–1812
29 Costello JF, Plass C, Arap W, et al. Cyclin-dependent kinase 6 (CDK6) amplification in human gliomas identified using two-dimensional separation of genomic DNA. Cancer Res 1997;57 (07):1250–1254
30 Ohgaki H, Kleihues P. The definition of primary and secondary glioblastoma. Clin Cancer Res 2013;19(04):764–772
31 Richardson TE, Snuderl M, Serrano J, et al. Rapid progression to glioblastoma in a subset of IDH-mutated astrocytomas: a genome-wide analysis. J Neurooncol 2017;133(01):183–192
32 Szopa W, Burley TA, Kramer-Marek G, Kaspera W. Diagnostic and therapeutic biomarkers in glioblastoma: current status and future perspectives. BioMed Res Int 2017;2017:8013575
33 Suzuki H, Aoki K, Chiba K, et al. Mutational landscape and clonal architecture in grade II and III gliomas. Nat Genet 2015;47(05): 458–468
34 Nobusawa S, Watanabe T, Kleihues P, Ohgaki H. IDH1 mutations as molecular signature and predictive factor of secondary glioblastomas. Clin Cancer Res 2009;15(19):6002–6007
35 Aldape K, Zadeh G, Mansouri S, Reifenberger G, von Deimling A. Glioblastoma: pathology, molecular mechanisms and markers. Acta Neuropathol 2015;129(06):829–848
36 Jordan JT, Gerstner ER, Batchelor TT, Cahill DP, Plotkin SR. Glioblastoma care in the elderly. Cancer 2016;122(02):189–197
37 Roth P, Gramatzki D, Weller M. Management of elderly patients with glioblastoma. Curr Neurol Neurosci Rep 2017;17(04):35
38 Zawlik I, Vaccarella S, Kita D, Mittelbronn M, Franceschi S, Ohgaki H. Promoter methylation and polymorphisms of the MGMT gene in glioblastomas: a population-based study. Neuroepidemiology 2009;32(01):21–29
39 Sanson M, Marie Y, Paris S, et al. Isocitrate dehydrogenase 1 codon 132 mutation is an important prognostic biomarker in gliomas. J Clin Oncol 2009;27(25):4150–4154
40 Wick W, Hartmann C, Engel C, et al. NOA-04 randomized phase III trial of sequential radiochemotherapy of anaplastic glioma with procarbazine, lomustine, and vincristine or temozolomide. J Clin Oncol 2009;27(35):5874–5880
41 Yan H, Parsons DW, Jin G, et al. IDH1 and IDH2 mutations in gliomas. N Engl J Med 2009;360(08):765–773
42 Weller M, Weber RG, Willscher E, et al. Molecular classification of diffuse cerebral WHO grade II/III gliomas using genome- and transcriptome-wide profiling improves stratification of prognostically distinct patient groups. Acta Neuropathol 2015;129(05):679–693
43 Pekmezci M, Rice T, Molinaro AM, et al. Adult infiltrating gliomas with WHO 2016 integrated diagnosis: additional prognostic roles of ATRX and TERT. Acta Neuropathol 2017;133(06):1001–1016
44 Dali R, Verginelli F, Pramatarova A, Sladek R, Stifani S. Characterization of a FOXG1:TLE1 transcriptional network in glioblastoma-initiating cells. Mol Oncol 2018;12(06):775–787
45 Han F, Hu R, Yang H, et al. PTEN gene mutations correlate to poor prognosis in glioma patients: a meta-analysis. OncoTargets Ther 2016;9(09):3485–3492
46 Mosrati MA, Malmström A, Lysiak M, et al. TERT promoter mutations and polymorphisms as prognostic factors in primary glioblastoma. Oncotarget 2015;6(18):16663–16673
47 Labussière M, Di Stefano AL, Gleize V, et al. TERT promoter mutations in gliomas, genetic associations and clinico-pathological correlations. Br J Cancer 2014;111(10):2024–2032
48 Fan X, Wang Y, Liu Y, et al. Brain regions associated with telomerase reverse transcriptase promoter mutations in primary glioblastomas. J Neurooncol 2016;128(03):455–462
49 Fan X, Wang Y, Wang K, et al. Anatomical specificity of vascular endothelial growth factor expression in glioblastomas: a voxel-based mapping analysis. Neuroradiology 2016;58(01):69–75
50 Reich TR, Switzeny OJ, Renovanz M, et al. Epigenetic silencing of XAF1 in high-grade gliomas is associated with IDH1 status and improved clinical outcome. Oncotarget 2017;8(09): 15071–15084
51 Mansouri A, Karamchandani J, Das S. Molecular Genetics of Secondary Glioblastoma. In: De Vleeschouwer S, ed. Glioblastoma. Brisbane, Australia: Codon Publications; 2017:27–42
52 Schindler G, Capper D, Meyer J, et al. Analysis of BRAF V600E mutation in 1,320 nervous system tumors reveals high mutation frequencies in pleomorphic xanthoastrocytoma, ganglioglioma and extra-cerebellar pilocytic astrocytoma. Acta Neuropathol 2011;121 (03):397–405
53 Verhaak RG, Hoadley KA, Purdom E, et al; 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 2010;17(01):98–110
54 Phillips HS, Kharbanda S, Chen R, et al. Molecular subclasses of high-grade glioma predict prognosis, delineate a pattern of disease progression, and resemble stages in neurogenesis. Cancer Cell 2006;9(03):157–173
55 Turcan S, Rohle D, Goenka A, et al. IDH1 mutation is sufficient to establish the glioma hypermethylator phenotype. Nature 2012; 483(7390):479–483
56 Stupp R, Mason WP, van den Bent MJ, et al; European Organisation for Research and Treatment of Cancer Brain Tumor and Radiotherapy Groups; National Cancer Institute of Canada Clinical Trials Group. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. N Engl J Med 2005;352(10):987–996
57 Chen R, Cohen AL, Colman H. Targeted therapeutics in patients with high-grade gliomas: past, present, and future. Curr Treat Options Oncol 2016;17(08):42
58 Chinot OL, Wick W, Mason W, et al. Bevacizumab plus radiotherapy-temozolomide for newly diagnosed glioblastoma. N Engl J Med 2014;370(08):709–722
59 Gilbert MR, Dignam JJ, Armstrong TS, et al. A randomized trial of bevacizumab for newly diagnosed glioblastoma. N Engl J Med 2014;370(08):699–708
60 Gilbert MR, Wang M, Aldape KD, et al. Dose-dense temozolomide for newly diagnosed glioblastoma: a randomized phase III clinical trial. J Clin Oncol 2013;31(32):4085–4091
61 Marosi C, Preusser M. Milestones of the last 10 years: CNS cancer. Memo 2017;10(01):18–21
62 Nabors LB, Fink KL, Mikkelsen T, et al. Two cilengitide regimens in combination with standard treatment for patients with newly diagnosed glioblastoma and unmethylated MGMT gene promoter: results of the open-label, controlled, randomized phase II CORE study. Neuro Oncol 2015;17(05):708–717
63 Sampson JH, Aldape KD, Archer GE, et al. Greater chemotherapy-induced lymphopenia enhances tumor-specific immune responses that eliminate EGFRvIII-expressing tumor cells in patients with glioblastoma. Neuro Oncol 2011;13(03):324–333
64 Schulte A, Liffers K, Kathagen A, et al. Erlotinib resistance in EGFR-amplified glioblastoma cells is associated with upregulation of EGFRvIII and PI3Kp110δ. Neuro Oncol 2013;15(10):1289–1301
65 Stupp R, Hegi ME, Gorlia T, et al; European Organisation for Research and Treatment of Cancer (EORTC); Canadian Brain Tumor Consortium; CENTRIC study team. Cilengitide combined with standard treatment for patients with newly diagnosed glioblastoma with methylated MGMT promoter (CENTRIC EORTC 26071-22072 study): a multicentre, randomised, open-label, phase 3 trial. Lancet Oncol 2014;15(10):1100–1108

66 van den Bent MJ, Brandes AA, Rampling R, et al. Randomized phase II trial of erlotinib versus temozolomide or carmustine in recurrent glioblastoma: EORTC brain tumor group study 26034. J Clin Oncol 2009;27(08):1268–1274
67 Taylor JW, Schiff D. Treatment considerations for MGMT-unmethylated glioblastoma. Curr Neurol Neurosci Rep 2015;15(01):507
68 Binabaj MM, Bahrami A, ShahidSales S, et al. The prognostic value of MGMT promoter methylation in glioblastoma: a meta-analysis of clinical trials. J Cell Physiol 2018;233(01):378–386
69 Azoulay M, Santos F, Shenouda G, et al. Benefit of re-operation and salvage therapies for recurrent glioblastoma multiforme: results from a single institution. J Neurooncol 2017;132(03):419–426
70 Franceschi E, Bartolotti M, Tosoni A, et al. The effect of re-operation on survival in patients with recurrent glioblastoma. Anticancer Res 2015;35(03):1743–1748
71 Roth P, Preusser M, Weller M. Immunotherapy of brain cancer. Oncol Res Treat 2016;39(06):326–334
72 Weller M, Butowski N, Tran DD, et al; 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 2017;18 (10):1373–1385
73 Felsberg J, Hentschel B, Kaulich K, et al; 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 2017;23 (22):6846–6855
74 Tanguturi SK, Trippa L, Ramkissoon SH, et al. Leveraging molecular datasets for biomarker-based clinical trial design in glioblastoma. Neuro Oncol 2017;19(07):908–917
75 Ghosh D, Nandi S, Bhattacharjee S. Combination therapy to checkmate Glioblastoma: clinical challenges and advances. Clin Transl Med 2018;7(01):33
76 Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. Nat Rev Cancer 2012;12(04):252–264
77 Zeng J, See AP, Phallen J, et al. Anti-PD-1 blockade and stereotactic radiation produce long-term survival in mice with intracranial gliomas. Int J Radiat Oncol Biol Phys 2013;86(02):343–349
78 Jenkinson MD, Barone DG, Bryant A, et al. Intraoperative imaging technology to maximise extent of resection for glioma. Cochrane Database Syst Rev 2018;1:CD012788
79 Lin CA, Rhodes CT, Lin C, Phillips JJ, Berger MS. Comparative analyses identify molecular signature of MRI-classified SVZ-associated glioblastoma. Cell Cycle 2017;16(08):765–775