Review

New Molecularly Targeted Therapies for Glioblastoma Multiforme

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Abstract. *Glioblastoma multiforme (GBM) is the most malignant brain tumor in adults, exhibiting high mortality. Standard therapy (surgery, radiotherapy and chemotherapy with temozolomide) has only limited effectiveness. The progress in genomics regarding GBM, in the detection of new markers of oncogenesis, abnormalities in signalling pathways, tumor microenvironment, and pathological angiogenesis over the past decade are briefly discussed. The role of novel prognostic in this review biomarkers [isocitrate dehydrogenases 1 and 2, CpG island methylator phenotype, promoter methylation status of the MGMT (O-6-methylguanine-methyltransferase) gene] is also discussed. New targeted therapeutic approaches are classified into several functional subgroups, such as inhibitors of growth factors and their receptors, inhibitors of proteins of intracellular signaling pathways, epigenetic gene-expressing mechanisms, inhibitors of tumor angiogenesis, tumor imunotherapy and vaccines. Finally novel possibilities for GBM treatment are summarized in this review.*

Glioblastoma multiforme (GBM) is the most common and most malignant primary brain tumor in adults, with an incidence of 3-4/100,000/year (1). GBM is extremely invasive and difficult to treat surgically, characterized by intense and aberrant vascularization and high resistance to radiotherapy (RT) and chemotherapy. The current standard of care for patients with newly diagnosed GBM is neurosurgery, followed by fractionated external beam RT and chemotherapy with systemic temozolomide (2). The median survival of patients with GBM is 12.1-14.6 months (3) and only 3-5% of patients survive longer than 3 years (4). The progress in genomics of GBM over the past 10 years, has revealed several abnormalities in signaling pathways and a diversity of mutated genes. The importance of the microenvironment in GBM, especially of tumor angiogenesis and the role of tumor biomarkers have also been studied. The use of this new knowledge regarding the diversity of GBM on molecular and genetic levels could lead to individual patient tumor analysis and treatment management. This review focuses on novel therapeutic approaches to GBM, facilitated by these findings.

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Pathology of Malignant Glioma

The application of pathology, as well as genetics and molecular biology, is required in order for one to understand the complexity of gliomas. These tumors represent primary brain malignancies originating from glia, the brain tissue which provides supportive functions to neural cells (nutrients, oxygen, mechanical support, guidance in development and immune functions) but also acts in very complex processes (signal transduction and neurotransmission). GBM is the most common form of high-grade glial tumor, which is defined by specific histopathological criteria namely hyper-cellularity, necrosis, pleomorphism, vascular proliferation and pseudopallisading (5). GBMs can be categorized into two subgroups, as primary and secondary. Primary GBMs are diagnosed as advanced cancer,

whereas secondary cases have clinical, radiological or histopathological evidence of progression from a pre-existing lower-grade tumor (6). There are some clinical differences between the two groups. Secondary GBMs occur less frequently (5% of GBMs) and among younger patients (with a median age of 45 years). Histopathological differentiation between primary and secondary GBMs is not possible (7). However there are distinctions between primary and secondary tumors at the genetic level (8), but none of the alterations is specific enough to distinguish between these two subgroups.

Genetics of Malignant Glioma

The origin of cancer is presently understood as the accumulation of hereditary or somatic alterations in genes that control critical biological processes, such as regulation of apoptosis, cell cycle progression and proliferation. The changes could be manifested by the activation of oncogenes, and by the silencing of tumor suppressor genes, which leads to the different gene expression profile of cancer cells. However it is not only genetic alterations that are immediately essential for malignant transformation. Epigenetic mechanisms of modification of gene expression, such as DNA methylation status, imprinting, chromatin changes, and the role of micro-RNAs, are also being frantically discussed.

Comprehensive analysis of genetic and epigenetic alterations in high grade glioma in comparison to normal brain tissue is now absolutely essential. This molecular and genomic approach could provide novel targets for diagnostic, prognostic or therapeutic purposes. It could also be helpful in the identification of subgroups of patients who have better prognosis on standard therapy or preferentially respond to certain single or combined novel targeted therapies.

Some of the first genetic studies of malignant glioma described the presence of an extra copy of chromosome 7 and an amplification of the receptor of epidermal growth factor (*EGFR*) gene was identified (9). Further karyotypic and loss of heterozygosity studies identified the positions of tumor suppressor genes on chromosomes 9, 10 and 17 (10). The main gene which was altered on chromosome 17 in GBM, was identified as tumor suppressor TP53, which has a critical role in the inspection of the genome for DNA damage and can arrest the cell cycle and trigger apoptosis (11). Owing to further progress in genetics, the loss of tumor suppressors from chromosomes 19 (p16 cell-cycle inhibitor) and chromosome 10 (phosphatase and tensin homolog, *PTEN*) were described in 1993 and 1997, respectively. The role of p16 is to arrest cell-cycle progression, whereas PTEN is a negative regulator of the phosphoinositide 3-kinase (PI3K) pathway (12).

The unprecedented progress of recent years in all 'omics' disciplines (such as genomics, transcriptomics, proteomics and others), together with improvements in bioinformatics technologies, has provided new opportunities in current brain cancer research. The human genome was fully sequenced and the improvements of sequencing methods have lately permitted genome-wide association studies of human cancer, including those of high-grade glioma. One of the most important genome-wide analyses of 20,661 protein coding genes in GBM tumors was completed in 2008. This study examined 22 genome samples from GBM and probably identified the most important alterations at the genetic level that drive glioblastoma formation (13). Most of the common alterations in DNA were identified, such as point mutations, small insertions and deletions, as well as larger copy number changes, genomic amplifications and deletions.

The alterations of several important pathways which are involved in GBM development and growth were uncovered. Among the most important ones are i) RAS and PI3K-AKT oncogenic pathway with alterations in EGFR/PI3K/PTEN/NF1/RAS; ii) the p53 pathway with changes in TP53/MDM2/MDM4/$p14^{ARF}$ changes; iii) cell-cycle regulatory pathway, with alterations in RB1/CDK4/ $p16^{INK4A}$/CDKN2B, and iv) the newly discovered alterations in metabolic pathways including isocitrate dehydrogenases IDH1/IDH2. The alterations in IDH1/IDH2 could also serve as independent prognostic factor, which will be discussed later (13, 14).

New Classification of Human Glioblastoma

Another exciting work in this area is being conducted by The Cancer Genome Atlas (TCGA), which is sponsored by the National Institutes of Health (NIH) of the USA. This consortium studies the nature of cancer through the integration of genetic data with the gene expression profiles. The TCGA consortium is carrying out research in more than 20 types of human cancer, including GBM. A total of 500 specimens of primary untreated GBM are being utilized for the DNA (gene copy number, gene sequencing, epigenetic methylation), mRNA (gene expression profile) and microRNA (regulation of expression) assessment (15).

The current findings from this activity have uncovered some novel genetic alterations, together with the possibility that GBM can be divided into different subtypes (16). By this approach, GBMs still remain one pathological unit but are subdivided by their genetic alterations and gene expression profiles. This new division also has some clinical relevance. The novel four subgroups of GBM are called Classical, Mesenchymal, Proneural and Neural, especially because of the differently elevated expression of some 'signature' genes across the subgroups (16). This novel molecular classification of GBMs could be highly useful in the future for finding important molecular targets within each group, suitable for therapeutic intervention, as well as for the selection of the best targeted therapy for each patient.

Novel Prognostic Biomarkers for Malignant Glioma

Not only a new classification for GBMs but also novel prognostic biomarkers, have emerged in the recent years. Three of the most important markers of GBMs in relation to the prediction of clinical outcome are discussed here. These are the IDH mutations, the CpG island methylator phenotype (CIMP) and the promoter methylation status of the *MGMT* gene.

Isocitrate dehydrogenases (IDH1 and IDH2) serve as the enzymes that convert isocitrate into alpha-ketoglutarate and reduce nicotinamide adenine dinucleotide phosphate (NADP) to the reduced form NADPH. The genes for IDH1 and IDH2 were found to carry specific mutations in a significant portion of lower grade gliomas and a subset of glioblastomas (mainly the proneural type of GBM) (13, 17). The mutation is very distinctive, namely a single amino acid change – R132H, in the IDH1 active site which leads to the loss of regular enzyme function. The mutations in *IDH1* and *IDH2* are present in about 70%-80% of low-grade gliomas, in 50% of anaplastic gliomas and in approximately 5% of glioblastomas (18). The aberrant function of mutated IDH1 is the conversion of alpha-ketoglutarate to 2-hydroxyglutarate. (17). The latter is an inhibitor of alpha-ketoglutarate-dependent dioxygenases, which leads to genome-wide epigenetic changes in human glioma (19). The genome-wide changes associated with the mutated IDH1 predict a better prognosis and can be used for another subclassification of human GBMs. It would appear that there is a sequential pattern of epigenetic changes (CIMP, MGMT) regarding the *IDH1* alterations. The mutation of *IDH1* is the first step, followed by the production of 2-hydroxyglutarate, which leads to the CIMP profile, along with proneural gene expression changes (19).

A study of three different molecular alterations in low-grade gliomas (*IDH1*/*IDH2* point mutations, P53 expression and 1p/19q deletion status), demonstrated that only the *IDH1* mutation was an independent prognostic marker of favorable prognosis (20). In the next study, glioblastoma tissues were analyzed for prognostic markers, such as CIMP (6 CIMP markers) and *IDH1* mutations. The data came from the M.D. Anderson Cancer Center and were evaluated in the RTOG 0525 study with more than 800 newly diagnosed GBM patients. Based on multivariate analyses, both the *IDH1* mutations and the CIMP status were determined as being independent prognostic factors. The patients were subdivided into three prognostic groups according to the number of positive CIMP markers. The first group, with 0-1 CIMP (regarded as being CIMP-negative), had a median survival of 13.8 months; the second group with 2-4 CIMPs (CIMP-intermediate) had a median survival of 20.1 months, and the third group with more than 5 CIMPs (CIMP-positive) had a median survival of 90.6 months (21, 22). Naturally, there are many more studies that address the impact of *IDH* mutations in progression-free survival (PFS) and overall survival (OS) of patients with glioma (23, 24).

The current standard of care for GBM includes surgery, RT and the use of the chemotherapeutic agent temozolomide, which is the oral alkylating agent that causes DNA damage by alkylation of the 0-6 position of guanine and the production of DNA interstrand cross-links. In a large, randomized, phase III trial in newly diagnosed patients with GBM, the therapeutic interventions were divided into two subgroups: RT alone *vs*. RT and concurrent daily temozolomide followed by adjuvant temozolomide. The subgroup of patients treated with RT plus temozolomide had a median survival benefit of 2.5 months and the proportion of 2-year survivors increased from 10.4% to 26.5% (25). There is a proportion of patients who have a better response to temozolomide, but the majority of patients become rapidly resistant to this chemotherapeutic agent. One of the strongest predictive biomarkers for the chemotherapy response is the alteration in the *MGMT* gene. The enzyme *O*-6-methylguanine-methyltransferase (the product of MGMT) is able to repair the DNA damage caused by temozolomide. The presence of *MGMT* leads to reduction in the effect of temozolomide chemotherapy. The silencing of the *MGMT* gene can be caused by epigenetic mechanisms, the DNA hypermethylation of CpG islands in the promoter region of the *MGMT* gene. This alteration leads to a decrease in the transcription level of the MGMT gene and in the amount of gene product. Methylation of the *MGMT* promoter was observed in 47.7% patients with GBM (more in the subgroup with secondary GBM) (26). The subset analyses of the large, randomized, phase III trial mentioned above (25) showed that the patients with hypermethylated *MGMT* promoter had a significantly better median survival after therapy with temozolomide compared with those that did not (21.7 *vs*. 15.3 months) (25, 27). In another study, *MGMT* promoter hypermethylation was the predictive biomarker for a better response to RT independently of treatment with temozolomide. Therefore, the *MGMT* methylation status could be potentially considered as a general biomarker of better therapeutic response in GBM (28). The strong correlation between *MGMT* methylation and the CIMP profile was also observed in one study. This finding could signify that *MGMT* hypermethylation is the epiphenomenon of the genome-wide methylation status associated with the CIMP (29).

Other non-genetic prognostic biomarkers for GBM have also been reported. One study, which examined the prognostic significance of individual angiogenic factors, collected the serum samples from 36 patients with GBM and simultaneously assayed them for 48 angiogenic factors using protein microarrays. Two different subtypes of GBMs were revealed by cluster analysis and a low serum level of tissue inhibitor of metalloproteinase-1(TIMP1) was established as an independent predictor of better survival (30). Another article discussed the predictive value of serum α2-Heremans-

Schmid glycoprotein (AHSG) in patients with glioblastoma. The median survival was longer (51 *vs*. 29 weeks) in patients with normal (more than 285 mg/l) *vs*. low serum AHSG concentrations. This finding was independent of age and Karnofsky score, and the serum AHSG level inversely correlated with the Ki-67 proliferative index (31). The study of serum concentrations of extracellular matrix glycoprotein (YKL-40) and matrix metalloproteinase-9 (MMP-9) concluded that these two biomarkers could be monitored in the serum of patients with GBM and help confirm the absence of active disease. YKL-40 was also used as a predictive biomarker of overall survival in patients with high-grade glioma (32). On the other hand, a more recent study failed to prove any clinical relevance of serum MMP-9 as a biomarker of disease status or overall survival in a large group of patients with glioma (33).

Some of the previously mentioned biomarkers, together with the novel stratifications of a molecular and genetic level, could be potentially useful in the near future for treatment strategies for patients with GBM and other types of high-grade glioma. Improved insight into the therapeutic responses, as well as the biology of these tumors, are urgently needed for more effective therapeutic management of patients with high-grade gliomas.

Novel Targeted Therapies for Malignant Glioma

The standard therapeutic options for the treatment of GBM and other types of high-grade glioma have only limited benefits, as discussed earlier. The new targeted therapies which have recently emerged are directed against certain tumoral features, such as altered signaling and metabolic pathways, aberrant tumor vessels, angiogenesis and the tumor microenvironment. Recent genome-wide studies and the molecular characterization of GBM has enabled the identification of potential new targets, development of novel therapeutic small molecules and monoclonal antibodies and initiation of clinical trials with these targeted drugs. However, there is a wide molecular diversity and heterogeneity associated with the aberrant GBM signaling pathways. This could be the reason for the relative lack of success of these new approaches in the treatment of GBM. Only a small clinical benefit has been demonstrated with the novel therapeutics so far. Overcoming these barriers will require the use of individualized molecular profiling of each GBM tumor and application of personalized medicine in combinatorial targeted therapies for high-grade gliomas.

The most important molecular and genetic alterations in GBM can cause increased tumor invasiveness, cell survival, proliferation, evasion of apoptosis, angiogenesis and immune response weakening. Novel therapeutic approaches targeting such changes in high-grade glioma can be classified into several functional subgroups (34, 35).

Growth factor receptors and their inhibitors in GBM. The first subgroup, inhibitors of growth factors and their receptors, includes the therapeutics directed to the aberrant growth factor pathways presented in GBM including EGFR, platelet-derived growth factor receptor (PDGFR), insulin-like growth factor (IGF), fibroblast growth factor (FGF). These receptors and their ligands are overexpressed or mutated in high proportion of GBM (13, 14).

The amplification, as well as the overexpression, of the EGFR family are described in approximately 50% of GBM cases (14, 36). More than 40% of the tumors carry the unique deletion mutant called *EGFR*vIII. This *EGFR* gene has the deletion of exons 2-7, which causes constitutive ligand-independent constitutive receptor activation (14, 37). *EGFR*vIII could be an ideal tumor-specific target for novel therapeutics and will be discussed later. One of the new drugs directed against EGFR function is gefitinib (Iressa; AstraZeneca). In a phase II study of gefitinib, patients with GBM had partial tumor regression in 12.7% of cases (38). The PFS at 6 months was 13% and the median OS was 10 months in another study of recurrent GBM (39). There are more recent studies with gefitinib in GBM, with results of minimal efficicacy compared to standard RT/temozolomide treatment (40, 41). Another new EGFR inhibitor also examined as a possible treatment for GBM is erlotinib (Tarceva; Genentech). Some phase II trials of erlotinib as a single agent showed only minimal benefit for glioblastoma treatment and modest survival benefit in combination with temozolomide and RT, or with other agents (42, 47). A better therapeutic response to these agents was achieved by stratifying patients based on their own molecular profile (48). Another promising EGFR inhibitor is lapatinib (Tyverb; GlaxoSmithKline). According to a phase II study, lapatinib is distributed into the tumor tissue (49). However, in a subsequent trial with a small number of patients with recurrent GBM, no efficacy was observed (50). Cetuximab (Erbitux; ImClone Systems) is a chimeric monoclonal antibody which can inhibit EGFR. A small group of patients responded to this agent in a phase II study (51). In another phase II study, the patients with recurrent high-grade glioma were stratified according to amplification of the *EGFR* gene. Cetuximab had limited activity and a median overall survival of 5 months (52). Little improvement was observed in the phase II study with the combination of cetuximab, irinotecan (Camptosar; Pfizer) and bevacizumab (53).

The PDGF receptor is often overexpressed and activated in GBM, especially in the proneural subtype (14, 16). The changes leading to aberrant activation of PDGFR assist in the transition from grade II-III glioma to glioblastoma. The PDGF ligand is able to stimulate GBM growth and angiogenesis (54, 55). The kinase inhibitor of PDGRF, c-KIT and oncogene fusion protein BCR-ABL imatinib (Gleevec; Novartis Pharmaceuticals) has been extensively examined in the GBM setting. The modest response of PFS at 6 months of 15.7%

was observed in one phase II trial of patients with recurrent disease (56). A better result in PFS at 6 months of 32% was recorded in a study with stratification of patients by their PDGFR expression 57). There are more studies with imatinib in combination with hydroxyurea. After an initial promising phase II trial, further multicenter studies did not confirm the preliminary results, and other trials with combinatorial therapy are ongoing (58, 59, 60). In the most recent study, imatinib had limited activity in patients with recurrent oligodendroglioma and mixed oligoastrocytoma, with median survival of 16.6 months, but with a moderate toxicity profile (61). Another PDGFR inhibitor, tandutinib (MLN 518; Millennium Pharmaceuticals), is in phase II trials as a single agent, or in combination with bevacizumab. Among multikinase inhibitors with the potential to block PDGFR, are sunitinib (Sutent; Pfizer), sorafenib (Nexavar; Bayer and Onyx Pharmaceuticals), vandetanib (Caprelsa; AstraZeneca) and others, most of which are used in the trials as antiangiogenic drugs for GBM (62, 63).

Inhibitors of intracellular signaling pathways. Intracellular components in signaling pathways mediate the response of cells to the growth factors and their interactions with cell surface receptors. Inhibition of such aberrant signaling components is a promising targeted therapeutic approach for the treatment of many types of cancer including high-grade glioma.

Mutations of RAS protein in GBM are rare (13, 14). On the other hand, the inhibition of RAS could be effective because of its involvement in the deregulated signaling pathways through growth factor receptors. The RAS protein must be post-translationaly modified by farnesyltransferase before translocation to the cell membrane. The inhibitors of this process have also been tested in GBM. Tipifarnib (Zanestra; Johnson and Johnson) had modest activity in patients with recurrent glioblastoma, with a PFS at 6 months of 12% in a phase II trial (64). Another inhibitor of farnesyltransferase is lonafarnib (SCH66336; Schering-Plough), which was examined in a phase I study (65).

Activation of protein kinase C (PKC) contributes to the signal propagation from several growth factors, such as EGF and PDGF, which stimulate glioma cell proliferation. The targeting of PKC with the well-known anti-estrogen drug tamoxifen was examined, but with only little or no clear benefit in clinical trials for GBM (66, 67). A novel specific PKC inhibitor is enzastaurin (LY317615; Eli Lilly and Company). Its effect on recurrent malignant glioma was reported, with 22% of patients achieving radiographic response and 5% achieving stable disease (68). More recent studies of enzastaurin showed some limited efficacy in recurrent GBM (69, 70).

Another protein, (mTOR), is involved in cell growth signaling. It transduces the signals from PI3/AKT, as well as, the RAS pathway. Overexpression of growth factors or deletion of *PTEN* increases the mTOR activation in GBM (14, 36). There are some selective mTOR inhibitors that have been examined in GBM settings. The small molecule sirolimus (Rapamune; Wyeth) was not effective as a single agent. It had limited efficacy in a phase II trial with erlotinib (47, 71). Temsirolimus (Toricel; Wyeth) had some efficacy as a single agent for recurrent GBM. There are now some ongoing trials using it in combination with EGFR/PI3K pathway inhibitors or bevacizumab (72). The derivative of sirolimus, mTOR inhibitor everolimus (Zortress; Novartis) had no clear clinical benefit in combination with gefitinib for recurrent GBM (41).

Other intracellular molecular targets for GBM therapy. One of the recently defined molecular targets now being examined in the treatment of various types of cancer is the family of proteins called polyADP ribose polymerases (PARPs). The PARP protein family acts in DNA repair. Its main role is the detection and signaling of single-strand DNA breaks. PARP inhibitors have been widely examined in clinical trials for therapy of tumors with specific genetic deficits in DNA repair pathways such as *BRCA1* and *BRCA2* (73). In the case of GBM, two new drugs are being examined for the treatment of initial as well as recurrent disease: the specific PARP inhibitors iniparib (BSI 201; Sanofi-Aventis) and veliparib (ABT 888; Abbott).

The mechanisms of epigenetic modifications of genes and their aberrant functions are also very important in cell transformation in the case of malignant glioma. Histone acetylation (by histone acetyltransferases) and deacetylation (by histone deacetylases, HDACs) play fundamental roles in the regulation of gene expression. There are some HDAC inhibitors that were examined for GBM, such as phenylbutyrate, valproic acid, depsipeptide (FK228) and vorinostat (Zolinza; Merck) (74). A recent phase II study of vorinostat as a monotherapy for recurrent GBM showed modest activity with a median OS of 5.7 months (75).

The proteasome complex inhibitors are other prospective anticancer agents. The proteasome complex is involved in important cellular functions, such as protein homeostasis, apoptosis and cell cycle progression, and in resistance to anticancer therapy. The usage of proteasome inhibitors can induce cancer cell apoptosis or growth arrest (76). The proteasome inhibitor bortezomib (Velcade; Millenium) was examined for the treatment of recurrent GBM. It had a low response rate but led to better results in combination with standard therapy for patients with newly diagnosed GBM (77, 78).

Inhibition of angiogenesis in GBM. The role of the tumor microenvironment and angiogenesis has been widely studied in the case of glioblastoma. Extensive microvascular proliferation denotes poor survival and increased risk of

recurrence in GBM (79). The role of vascular growth factors (VEGF, especially VEGF-A) is well established in aberrant angiogenesis. In the case of GBM, the plasma and the tumor level of VEGF has been found to be relatively high and the elevated intracavitary level of these growth factors was discovered in patients with recurrents in comparison to those with non-recurrent GBM (80, 81). VEGF overexpression in tumor histology also correlates with a poor prognosis (82, 83). Therefore, a great effort is being made with the evaluation of antiangiogenic and anti-VEGF agents in GBM settings.

One of the most common used inhibitors of angiogenesis in cancer treatment is bevacizumab (Avastin; Genentech). It is a humanized monoclonal antibody against VEGF-A. Bevacizumab has also been examined in clinical trials for treatment of recurrent, as well as non-recurrent GBM, as a single agent, and in various combinations with chemotherapy and other targeted therapeutics. In combination with irinotecan, the 6-month PFS among 35 patients was 46% and the median OS was 42 weeks, in one of the first prospective phase II trials for patients with recurrent disease. The 4-year OS was reported to be 11% (84, 85). In the phase II BRAIN study, the use of bevacizumab with or without irinotecan was examined in 167 patients with recurrent GBM. In the bevacizumab plus irinotecan arm, 6-month PFS was 50.3% and the median OS was 8.9 months. The 12-, 18-, 24- and 30-month survival rates were 38%, 18%, 17% and 16%, respectively. For the bevacizumab monotherapy arm, the 6-month PFS was 42.6% and the median OS was 9.3 months. The 12-, 18-, 24- and 30-month survival rates were 38%, 24%, 16% and 11%, respectively (86, 87). There are additional phase II studies among patients with recurrent glioblastoma that support the treatment effect of combining bevacizumab with chemotherapy (88, 89, 90, 91, 92). Bevacizumab was approved in 2009 by the US FDA as a monotherapy for treating recurrent GBM due to high response rates and modest survival benefit (86, 93, 94).

There are other antiangiogenic therapies that are being studied as single-agent treatment for recurrent GBM. In one phase II study, the integrin inhibitor cilengitide (Merck) was examined for patients with recurrent disease. In the arm with the higher dose (2000 mg of cilengitide twice weekly), the median OS was 9.9 months and the OS rates were 37%, 23%, 15% and 10% at 12, 24, 36 and 48 months, respectively, cilengitide was also well-tolerated (95, 96). Another antiangiogenic drug, aflibercept (Zaltrap; Sanofi and Regeneron Pharmaceuticals), is a recombinantly prepared fusion protein that can bind VEGF-A, VEGF-B and placental growth factor (PGF). In the ongoing NABTC 0601 phase II study with aflibercept, the preliminary ORR was 30% for recurrent GBM (97). There is also a phase I trial with aflibercept and standard RT/temozolomide therapy for initial GBM (98). The oral inhibitor of MET/VEGFR2 cabozantinib (XL184; Exelixis) was examined in a phase II study in patients with previously treated recurrent GBM. The median PFS of patients without previous antiangiogenic treatment was 16 weeks. Furthermore, 61% of patients on corticosteroids had a more than 50% reduction in corticosteroid dose (99). Another small-molecule kinase inhibitor, cediranib (Recentin; AstraZeneca), led to normalization of tumor vessels and reduction of brain edema among glioblastoma patients (100). On the other hand, cediranib increased tumor infiltration in one phase II study of recurrent GBM (101). One hypothesis is that there is an angiogenesis-independent tumor population, or mechanism, in GBM which can be promoted by antiangiogenic treatment and which limits the efficacy of these new therapeutics (102). The potential for recurrent infiltrative as well as invasive tumor growth after the use of antiangiogenic agents has been reported in some studies (103, 104, 105). Other recent trials reported that there were no significantly changed patterns of relapse of GBM after the antiangiogenic treatments (106-109).

Combinations of antiangiogenic agents and chemoradiaton for newly diagnosed as well as recurrent GBM were also examined. In one study of standard RT/temozolomide treatment in combination with bevacizumab, for patients with newly diagnosed high-grade glioma, the 12-month PFS and OS were 59.3% and 86.7%, respectively (110). The combination of chemotherapy and bevacizumab for patients with newly diagnosed GBM approximately doubled the median PFS compared to standard therapy (14 *vs*. 6.9 months) (111). The combination of cilengitide with RT/temozolomide was examined in a phase I/II trial of newly diagnosed GBM. The median PFS was 8.0 months and the 12- and 24- month OS were 68% and 35%. The median OS was 16.1 months, with no additional toxicities (112). There are two large phase III studies that will evaluate bevacizumab-containing regimes for newly diagnosed GBMs and that have recently begun enrolling patients [AVAglio (NCT00943826) and RTOG-0825 (NCT00884741).

Immunotherapy and vaccines for treatment of GBM. Immunotherapy is a promising new area of multimodal anticancer treatment for many types of human malignancies. The dramatic change in the efficacy of such approaches after decades of relative disappointment was brought about by the recent introduction of vaccine sipuleucel-T and the monoclonal antibody ipilimumab, for the treatment of hormone-refractory prostate cancer and metastatic melanoma, respectively. These two immunotherapeutic agents mean real survival benefit for patients with cancer (113, 114). There has also been great progress in immunotherapy of GBM over the past few years. Although there is no approved anticancer vaccine for GBM at the moment, there is one hot candidate and many others in the pipeline.

Among the immunotherapeutic approaches in GBM research are passive immunotherapy with antibodies, utilization of autologous stimulated lymphocytes and immunotherapy with

cytokines, and active immunotherapy with tumor-based, peptide or dendritic cell (DC) vaccines. Among the peptide vaccines, there is one strong candidate for near future use in GBM treatment, Rindopepimut (CDX-110; Celldex Therapeutics) which is a peptide-based vaccine (13 amino acid sequence) against the antigen EGFRvIII. This specific *EGFR* mutant variant is constitutively activated and expressed in almost 30% of glioblastomas. One phase I/II multicenter study in patients with newly diagnosed GBM who were treated with rindopepimut led to a median PFS of 15.2 months and an OS of 23.6 months (115). Another phase II trial, ACT III, examined rindopepimut in combination with standard RT/temozolomide in 65 patients with newly diagnosed glioblastoma with *EGFR*vIII positivity. The median survival was 21 months from the time of initiating therapy and 24 months from the initial diagnosis. Patients with unmethylated *MGMT* had an OS of 20.9 months from diagnosis, whereas those with methylated *MGMT* had an OS of 40 months from diagnosis (116). The new double-blind, randomized, multicenter phase III study of rindopepimut in patients with newly diagnosed glioblastoma (ACT IV) is now enrolling patients (NCT01480479). In another phase II trial, the HLA-restricted, Wilms tumor 1 (WT1) 9-mer peptide vaccine was examined in patients with recurrent GBM. Partial response was seen in 2 out of 21 patients and the vaccine was well-tolerated (117). The most recent phase II trial with HSPPC-96 (vitespen), an autologous heat-shock protein-peptide vaccine, has shown promise in patients with recurrent GBM. The median OS was 47.6 weeks for vaccine-treated patients, compared to 32.8 weeks for the non-vaccinated group; 6-month OS was 93% for the vaccinated group compared to 68% for the non vaccinated group. There were no grade 3 or 4 side-effects (118).

Vaccines employing dendritic cells are other prospective approaches to GBM treatment. In a trial of relapsed GBM, the use of a vaccine with DCs loaded with autologous tumor lysate was examined in 56 patients. The median PFS was 3 months and the median OS was 9.6 months (119). The same group is investigating the integration of the vaccine in the primary treatment of patients with newly diagnosed GBM (120). Very promising data from a large, double-blind, randomized phase II trial of a DC vaccine in patients with newly diagnosed GBM showed a median survival of 3 years, with 4-year survival reaching 33% of patients and 27% of patients exceeding 6-years survival from initial surgery (121). Another phase I/II trial with DCs pulsed with specific tumor-associated peptides showed a PFS of 6.8 months and median OS of 18.7 months from the time of vaccination in patients with newly diagnosed GBM (122).

New approaches to the treatment of malignant glioma with immunotherapy are emerging and are demonstrating some promise for the near future for significant improvement of GBM therapy.

Conclusion

The prognosis of GBM still remains poor, despite aggressive surgery, RT and chemotherapies. On the other hand, there have been many novel discoveries in basic and translational research made in recent years. Besides the common predictors of the responsiveness to therapy and outcome, such as functional status or simple demographics, there are important underlying molecular characteristics of the tumor which could play a major role in disease evolution and prognosis.

New prognostic biomarkers, such as IDH 1and 2, the CIMP, promoter methylation status of the *MGMT* gene, and others, could be helpful for the determination of prognosis of the disease, as well as for the prediction of outcome of current standard GBM therapy for individual patients. The novel GBM classification according to genetic alterations and gene expression profiles into the Classical, Mesenchymal, Proneural and Neural subtypes could be very useful in the near future for finding important molecular targets within each group, suitable for therapeutic intervention, as well as for the selection of the best targeted therapy for each patient.

The new targeted therapies that are directed against certain tumoral features, such as altered signaling and metabolic pathways, aberrant tumor vessels, angiogenesis and the tumor microenvironment, are being widely examined in clinical trials. Due to the wide molecular diversity and heterogeneity of GBM, there has been a relative lack of success of these new treatment approaches. At the moment, there is only one targeted drug, bevacizumab, approved by the US FDA for the treatment of recurrent GBM, as a single agent. On the other hand, there has been significant progress in immunotherapy for GBM. The most promising agent, currently in phase III clinical trial for newly diagnosed glioblastoma, is the peptide-based vaccine rindopepimut. There are also other promising immunotherapies on the way.

Further progress in GBM treatment will probably be based on the patient's individual tumor analysis and the selection of the best combination of novel targeted agents together with another multimodal therapy for each individual patient, within the actual application of personalized medicine.

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