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Chapter 14

Mechanisms of Aggressiveness in Glioblastoma: Prognostic and Potential Therapeutic Insights

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Additional information is available at the end of the chapter

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1. Introduction

1.1. Glioblastoma

Glioblastoma (GBM) is the most prevalent and most malignant (WHO grade IV) type of brain tumor in adults [1, 2]. In the United States, there are ~10,000 new cases diagnosed annually, and >50,000 patients living with the disease [2, 3]. The clinical responses of patients are particularly poor and vary greatly among individuals [4], and ~32% of all diagnosed cases survive less than a year [3]. This highly aggressive tumor develops either *de novo* (primary GBM), or as the result of the malignant progression from a lower-grade glioma (secondary GBM). In both cases, prognosis is very poor, and the median survival when radiotherapy and chemotherapy are combined is approximately 15 months [5]. Importantly, GBM is also characterized by extensive heterogeneity at the cellular and molecular levels. These tumors are highly diffuse, with extensive dissemination of tumor cells within the brain, which hinders complete surgical resection. These aggressive characteristics are associated with a remarkable resistance to therapies available today [6], which unfortunately are mostly palliative. In the context of their highest incidence of all malignant brain tumors in adults, their highly aggressive behavior and therapy-insensitive nature, which together account for a very poor prognosis of GBM patients, this chapter will focus specifically on GBM. In particular, it will review the different hypotheses of glioma/GBM-initiating cells, the major alterations at the levels of gene expression and signaling pathways found in GBM, as well as putative biomarkers of GBM prognosis, and current therapies currently available or under investigation for dealing with these tumors.

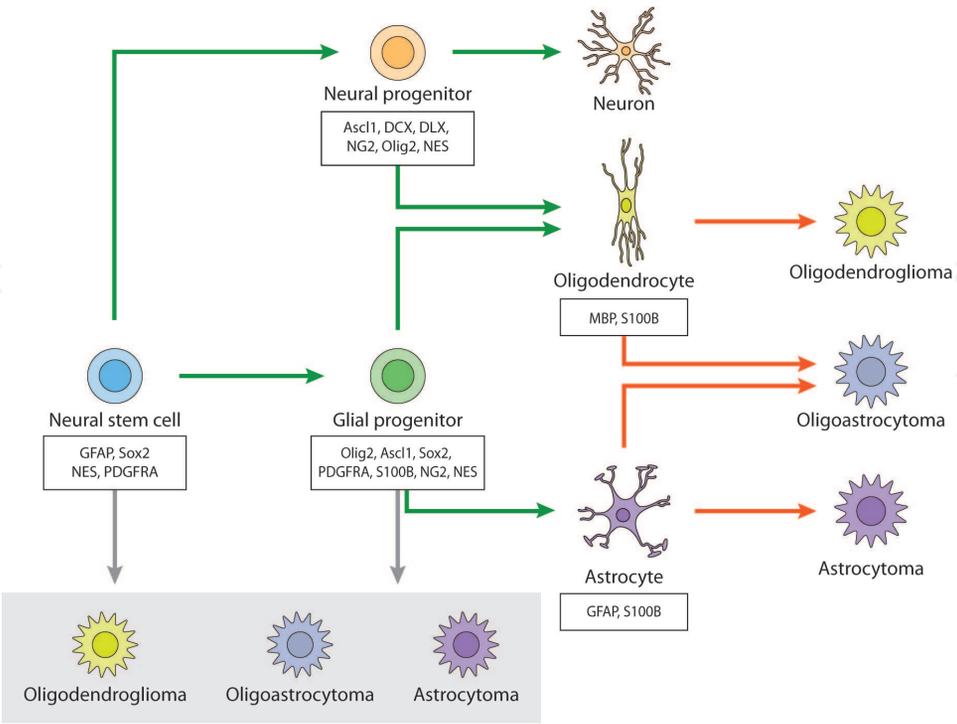

Figure 1. Schematic representation of the differentiation process of neural stem cells into different cell lineages of the CNS and putative cells of origin of gliomas. Protein markers for neural stem cells, progenitors cells, and differentiated cells are indicated in boxes. The normal differentiation process (green arrows) originates three main types of cells in the mature CNS, including neurons and glial cells (particularly oligodendrocytes and astrocytes; ependymal cells are not represented). The most classical hypothesis on the origin of glioma cells is represented by orange arrows (differentiated glial cells are malignantly transformed through a dedifferentiation process). The most recent hypothesis postulating that gliomas originate from the direct transformation of neural stem cells or glial progenitor cells is represented by grey arrows.

1.2. Glioma/GBM-Initiating cells

The true cellular origin of gliomas, including GBM, is still a debatable question. It is generally accepted that identifying such tumor-initiating cells may allow a better understanding of tumor biology, and ultimately help in designing improved therapies for GBM. All human tumors arise from a series of molecular alterations that occur in a small number, or even single, founder cells. These tumor cells present a clonal nature due to the sequential accumulation of multiple rare genetic and epigenetic events. The critical importance of the tumor microenvironment in influencing tumor cells behavior and evolution has been recently recognized [7]. Indeed, the tumor microenvironment has been associated with the generation and maintenance of tumor heterogeneity; thus, understanding not only the surrounding microenvironment but also tumor heterogeneity, as well as their relationships, may be crucial in understanding the biology of these tumors. In the case of the brain tissue, a highly complex microenvironment with extreme phenotypic and functional diversity, the multiplicity of putative brain tumor cells of origin, and the variety of niches in which the malignant cells may evolve, is even more challenging. Thus, understanding this complexity is crucial to provide firm evidence for the cellular origin of gliomas [8-10]. Two different hypotheses for the origin of glioma cells, or tumor cells in general, have been proposed (Figure 1), as detailed below.

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One classical hypothesis postulates that cancer cells arise from the accumulation of alterations that occur in differentiated mature cells (glial cells in the case of glioma tumors, including GBM), which would result in a dedifferentiation of these cells along the carcinogenic process. This concept is supported, for instance, by the histological similarities between functional and differentiated glial cells and tumor cells from gliomas. In addition, before the experimental identification of the adult neural stem cells (NSCs), glial cells were the only known replication-competent population of cells in the adult brain, which further supported the idea that highly-proliferative glioma cells could derive from accumulated alterations in differentiated and proliferative glial cells. A landmark study supporting this theory showed that differentiated cells could be transformed into a pluripotent embryonic stem cell phenotype by using a cocktail of transcription factors [11]. However, this hypothesis has never been adequately tested, as there have been experimental limitations that preclude its validation, including: (i) the absence of good mature "astrocyte" markers in i*n vivo* experiments [12], as it is now well known that the commonly used astrocyte marker GFAP is also expressed by adult NSCs; (ii) *in vitro*, the culture of mature astrocytes is particularly difficult; (iii) culturing astrocytes from neonatal mouse cortex has been described to contain also immature progenitor cells [13].

The second and most recent hypothesis assumes that cancer cells arise from the accumulation of alterations that occur directly in stem cells, or progenitor (multipotent) undifferentiated cells, that are present in different tissues throughout the entire lifetime (neural stem cells or glial progenitor cells in the case of brain gliomas). According to this rationale, the tumorigenic process would not be accompanied by a dedifferentiation mechanism, as the molecular alterations would accumulate directly in undifferentiated cells [7-9, 14]. In support of this hypothesis is the concept of cancer stem cells (CSCs), which is a subpopulation of cells in the tumor that displays self-renewal capacity, and which can give rise to heterogeneous cancer cells that constitute the tumor. However, it should be noted that the concepts of CSCs and tumor-initiating cells have been frequently confused. The term ''tumor-initiating cells'' refers to the cells of origin of the tumor, whose alterations support tumor establishment and progression; in contrast, CSCs would more accurately be referred to as tumor-propagating cells, with stem cell-like properties, which are not necessarily the cells of origin [8, 14, 15]. A study by Chen and colleagues (2010) may help to distinguish these different cell populations and their role on tumor development, particularly in GBM [16]. They demonstrated a hierarchical organization of brain tumor-initiating cells by identifying subpopulations of clonal and long-term proliferating cells in GBM specimens. These subpopulations were shown to be hierarchically organized and to give rise to tumors with different molecular and histopathological features [16]. There are specific and very well delimited regions in the brain where neural stem cells and progenitor cells exist, particularly the subventricular zone (SVZ) of the fore brain lateral ventricles, and the subgranular zone (SGZ) in the dentate gyrus of the hippocampus [8-10]. It has been hypothesized that these are favorable regions where the process of gliomagenesis may originate, as these regions present an attractive microenvironment that has been described as propitious for the growth of stem cells, namely in the SVZ [8-10]. There is increasing experimental evidence that the SVZ is one of the most important regions of origin for malignant gliomas [10] as it may present ideal conditions for gliomagenesis, like the exposure to a transcription factor cocktail ideal for their growth. When compared

to any other brain regions, stem cell-containing compartments have been shown to be more susceptible to tumor transformation [10], which additionally may argue in favor of this hypothesis of tumors arising from changes in stem/progenitor cells. Additionally, while it may be coincidence, there is a great similarity between the SVZ stem/progenitor cells and glioma cells. For instance, malignant astrocytic tumors in the brain typically appear close to the lateral ventricles [9, 10].

In the recent years, the notable therapy resistance of gliomas, namely GBM, has been associated with the presence of glioma stem cells (GSCs). These cells present characteristics of stem cells, including: (i) self-renewal; (ii) multipotency, i.e., the capacity to differentiate into other cell lineages; and (iii) high replicative potential. GSCs are predicted to be difficult to target by anti-cancer therapeutics because they have a slow cell cycle, present high levels of proteins involved in drug efflux, and do not express or are dependent on particular oncoproteins for which targeted therapies are currently available [17]. GSCs were one of the first types of cancer stem cells isolated from solid tumors [18]. It was shown that as few as 100 GSCs could give rise to tumors that recapitulated the parental tumor when implanted in xenografted immunodeficient mice, whereas as many as 1,000,000 non-GSCs could not [18]. This suggests that neoplastic clones are maintained exclusively by a little fraction of cells with stem cell properties [18]. Of note, studies involving the use of GSCs face many difficulties, particularly in isolating such cells directly from biopsies, partly because of the high cellular heterogeneity composition of the specimen. On the other hand, currently there are no standardized methods available for cell sorting and assessment of "stemness" [8]. Indeed, there is a relevant discussion regarding the best methodology for culturing GSCs isolated from human GBM specimens. It has been argued by several authors that adherent monolayer cultures of glioma cells allow a more homogeneous exposure to the culture conditions (e.g., nutrients and oxygen levels) than nonadherent cultures, thus increasing the homogeneity of the cell population, reviewed in [8]. In contrast, the sphere-forming assay has been widely used for this purpose. The fidelity and benefits of these assays are still under debate. Thus, there is an exigency to standardize methods for identifying and isolating GSCs with unequivocal markers. It is believed that the use of NSCs markers is a good principle for identifying GSCs, as NSCs are now known to exist in very restricted areas of the brain, and can be unambiguously identified with specific markers [8]. Indeed, in the last decade, putative markers of GSCs have been identified, including Nestin, CD133, L1CAM, CD15, CD44, Id1, and integrin-α6 [8, 10, 14, 19-21]. Nonetheless, none of these markers is sufficient to, independently, identify specifically GSCs, implicating that a functional identification of GSCs (including their ability to (i) be tumorigenic in *in vivo* models, (ii) form neurospheres in culture; (iii) be multipotent) is still mandatory.

2. Gene expression and signaling in GBM

GBM, like other cancers, is a disease that presents several alterations, including DNA mutations, copy number aberrations, and chromosomal rearrangements, but also DNA and histones epigenetic modifications, ultimately resulting in alterations in the gene expression profiles [22]. Molecular studies from the last decades have identified critical genetic alterations that affect

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many key pathways involved in the regulation of typical cancer hallmarks, such as alterations in cell cycle, migration, proliferation, survival, angiogenesis, invasion and apoptosis [22]. While several alterations in signaling pathways occur in GBM, such as Wnt, Notch and Shh pathways (particularly relevant due to their associations with cancer stem-cells and resistance to radiochemotherapy) [23, 24], the most frequent aberrations in GBM occur in three critical signaling pathways: (i) retinoblastoma (RB), (ii) p53, and (iii) RTK/RAS/PI3K pathways [22, 25, 26], as detailed in Figure 2 and below.

2.1. Retinoblastoma (RB) pathway

Mutations in genes implicated in cell cycle regulation that allow cells to proliferate uncontrollably have been frequently identified in GBM, as in other human tumors [26-28]. The RB pathway, which is important in the G1/S transition, is aberrantly inactivated in GBM through the alteration of several genes and proteins [28].

In a normal condition, the RB protein (encoded by *RB1* gene, the first tumor suppressor gene described), a negative regulator of the cell cycle, is recruited to specific promoters through its interactions with E2F transcription factors. RB inhibits the transcription of genes by directly suppressing the transactivating function of E2F, and by recruiting factors that mediate transcriptional repression [27, 28]. E2F regulates the promoters' activity of several genes related to (i) cell cycle, such as Cyclin E (*CCNE*) and A (*CCNA*), (ii) DNA replication, such as minichromosome maintenance complex component 7 (MCM7) and cell division cycle 6 (*CDC6*), (iii) nucleotide byosynthesis, such as ribonucleotide reductase (*RRM*), (iv) mitotic progression, such as Cyclin B1 (*CCNB1*) and cyclin-dependent kinase 1 (*CDK1*), and (v) apoptosis activation, such as apoptotic peptidase activating factor 1 (*APAF-1*) and caspases, such as caspase 3 (*CASP3*) [27, 28]. The interaction between RB and E2F can be disrupted due to the phosphorylation of the RB protein by Cdk4/6 kinases [27, 28]. To be active, these kinases are dependent of Cyclin proteins, namely CCND2 that competes for the binding site with Ink4 proteins [27]. Thus, the function of Ink4 is to prevent the formation of the active kinase complex (CCND2/Cdk4/6) [27]. This process is ultimately regulated by external signals, such as growth factors, which induce the cell to progress to the S phase [27].

In GBM, the *RB1* gene is frequently mutated [26]. However, the loss of function of RB is also reported to be a consequence of the amplification of *CDK4* and *CDK6*, as well as by the inactivation of the INK4A/B (isoforms of CDKN2A/B) and INK4C (encoded by CDKN2C), which are inhibitors of Cdk4/6 [26]. Ultimately, these alterations lead to E2F accumulation and the consequent progression to S phase mediated by E2F-target genes [26, 27].

2.2. p53 Pathway

The *TP53* gene encodes a protein (p53) that also controls the cell cycle by regulating target genes involved in cell cycle arrest, apoptosis and senescence [27]. Moreover, p53 has been named as the "guardian of the genome" because it leads to the arrest of cells with DNA damage in G1 phase, in order to promote DNA repair processes [29]. On the other hand, if irreparable genetic injuries occur, p53 induces cell death by activating the apoptotic machinery [29]. In

normal unstressed cycling cells, some proteins, such as the ubiquitin ligase Mdm2, bind to p53 to promote its degradation via the ubiquitin/proteasome pathway [29-31]. The p53-mediated upregulation of *MDM2* gene leads to a negative feedback that will maintain the levels of p53 very low in these cells [30, 31]. In this context, p53 loss of function may lead, for example, to uncontrolled growth and increased genetic instability. Its loss of function may be due to several reasons, including: (i) inactivating mutation [26], (ii) amplification of *MDM2* and *MDM4* [26, 31], and (iii) loss of function of ARF product encoded by *CDKN2A*, which interacts with and sequesters Mdm2 [26, 31, 32]. Unlike Mdm2, which degrades p53, Mdm4 inhibits p53 by binding to its transcriptional activation domain [31]. Moreover, Mdm4 also inhibits the degradation of Mdm2 [31].

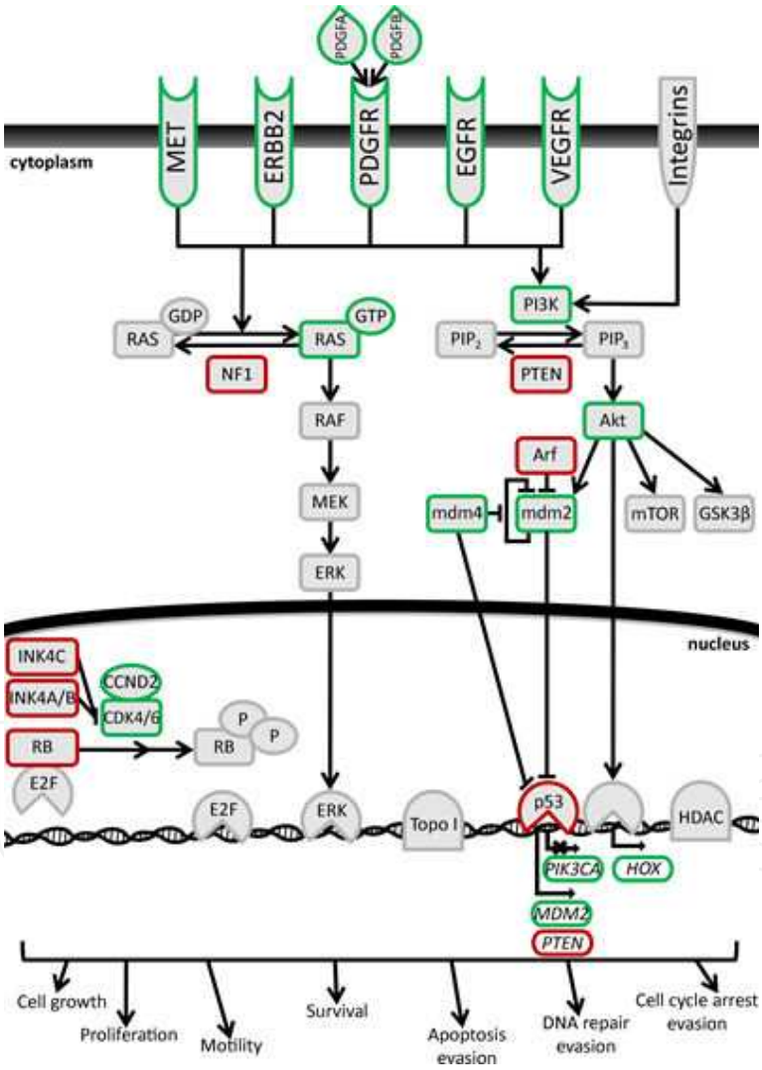

Figure 2 Common genetic alterations in GBM affect the RB, p53 and RTKs pathways. The aberrant deregulation of these pathways in GBM leads to alterations in cell cycle, migration, proliferation, angiogenesis, and apoptosis. Known proto-oncogenes or growth-promoting genes (shown in green), such as *EGFR*, *PIK3CA* (p110α) and *AKT*, are activated by mutations, overexpression and amplification, while tumor suppressor genes (show in red), such as PTEN, Arf and p53, are lost or inactivated by mutations, deletions, loss of heterozigosity, and epigenetic changes.

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2.3. Receptor Tyrosine Kinase (RTK) pathways

GBM cells also commonly present a constitutive activation of cell growth signaling pathways by the overexpression of several mitogens and their specific membrane receptors [22, 24, 26, 33]. Glioma cells can also acquire mutations in the membrane receptors becoming independent of exogenous growth stimulation, increasing survival and motility [22, 24, 26, 33, 34]. In GBM, the deregulation of growth factor signaling occurs frequently by the amplification and/or activating mutations of RTKs [22, 26]. These play critical roles in several cellular processes, including cell growth, motility, survival and proliferation, and are tightly controlled by various physiological mechanisms (e.g., autocrine loops in which RTK ligands are produced in result of receptor activation) [26]. One of the most described RTK alteration in GBM is the deletion of exons 2-7 of epidermal growth factor receptor (*EGFR*) gene that results in the loss of the extracellular domain (EGFR-vIII mutant) [26]. Notwithstanding, other genetic alterations affecting *EGFR*, such as amplifications, activating point mutations that affect the extracellular domain, and other deletions in the region coding for the cytoplasmic domain, have also been described [26]. Moreover, alterations of other RTKs also occurs frequently in GBM, including: (i) overexpression of platelet-derived growth factor receptor (*PDGFR*) and its ligands *PDGFA* and *PDGFB*, suggesting an autocrine or paracrine loop activation, (ii) activating mutations in *ERBB2* (member of the EGFR family), and (iii) activating mutations in hepatocyte growth factor receptor (*MET)* [22, 26, 33, 34]. RTKs mediate its functions by downstream effectors, namely *phosphatidylinositol 3-kinase* (PI3K), *mitogen-activated protein kinase* (MAPK) and *signal transducer and activator of transcription* (STAT) signaling cascades [34]. Although genetic alterations in RTKs may potentially activate these pathways, they can also be specifically activated due to other aberrations. Among them, the PI3K pathway is the most described in GBM and is involved in cell growth, proliferation, differentiation, motility and survival [26, 34]. The most frequent alterations involve inactivating mutations and homozygous deletions of *PTEN* [26]. This gene encodes the enzyme phosphatidylinositol (3,4,5)-trisphosphate 3-phosphatase, which removes a phosphate from phosphatidylinositol-(3,4,5)-triphosphate (PIP_3), converting it to phosphatidylinositol-(4,5)-bisphosphate (PIP_2) [22]. Thus, PTEN counteracts the action of the PI3K, which catalyzes the addition of a phosphate to PIP_2 at the 3 position, converting it to PIP_3 [22]. The accumulation of PIP_3 recruits Akt to the plasma membrane. Here, Akt is activated by phosphorylation, promoting cell survival and proliferation [22]. The PI3K enzymatic complex is formed by 2 subunits, one regulatory protein (p85α), encoded by *PIK3R1*, and one catalytic protein (p110α), encoded by *PIK3CA* [22]. Note that other variants of this complex exist, but the referred subunits are the most expressed in GBM and the most widely-studied. Activating missense mutations and in-frame deletions have been detected in the *PIK3CA* [26]. One deletion was identified in the adaptor binding domain, raising the hypothesis that it may disrupt the normal interaction between p110α and its regulatory subunit, p85α [26]. Interestingly, in a few percentage of samples without activating mutations in the catalytic subunit, inactivating mutations were detected in the regulatory subunit [26]. This suggests a functional redundancy of these mutations as they individually activate PI3K. Again, the amplification of *AKT3* gene, which encodes one of the Akt proteins, was described recently in a small fraction of GBM samples [26]. Other known mutation that ultimately leads to activation of PI3K and MAPK is the activating mutation of *RAS* [26]. RAS is indirectly

activated by RTKs through the dissociation of a GDP molecule and association to a GTP. However, in a normal condition, this activation is quickly reverted from RAS-GTP to RAS-GDP. This *RAS* mutation impedes the dissociation of GTP, remaining RAS constitutively active. Neurofibromin 1 (NF1), which negatively regulates RAS signaling, is also downregulated in GBM by *NF1* mutations or deletions, resulting in increased RAS signaling. Moreover, loss of expression of NF1 without evidence of genomic alteration was also observed [26]. In addition to its critical effects in cell growth, motility, and survival, the PI3K pathway seems to be also important in the activation of *HOX* genes, which were recently described to be important for the malignant phenotype of GBMs [35-37] (see section 3.3 for details).

2.4. Crosstalk between RB, p53, and RTK pathways in GBM

The development of new platforms of genome-scale screenings has allowed a more robust identification of the accumulation of genetic and epigenetic alterations. The Cancer Genome Atlas (TCGA) project, for example, was established with the aim of using genome-wide analysis technologies, which include DNA copy number, gene expression, DNA methylation, and nucleotide sequencing, to understand the molecular basis of cancer [26]. With this multiplatform profiling and using an integrative analysis, they identified a highly interconnected network of aberrations in GBM that include the pathways described above (RB, p53, RTKs and PI3K pathways) [26]. Interestingly, this integrative analysis showed a statistical tendency to mutual exclusivity for the specific alterations of components within each pathway. Nonetheless a great percentage of samples harbored aberrations in all signaling pathways [26], which is in agreement with the hypothesis that these pathways are a core prerequisite for GBM disease.

2.5. Other key alterations in GBM

In addition to the most common genetic alterations found in GBM, several other aberrations have been described. For example, mutations in *IDH1* and *IDH2* genes, which encode the metabolic enzymes isocitrate dehydrogenases were described. These reports suggest that these mutations lead to a new pro-oncogenic activity of IDH1/2 with the production of R(-)-2-hydroxyglutarate, an onco-metabolite [38, 39] (see section 3.2 for details). Other classes of proteins extremely important in GBM are DNA repair proteins, as they increase the probability of mutations. In fact, at least one of the MMR genes (*MLH1*, *MSH2*, *MSH6* or *PMS2*) is mutated in hypermutated GBM samples [26], decreasing DNA repair competencies in these cells.

2.6. Molecular subclasses of GBM

Using an unsupervised hierarchical clustering analysis, Verhaak *et al.* [40] used TCGA data to successfully classify GBM into four subtypes - classical, mesenchymal, proneural and neural - improving and validating previous classifications of GBM [37, 41-47].

The identity of the classical subtype was defined by displaying the most common genomic aberrations of GBM, with 93% of samples presenting amplifications in chromosome 7 paired with loss of chromosome 10, 95% showing high levels of *EGFR* amplification and/or expres-

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sion, and *EGFR-vIII* activating point mutations. These amplifications of *EGFR* co-occurred with focal homozygous deletions targeting *CDKN2A,* which in turn was almost mutually exclusive with other alterations in RB pathway components, such as *RB1*, *CDK4* and *CCDN2*. However, this subtype does not present *TP53* mutations. Additionally, the Notch (*NOTCH3*, *JAG1*, *LFNG*) and Sonic hedgehog (*SMO*, *GAS1*, *GLI2*) signaling pathways, as well as the neural precursor and stem cell marker *NES*, were highly expressed in this subtype [40].

The mesenchymal subtype presents a focal hemizygous deletions of 17q11.2 region that contains the *NF1* gene. In fact, this deletion was associated with lower expression of *NF1* in most cases. However, *NF1* was also found to be mutated predominantly in this subtype, and sometimes this mutation co-occurred with *PTEN* inactivating mutations. Moreover, *TRADD*, *RELB* and *TNFRSF1A* genes, belonging to the tumor necrosis factor (*TNF*) superfamily, and genes encoding proteins from the NF-κB pathways, are highly expressed. Additionally, mesenchymal markers, such as *CHI3L1* and *MET,* were expressed [40].

The most relevant features of the proneural subtype were high levels of *PDGFRA* gene expression in combination with its focal amplification, and point mutations in *IDH1.* Importantly, these aberrations seem to be mutually exclusive. Loss of heterozygosity and inactivating mutations of *TP53* were frequent in this subtype. While less frequent than in classical GBM samples, half of proneural samples also manifested amplification in chromosome 7, paired with loss of chromosome 10. *PIK3CA* and *PIK3R1* activating and inactivating mutations, respectively, were observed mostly in samples without *PDGFRA* aberrations. Oligodendrocytic development genes, such as *PDGFRA*, *NKX2-2* and *OLIG2,* were highly expressed. Lower expression of *CDKN1A* was observed, probably due to *OLIG2* overexpression, which was described to be able to downregulate *CDKN1A*. Additionally, this subtype also presents the expression of proneural developmental genes, such as *SOX* genes, as well as *DCX*, *DLL3*, *ASCL1* and *TCF4* [40].

In what concerns the neural subtype, few characteristics were reported, and it was almost merely classified based on neuron markers expression, including neurofilament light chain polypeptide (NEFL), gamma-aminobutyric acid A receptor (GABRA1), synaptotagmin I (*STY1*), and solute carrier family 12 (SLC12A5) [40].

3. Molecular prognostic factors of GBM

It is widely recognized that the molecular stratification of GBM patients may prove crucial in rationalizing treatment decisions, for which a set of molecular markers predictive of tumor response to specific therapies and/or patient outcome are required. The most well established prognostic factors in GBM patients include age, general performance status, tumor histological features and the extent of tumor resection [48]. Recently, several studies have identified biological and molecular features of GBMs that present prognostic value [37, 46, 49-58] and may help in therapeutic decisions. The work performed so far presents reasons for both optimism and caution regarding the improvements in the diagnosis and treatment of patients, but also demand validation in prospectively followed and in uniformly treated patients.

Therefore, the focus remains in the identification of biomarkers that truly foster patient distinction in ways that may improve therapeutic decisions. The current most relevant prognostic biomarkers for GBM are summarized in Table 1, of which the most promising are briefly discussed below.

Molecular Prognostic Marker	References
MGMT promoter methylation	[52]
IDH1 and *IDH2* mutations	[57]
Loss of chromosome 10	[56]
Activation of the PI3K/AKT pathway	[50, 58]
HOX genes signature	[36, 37]
HOXA9 overexpression	[35, 36]
CHI3L1 (*YKL40*) expression	[53, 56]
miRNA expression signatures	[59]
EGFR expression	[37]
EGFR mutation (EGFR-vIII)	[51, 55]
PTEN expression (wild-type)	[55]
Molecular signatures	[40, 46]
High expression of angiogenic genes	[46]
Stem-cell like gene expression signatures	[37, 49, 54]
Activation of MAPK members	[58]
PTEN and *DLL3* expression	[46]

Table 1. Selected molecular prognostic markers for glioblastoma.

3.1. *MGMT* promoter methylation

Many studies have shown that the methylation status of *MGMT* (O^6-methylguanine-DNA methyltransferase) gene is currently one of the most promising biomarkers of prognosis in GBM patients, although it has not yet reached broad clinical applicability [52, 60]. *MGMT* encodes a DNA-repair protein that removes alkyl groups from the O^6 position of guanine, an important site for DNA alkylation. When DNA is left unrepaired, the lesions induced by chemotherapy trigger apoptosis and cytotoxicity [61]. Hegi and co-workers [52] showed that the epigenetic silencing of *MGMT* by promoter methylation leads to the loss of *MGMT* expression and reduced DNA-repair activity, resulting in increased sensitivity of the tumor

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cells to temozolomide (TMZ) treatment. In fact, they reported that this increased sensitivity is translated into differences in patient survival, as the methylation of the *MGMT* promoter is associated with longer overall survival (OS) in patients with GBM. Indeed, patients whose *MGMT* promoter is methylated and are treated with TMZ have an increased OS (median of 21.7 months), as well as a higher 2-year survival rate (46%), in comparison to patients treated with TMZ but with unmethylated *MGMT* promoter (median survival of 12.7 months and 2-year survival of 13.8%), suggesting that GBM patients whose tumors present *MGMT* expression do not benefit from TMZ treatment [52]. These results suggest *MGMT* promoter methylation as an independent and favorable predictive factor to patients' response to TMZ therapy [52]. Despite these remarkable findings suggesting *MGMT* as a prognostic biomarker and as a specific predictor of response to TMZ-based chemotherapy, there is still a significant body of controversy surrounding them. Such controversy is mainly due to the heterogeneity of the patients enrolled in the study groups, as they present different glioma histologies, grades and treatment regimens, as well as the fact that different studies analyzed *MGMT* at different levels, including mRNA expression, methylation status and protein levels (as summarized in [62]). In an attempt to replicate Hegi's findings, Costa and co-workers [62] analyzed a set of 90 GBM patients treated with postoperative TMZ-based chemoradiation regarding *MGMT* methylation. Despite a trend for longer overall and progression-free survival in GBM patients with *MGMT* promoter methylation, the differences did not reach statistical significance [62]. Moreover, sample classification as methylated or unmethylated for a certain gene is still controversial, as the relationship between the overall CpG island methylation, CpG methylation at individual sites, and their effects on gene silencing, is highly dependent on the location within the gene [63]. In this sense, Bady and co-workers [64] evaluated the relationship between the specific location of CpG methylation, *MGMT* expression and the outcome of patient in a population homogenously treated with alkylating agents. They reported two regions of methylated CpG's that present strong association with patient longer survival, which negatively correlate with *MGMT* gene expression [64]. This is consistent with *MGMT* expression silencing via CpG methylation, resulting in sensitization to alkylating agents [64]. Similarly, Shah and colleagues also identified three regions of methylated CpGs on *MGMT*, associated with favorable patient progression-free survival, within a population of 44 GBM patients treated with radiotherapy and concomitant and adjuvant TMZ [65]. Nonetheless, the value of *MGMT* methylation status is also supported by a recent clinical trial that compares radiotherapy and TMZ treatment in elder patients, and reported an association between *MGMT* methylation and good outcome in the TMZ cohort, but not in the radiotherapy cohort [66]. Similarly, a meta-analysis performed by Olson and co-workers [67] that included 2018 patients from 20 different studies, showed that the silencing of *MGMT* was highly associated with improved OS in patients receiving chemotherapy as a part of the adjuvant treatment, a mild association in patients that received adjuvant radiotherapy, and no benefit in those submitted to surgery alone.

3.2. *IDH1* and *IDH2* mutations

Other important prognostic factors for GBM have been revealed by recent genomic studies and concern the presence of mutations in isocitrate dehydrogenase 1 and 2 genes

(*IDH1* and *IDH2*; *IDH* when referring to both) [26, 57, 68]. These are NADP-dependent enzymes that catalyze the oxidative decarboxylation of isocitrate to α-ketoglutarate, with the simultaneous production of NADPH [69]. The high-throughput sequencing of GBM revealed that *IDH1* mutations occur in 12% of GBM, are somatic and heterozygous, and a consequence of the change of a guanine to an adenine at position 395 of the *IDH1* gene (G395A), leading to the replacement of an arginine with a histidine at amino acid residue 132 of the protein (R132H) [57]. Similarly, sequence evaluation of *IDH2* exons revealed a mutation in a histidine at amino acid residue 172 (R172), which is the exact analogue of the R132 residue in *IDH1* [68]. Overexpression of $IDH1^{R132H}$ reduces the formation of α-ketoglutarate and increases the levels of HIF-1α [70]. As stated above, a recent study suggested that mutant IDH1 reduces α-ketoglutarate to R(-)-2-hydroxyglutarate, while converting NADPH to $NADP^+$ [38, 39]. Even though the mechanism is yet to be clarified, it seems probable that the increased capacity to produce 2-hydroxyglutarate of cells presenting $IDH1^{R132H}$ mutation contributes to tumorigenesis [38]. *IDH* mutations are highly frequent in secondary GBM (up to 80%), but are rare in primary GBM (less than 10%) [68, 71]. *IDH* mutations are correlated with younger age at diagnosis, and with GBM patients' longer survival when compared to patients with IDH^{wt} genes [68, 72]. Mutations in *IDH1* and *IDH2* are mutually exclusive, which indicates that they might independently confer a growth advantage to mutated cells [73]. Moreover, *IDH* mutations generally associate with specific genetic and clinical characteristics when compared to gliomas that have IDH^{wt}. In particular, it was shown that *IDH* mutations and amplification of *EGFR* in GBM are mutually exclusive events [74], and that the methylation of the *MGMT* gene promoter is often associated with *IDH* mutations [74, 75]. However, this association is yet to be clarified as it may represent a direct consequence of the activity of the mutant IDH, or an alternative marker for epigenetic changes in tumors presenting *IDH* mutations (reviewed in [76]). So, the deep understanding of the link between *IDH* mutations and common genetic events in GBM might furnish insights into their roles on gliomagenesis [40, 68]. Furthermore, a recent study evaluated the response of a series of 86 secondary GBM to TMZ treatment, and correlated several markers of GBM (including *IDH* mutations, 1p19q co-deletion, *MGMT* promoter methylation status, and *TP53* expression) with progression-free survival and OS [77]. This study showed that *IDH* mutations were present in 73.4% of the analyzed patients, and that these mutations were associated with higher progression-free survival [77]. The authors also evaluated the response of patients presenting *IDH* mutations and *MGMT* promoter methylation, and found that patients presenting this combination had the best response to TMZ treatment, reporting also that IDH mutations seems to be a significant marker for positive chemosensitivity in secondary GBM [77].

3.3. Molecular subclasses and prognostic value

Strikingly, as stated above, mutations in *IDH1* have been included in a GBM signature that allowed the division of GBMs into subtypes according to their recurrent genomic alterations [40] (see section 2.6 for details). The importance in the division of GBM into subtypes lies on the possible application of different therapeutic approaches, as treatments efficacy differs per subtype [40]. Aggressive therapy significantly delayed mortality in classical and mesenchymal

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subtypes, and a tendency to longer outcome was observed for the neural subtype, yet patients whose GBM present proneural features, associated with younger age, do not seem to benefit from highly aggressive therapies although presenting longer survival [40]. In this sense, some of the genetic events underlying the different GBM subtypes could become part of the clinical routine to rationalize therapeutic decisions, and ultimately lead to more personalized therapies for groups of patients with GBM.

3.4. *HOX* genes signature

Recent evidences have been revealing a remarkable resemblance between tumorigenic and developmental processes, indicating the relevance of molecular regulatory mechanisms crucial on normal development and on the tumorigenic process. Homeobox (*HB*) genes encode transcription factors that primarily play a crucial role during normal development, and are divided into two classes: class I comprises clustered homeobox (*HOX*) genes, and class II includes non-*HOX* genes, which are dispersed through the genome, and mainly serve as cofactors for HOX proteins [78]. During embryonic development, *HOX* genes are sequentially expressed from 3′ to 5′ along the anterior-posterior axis contributing to the temporospatial development of limbs and organs [79]. The mechanisms underlying *HOX* genes control in normal development occur according to three main principles: spatial collinearity, posterior prevalence, and temporal collinearity [80]. These were found to be altered in cancer as a consequence of three major mechanisms proposed by Abate-Shen [81]: temporospatial deregulation, gene dominance and epigenetic regulation. Different groups have been reporting the deregulation of these mechanisms in different *HOX* genes, and in different tumors (reviewed in [80]).

The aberrant expression of *HOX* genes have been reported as crucial in several hallmarks of cancer, including increased proliferation, angiogenesis and invasion, and apoptosis resistance in leukemia and in several solid tumors [80, 82-85]. Interestingly, in recent years, *HOX* genes aberrant expression has been implicated in gliomagenesis. Abdel-Fattah and co-workers [86] evaluated the expression of all *HOX* genes in primary astrocytomas and in non-tumor brain specimens, reporting that some *HOX* genes are abnormally expressed in malignant astrocytomas. A subsequent report by Murat *et al.* [37] identified a *HOX*-dominated gene cluster, suggestive of a signature that displays srm cell-like self-renewal properties. These authors argue show that the expression of *HOXA10* gene in GBM neurospheres is consistent with a role of *HOX* genes in glioma stem-like cell compartments [37]. Interestingly, the *HOX*-dominated gene signature arises along malignant progression to GBM, and is an independent predictive factor of chemo-radiotherapy resistance in patients [37]. Later, Costa and co-workers [35] showed that *HOXA* genes are predominantly activated in GBM, as compared to lower-grade gliomas and normal brain tissue, suggesting they may be a useful component of a molecular classification of gliomas. By analyzing expression microarrays data from 100 GBMs, they identified tumors with abnormal chromosomal domains of transcriptional activation, which comprise the *HOXA* cluster, and is reversibly regulated by the PI3K pathway [35]. Of all *HOXA* genes, *HOXA9* expression was predictive of worse GBM patient outcome, and associated with pro-proliferative and anti-apoptotic functions, which may explain the

unfavorable prognosis of GBM patients with *HOXA9* reactivation [35]. More recently, Gaspar *et al.* [36], showed pediatric GBM cell lines that are resistant to TMZ present the coordinated expression of several *HOX* genes, of which *HOXA9* and *HOXA10* were highlighted as crucial effectors in this resistance [36]. In line with Costa *et al.* [35] report, Gaspar suggested that the *HOX*-enriched signature is regulated by the PI3K pathway, and interestingly, is associated with resistance to TMZ in pediatric GBM cell lines [36]. Moreover, pediatric patients with high-grade gliomas that express *HOXA9* and *HOXA10* presented shorter survival [36].

3.5. *CHI3L1* (*YKL40*) expression

The molecular prognostic biomarkers currently available require the evaluation of tumor tissue in order to assess gene expression and promoter methylation levels. Moreover, tumor progression and treatment responses are monitored using imaging techniques, which do not distinguish the effects of treatment and tumor regrowth. In fact, patients who are submitted to magnetic resonance imaging (MRI) shortly after radiotherapy show increased volume of the tumor, which in up to 50% of the cases, is a consequence of the increased blood vessel permeability due to radiotherapy, an effect called pseudoprogession [87]. As it is difficult to distinguish between the therapeutic effects and real growth of the tumor [88], in addition to the impossibility of multiple tumor sampling during the course of the malignancy [89, 90], demand the establishment of less invasive prognostic and predictive markers. Serum markers that correlate with tumor biological properties might prove crucial in providing prognostic information and response to treatment, therefore allowing the proper adjustment of therapeutics, and improve care of patients with GBM. A study conducted by Tanwar [91] analyzed gene expression microarray data of tumor tissue from glioma patients, and showed that chitinase 3-like 1 (*CHI3L1* or *YKL40*) was the most highly expressed among 10000 genes, when comparing to normal brain tissue [91]. The function of YKL-40 in gliomas and other tumors is yet to be fully clarified; however, it is thought to be involved in increased cell proliferation, differentiation, angiogenesis, decreased apoptosis, and extracellular matrix remodeling [92-94]. Interestingly, YKL-40 is secreted both by tumor cells and by tumor-associated macrophages in the bloodstream, therefore allowing its quantification in the blood. YKL-40 was found to be increased in the serum of patients with several solid tumor types, as breast, colorectal, ovary, small cell lung cancer and GBM (reviewed in [93]). Particularly in GBM, YKL-40 serum concentrations seem to be a strong predictor of an aggressive phenotype [53, 91], as the increased expression of YKL-40 appears to be associated with glioma grade, resistance to radiotherapy, shorter time to progression, and worse patient OS [53, 95-97]. However, to establish YKL-40 serum levels as a prognostic marker, there is still the need to perform further prospective studies that concern repeated determinations of YKL-40 levels before and after surgery. As YKL-40 can be reproducibly measured in the serum, and this biomarker is already well established for routine use, its inclusion in the clinical practice should be relatively straightforward, and might provide crucial information on tumor progression.

In conclusion, the identification of molecular biomarkers that truly aid in the distinction of patients and therapeutic decisions still requires much effort. The integration of clinical and molecular data is becoming more frequent, and easier to perform and analyze, which will

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probably lead to more targeted and effective treatments. Moreover, it seems probable that sets of molecular biomarkers for GBM will be established in the next few years, and will become part of the clinical routine, leading to tailored therapies for subgroups of GBM patients. Importantly, the timely identification of patients who are not likely to respond to a certain therapy would allow their integration in clinical trials with novel therapies, but also to avoid the possible adverse side effects of a therapy that may not prove beneficial. Equally interesting, the establishment of molecular biomarkers of tumor therapy resistance may lead to a more guided and rational design of novel therapeutic agents and clinical trials for GBM patients. In the search for GBM patient individualized therapy, the discovery of particular tumor molecular features, as the status of *MGMT* promoter methylation status, the mutation status of *IDH1* and *IDH2*, the expression of *HOXA* genes, and the serum levels of YKL-40, may prove crucial as initial building blocks of a panel of molecular biomarkers that may have real clinical implications. The challenge ahead is to discover further molecular markers of GBM, but also to integrate all the knowledge in an interdisciplinary way, considering different GBM subtypes, which altogether might allow a more rational and efficient fight against GBM.

4. New molecular targets and treatments

As described throughout this chapter, the molecular and cellular heterogeneity of GBM represents a major therapeutic challenge, but also offers a large number of opportunities to specific targeting of tumor cells' alterations. Furthermore, the unsatisfactory prognosis of GBM patients, independently of the used treatment approaches, and the absence of a cure or significant advances in the treatment of GBM, are the major drivers of GBM therapeutics research.

4.1. Classic therapeutics

The current standard therapy for the treatment of GBM includes maximal surgical resection, followed by radiotherapy (RT) with concomitant and adjuvant administration of alkylating agents [98]. Administration of RT is usually given after the surgical removal of the tumor in order to eliminate residual tumor cells [99]. Alkylating agents act by introducing methyl groups in different positions in the DNA, resulting in DNA damage and specific cytotoxicity, that ultimately leads to apoptosis and cell death [100]. Before 1999, only nitrosourea-based chemotherapeutics were approved for the treatment of GBM, which includes oral lomustine (CCNU) and intravenous carmustine (BCNU) [101]. In 1999, FDA approved Gliadel® that consists in a polymeric biodegradable wafer that is able to release carmustine during 2-3 weeks after implantation in the gap where the tumor was removed during surgery [101-103]. Furthermore, in this same year, FDA granted accelerated approval to the imidazole derivative of the second-generation class of alkylating agents, TMZ, mainly because of its efficient absorption after oral administration and its ability to easily cross the blood-brain barrier [101, 104].

TMZ was regularly approved by the FDA in 2005, and became the standard chemotherapeutic agent for the treatment of GBM [5]. The approval of TMZ was mainly due to the improvement in the OS of patients observed in a landmark study by Stupp *et al.* [5]. This clinical trial involving 573 patients with newly diagnosed GBM showed an increase in OS from 12.1 months to 14.6 months when patients were treated with RT plus TMZ comparing with RT alone [5]. In 2009, the 5-years retrospective analysis from this phase III clinical trial reported that, in addition to the improvement in OS, the 5-year survival rate was also higher in the group of patients treated with RT and TMZ, showing again the benefits of this treatment [105]. Nevertheless, some molecular mechanisms of resistance to this agent were identified, like the methylation status of the *MGMT* gene, which encodes a protein that repairs the damage induced by TMZ, and alkylating agents in general, resulting in chemoresistance [106].

Besides TMZ, bevacizumab (BVZ, also known as Avastin®) was also conceded accelerated approval by the FDA in 2009 as monotherapy for patients with progressive GBM that did not respond to standard care (TMZ + RT) [101, 107]. This drug is a monoclonal antibody that targets VEGF, which is involved in the formation of new blood vessels [99]. Since GBM are highly vascularized tumors, this drug presented an attractive way to target tumor-associated increased angiogenesis [108]. When BVZ was combined with TMZ + RT for the treatment of newly diagnosed GBM patients in a phase II clinical trial, an improvement in OS (19.6 vs. 14.6 months) and progression-free survival (PFS, 13.6 vs. 6.9 months) was reported, when compared to the control cohort of the European Organization for Research and Treatment of Cancer-National Cancer Institute of Canada (EORTC/NCIC), in which patients were treated only with RT and TMZ [109]. BVZ also showed good radiographic responses in patients with recurrent GBM (71% and 35%, according to Levin and Macdonald criteria, respectively) when used first as a single agent, and later combined with irinotecan (topoisomerase I inhibitor) in a phase II clinical trial [110]. Although some exciting clinical results were described, several *in vitro* and *in vivo* studies have been unmasking unpredictable consequences of BVZ treatment. The treatment of intracranial xenograft mouse models of GBM with this VEGF inhibitor showed a decrease in the vascular network and contrast enhancement in MRI, but also a 68% increase in the infiltration of tumor cells trough the brain parenchyma [111, 112]. Furthermore, BVZ treatment increased the hypoxic microenvironment which is also implicated in increased invasion ability of tumor cells [24, 112].

4.2. Novel molecular targeted therapeutics

Conceptually, the development of targeted therapies for the treatment of GBM represents a significant advance in the search for a cure for this devastating disease. First, the specificity of these therapies has the potential to reduce toxic side effects. Second, the direct blockade of altered oncogenic signaling cascades may allow the reduction of tumor cell proliferation [113]. This next part will review some of the most promising therapeutic molecular and targeting strategies, including membrane proteins and growth factor receptors (e.g. RTK), and intracellular signaling pathways.

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4.2.1. Therapeutic targeting of membrane protein/growth factor receptors

RTKs represent attractive targets for this therapeutic approach, since they are associated with GBM oncogenesis, and the binding of growth factors to these receptors activate signaling pathways that drive GBM cells survival and proliferation [113, 114] (see section 2.3 for information). There are two kinds of inhibitors for RTKs: (i) inhibitors targeting the intracellular tyrosine kinase domain (TKD), and (ii) monoclonal antibodies that can block RTK activation or target the RTK-expressing cells [115].

a. EGFR

As stated above, *EGFR* amplification, overexpression and mutation are frequent events in GBM cells and increased EGFR signaling is known to increase tumor proliferation, invasion ability, angiogenesis and blocking apoptosis [22, 116]. Several small molecule inhibitors targeting EGFR have been developed and approved for the treatment of particular cancers, as erlotinib and gefitinib in the treatment of advanced metastatic non-small cell lung cancer [24, 117]. This RTK can be targeted with a large number of inhibitors, like lapatinib (EGFR2, ErbB2), vandetanib (EGFR, VEGFR-2), PF-00299804 (EGFR, ERBB2 and ERBB4), BIBW2992 (EGFR, ERBB2, ERBB4), AEE 788 (EGFR, ERBB2, VEGFR), and monoclonal antibodies, as cetuximab (EGFR) and nimotuzumab (EGFR) [98, 116]; however, this section focus on the most reviewed and clinically tested drugs for the treatment of GBM (erlotinib, gefitinib and cetuximab). Erlotinib and gefitinib although, extensively tested in clinical trials for GBM patients (either already completed or currently ongoing), have not shown a significant benefit, and thus failed to reach clinical applicability (Table 2) [118]. The chimerical monoclonal antibody cetuximab (Erbitux) can also inhibit EGFR, and was shown to inhibit the mutant EGFR-vIII in glioma cells [119, 120]. Furthermore, preclinical studies using GBM xenograft models suggest that cetuximab could be effective for the treatment of invasive GBM [121]. The clinical evaluation of the administration of cetuximab in phase II trials for recurrent GBM patients has shown mixed results. The combination of cetuximab with BVZ and irinotecan resulted in 5% complete responses (CR), 21% partial responses (PR) and 40% of the patients with stable disease (SD), with only 9% of the GBM patients presenting signs of progressive disease (PD); the 6 months progression-free survival (6-PFS) of 33% obtained in this trial was also surprising [108]. In another phase II clinical trial for recurrent GBM patients, treatment with cetuximab showed worse outcomes, with a median time-to-progression (TTP) of only 1.9 months, and only 7.3% of the patients being progression free at 6 months after treatment [122].

Most of the *EGFR* amplified GBMs also present expression of the mutant EGFR-vIII [116]. Since this mutated form of EGFR is absent in normal tissues, an immunotherapy-based approach to target EGFR-vIII was developed and is now under clinical trials (phase I, II and III) [118, 123]. This vaccine, called rindopepimut (CDX-110, PEPvIII) consists in a 14 aminoacids peptide that specifically recognizes EGFR-vIII, combined with an immunoadjuvant (keyhole limpet hemocyanin), that will potentiate an immune response against EGFR-vIII-positive tumor cells [124]. The clinical applicability of this vaccine was already tested in different clinical trials showing the benefits of this strategy (Table 2). Newly diagnosed GBM EGFR-vIII positive had a significant improvement in OS from 15.2 months (treated with TMZ + RT) to 23.2 months

(CDX-110 + granulocyte macrophage-colony stimulating factor, GM-CSF, and TMZ, after RT), consistent with the benefit of this vaccine alone in other studies (OS 26 months vs. 15 months) [124, 125].

b. PDGFR

As referred previously, *PDGFR* is also frequently overexpressed in GBM [114]. As described for other RTK, PDGFR can also be blocked with different pharmacological inhibitors, such as imatinib mesylate (PDGFR, c-KIT, BCR-ABL), sunitinib (PDGFR, VEGFR, c-KIT), sorafenib (PDGFR, VEGFR, RAF), tandutinib (PDGFR, FLT3, c-KIT), vatalanib (PDGFR, VEGFR, c-KIT), IMC3G3 (PDGRFα), pazopanib (PDGFR, c-KIT, EGFR) or dasatinib (PDGFRβ, Src, BCR/Abl, c-KIT, ephrin A2) [98, 116]. However, this part will focus on the best characterized PDGFR inhibitor, imatinib mesylate (Gleevec or Livec), already evaluated in phase I/II clinical trials with GBM patients, which was originally FDA approved for the treatment of acute myeloid leukemia [106, 126]. *In vitro* treatments of GBM cells with imatinib have already shown inhibitory effects on cell proliferation, as a result of cell cycle arrest, increase apoptotic population and decreased clonogenic ability [127]. Its administration in mice models of GBM also showed an improvement in survival [128]. In clinical studies, imatinib mesylate was usually combined with hydroxyurea (HU), a ribonucleotide reductase inhibitor that blocks DNA synthesis [126, 129]. Treatment of recurrent GBM in phase II clinical trials was mostly disappointing, with 6-months PFS (6-PFS) of only 3% and 16% [130, 131]. Combination with HU, although showing a mild increase in OS and 6-PFS rates, again showed a lack of efficacy as compared to RT + TMZ [126]. The best result using imatinib was achieved in a phase I clinical trial for recurrent malignant glioma (MG), where imatinib was combined with HU and vatalanib (VEGFR inhibitor), with 24% of GBM patients revealing a radiographic partial response, 49% showing signals of stable disease, however 27% of the patients had progressive disease [132] (Table 2).

c. VEGFR

The therapeutic targeting of GBM-associated angiogenesis is already an approved strategy through VEGF inhibition with BVZ, but can also be achieved through inhibition of VEGF receptors using specific inhibitors, like cediranib, sorafenib, sunitinib, pazopanib, vandetanib, CT-332 (all VEGFR), XL-184 (VEGFR2, Met, RET, c-KIT, Flt3, Tie-2), semaxanib or AEE 788 [98, 116, 133]. For instance, cediranib (AZD2171) inhibits all VEGFR subtypes and was explored in phase I, II and III clinical trials [116]. The outcomes of cediranib (AZD2171) treatment in GBM patients are described as similar to the ones observed for BVZ, although only one of the completed trials has published results (Table 2) [116]. As reported for BVZ, also cediranib was associated with infiltrative cells not visible with contrast-enhanced MRI [112, 134]. In orthotopic mouse models of GBM, this VEGFR inhibitor induced alterations in the permeability and diameter of blood vessels, alleviating edema and increasing the survival of the mice [135].

d. Met

Met is an RTK for hepatocyte growth factor (HGF) that activates a series of signaling pathways, as referred above in section 2.3, similar to what is observed for EGFR or PDGFR activation,

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which ultimately leads to proliferative and invasive behaviors of cancer cells [106, 136]. In a series of 62 GBM patient samples, Met was found to be overexpressed and associated with poor prognosis, and with an invasive phenotype, supported by invasive multifoci lesions and expression of metalloproteinases 2 and 9 [137]. Inhibitors targeting Met include tivantinib, and cabozantinib (XL184) a potent inhibitor of several kinases, cabozantinib (XL184), which hase shown significant inhibitory effect on GBM tumor growth [138]. Furthermore, three phase I and II clinical trials for the evaluation of cabozantinib on the treatment of newly diagnosed GBM (monotherapy or combined with RT + TMZ) and recurrent GBM (monotherapy) (NCT00960492, NCT00704288 and NCT01068782) are now ongoing [118]. Another therapeutic approach to target HGF/Met axis is the use of the monoclonal antibody against HGF, rilotumumab (AMG-102), which was already tested during a phase II [116] clinical trial for recurrent GBM (Table 2); a second phase II trial to test the combination of rilotumumab with Avastin in patients with recurrent MG is now recruiting patients (NCT01113398) [118].

e. Integrins

Therapeutic targeting of the cell adhesion receptors integrins, which are transmembrane glycoproteins that attach cells to extracellular matrix proteins of the basement membrane or to ligands on other cells, have also proved to be a valuable therapeutic strategy for the treatment of GBM, with several recent clinical trials testing the success of the integrin inhibitor cilengetide (EMD 121974) as a monotherapy or in combination with RT + TMZ (Table 2) [139]. Cilengitide is an RGD (Asp-Gly-Asp) synthetic peptide that inhibits integrins $\alpha V\beta 3$ and $\alpha V\beta 5$ by receptor binding competition [139]. *In vitro* studies have shown an anti-angiogenic effect of this inhibitor by inhibiting proliferation and differentiation of endothelial progenitor cells, without affecting apoptosis [140]. In GBM cells, cilengitide exerted only a moderate loss of viability and was unable to sensitize GBM cells to radiotherapy and TMZ treatment [141]. Clinical studies with this drug have shown limited toxicity, but also reduced beneficial effect, when administered in newly diagnosed patients of GBM with RT+ TMZ (Table 2).

4.2.2. Therapeutic targeting of intracellular signaling pathways

a. PI3K/AKT/mTOR pathway

As already mentioned the PI3K/Akt/mTOR pathway represents one of the most altered pathways in cancer, including GBM [113, 116]. Several inhibitors targeting different elements of this pathway are available and being tested both pre-clinically and at the clinical level. Enzastaurin is a specific inhibitor of protein kinase C (PKC) proteins, thus indirectly inhibiting Akt [104, 113, 142]. In preclinical studies, this inhibitor was able to suppress proliferation of GBM cells and tumor growth in GBM xenograft mice models [143]. In clinical studies, especially for recurrent GBM patients this drug failed to improve patient outcome, with PFS, OS and 6-PFS inferior to that of patients treated with lomustine in phase III clinical trials (Table 2) [144]. Inhibition of Akt can also be achieved using perifosine (KRX-0401), which affects the interaction of PIP_3 with the PH domain of Akt [24]. When this drug was compared to mTOR inhibition in *in vivo* models with differential expression of *PTEN*, the treatment with perifosine did not alter tumor volume; on the other hand, treatment with mTOR inhibitor resulted in decrease tumor volume [145]. Furthermore, only a clinical trial phase II for patients with

recurrent MG is under evaluation and no results are available until now (NCT00590954) [118]. A HIV type I (HIV-1) protease inhibitor called nelfinavir with applications in HIV infections is also able to downregulate Akt, and was proposed as an Akt inhibitor [146, 147]. Preclinical studies showed that treating GBM cells and xenograft mouse models with nelfinavir is able to sensitize tumor cells to RT and TMZ treatment [148]. Furthermore, this protease inhibitor decreases VEGF levels and angiogenesis, as well as HIF-1 expression levels and can cause endoplasmic reticulum stress and autophagy [146, 149]. Three phase I clinical trials to assess the toxicity of this treatment combined with RT + TMZ in newly diagnosed GBM are currently recruiting patients or active and ongoing (NCT01020292, NCT00694837, NCT00915694) [118].

Several inhibitors of PI3K are also available, but the clinical evaluation of their efficacy is still very preliminary [150]. The class of pan-PI3K inhibitors (inhibit the catalytic p110 subunit) include LY294002, ZSTK474, and wortmannin. Derivatives of LY294002 and wortmannin, include SF1126 (LY294002 conjugated with an RGD peptide), PWT-458 and PX-866 (the first is a PEGylated derivate of wortmannin and the second is a wortmannin analog) [150]. From this group of specific PI3K inhibitors, only evaluation of PX-866 is proposed in a phase II clinical trial for the treatment of recurrent GBM patients, and is currently recruiting patients (NCT01259869) (Table 2) [118]. XL147 and GDC-0941 are also class I PI3K inhibitors, and IC877114 (targets p110δ) and TG100-115 (targets p110δ and p110γ) are PI3K isoform-specific inhibitors [150]. In turn, LY294002 was able to potentiate the citotoxicity of TMZ in glioma cells [151, 152]. Besides these agents that only target PI3K there are several dual PI3K/mTOR inhibitors, as PI-103, PI-540, PI-620, XL765, BEZ235 and BGT226 [150]. XL765 and XL147 were already tested in a phase I clinical trial with recurrent GBM patients (Table 2). Some preclinical studies support the theory of targeting these pathways in GBM therapeutics. Combination of LY294002 with the mTOR inhibitor rapamycin (or sirolimus) was able to diminish the self-renewal capacity of GBM cells and induce differentiation of cancer stem cell like cells (CSC); the same effect was achieved using a dual PI3K/mTOR inhibitor, NVP-BEZ235, which additionally reduced the ability of GBM CSLC to form tumors *in vivo* [153].

For specific targeting of mTOR, several inhibitors were developed and tested clinically, like sirolimus (rapamycin), everolimus (RAD001) and temsirolimus (CCI-779) [106, 133]. All of these agents were already evaluated for the treatment of GBM in phase I and II clinical trials, but no significant improvements were seen (Table 2). A preclinical study showed that the outcome of mTOR inhibitory treatments could be efficiently monitored by Positron Emission Tomography (PET) based only in glucose and thymidine metabolism, through the uptake of [^{18}F]FDG and [^{18}F]FLT [154]. Furthermore, combination with other kinase inhibitors like AEE788 (inhibits both EGFR and VEGFR2) also showed some preclinical promising results, since its combination with everolimus (RAD001) resulted in increased effect on cell cycle arrest, proliferation and apoptosis, and impact tumor growth and survival *in vivo* [155]. This combination was tested in a phase I/II trial in 2006 for the treatment of recurrent GBM (NCT00107237) [118]. One liability of these therapies is that they only target mTORC1, and although this is the best characterized mTOR isoform, it is also known that full activation of PI3K/AKT pathway also requires mTORC2 [156]. Consequently, it is argued that dual inhibition of mTOR complexes 1 and 2 will be more effi-

cient [156]. Preclinical studies have shown a significant decrease in tumor volume and growth in xenograft mouse models of GBM treated with PI3K/mTOR inhibitor AZD8055 [157]. Furthermore, there are three phase I clinical trials recruiting patients with MG (drug/trial reference: AZD8055/NCT01316809; CC-223/NCT01177397; OSI-027/NCT00698243) to test this possibility [118].

b. RAS/RAF/MEK/ERK/MAPK pathway

Another important pathway contributing to the neoplastic process is the one mediated by RAS/RAF/MEK/MAPK [106]. Inhibitors targeting members of this pathway include the farnesil transferase inhibitors of RAS, such as tipifarnib (Zanestra or R115777) and lonafarnib (Sarasar or SCH 66336) or multiple kinases inhibitors that target this pathway, like sorafenib [98, 116]. Some of the more significant clinical results of tipifarnib are summarized in Table 2. A phase I clinical trial to test the effectiveness of combining tipifarnib with TMZ and RT for newly diagnosed GBM or gliosarcoma is now ongoing (NCT00049387) [118]. Sorafenib is described as an inhibitor of EGFR, PDGFR and RAF, that can block MEK activation and, in preclinical studies, was able to induce apoptosis, and decreased proliferation of GBM cells [98, 158]. At the clinical level, it has been extensively studied in 12 clinical trials with completed, ongoing or recruiting status [118]; however, the results have still been somewhat different, with good results for newly diagnosed GBM and recurrent GBM, but when combined with BVZ for the treatment of recurrent GBM, it failed to improve survival, showing a high percentage of patients with progressive disease (Table 2) [118].

c. Histone deacetylases (HDACs)

Epigenetic events are crucial during the carcinogenic process, in which the chromatin state and remodeling are important mediators. Histone deacetylases (HDAC) are responsible for chromatin condensation and repression of transcription [159, 160]. Mechanistically they catalyze the elimination of acetyl groups from lysine residues in N-terminal tails of histone proteins [161]. The use of specific HDAC inhibitors has been described as an attractive opportunity to alter cancer-related epigenetic modifications [159]. These inhibitors are also reported as being able to block angiogenesis and invasion, promote cell cycle arrest and apoptosis, and to act as immunomodulators [116, 159, 160]. Valproic acid (VPA) is a short chain fatty acid, class I and IIa HDAC inhibitor, used as an anticonvulsant drug and frequently administered to treat glioma-associated seizures [159, 162, 163]. So, when the results of the EORTC/NCIC TMZ trial were analyzed taking in consideration the anti-epileptic drugs used, an interesting result showing a benefit in OS of the patients treated with TMZ + RT that were under VPA treatment was observed, suggesting that this drug could enhance the effects of TMZ + RT treatment [162]. VPA in combination with TMZ *in vitro* showed an increase in TMZ cytotoxicity, even for TMZ resistant cell lines, through downregulation of MGMT [164-166]; *in vivo*, this combination had also a benefit in tumor growth inhibition [164]; and increased the effects of γ-radiation in glioma cells [165]. Clinically, the evaluation of VPA for the treatment of GBM is proposed in two clinical trials: a phase II trial to evaluate the efficacy of VPA + RT fol-

lowed by combination of VPA + BVZ for the treatment of children with high-grade gliomas (HGG) (NCT00879437) and a phase II trial to test the combination of VPA with TMZ and RT in adult HGG (NCT00302159) [118]. Vorinostat is also an inhibitor of class I and II HDACs, tested in several clinical trials now recruiting patients (NCT01378481, NCT01266031, NCT00731731, NCT01110876, NCT00555399) [118, 163]. The results of some of the already completed clinical trials for HDAC inhibitors as GBM therapeutics are in some way disappointing, with no radiographic responses observed when recurrent GBM patients treated with romidepsin and vorinostat failing to improve survival outcomes in different combinatory strategies (Table 2).

Drug; Target(s)	**Clinical Trials/Population/Results**	**Refs**
Erlotinib (Tarceva®); EGFR	Phase I, and II clinical trials Acceptable toxicity and tolerable treatment with daily administrations of 150-200 mg/day dose Newly diagnosed GBM: combined with TMZ showed a PFS of 7.2 months and OS of 15.3 months; worse outcome for patients older than 70 years old; combined with standard care (RT + TMZ), the OS was 19.3 months, and correlated with *MGMT* promoter methylation and PTEN expression; combinations with other drugs are also under clinical trials (BVZ, administration after TMZ + RT, RT and erlotinib in younger patients) (NCT00124657, NCT00720356). Recurrent GBM: erlotinib as a single agent was not able to improve PFS compared to standard treatment (TMZ or carmustine + RT); combined with mTOR inhibitor sirolimus, treatment was well tolerated and OS was 33.8 weeks; combination with carboplatin showed a 30 weeks OS; Recurrent MG: combination with BVZ resulted in partial or total radiographic response for 48% of GBM patients and association with PFS; GBM tumors showing high levels of HIF-2α and VEGFR2 expression presented a worst prognosis. Recruiting or ongoing clinical trials combining erlotinib with isotretinoin, sirolimus and vorinostat, and also single agent administration for patients harboring the EGFR-vIII mutation (NCT01110876, NCT01103375, NCT01257594, NCT00509431). Nonprogressive GBM: as single agent, 1-year PFS was only 9% and less than 53% of 2 months, and less than 57% of the patients were alive after 1 year.	[118, 167-175]
Gefitinib (Iressa or ZD1839); EGFR	Phase I, and II clinical trials Recurrent GBM: as single agent, the treatment was well tolerated and resulted in OS of 39.4 weeks and PFS of 8.1 weeks. In a phase II study, OS did not overcome 8.8 months. Newly diagnosed GBM: 1-year OS (54.2%) and 1-year PFS (16.7%) were not significantly different from controls of other clinical trials.	[176-178]
Rindopepimut (CDX-110, PEP-3); EGFR-vIII	Phase I, II, and III clinical trials EGFR-vIII-positive newly diagnosed GBM: given with GM-CSF, TTP of 14.2 months (vs. 6.3 months of historical controls) and OS of 26 months (vs. 15 months of historical controls); administration with TMZ also improved TTP (15.2 months vs. 6.4 months) and OS (23.2	[118, 124]

Drug; Target(s)	Clinical Trials/Population/Results	Refs
	months vs. 15.2 months); phase III trial (recruiting status) is projected to test the efficacy of rindopepimut with TMZ (NCT01480479). Newly diagnosed GBM: TTP was 10.2 months and OS was 22.8 months (vaccine given with DC); Phase II clinical trial is recruiting patients with relapsed GBM EGFR-vIII positive to test the efficacy of rindopepimut with BVZ (NCT01498328).	
Imatinib mesylate; PDGFR, KIT, ABL	Phase I, II, and III clinical trials Newly diagnosed GBM: a phase II study with 20 patients showed a OS of 6.2 months. Recurrent GBM or MG: as single agent was well tolerated until doses of 800-1200 g/day, but very poor outcome with 6-PFS of 3%, only 2/34 patients with PR, and 6/34 with SD; when combined with HU, 6-PFS (27%) improved, but still very poor; combination with HU and vatalanib was well tolerated and resulted in OS of 48 weeks, PFS of 12 weeks and 6-PFS of 25%. In another phase II study the outcome of patients treated with imatinib as single agent was also (in newly diagnosed GBM) very poor (6-PFS: 16%); when combined with HU, imatinib also lacked efficacy. A phase III clinical trial showed no differences in TMZ resistant GBM patients treated with imatinbib + HU or HU alone (NCT00154375); phase II clinical trials combining imatinib with HU and Zactima were also performed but no results have been published (NCT00613054).	[118, 126, 129-132, 179, 180]
Cediranib (AZD2171); VEGFR	Phase I, II, and III clinical trials Recurrent GBM: as a single agent showed a PFS was 117 days and OS was 227 days (phase II); phase I trials to test cediranib + lomustine to treat GBM is already completed but without published results (NCT00503204); a phase III trial with the same combinatory approach for the treatment of recurrent GBM in currently ongoing (NCT00777153); recruiting trials include combination with gefitinib (NCT01310855) and with cilengitide (NCT00979862). Newly diagnosed GBM: all clinical trials are currently ongoing or recruiting – phase I and phase I/II cediranib + RT + TMZ (NCT01062425 and NCT00662506); phase I combination with BVZ (NCT00458731); phase I combination with gamma secretase inhibitor RO4929097 (NCT0130855).	[118, 181, 182]
Rilotumumab (AMG-102); HGF	Phase II clinical trial Recurrent GBM: when combined with prior BVZ treatment, did not affect PFS (4-4.1 weeks vs. 4.1-4.7 weeks), but OS was significantly different (3.4-3.6 months vs. 10.9-11.4 months).	[183]
PX-866; PI3K	Phase I, and II clinical trials Completed a phase I clinical study in patients with solid tumors (NCT00726583); Recruiting recurrent GBM patients for a phase II clinical trial (NCT01259869).	[118]
XL765; PI3K/mTOR	Phase I clinical trial Recurrent GBM: combination with a PI3K inhibitor XL147 already completed phase I trial (NCT0124460). Recruiting for a phase I trial for combination with TMZ to treat MG (NCT00704080).	[118]

Drug; Target(s)	Clinical Trials/Population/Results	Refs
Enzastaurin; PKCβ (indirect inhibition of Akt)	Phase I, II, and III clinical trials Recurrent or progressive MG: in recurrent HGG, monotherapy had no significant impact in 6-PFS (7%); when compared with lomustine in a phase III clinical trial, no improvement in OS or PFS was achieved.	[118, 142, 144, 184, 185]
Everolimus (RAD001); mTOR	Phase I, and II clinical trials Phase I clinical trials showed that everolimus was well tolerated even when combined with RT + TMZ, BVZ or erlotinib. Changes in metabolism detected with FDG positron emission tomography days after administration of everolimus. Newly diagnosed GBM: combination with TMZ + RT + BVZ followed by BVZ + everolimus in a phase II clinical trial resulted in 57% PR, 1 CR, 18-months OS of 44%, and 18-months PFS of 29%.	[186-188]
Temsirolimus (CCI779); mTOR	Phase I, and II clinical trials Phase I trial showed that temsirolimus combined with TMZ and RT increased the risk of infectious diseases (3/25 fatal infections). Recurrent GBM: it was well tolerated as a single agent, and 36% radiographic responses were observed; 6-PFS was 7.8%, and OS was 4.4 months (phase II).	[189, 190]
Sirolimus; mTOR	Phase I, and II clinical trials Recurrent GBM or MG: in tumors without PTEN, mTOR inhibition correlated with decreased proliferation of the tumors (phase I/II); combination with erlotinib (phase II) resulted in 47% SD, no CR or PR and 6-PFS of 3.1%; phase I/II trial combinatory treatment with erlotinib is currently ongoing (NCT00509431); phase I trial is recruiting patients to test combinatory treatment with vandetanib (NCT00821080). Recruiting patients with solid tumors to test combination with a vaccine (NCT01522820).	[118, 172, 191]
Tipifarnib (Zarnestra, R115777); RAS	Phase I, and II clinical trials Newly diagnosed GBM: combined with RT and with or without TMZ, this treatment was well tolerated until doses of 300 mg (4-week cycle) (phase I). Administration with RT well tolerated until 200 mg/day, OS of 12 months and 1/9 PR, 4/9 SD, and 3/9 rapid progression. No significant improvement in survival with tipifarnib before RT (OS of 7.7 months). Recurrent GBM: treatment well tolerated, but 6-PFS (11.9%) and PFS (8 weeks) very poor, although one GBM patient remained progression-free for 36 months.	[192-195]
Sorafenib; RAF, VEGFR, PDGFR	Phase I, and II clinical trials Newly diagnosed GBM: combination of TMZ and sorafenib after RT + TMZ showed 13% PR, 53% SD, and 28% PD. OS was 12 months, 1-year PFS was 16%, and PFS was 6 months (phase II); also tested in combination with erlotinib/tipifarnib/temsirolimus (NCT00335764). Recurrent GBM: combination with TMZ resulted in OS of 41.5 weeks, 1-year OS of 34.4%, PFS of 6.4 weeks (6-PFS: 9.4%); 3% of the patients had PR, 4.7% SD, and 50% PD (phase II); combination with BVZ was also tested (NCT00621686).	[118, 196, 197]

Drug; Target(s)	Clinical Trials/Population/Results	Refs
	Ongoing or recruiting clinical trials: NCT00734526 (phase I/II: sorafenib + RT + TMZ for the treatment of newly diagnosed GBM), NCT00884416 (phase I single agent HGG), NCT00329719 (phase I/II: combination with temsirolimus for recurrent GBM).	
Cilengitide (EMD 121974); Integrins	Phase I, II, and III clinical trials Well tolerated until doses of 2400 mg/m^2 Newly diagnosed GBM: when combined with RT + TMZ, the OS was 16.1 months and patients with *MGMT* promoter methylation tend to show a higher PFS and OS; clinical trials testing the efficacy of cilengitide with TMZ + RT in patients with or without *MGMT* methylation are now recruiting or ongoing (NCT00813943, NCT0068922). Recurrent GBM: as a single agent no complete responses were observed, but median OS was at least 6.5-9.9 months.	[118, 198-200]
Vorinostat; HDAC	Phase I, and II clinical trials Progressive or recurrent GBM/MG: combination with bortezomib in a phase II trial resulted in very poor results (6-PFS 0%, OS 3.2 months, TTP 1.5 months); phase II monotherapy showed a 6-PFS of 15.2%, TTP of 1.9 months, PFS of 11.2 months, and OS of 5.7 months; Ongoing trials: phase I/II combination with BVZ and TMZ for recurrent MG (NCT00939991), phase I combination with TMZ for MG (NCT00268385), phase I combination with BVZ and irinotecan for recurrent GBM (NCT00762255).	[118, 201, 202]
Romidepsin; HDAC	Phase II clinical trial Recurrent MG: no radiographic responses, 72% PD and 28% SD; 6-PFS of 3%, PFS of 8 weeks, and OS of 34 weeks; 83% of the patients stopped treatment due to tumor progression, and 11% due to treatment toxicity.	[203]

PFS (median Progression-Free Survival); OS (median Overall Survival); TTP (median Time-to-Progression); PR (Partial Response); SD (Stable Disease); PD (Progressive Disease); 6-PFS (6 month PFS); BVZ (Bevacizumab); RT (Radiotherapy); TMZ (Temozolomide); GM-GSF (granulocyte macrophage-colony stimulating factor); DC (Dendritic Cells); HU (Hydroxyurea); MG (Malignant Glioma); HGG (High Grade Glioma).

Table 2. Examples of clinical trials with molecularly targeted therapies directed to the most commonly altered signalling pathways in GBM.

4.3. Novel therapeutic approaches

As stated above, a small population of cells within the tumor, called cancer stem-cells, presents self-renewal capacity, ability to differentiate and initiate tumorigenesis, and express several markers of neural stem cells [24, 33, 116]. Furthermore, these cells are increasingly recognized as a niche of radiochemotherapy-resistant cells, making then attractive targets for new therapies [24, 49, 204]. There are several signaling pathways altered in cancer stem cells and that represent possible targets, such as PI3K, OLIG2, Shh, Wnt and Notch signaling pathways [24, 116].

Another novel therapeutic strategy to treat cancer-related diseases is gene therapy (GT). GT was proposed for a long time as a molecular strategy that may help circumvent the non-specific cytotoxicity of the current pharmacological inhibitors, through specific delivery of suicide, pro-apoptotic, *TP53*, and other genes to tumor cells that, ultimately, lead to cancer regression or cure [106, 205]. GT can be performed delivering conditional or toxic transgenes using viral or non-viral delivery systems, including exosomes and stem cells [205, 206]. In GBM, the delivery of the thymidine kinase (TK) gene, produced by the herpes simplex virus type 1 (HSV1), in combination with the prodrug ganciclovir (GCV), using both retrovirus and adenovirus, was already tested at clinical level. The advantage of using retroviruses to deliver viral vectors is the specificity, since they target only highly proliferating cells. On the other hand, adenoviruses infect both quiescent cells and rapidly dividing cells [207]. A retroviralmediated delivery was already applied to newly diagnosed GBM patients until phase III clinical trial, but it was rejected after failing to improve survival compared to RT + TMZ [208]. Another promising strategy is the combination of viral vectors with factors that stimulate the immune system, as, for example, the delivery of the suicide gene HSV1 *TK* gene, with the cytokine IL-2. This strategy was already tested in 12 patients with recurrent GBM, where it was proved to be safe and well tolerable [209]. However, in terms of outcome, there were no patients with complete response and the PFS and OS were only 4.5 and 7.5 months, respectively [209].

The induction of an immune response against tumor cells, called immunotherapy, is also a novel approach for the treatment of cancer, including GBM [210]. Immunotherapy can be performed with two different approaches: increasing the immune response to the tumor (active immunotherapy) with long term immunization, or delivering immune effectors to an immediate immune response (passive immunotherapy) [106]. Potent anti-tumor immunity is achieved through antigen-presenting cells, of which dendritic cells (DC) are the most promising [210, 211]. In a phase I clinical trial with 12 GBM patients (7 newly diagnosed GBM and 5 recurrent GBM) the administration of autologous DC vaccines showed that this treatment was well tolerated and minimally toxic. Additionally, it revealed promising outcome results, such as 2 long term-survivors (≥4 years) and OS of 23.4 months; however, the benefit in clinical outcomes were mainly observed in patients with stable disease and low levels of TGF-β2, who also had a higher number of infiltrating cytotoxic T-cells in the tumor bulk, suggesting that this treatment may favor particularly these patients [212]. In another phase I/II clinical trial with patients with recurrent GBM, it was found more beneficial the treatment with mature DC vs. non-mature DC, as well as intradermal and intratumoral administration of the DC pulsed with autologous tumor lysate, compared to intradermal approach alone [213]. The transfer of *ex vivo* maturated immune cells like effector T cells or lymphokine activated killer cells (LAK) is also under clinical evaluation for GBM immunotherapy [214].

4.4. Current challenges and future trends

As illustrated by the vast panoply of drugs and therapeutic strategies under investigation for the treatment of GBM, there is a major effort to develop more effective therapies to treat this highly malignant and therapy-insensitive disease. Unfortunately, the success

of these new therapies has mostly been somewhat disappointing. Nevertheless, the efficacy of some of these approaches has yet to be determined. Of note, in addition to the strategies reviewed here, therapies targeting apoptotic elements (like Bcl-2, and inhibitor of apoptosis proteins), the mechanisms of resistance to TMZ (such as PARP and MGMT), or gene therapy to *TP53*, are also some examples of the search for an effective therapy for GBM [33]. Additionally, several developments were also made in helping surgeons with fluorescence-guided resection of the tumor and in radiotherapy [116]. In conclusion, the relevance of the effort to find a cure for GBM is unquestionable. However, despite the hard working search for a therapeutic strategy to reverse the poor outcomes of these patients, the standard treatment with TMZ and RT remains presently the best option. Future therapeutic trends for the treatment of GBM will have to: (i) include the new molecular classification of GBM; (ii) incorporate more efficient drug delivery systems to overcome blood-brain barrier restraints; and (iii) redirect the therapeutic choices to each patient, considering the specific molecular alterations of each tumor.

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References

[1] Ohgaki, H, & Kleihues, P. Genetic pathways to primary and secondary glioblastoma. The American journal of pathology. (2007). May; , 170(5), 1445-53.

[2] Porter, K. R, Mccarthy, B. J, Freels, S, Kim, Y, & Davis, F. G. Prevalence estimates for primary brain tumors in the United States by age, gender, behavior, and histology. Neuro Oncol. (2010). Jun; , 12(6), 520-7.

[3] CBTRUSPrimary Brain and Other Nervous System Tumors, Estimated Number of Cases Overall and by Behavior by State, (2012). Primary Malignant Brain and Other Nervous System Tumors, Estimated Number of Deaths by State, 2012. http:// wwwcbtrusorg/ html2012.

[4] Burnet, N. G, Jefferies, S. J, Benson, R. J, Hunt, D. P, & Treasure, F. P. Years of life lost (YLL) from cancer is an important measure of population burden--and should be

considered when allocating research funds. British journal of cancer. (2005). Jan 31; , 92(2), 241-5.

[5] Stupp, R, & Mason, W. P. van den Bent MJ, Weller M, Fisher B, Taphoorn MJ, et al. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. N Engl J Med. (2005). Mar 10; , 352(10), 987-96.

[6] Louis, D. N, Ohgaki, H, Wiestler, O. D, Cavenee, W. K, Burger, P. C, Jouvet, A, et al. The 2007 WHO classification of tumours of the central nervous system. Acta neuropathologica. (2007). Aug; , 114(2), 97-109.

[7] Nguyen, L. V, Vanner, R, Dirks, P, & Eaves, C. J. Cancer stem cells: an evolving concept. Nat Rev Cancer. (2012). Feb; , 12(2), 133-43.

[8] Dirks, P. B. Brain tumor stem cells: bringing order to the chaos of brain cancer. J Clin Oncol. (2008). Jun 10; , 26(17), 2916-24.

[9] Charles, N. A, Holland, E. C, Gilbertson, R, Glass, R, & Kettenmann, H. The brain tumor microenvironment. Glia. (2012). Mar; , 60(3), 502-14.

[10] Heywood, R. M, Marcus, H. J, Ryan, D. J, Piccirillo, S. G, Al-mayhani, T. M, & Watts, C. A review of the role of stem cells in the development and treatment of glioma. Acta Neurochir (Wien). (2012). Jun; discussion 69., 154(6), 951-69.

[11] Bachoo, R. M, Maher, E. A, Ligon, K. L, Sharpless, N. E, Chan, S. S, You, M. J, et al. Epidermal growth factor receptor and Ink4a/Arf: convergent mechanisms governing terminal differentiation and transformation along the neural stem cell to astrocyte axis. Cancer cell. (2002). Apr; , 1(3), 269-77.

[12] Doetsch, F, Caille, I, Lim, D. A, Garcia-verdugo, J. M, & Alvarez-buylla, A. Subventricular zone astrocytes are neural stem cells in the adult mammalian brain. Cell. (1999). Jun 11; , 97(6), 703-16.

[13] Laywell, E. D, Rakic, P, Kukekov, V. G, Holland, E. C, & Steindler, D. A. Identification of a multipotent astrocytic stem cell in the immature and adult mouse brain. Proc Natl Acad Sci U S A. (2000). Dec 5; , 97(25), 13883-8.

[14] Chen, J, Mckay, R. M, & Parada, L. F. Malignant glioma: lessons from genomics, mouse models, and stem cells. Cell. (2012). Mar 30; , 149(1), 36-47.

[15] Vescovi, A. L, Galli, R, & Reynolds, B. A. Brain tumour stem cells. Nat Rev Cancer. (2006). Jun; , 6(6), 425-36.

[16] Chen, R, Nishimura, M. C, Bumbaca, S. M, Kharbanda, S, Forrest, W. F, Kasman, I. M, et al. A hierarchy of self-renewing tumor-initiating cell types in glioblastoma. Cancer cell. (2010). Apr 13; , 17(4), 362-75.

[17] Stiles, C. D, & Rowitch, D. H. Glioma stem cells: a midterm exam. Neuron. (2008). Jun 26; , 58(6), 832-46.

[18] Singh, S. K, Hawkins, C, Clarke, I. D, Squire, J. A, Bayani, J, Hide, T, et al. Identification of human brain tumour initiating cells. Nature. (2004). Nov 18; , 432(7015), 396-401.

[19] Dirks, P. B. Brain tumor stem cells: the cancer stem cell hypothesis writ large. Molecular oncology. (2010). Oct; , 4(5), 420-30.

[20] Brescia, P, Richichi, C, & Pelicci, G. Current strategies for identification of glioma stem cells: adequate or unsatisfactory? Journal of oncology. (2012).

[21] Jhanwar-uniyal, M, Albert, L, Mckenna, E, Karsy, M, Rajdev, P, Braun, A, et al. Deciphering the signaling pathways of cancer stem cells of glioblastoma multiforme: role of Akt/mTOR and MAPK pathways. Advances in enzyme regulation. (2011). , 51(1), 164-70.

[22] Dunn, G. P, Rinne, M. L, Wykosky, J, Genovese, G, Quayle, S. N, Dunn, I. F, et al. Emerging insights into the molecular and cellular basis of glioblastoma. Genes Dev. (2012). Apr 15; , 26(8), 756-84.

[23] Cheng, L, Bao, S, & Rich, J. N. Potential therapeutic implications of cancer stem cells in glioblastoma. Biochem Pharmacol. (2010). Sep 1; , 80(5), 654-65.

[24] Ohka, F, Natsume, A, & Wakabayashi, T. Current trends in targeted therapies for glioblastoma multiforme. Neurol Res Int. (2012).

[25] Lim, S. K, Llaguno, S. R, Mckay, R. M, & Parada, L. F. Glioblastoma multiforme: a perspective on recent findings in human cancer and mouse models. BMB Rep. (2011). Mar; , 44(3), 158-64.

[26] TCGAComprehensive genomic characterization defines human glioblastoma genes and core pathway. Nature. 2008 23 October (2008). , 455(7216), 1061-8.

[27] Sherr, C. J, Mccormick, F, & The, R. B. and pathways in cancer. Cancer Cell. (2002). Aug;2(2):103-12., 53.

[28] Shapiro, G. I. Cyclin-dependent kinase pathways as targets for cancer treatment. J Clin Oncol. (2006). Apr 10; , 24(11), 1770-83.

[29] Mao, H, Lebrun, D. G, Yang, J, Zhu, V. F, & Li, M. Deregulated signaling pathways in glioblastoma multiforme: molecular mechanisms and therapeutic targets. Cancer Invest. (2012). Jan; , 30(1), 48-56.

[30] Deb, S. P. Cell cycle regulatory functions of the human oncoprotein MDM2. Mol Cancer Res. (2003). Dec; , 1(14), 1009-16.

[31] Iwakuma, T, & Lozano, G. MDM2, an introduction. Mol Cancer Res. (2003). Dec; , 1(14), 993-1000.

[32] Zhang, Y, Xiong, Y, & Yarbrough, W. G. ARF promotes MDM2 degradation and stabilizes ARF-INK4a locus deletion impairs both the Rb and p53 tumor suppression pathways. Cell. (1998). Mar 20;92(6):725-34., 53.

[33] Krakstad, C, & Chekenya, M. Survival signalling and apoptosis resistance in glioblastomas: opportunities for targeted therapeutics. Mol Cancer. (2010).

[34] Moscatello, D. K, Holgado-madruga, M, Emlet, D. R, Montgomery, R. B, & Wong, A. J. Constitutive activation of phosphatidylinositol 3-kinase by a naturally occurring mutant epidermal growth factor receptor. J Biol Chem. (1998). Jan 2; , 273(1), 200-6.

[35] Costa, B. M, Smith, J. S, Chen, Y, Chen, J, Phillips, H. S, Aldape, K. D, et al. Reversing HOXA9 oncogene activation by PI3K inhibition: epigenetic mechanism and prognostic significance in human glioblastoma. Cancer Res. (2010). Jan 15; , 70(2), 453-62.

[36] Gaspar, N, Marshall, L, Perryman, L, Bax, D. A, Little, S. E, Viana-pereira, M, et al. MGMT-independent temozolomide resistance in pediatric glioblastoma cells associated with a PI3-kinase-mediated HOX/stem cell gene signature. Cancer Res. (2010). Nov 15; , 70(22), 9243-52.

[37] Murat, A, Migliavacca, E, Gorlia, T, Lambiv, W. L, Shay, T, Hamou, M. F, et al. Stem cell-related "self-renewal" signature and high epidermal growth factor receptor expression associated with resistance to concomitant chemoradiotherapy in glioblastoma. J Clin Oncol. (2008). Jun 20; , 26(18), 3015-24.

[38] Dang, L, White, D. W, Gross, S, Bennett, B. D, Bittinger, M. A, Driggers, E. M, et al. Cancer-associated IDH1 mutations produce 2-hydroxyglutarate. Nature. (2009). Dec 10; , 462(7274), 739-44.

[39] Sasaki, M, Knobbe, C. B, Munger, J. C, Lind, E. F, Brenner, D, Brustle, A, et al. IDH1(R132H) mutation increases murine haematopoietic progenitors and alters epigenetics. Nature. (2012). Jul 4.

[40] Verhaak, R. G, Hoadley, K. A, Purdom, E, Wang, V, Qi, Y, Wilkerson, M. D, et al. Integrated genomic analysis identifies clinically relevant subtypes of glioblastoma characterized by abnormalities in PDGFRA, IDH1, EGFR, and NF1. Cancer Cell. (2010). Jan 19; , 17(1), 98-110.

[41] Liang, Y, Diehn, M, Watson, N, Bollen, A. W, Aldape, K. D, Nicholas, M. K, et al. Gene expression profiling reveals molecularly and clinically distinct subtypes of glioblastoma multiforme. Proc Natl Acad Sci U S A. (2005). Apr 19; , 102(16), 5814-9.

[42] Mischel, P. S, Shai, R, Shi, T, Horvath, S, Lu, K. V, Choe, G, et al. Identification of molecular subtypes of glioblastoma by gene expression profiling. Oncogene. (2003). Apr 17; , 22(15), 2361-73.

[43] Shai, R, Shi, T, Kremen, T. J, Horvath, S, Liau, L. M, Cloughesy, T. F, et al. Gene expression profiling identifies molecular subtypes of gliomas. Oncogene. (2003). Jul 31; , 22(31), 4918-23.

[44] Freije, W. A, Castro-vargas, F. E, Fang, Z, Horvath, S, Cloughesy, T, Liau, L. M, et al. Gene expression profiling of gliomas strongly predicts survival. Cancer Res. (2004). Sep 15; , 64(18), 6503-10.

[45] Nutt, C. L, Mani, D. R, Betensky, R. A, Tamayo, P, Cairncross, J. G, Ladd, C, et al. Gene expression-based classification of malignant gliomas correlates better with survival than histological classification. Cancer Res. (2003). Apr 1; , 63(7), 1602-7.

[46] Phillips, H. S, Kharbanda, S, Chen, R, Forrest, W. F, Soriano, R. H, Wu, T. D, et al. Molecular subclasses of high-grade glioma predict prognosis, delineate a pattern of disease progression, and resemble stages in neurogenesis. Cancer Cell. (2006). Mar; , 9(3), 157-73.

[47] Tso, C. L, Freije, W. A, Day, A, Chen, Z, Merriman, B, Perlina, A, et al. Distinct transcription profiles of primary and secondary glioblastoma subgroups. Cancer Res. (2006). Jan 1; , 66(1), 159-67.

[48] Curran, W. J. Jr., Scott CB, Horton J, Nelson JS, Weinstein AS, Fischbach AJ, et al. Recursive partitioning analysis of prognostic factors in three Radiation Therapy Oncology Group malignant glioma trials. J Natl Cancer Inst. (1993). May 5; , 85(9), 704-10.

[49] Bao, S, Wu, Q, Mclendon, R. E, Hao, Y, Shi, Q, Hjelmeland, A. B, et al. Glioma stem cells promote radioresistance by preferential activation of the DNA damage response. Nature. (2006). Dec 7; , 444(7120), 756-60.

[50] Chakravarti, A, Zhai, G, Suzuki, Y, Sarkesh, S, Black, P. M, Muzikansky, A, et al. The prognostic significance of phosphatidylinositol 3-kinase pathway activation in human gliomas. J Clin Oncol. (2004). May 15; , 22(10), 1926-33.

[51] Haas-kogan, D. A, Prados, M. D, Lamborn, K. R, Tihan, T, Berger, M. S, & Stokoe, D. Biomarkers to predict response to epidermal growth factor receptor inhibitors. Cell Cycle. (2005). Oct; , 4(10), 1369-72.

[52] Hegi, M. E, Diserens, A. C, Gorlia, T, Hamou, M. F, De Tribolet, N, Weller, M, et al. MGMT gene silencing and benefit from temozolomide in glioblastoma. N Engl J Med. (2005). Mar 10; , 352(10), 997-1003.

[53] Hormigo, A, Gu, B, Karimi, S, Riedel, E, Panageas, K. S, Edgar, M. A, et al. YKL-40 and matrix metalloproteinase-9 as potential serum biomarkers for patients with high-grade gliomas. Clin Cancer Res. (2006). Oct 1; , 12(19), 5698-704.

[54] Liu, G, Yuan, X, Zeng, Z, Tunici, P, Ng, H, Abdulkadir, I. R, et al. Analysis of gene expression and chemoresistance of CD133+ cancer stem cells in glioblastoma. Mol Cancer. (2006).

[55] Mellinghoff, I. K, Wang, M. Y, Vivanco, I, Haas-kogan, D. A, Zhu, S, Dia, E. Q, et al. Molecular determinants of the response of glioblastomas to EGFR kinase inhibitors. N Engl J Med. (2005). Nov 10; , 353(19), 2012-24.

[56] Nigro, J. M, Misra, A, Zhang, L, Smirnov, I, Colman, H, Griffin, C, et al. Integrated array-comparative genomic hybridization and expression array profiles identify clinically relevant molecular subtypes of glioblastoma. Cancer Res. (2005). Mar 1; , 65(5), 1678-86.

[57] Parsons, D. W, Jones, S, Zhang, X, Lin, J. C, Leary, R. J, Angenendt, P, et al. An integrated genomic analysis of human glioblastoma multiforme. Science. (2008). Sep 26; , 321(5897), 1807-12.

[58] Pelloski, C. E, Lin, E, Zhang, L, Yung, W. K, Colman, H, Liu, J. L, et al. Prognostic associations of activated mitogen-activated protein kinase and Akt pathways in glioblastoma. Clin Cancer Res. (2006). Jul 1; , 12(13), 3935-41.

[59] Srinivasan, S, Patric, I. R, & Somasundaram, K. A ten-microRNA expression signature predicts survival in glioblastoma. PLoS One. (2011). e17438.

[60] Colman, H, & Aldape, K. Molecular predictors in glioblastoma: toward personalized therapy. Arch Neurol. (2008). Jul; , 65(7), 877-83.

[61] Liu, L, Markowitz, S, & Gerson, S. L. Mismatch repair mutations override alkyltransferase in conferring resistance to temozolomide but not to 1,3-bis(2-chloroethyl)nitrosourea. Cancer Res. (1996). Dec 1; , 56(23), 5375-9.

[62] Costa, B. M, Caeiro, C, Guimaraes, I, Martinho, O, Jaraquemada, T, Augusto, I, et al. Prognostic value of MGMT promoter methylation in glioblastoma patients treated with temozolomide-based chemoradiation: a Portuguese multicentre study. Oncol Rep. (2010). Jun; , 23(6), 1655-62.

[63] Van Vlodrop, I. J, Niessen, H. E, Derks, S, Baldewijns, M. M, Van Criekinge, W, Herman, J. G, et al. Analysis of promoter CpG island hypermethylation in cancer: location, location, location! Clin Cancer Res. (2011). Jul 1; , 17(13), 4225-31.

[64] Bady, P, Sciuscio, D, Diserens, A. C, & Bloch, J. van den Bent MJ, Marosi C, et al. MGMT methylation analysis of glioblastoma on the Infinium methylation BeadChip identifies two distinct CpG regions associated with gene silencing and outcome, yielding a prediction model for comparisons across datasets, tumor grades, and CIMP-status. Acta Neuropathol. (2012). Jul 19.

[65] Shah, N, Lin, B, Sibenaller, Z, Ryken, T, Lee, H, Yoon, J. G, et al. Comprehensive analysis of MGMT promoter methylation: correlation with MGMT expression and clinical response in GBM. PLoS One. (2011). e16146.

[66] Wick, W, Platten, M, Meisner, C, Felsberg, J, Tabatabai, G, Simon, M, et al. Temozolomide chemotherapy alone versus radiotherapy alone for malignant astrocytoma in the elderly: the NOA-08 randomised, phase 3 trial. Lancet Oncol. (2012). Jul; , 13(7), 707-15.

[67] Olson, R. A, Brastianos, P. K, & Palma, D. A. Prognostic and predictive value of epigenetic silencing of MGMT in patients with high grade gliomas: a systematic review and meta-analysis. J Neurooncol. (2011). Nov; , 105(2), 325-35.

[68] Yan, H, Parsons, D. W, Jin, G, Mclendon, R, Rasheed, B. A, Yuan, W, et al. IDH1 and IDH2 mutations in gliomas. N Engl J Med. (2009). Feb 19; , 360(8), 765-73.

[69] Koshland, D. E. Jr., Walsh K, LaPorte DC. Sensitivity of metabolic fluxes to covalent control. Curr Top Cell Regul. (1985). , 27, 13-22.

[70] Zhao, S, Lin, Y, Xu, W, Jiang, W, Zha, Z, Wang, P, et al. Glioma-derived mutations in IDH1 dominantly inhibit IDH1 catalytic activity and induce HIF-1alpha. Science. (2009). Apr 10; , 324(5924), 261-5.

[71] Balss, J, Meyer, J, Mueller, W, Korshunov, A, Hartmann, C, & Von Deimling, A. Analysis of the IDH1 codon 132 mutation in brain tumors. Acta Neuropathol. (2008). Dec; , 116(6), 597-602.

[72] Ichimura, K, Pearson, D. M, Kocialkowski, S, Backlund, L. M, Chan, R, Jones, D. T, et al. IDH1 mutations are present in the majority of common adult gliomas but rare in primary glioblastomas. Neuro Oncol. (2009). Aug; , 11(4), 341-7.

[73] Hartmann, C, Meyer, J, Balss, J, Capper, D, Mueller, W, Christians, A, et al. Type and frequency of IDH1 and IDH2 mutations are related to astrocytic and oligodendroglial differentiation and age: a study of 1,010 diffuse gliomas. Acta Neuropathol. (2009). Oct; , 118(4), 469-74.

[74] Sanson, M, Marie, Y, Paris, S, Idbaih, A, Laffaire, J, Ducray, F, et al. Isocitrate dehydrogenase 1 codon 132 mutation is an important prognostic biomarker in gliomas. J Clin Oncol. (2009). Sep 1; , 27(25), 4150-4.

[75] Noushmehr, H, Weisenberger, D. J, Diefes, K, Phillips, H. S, Pujara, K, Berman, B. P, et al. Identification of a CpG island methylator phenotype that defines a distinct subgroup of glioma. Cancer Cell. (2010). May 18; , 17(5), 510-22.

[76] Yen, K. E, Bittinger, M. A, Su, S. M, & Fantin, V. R. Cancer-associated IDH mutations: biomarker and therapeutic opportunities. Oncogene. (2010). Dec 9; , 29(49), 6409-17.

[77] SongTao QLei Y, Si G, YanQing D, HuiXia H, XueLin Z, et al. IDH mutations predict longer survival and response to temozolomide in secondary glioblastoma. Cancer Sci. (2012). Feb; , 103(2), 269-73.

[78] Mcginnis, W, & Krumlauf, R. Homeobox genes and axial patterning. Cell. (1992). Jan 24; , 68(2), 283-302.

[79] Wellik, D. M. Hox genes and vertebrate axial pattern. Curr Top Dev Biol. (2009). , 88, 257-78.

[80] Shah, N, & Sukumar, S. The Hox genes and their roles in oncogenesis. Nat Rev Cancer. (2010). May; , 10(5), 361-71.

[81] Abate-shen, C. Deregulated homeobox gene expression in cancer: cause or consequence? Nat Rev Cancer. (2002). Oct; , 2(10), 777-85.

[82] Care, A, Felicetti, F, Meccia, E, Bottero, L, Parenza, M, Stoppacciaro, A, et al. HOXB7: a key factor for tumor-associated angiogenic switch. Cancer Res. (2001). Sep 1; , 61(17), 6532-9.

[83] Hu, Y. L, Fong, S, Ferrell, C, Largman, C, & Shen, W. F. HOXA9 modulates its oncogenic partner Meis1 to influence normal hematopoiesis. Mol Cell Biol. (2009). Sep; , 29(18), 5181-92.

[84] Raman, V, Martensen, S. A, Reisman, D, Evron, E, Odenwald, W. F, Jaffee, E, et al. Compromised HOXA5 function can limit expression in human breast tumours. Nature. (2000). Jun 22;405(6789):974-8., 53.

[85] Wu, X, Chen, H, Parker, B, Rubin, E, Zhu, T, Lee, J. S, et al. HOXB7, a homeodomain protein, is overexpressed in breast cancer and confers epithelial-mesenchymal transition. Cancer Res. (2006). Oct 1; , 66(19), 9527-34.

[86] Abdel-fattah, R, Xiao, A, Bomgardner, D, Pease, C. S, Lopes, M. B, & Hussaini, I. M. Differential expression of HOX genes in neoplastic and non-neoplastic human astrocytes. J Pathol. (2006). May; , 209(1), 15-24.

[87] Brandsma, D, Stalpers, L, Taal, W, & Sminia, P. van den Bent MJ. Clinical features, mechanisms, and management of pseudoprogression in malignant gliomas. Lancet Oncol. (2008). May; , 9(5), 453-61.

[88] Wen, P. Y, & Kesari, S. Malignant gliomas in adults. N Engl J Med. (2008). Jul 31; , 359(5), 492-507.

[89] Sorensen, A. G, Batchelor, T. T, Wen, P. Y, Zhang, W. T, & Jain, R. K. Response criteria for glioma. Nat Clin Pract Oncol. (2008). Nov; , 5(11), 634-44.

[90] van den Bent MJVogelbaum MA, Wen PY, Macdonald DR, Chang SM. End point assessment in gliomas: novel treatments limit usefulness of classical Macdonald's Criteria. J Clin Oncol. (2009). Jun 20; , 27(18), 2905-8.

[91] Tanwar, M. K, Gilbert, M. R, & Holland, E. C. Gene expression microarray analysis reveals YKL-40 to be a potential serum marker for malignant character in human glioma. Cancer Res. (2002). Aug 1; , 62(15), 4364-8.

[92] Bhat, K. P, Pelloski, C. E, Zhang, Y, & Kim, S. H. deLaCruz C, Rehli M, et al. Selective repression of YKL-40 by NF-kappaB in glioma cell lines involves recruitment of histone deacetylase-1 and-2. FEBS Lett. (2008). Sep 22;582(21-22):3193-200.

[93] Johansen, J. S, Schultz, N. A, & Jensen, B. V. Plasma YKL-40: a potential new cancer biomarker? Future Oncol. (2009). Sep; , 5(7), 1065-82.

[94] Shao, R, Hamel, K, Petersen, L, Cao, Q. J, Arenas, R. B, Bigelow, C, et al. YKL-40, a secreted glycoprotein, promotes tumor angiogenesis. Oncogene. (2009). Dec 17; , 28(50), 4456-68.

[95] Bernardi, D, Padoan, A, Ballin, A, Sartori, M, Manara, R, Scienza, R, et al. Serum YKL-40 following resection for cerebral glioblastoma. J Neurooncol. (2012). Apr; , 107(2), 299-305.

[96] Iwamoto, F. M, Hottinger, A. F, Karimi, S, Riedel, E, Dantis, J, Jahdi, M, et al. Serum YKL-40 is a marker of prognosis and disease status in high-grade gliomas. Neuro Oncol. (2011). Nov; , 13(11), 1244-51.

[97] Pelloski, C. E, Mahajan, A, Maor, M, Chang, E. L, Woo, S, Gilbert, M, et al. YKL-40 expression is associated with poorer response to radiation and shorter overall survival in glioblastoma. Clin Cancer Res. (2005). May 1; , 11(9), 3326-34.

[98] Bai, R. Y, Staedtke, V, & Riggins, G. J. Molecular targeting of glioblastoma: Drug discovery and therapies. Trends Mol Med. (2011). Jun; , 17(6), 301-12.

[99] Jones, T. S, & Holland, E. C. Standard of care therapy for malignant glioma and its effect on tumor and stromal cells. Oncogene. (2012). Apr 19; , 31(16), 1995-2006.

[100] Villano, J. L, Seery, T. E, & Bressler, L. R. Temozolomide in malignant gliomas: current use and future targets. Cancer Chemother Pharmacol. (2009). Sep; , 64(4), 647-55.

[101] Cohen, M. H, Shen, Y. L, Keegan, P, & Pazdur, R. FDA drug approval summary: bevacizumab (Avastin) as treatment of recurrent glioblastoma multiforme. The oncologist. (2009). Nov; , 14(11), 1131-8.

[102] Pan, E, Mitchell, S. B, & Tsai, J. S. A retrospective study of the safety of BCNU wafers with concurrent temozolomide and radiotherapy and adjuvant temozolomide for newly diagnosed glioblastoma patients. J Neurooncol. (2008). Jul; , 88(3), 353-7.

[103] Preusser, M, De Ribaupierre, S, Wohrer, A, Erridge, S. C, Hegi, M, Weller, M, et al. Current concepts and management of glioblastoma. Ann Neurol. (2011). Jul; , 70(1), 9-21.

[104] Mrugala, M. M, & Chamberlain, M. C. Mechanisms of disease: temozolomide and glioblastoma--look to the future. Nature clinical practice Oncology. (2008). Aug; , 5(8), 476-86.

[105] Stupp, R, Hegi, M. E, & Mason, W. P. van den Bent MJ, Taphoorn MJ, Janzer RC, et al. Effects of radiotherapy with concomitant and adjuvant temozolomide versus radiotherapy alone on survival in glioblastoma in a randomised phase III study: 5-year analysis of the EORTC-NCIC trial. Lancet Oncol. (2009). May; , 10(5), 459-66.

[106] Clarke, J, Butowski, N, & Chang, S. Recent advances in therapy for glioblastoma. Archives of neurology. (2010). Mar; , 67(3), 279-83.

[107] (FDA) USFaDABevacizumab Injection. [updated 03/08/(2012). Available from: http://www.fda.gov/AboutFDA/CentersOffices/OfficeofMedicalProductsandTobacco/CDER/ucm149364.htm.

[108] Hasselbalch, B, Lassen, U, Hansen, S, Holmberg, M, Sorensen, M, Kosteljanetz, M, et al. Cetuximab, bevacizumab, and irinotecan for patients with primary glioblastoma and progression after radiation therapy and temozolomide: a phase II trial. Neuro Oncol. (2010). May; , 12(5), 508-16.

[109] Lai, A, Tran, A, Nghiemphu, P. L, Pope, W. B, Solis, O. E, Selch, M, et al. Phase II study of bevacizumab plus temozolomide during and after radiation therapy for patients with newly diagnosed glioblastoma multiforme. J Clin Oncol. (2011). Jan 10; , 29(2), 142-8.

[110] Kreisl, T. N, Kim, L, Moore, K, Duic, P, Royce, C, Stroud, I, et al. Phase II trial of single-agent bevacizumab followed by bevacizumab plus irinotecan at tumor progression in recurrent glioblastoma. J Clin Oncol. (2009). Feb 10; , 27(5), 740-5.

[111] De Groot, J. F, Fuller, G, Kumar, A. J, Piao, Y, Eterovic, K, Ji, Y, et al. Tumor invasion after treatment of glioblastoma with bevacizumab: radiographic and pathologic correlation in humans and mice. Neuro Oncol. (2010). Mar; , 12(3), 233-42.

[112] Keunen, O, Johansson, M, Oudin, A, Sanzey, M, Rahim, S. A, Fack, F, et al. Anti-VEGF treatment reduces blood supply and increases tumor cell invasion in glioblastoma. Proc Natl Acad Sci U S A. (2011). Mar 1; , 108(9), 3749-54.

[113] Wick, W, Weller, M, Weiler, M, Batchelor, T, Yung, A. W, & Platten, M. Pathway inhibition: emerging molecular targets for treating glioblastoma. Neuro Oncol. (2011). Jun; , 13(6), 566-79.

[114] Kanu, O. O, Hughes, B, Di, C, Lin, N, Fu, J, Bigner, D. D, et al. Glioblastoma Multiforme Oncogenomics and Signaling Pathways. Clin Med Oncol. (2009). Apr 8; , 3, 39-52.

[115] Lemmon, M. A, & Schlessinger, J. Cell signaling by receptor tyrosine kinases. Cell. (2010). Jun 25; , 141(7), 1117-34.

[116] Van Meir, E. G, Hadjipanayis, C. G, Norden, A. D, Shu, H. K, Wen, P. Y, & Olson, J. J. Exciting new advances in neuro-oncology: the avenue to a cure for malignant glioma. CA Cancer J Clin. (2010). May-Jun; , 60(3), 166-93.

[117] Cohen, M. H, Johnson, J. R, Chen, Y. F, Sridhara, R, & Pazdur, R. FDA drug approval summary: erlotinib (Tarceva) tablets. The oncologist. (2005). Aug; , 10(7), 461-6.

[118] ClinicalTrialsgov- a service of the U.S. National Institutes of Health. [27 July (2012). Available from: www.clinicaltrials.gov.

[119] Polivka, J. Jr., Polivka J, Rohan V, Topolcan O, Ferda J. New molecularly targeted therapies for glioblastoma multiforme. Anticancer Res. (2012). Jul; , 32(7), 2935-46.

[120] Fukai, J, Nishio, K, Itakura, T, & Koizumi, F. Antitumor activity of cetuximab against malignant glioma cells overexpressing EGFR deletion mutant variant III. Cancer Sci. (2008). Oct; , 99(10), 2062-9.

[121] Martens, T, Laabs, Y, Gunther, H. S, Kemming, D, Zhu, Z, Witte, L, et al. Inhibition of glioblastoma growth in a highly invasive nude mouse model can be achieved by targeting epidermal growth factor receptor but not vascular endothelial growth factor receptor-2. Clin Cancer Res. (2008). Sep 1; , 14(17), 5447-58.

[122] Neyns, B, Sadones, J, Joosens, E, Bouttens, F, Verbeke, L, Baurain, J. F, et al. Stratified phase II trial of cetuximab in patients with recurrent high-grade glioma. Ann Oncol. (2009). Sep; , 20(9), 1596-603.

[123] Gan, H. K, Kaye, A. H, & Luwor, R. B. The EGFRvIII variant in glioblastoma multiforme. J Clin Neurosci. (2009). Jun; , 16(6), 748-54.

[124] Heimberger, A. B, & Sampson, J. H. The PEPvIII-KLH (CDX-110) vaccine in glioblastoma multiforme patients. Expert Opin Biol Ther. (2009). Aug; , 9(8), 1087-98.

[125] Sampson, J. H, Heimberger, A. B, Archer, G. E, Aldape, K. D, Friedman, A. H, Friedman, H. S, et al. Immunologic escape after prolonged progression-free survival with epidermal growth factor receptor variant III peptide vaccination in patients with newly diagnosed glioblastoma. J Clin Oncol. (2010). Nov 1; , 28(31), 4722-9.

[126] Reardon, D. A, Egorin, M. J, Quinn, J. A, Rich, J. N, Gururangan, S, Vredenburgh, J. J, et al. Phase II study of imatinib mesylate plus hydroxyurea in adults with recurrent glioblastoma multiforme. J Clin Oncol. (2005). Dec 20; , 23(36), 9359-68.

[127] Ranza, E, Mazzini, G, Facoetti, A, & Nano, R. In-vitro effects of the tyrosine kinase inhibitor imatinib on glioblastoma cell proliferation. J Neurooncol. (2010). Feb; , 96(3), 349-57.

[128] Kilic, T, Alberta, J. A, Zdunek, P. R, Acar, M, Iannarelli, P, & Reilly, O. T, et al. Intracranial inhibition of platelet-derived growth factor-mediated glioblastoma cell growth by an orally active kinase inhibitor of the 2-phenylaminopyrimidine class. Cancer Res. (2000). Sep 15; , 60(18), 5143-50.

[129] Reardon, D. A, Dresemann, G, Taillibert, S, & Campone, M. van den Bent M, Clement P, et al. Multicentre phase II studies evaluating imatinib plus hydroxyurea in patients with progressive glioblastoma. Br J Cancer. (2009). Dec 15; , 101(12), 1995-2004.

[130] Raymond, E, Brandes, A. A, Dittrich, C, Fumoleau, P, Coudert, B, Clement, P. M, et al. Phase II study of imatinib in patients with recurrent gliomas of various histologies: a European Organisation for Research and Treatment of Cancer Brain Tumor Group Study. J Clin Oncol. (2008). Oct 1; , 26(28), 4659-65.

[131] Wen, P. Y, Yung, W. K, Lamborn, K. R, Dahia, P. L, Wang, Y, Peng, B, et al. Phase I/II study of imatinib mesylate for recurrent malignant gliomas: North American Brain Tumor Consortium Study 99-08. Clin Cancer Res. (2006). Aug 15; , 12(16), 4899-907.

[132] Reardon, D. A, Egorin, M. J, Desjardins, A, Vredenburgh, J. J, Beumer, J. H, Lagattuta, T. F, et al. Phase I pharmacokinetic study of the vascular endothelial growth factor receptor tyrosine kinase inhibitor vatalanib (PTK787) plus imatinib and hydroxyurea for malignant glioma. Cancer. (2009). May 15; , 115(10), 2188-98.

[133] Agarwal, S, Sane, R, Oberoi, R, Ohlfest, J. R, & Elmquist, W. F. Delivery of molecularly targeted therapy to malignant glioma, a disease of the whole brain. Expert Rev Mol Med. (2011). e17.

[134] Gerstner, E. R, Chen, P. J, Wen, P. Y, Jain, R. K, Batchelor, T. T, & Sorensen, G. Infiltrative patterns of glioblastoma spread detected via diffusion MRI after treatment with cediranib. Neuro Oncol. (2010). May; , 12(5), 466-72.

[135] Kamoun, W. S, Ley, C. D, Farrar, C. T, Duyverman, A. M, Lahdenranta, J, Lacorre, D. A, et al. Edema control by cediranib, a vascular endothelial growth factor receptor-targeted kinase inhibitor, prolongs survival despite persistent brain tumor growth in mice. J Clin Oncol. (2009). May 20; , 27(15), 2542-52.

[136] Eder, J. P. Vande Woude GF, Boerner SA, LoRusso PM. Novel therapeutic inhibitors of the c-Met signaling pathway in cancer. Clin Cancer Res. (2009). Apr 1; , 15(7), 2207-14.

[137] Kong, D. S, Song, S. Y, Kim, D. H, Joo, K. M, Yoo, J. S, Koh, J. S, et al. Prognostic significance of c-Met expression in glioblastomas. Cancer. (2009). Jan 1; , 115(1), 140-8.

[138] De Bono, J. S. Yap TA. c-MET: an exciting new target for anticancer therapy. Ther Adv Med Oncol. (2011). Nov;3(1 Suppl):S , 3-5.

[139] Tabatabai, G, Weller, M, Nabors, B, Picard, M, Reardon, D, Mikkelsen, T, et al. Targeting integrins in malignant glioma. Target Oncol. (2010). Sep; , 5(3), 175-81.

[140] Loges, S, Butzal, M, Otten, J, Schweizer, M, Fischer, U, Bokemeyer, C, et al. Cilengitide inhibits proliferation and differentiation of human endothelial progenitor cells in vitro. Biochem Biophys Res Commun. (2007). Jun 15; , 357(4), 1016-20.

[141] Maurer, G. D, Tritschler, I, Adams, B, Tabatabai, G, Wick, W, Stupp, R, et al. Cilengitide modulates attachment and viability of human glioma cells, but not sensitivity to irradiation or temozolomide in vitro. Neuro Oncol. (2009). Dec; , 11(6), 747-56.

[142] Kreisl, T. N, Kim, L, Moore, K, Duic, P, Kotliarova, S, Walling, J, et al. A phase I trial of enzastaurin in patients with recurrent gliomas. Clin Cancer Res. (2009). May 15; , 15(10), 3617-23.

[143] Graff, J. R, Mcnulty, A. M, Hanna, K. R, Konicek, B. W, Lynch, R. L, Bailey, S. N, et al. The protein kinase Cbeta-selective inhibitor, Enzastaurin (LY317615.HCl), suppresses signaling through the AKT pathway, induces apoptosis, and suppresses growth of human colon cancer and glioblastoma xenografts. Cancer Res. (2005). Aug 15; , 65(16), 7462-9.

[144] Wick, W, Puduvalli, V. K, & Chamberlain, M. C. van den Bent MJ, Carpentier AF, Cher LM, et al. Phase III study of enzastaurin compared with lomustine in the treatment of recurrent intracranial glioblastoma. J Clin Oncol. (2010). Mar 1; , 28(7), 1168-74.

[145] Pitter, K. L, Galban, C. J, Galban, S, Tehrani, O. S, Li, F, Charles, N, et al. Perifosine and CCI 779 co-operate to induce cell death and decrease proliferation in PTEN-intact and PTEN-deficient PDGF-driven murine glioblastoma. PLoS One. (2011). e14545.

[146] Gills, J. J, Lopiccolo, J, & Dennis, P. A. Nelfinavir, a new anti-cancer drug with pleiotropic effects and many paths to autophagy. Autophagy. (2008). Jan; , 4(1), 107-9.

[147] Pyrko, P, Kardosh, A, Wang, W, Xiong, W, Schonthal, A. H, & Chen, T. C. HIV-1 protease inhibitors nelfinavir and atazanavir induce malignant glioma death by triggering endoplasmic reticulum stress. Cancer Res. (2007). Nov 15; , 67(22), 10920-8.

[148] Jiang, Z, Pore, N, Cerniglia, G. J, Mick, R, Georgescu, M. M, Bernhard, E. J, et al. Phosphatase and tensin homologue deficiency in glioblastoma confers resistance to radiation and temozolomide that is reversed by the protease inhibitor nelfinavir. Cancer Res. (2007). May 1; , 67(9), 4467-73.

[149] Pore, N, Gupta, A. K, Cerniglia, G. J, & Maity, A. HIV protease inhibitors decrease VEGF/HIF-1alpha expression and angiogenesis in glioblastoma cells. Neoplasia. (2006). Nov; , 8(11), 889-95.

[150] Yap, T. A, Garrett, M. D, Walton, M. I, Raynaud, F, De Bono, J. S, & Workman, P. Targeting the PI3K-AKT-mTOR pathway: progress, pitfalls, and promises. Curr Opin Pharmacol. (2008). Aug; , 8(4), 393-412.

[151] Han, L, Yang, Y, Yue, X, Huang, K, Liu, X, Pu, P, et al. Inactivation of PI3K/AKT signaling inhibits glioma cell growth through modulation of beta-catenin-mediated transcription. Brain Res. (2010). Dec 17; , 1366, 9-17.

[152] Chen, L, Han, L, Shi, Z, Zhang, K, Liu, Y, Zheng, Y, et al. LY294002 enhances cytotoxicity of temozolomide in glioma by down-regulation of the PI3K/Akt pathway. Mol Med Report. (2012). Feb; , 5(2), 575-9.

[153] Sunayama, J, Sato, A, Matsuda, K, Tachibana, K, Suzuki, K, Narita, Y, et al. Dual blocking of mTor and PI3K elicits a prodifferentiation effect on glioblastoma stem-like cells. Neuro Oncol. (2010). Dec; , 12(12), 1205-19.

[154] Wei, L. H, Su, H, Hildebrandt, I. J, Phelps, M. E, Czernin, J, & Weber, W. A. Changes in tumor metabolism as readout for Mammalian target of rapamycin kinase inhibition by rapamycin in glioblastoma. Clin Cancer Res. (2008). Jun 1; , 14(11), 3416-26.

[155] Goudar, R. K, Shi, Q, Hjelmeland, M. D, Keir, S. T, Mclendon, R. E, Wikstrand, C. J, et al. Combination therapy of inhibitors of epidermal growth factor receptor/vascular endothelial growth factor receptor 2 (AEE788) and the mammalian target of rapamy-

cin (RAD001) offers improved glioblastoma tumor growth inhibition. Mol Cancer Ther. (2005). Jan; , 4(1), 101-12.

[156] Liu, P, Cheng, H, Roberts, T. M, & Zhao, J. J. Targeting the phosphoinositide 3-kinase pathway in cancer. Nat Rev Drug Discov. (2009). Aug; , 8(8), 627-44.

[157] Chresta, C. M, Davies, B. R, Hickson, I, Harding, T, Cosulich, S, Critchlow, S. E, et al. AZD8055 is a potent, selective, and orally bioavailable ATP-competitive mammalian target of rapamycin kinase inhibitor with in vitro and in vivo antitumor activity. Cancer Res. (2010). Jan 1; , 70(1), 288-98.

[158] Yang, F, Brown, C, Buettner, R, Hedvat, M, Starr, R, Scuto, A, et al. Sorafenib induces growth arrest and apoptosis of human glioblastoma cells through the dephosphorylation of signal transducers and activators of transcription 3. Mol Cancer Ther. (2010). Apr; , 9(4), 953-62.

[159] Bolden, J. E, Peart, M. J, & Johnstone, R. W. Anticancer activities of histone deacetylase inhibitors. Nature reviews Drug discovery. (2006). Sep; , 5(9), 769-84.

[160] Xu, W. S, Parmigiani, R. B, & Marks, P. A. Histone deacetylase inhibitors: molecular mechanisms of action. Oncogene. (2007). Aug 13; , 26(37), 5541-52.

[161] Wagner, J. M, Hackanson, B, Lubbert, M, & Jung, M. Histone deacetylase (HDAC) inhibitors in recent clinical trials for cancer therapy. Clin Epigenetics. (2010). Dec;1(3-4): 117-36.

[162] Weller, M, Gorlia, T, & Cairncross, J. G. van den Bent MJ, Mason W, Belanger K, et al. Prolonged survival with valproic acid use in the EORTC/NCIC temozolomide trial for glioblastoma. Neurology. (2011). Sep 20; , 77(12), 1156-64.

[163] Dokmanovic, M, Clarke, C, & Marks, P. A. Histone deacetylase inhibitors: overview and perspectives. Mol Cancer Res. (2007). Oct; , 5(10), 981-9.

[164] Ryu, C. H, Yoon, W. S, Park, K. Y, Kim, S. M, Lim, J. Y, Woo, J. S, et al. Valproic Acid Downregulates the Expression of MGMT and Sensitizes Temozolomide-Resistant Glioma Cells. J Biomed Biotechnol. (2012).

[165] Van Nifterik, K. A. Van den Berg J, Slotman BJ, Lafleur MV, Sminia P, Stalpers LJ. Valproic acid sensitizes human glioma cells for temozolomide and gamma-radiation. J Neurooncol. (2012). Mar; , 107(1), 61-7.

[166] Chen, C. H, Chang, Y. J, Ku, M. S, Chung, K. T, & Yang, J. T. Enhancement of temozolomide-induced apoptosis by valproic acid in human glioma cell lines through redox regulation. J Mol Med (Berl). (2011). Mar; , 89(3), 303-15.

[167] Krishnan, S, Brown, P. D, Ballman, K. V, Fiveash, J. B, Uhm, J. H, Giannini, C, et al. Phase I trial of erlotinib with radiation therapy in patients with glioblastoma multiforme: results of North Central Cancer Treatment Group protocol N0177. Int J Radiat Oncol Biol Phys. (2006). Jul 15; , 65(4), 1192-9.

[168] De Groot, J. F, Gilbert, M. R, Aldape, K, Hess, K. R, Hanna, T. A, Ictech, S, et al. Phase II study of carboplatin and erlotinib (Tarceva, OSI-774) in patients with recurrent glioblastoma. J Neurooncol. (2008). Oct; , 90(1), 89-97.

[169] Prados, M. D, Chang, S. M, Butowski, N, Deboer, R, Parvataneni, R, Carliner, H, et al. Phase II study of erlotinib plus temozolomide during and after radiation therapy in patients with newly diagnosed glioblastoma multiforme or gliosarcoma. J Clin Oncol. (2009). Feb 1; , 27(4), 579-84.

[170] Raizer, J. J, Abrey, L. E, Lassman, A. B, Chang, S. M, Lamborn, K. R, Kuhn, J. G, et al. A phase II trial of erlotinib in patients with recurrent malignant gliomas and nonprogressive glioblastoma multiforme postradiation therapy. Neuro Oncol. (2010). Jan; , 12(1), 95-103.

[171] Raizer, J. J, Abrey, L. E, Lassman, A. B, Chang, S. M, Lamborn, K. R, Kuhn, J. G, et al. A phase I trial of erlotinib in patients with nonprogressive glioblastoma multiforme postradiation therapy, and recurrent malignant gliomas and meningiomas. Neuro Oncol. (2010). Jan; , 12(1), 87-94.

[172] Reardon, D. A, Desjardins, A, Vredenburgh, J. J, Gururangan, S, Friedman, A. H, Herndon, J. E, et al. Phase 2 trial of erlotinib plus sirolimus in adults with recurrent glioblastoma. J Neurooncol. (2010). Jan; , 96(2), 219-30.

[173] Sathornsumetee, S, Desjardins, A, Vredenburgh, J. J, Mclendon, R. E, Marcello, J, Herndon, J. E, et al. Phase II trial of bevacizumab and erlotinib in patients with recurrent malignant glioma. Neuro Oncol. (2010). Dec; , 12(12), 1300-10.

[174] van den Bent MJBrandes AA, Rampling R, Kouwenhoven MC, Kros JM, Carpentier AF, 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). Mar 10; , 27(8), 1268-74.

[175] Brown, P. D, Krishnan, S, Sarkaria, J. N, Wu, W, Jaeckle, K. A, Uhm, J. H, et al. Phase I/II trial of erlotinib and temozolomide with radiation therapy in the treatment of newly diagnosed glioblastoma multiforme: North Central Cancer Treatment Group Study N0177. J Clin Oncol. (2008). Dec 1; , 26(34), 5603-9.

[176] Hegi, M. E, Diserens, A. C, Bady, P, Kamoshima, Y, Kouwenhoven, M. C, Delorenzi, M, et al. Pathway analysis of glioblastoma tissue after preoperative treatment with the EGFR tyrosine kinase inhibitor gefitinib--a phase II trial. Mol Cancer Ther. (2011). Jun; , 10(6), 1102-12.

[177] Rich, J. N, Reardon, D. A, Peery, T, Dowell, J. M, Quinn, J. A, Penne, K. L, et al. Phase II trial of gefitinib in recurrent glioblastoma. J Clin Oncol. (2004). Jan 1; , 22(1), 133-42.

[178] Uhm, J. H, Ballman, K. V, Wu, W, Giannini, C, Krauss, J. C, Buckner, J. C, et al. Phase II evaluation of gefitinib in patients with newly diagnosed Grade 4 astrocytoma:

Mayo/North Central Cancer Treatment Group Study N0074. Int J Radiat Oncol Biol Phys. (2011). Jun 1; , 80(2), 347-53.

[179] Razis, E, Selviaridis, P, Labropoulos, S, Norris, J. L, Zhu, M. J, Song, D. D, et al. Phase II study of neoadjuvant imatinib in glioblastoma: evaluation of clinical and molecular effects of the treatment. Clin Cancer Res. (2009). Oct 1; , 15(19), 6258-66.

[180] Dresemann, G, Weller, M, Rosenthal, M. A, Wedding, U, Wagner, W, Engel, E, et al. Imatinib in combination with hydroxyurea versus hydroxyurea alone as oral therapy in patients with progressive pretreated glioblastoma resistant to standard dose temozolomide. J Neurooncol. (2010). Feb; , 96(3), 393-402.

[181] Batchelor, T. T, & Duda, D. G. di Tomaso E, Ancukiewicz M, Plotkin SR, Gerstner E, et al. Phase II study of cediranib, an oral pan-vascular endothelial growth factor receptor tyrosine kinase inhibitor, in patients with recurrent glioblastoma. J Clin Oncol. (2010). Jun 10; , 28(17), 2817-23.

[182] Sorensen, A. G, Batchelor, T. T, Zhang, W. T, Chen, P. J, Yeo, P, Wang, M, et al. A "vascular normalization index" as potential mechanistic biomarker to predict survival after a single dose of cediranib in recurrent glioblastoma patients. Cancer Res. (2009). Jul 1; , 69(13), 5296-300.

[183] Wen, P. Y, Schiff, D, Cloughesy, T. F, Raizer, J. J, Laterra, J, Smitt, M, et al. A phase II study evaluating the efficacy and safety of AMG 102 (rilotumumab) in patients with recurrent glioblastoma. Neuro Oncol. (2011). Apr; , 13(4), 437-46.

[184] Butowski, N, Chang, S. M, Lamborn, K. R, Polley, M. Y, Pieper, R, Costello, J. F, et al. Phase II and pharmacogenomics study of enzastaurin plus temozolomide during and following radiation therapy in patients with newly diagnosed glioblastoma multiforme and gliosarcoma. Neuro Oncol. (2011). Dec; , 13(12), 1331-8.

[185] Kreisl, T. N, Kotliarova, S, Butman, J. A, Albert, P. S, Kim, L, Musib, L, et al. A phase I/II trial of enzastaurin in patients with recurrent high-grade gliomas. Neuro Oncol. (2010). Feb; , 12(2), 181-9.

[186] Hainsworth, J. D, Shih, K. C, Shepard, G. C, Tillinghast, G. W, Brinker, B. T, & Spigel, D. R. Phase II Study of Concurrent Radiation Therapy, Temozolomide, and Bevacizumab Followed by Bevacizumab/Everolimus as First-Line Treatment for Patients With Glioblastoma. Clin Adv Hematol Oncol. (2012). Apr; , 10(4), 240-6.

[187] Mason, W. P, Macneil, M, Kavan, P, Easaw, J, Macdonald, D, Thiessen, B, et al. A phase I study of temozolomide and everolimus (RAD001) in patients with newly diagnosed and progressive glioblastoma either receiving or not receiving enzyme-inducing anticonvulsants: an NCIC CTG study. Invest New Drugs. (2011). Dec 9.

[188] Sarkaria, J. N, Galanis, E, Wu, W, Peller, P. J, Giannini, C, Brown, P. D, et al. North Central Cancer Treatment Group Phase I trial N057K of everolimus (RAD001) and temozolomide in combination with radiation therapy in patients with newly diag-

nosed glioblastoma multiforme. Int J Radiat Oncol Biol Phys. (2011). Oct 1; , 81(2), 468-75.

[189] Galanis, E, Buckner, J. C, Maurer, M. J, Kreisberg, J. I, Ballman, K, Boni, J, et al. Phase II trial of temsirolimus (CCI-779) in recurrent glioblastoma multiforme: a North Central Cancer Treatment Group Study. J Clin Oncol. (2005). Aug 10; , 23(23), 5294-304.

[190] Sarkaria, J. N, Galanis, E, Wu, W, Dietz, A. B, Kaufmann, T. J, Gustafson, M. P, et al. Combination of temsirolimus (CCI-779) with chemoradiation in newly diagnosed glioblastoma multiforme (GBM) (NCCTG trial N027D) is associated with increased infectious risks. Clin Cancer Res. (2010). Nov 15; , 16(22), 5573-80.

[191] Cloughesy, T. F, Yoshimoto, K, Nghiemphu, P, Brown, K, Dang, J, Zhu, S, et al. Antitumor activity of rapamycin in a Phase I trial for patients with recurrent PTEN-deficient glioblastoma. PLoS Med. (2008). Jan 22;5(1):e8.

[192] Lustig, R, Mikkelsen, T, Lesser, G, Grossman, S, Ye, X, Desideri, S, et al. Phase II preradiation R115777 (tipifarnib) in newly diagnosed GBM with residual enhancing disease. Neuro Oncol. (2008). Dec; , 10(6), 1004-9.

[193] Nghiemphu, P. L, Wen, P. Y, Lamborn, K. R, Drappatz, J, Robins, H. I, Fink, K, et al. A phase I trial of tipifarnib with radiation therapy, with and without temozolomide, for patients with newly diagnosed glioblastoma. Int J Radiat Oncol Biol Phys. (2011). Dec 1; , 81(5), 1422-7.

[194] Cloughesy, T. F, Wen, P. Y, Robins, H. I, Chang, S. M, Groves, M. D, Fink, K. L, et al. Phase II trial of tipifarnib in patients with recurrent malignant glioma either receiving or not receiving enzyme-inducing antiepileptic drugs: a North American Brain Tumor Consortium Study. J Clin Oncol. (2006). Aug 1; , 24(22), 3651-6.

[195] Moyal, E. C, Laprie, A, Delannes, M, Poublanc, M, Catalaa, I, Dalenc, F, et al. Phase I trial of tipifarnib (R115777) concurrent with radiotherapy in patients with glioblastoma multiforme. Int J Radiat Oncol Biol Phys. (2007). Aug 1; , 68(5), 1396-401.

[196] Hainsworth, J. D, Ervin, T, Friedman, E, Priego, V, Murphy, P. B, Clark, B. L, et al. Concurrent radiotherapy and temozolomide followed by temozolomide and sorafenib in the first-line treatment of patients with glioblastoma multiforme. Cancer. (2010). Aug 1; , 116(15), 3663-9.

[197] Reardon, D. A, Vredenburgh, J. J, Desjardins, A, Peters, K, Gururangan, S, Sampson, J. H, et al. Effect of CYP3A-inducing anti-epileptics on sorafenib exposure: results of a phase II study of sorafenib plus daily temozolomide in adults with recurrent glioblastoma. J Neurooncol. (2011). Jan; , 101(1), 57-66.

[198] Nabors, L. B, Mikkelsen, T, Rosenfeld, S. S, Hochberg, F, Akella, N. S, Fisher, J. D, et al. Phase I and correlative biology study of cilengitide in patients with recurrent malignant glioma. J Clin Oncol. (2007). May 1; , 25(13), 1651-7.

[199] Reardon, D. A, Fink, K. L, Mikkelsen, T, Cloughesy, T. F, Neill, O, & Plotkin, A. S, et al. Randomized phase II study of cilengitide, an integrin-targeting arginine-glycine-

aspartic acid peptide, in recurrent glioblastoma multiforme. J Clin Oncol. (2008). Dec 1; , 26(34), 5610-7.

[200] Stupp, R, Hegi, M. E, Neyns, B, Goldbrunner, R, Schlegel, U, Clement, P. M, et al. Phase I/IIa study of cilengitide and temozolomide with concomitant radiotherapy followed by cilengitide and temozolomide maintenance therapy in patients with newly diagnosed glioblastoma. J Clin Oncol. (2010). Jun 1; , 28(16), 2712-8.

[201] Friday, B. B, Anderson, S. K, Buckner, J, Yu, C, Giannini, C, Geoffroy, F, et al. Phase II trial of vorinostat in combination with bortezomib in recurrent glioblastoma: a north central cancer treatment group study. Neuro Oncol. (2012). Feb; , 14(2), 215-21.

[202] Galanis, E, Jaeckle, K. A, Maurer, M. J, Reid, J. M, Ames, M. M, Hardwick, J. S, et al. Phase II trial of vorinostat in recurrent glioblastoma multiforme: a north central cancer treatment group study. J Clin Oncol. (2009). Apr 20; , 27(12), 2052-8.

[203] Iwamoto, F. M, Lamborn, K. R, Kuhn, J. G, Wen, P. Y, Yung, W. K, Gilbert, M. R, et al. A phase I/II trial of the histone deacetylase inhibitor romidepsin for adults with recurrent malignant glioma: North American Brain Tumor Consortium Study 03-03. Neuro Oncol. (2011). May; , 13(5), 509-16.

[204] Eramo, A, Ricci-vitiani, L, Zeuner, A, Pallini, R, Lotti, F, Sette, G, et al. Chemotherapy resistance of glioblastoma stem cells. Cell Death Differ. (2006). Jul; , 13(7), 1238-41.

[205] Verma, I. M, & Weitzman, M. D. Gene therapy: twenty-first century medicine. Annu Rev Biochem. (2005). , 74, 711-38.

[206] Kroeger, K. M, Muhammad, A. K, Baker, G. J, Assi, H, Wibowo, M. K, Xiong, W, et al. Gene therapy and virotherapy: novel therapeutic approaches for brain tumors. Discov Med. (2010). Oct; , 10(53), 293-304.

[207] Castro, M. G, Candolfi, M, Kroeger, K, King, G. D, Curtin, J. F, Yagiz, K, et al. Gene therapy and targeted toxins for glioma. Curr Gene Ther. (2011). Jun; , 11(3), 155-80.

[208] Lesniak, M. S. Gene therapy for malignant glioma. Expert Rev Neurother. (2006). Apr; , 6(4), 479-88.

[209] Colombo, F, Barzon, L, Franchin, E, Pacenti, M, Pinna, V, Danieli, D, et al. Combined HSV-TK/IL-2 gene therapy in patients with recurrent glioblastoma multiforme: biological and clinical results. Cancer Gene Ther. (2005). Oct; , 12(10), 835-48.

[210] Vanneman, M, & Dranoff, G. Combining immunotherapy and targeted therapies in cancer treatment. Nat Rev Cancer. (2012). Apr; , 12(4), 237-51.

[211] Yong, R. L, & Lonser, R. R. Immunotherapy trials for glioblastoma multiforme: promise and pitfalls. World Neurosurg. (2012). May;77(5-6):636-8.

[212] Liau, L. M, Prins, R. M, Kiertscher, S. M, Odesa, S. K, Kremen, T. J, Giovannone, A. J, et al. Dendritic cell vaccination in glioblastoma patients induces systemic and intra-

cranial T-cell responses modulated by the local central nervous system tumor microenvironment. Clin Cancer Res. (2005). Aug 1; , 11(15), 5515-25.

[213] Yamanaka, R, Homma, J, Yajima, N, Tsuchiya, N, Sano, M, Kobayashi, T, et al. Clinical evaluation of dendritic cell vaccination for patients with recurrent glioma: results of a clinical phase I/II trial. Clin Cancer Res. (2005). Jun 1; , 11(11), 4160-7.

[214] Jackson, C, Ruzevick, J, Phallen, J, Belcaid, Z, & Lim, M. Challenges in immunotherapy presented by the glioblastoma multiforme microenvironment. Clin Dev Immunol. 2011;(2011).

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