Anti-angiogenic therapy in glioblastoma multiforme

Paulina Kozakiewicz¹ (ABDEF), Izabela Kordzińska-Cisek¹ (BDEF), Katarzyna Król-Woch² (A,E) Paulina Stachyra¹ (AE)
1 Department of Oncology, Medical University of Lublin
2 Department of Radiation Oncology Center of the Lublin Region

SUMMARY

Primary brain tumors constitute a highly diversified group of neoplasms. In adults, the most common are tumors originating from the astro-glial lineage [1]. Glioblastoma multiforme is the most common brain malignancy worldwide. Median survival is 12–15 months from diagnosis in patients receiving standard treatment, which makes it one of the most malignant cancers. Only 3–5% of patients survive longer than 3 years [2]. The pathogenesis of glioblastoma is highly complex as mutations contributing to its occurrence result in alterations in not only one, but several cell signaling pathways responsible for cell proliferation, differentiation and apoptosis [2]. Glioblastoma multiforme (GBM) may be primary (de novo) or secondary, progressing from lower grade astrocytomas. Depending on the manner of their occurrence, these tumors have different genetic and epigenetic profiles that affect prognosis. Secondary GBM is more prevalent in younger patients with the mean age of diagnosis being 40 years of age. Moreover, its growth progresses more slowly and prognosis is better [3,4]. These tumors are characterized by more frequent occurrence of suppressor gene TP53 (tumor protein p53) mutation and, above all, gene IDH1 (isocitrate dehydrogenase (NADP(+)) 1) mutation, which results in greater Hif-1-alpha expression contributing to tumor progression, for instance via angiogenesis activation. IDH1 mutations are reported for over 80% of secon-dary tumors and less than 5% of primary tu-mors [3,5]. In about 90% of cases, glioblastoma multiforme is of de novo nature and is characterized by faster clinical manifestation. It is mainly detected in older individuals with the mean age of diagnosis being 55 years. The most common genetic disorders found in these tu-mors include: loss of chromosome 10q hetero-zygosity, EGFR (epidermal growth factor recep-tor) gene amplification, suppressor p16 deletion and mutation in the suppressor PTEN gene (phosphatase and tensin homolog deleted on chromosome ten) [4,6]. A more recent classifi-
cation of glioblastoma multiforme is based on a study on genetic signatures and distinguishes four subtypes: proneural, neural, classical and mesenchymal. They differ in gene mutations and thus the response to treatment [7].

Infiltrating and diffuse growth is an important feature of GBM as it prevents radical tumor resection and is conductive to its rapid regrowth [8]. GBM treatment requires an inter-disciplinary approach. Radical tumor resection with adjuvant radiotherapy combined with chemotherapy using temozolomide is aimed at as standard management [9,10]. Methylguanine methyltransferase (MGMT, O-6-methylguanine-DNA methyltransferase) promoter methylation is a positive prognostic and predictive factor. It is more common in secondary than in primary tumors (73% vs 43%) and may be associated, for instance, with concurrent IDH1 mutation [11]. GBM cells that have methylated MGMT promoter are characterized by better response to radiotherapy and chemotherapy with temozolomide [12,13]. Tumor vasculature increases with tumor grade. GBM is characterized by the richest vascular network of all brain tumors [14]. This rich vasculature is lin-ked with high expression of proangiogenic factors. High grade tumors are frequently ac-companied by overexpression of vascular endo-thelial growth factor (VEGF), transforming growth factor beta (TGF-B), cathepsin B and epidermal growth factor receptor (EGFR) inc-luding its activating mutations (EGFRvIII) [15]. In GBM, newly emerging blood vessels take the form of characteristic structures resembling renal glomeruli [16]. These vessels have an irregular course, abnormal interconnections and blind branches, thus being incapable of deliver-ring sufficient oxygen to glioblastoma cells. Hypoxia, on the other hand, creates a „vicious circle” in the form of abnormal angiogenesis [7]. Glioblastoma cells initially settle healthy host blood vessels which are used for GBM growth. Subsequently, they trigger the forma-tion of their own blood vessels [14]. There are reports stating that glioblastoma stem cells can imitate endothelial cells or pericytes [17,18,19]. These neoplastic pericytes are found in the brain, even beyond the tumorous lesion [19]. Tumor growth and development was for the first time corre-la-ted with the neoangiogenesis in 1971 by an American scientist, Jugah Folk-man [20]. Therapy targeted at proangiogenic factors in the treatment of glioblastoma helps normalize or reduce the abnormal vascular network and decrease tumor edema [21].

VEGF

Vascular endothelial growth factor (VEGF) is highly proangiogenic and plays a crucial role in the regulation of new vessel formation [22]. Glioblastoma cells exhibit significant overexpression of this factor compared with healthy tissues, which leads to irregular tumor vasculature. The main VEGF-stimulating factor is hypoxia. Chronic oxygen deficiency induces the production of hypoxia-inducible factor 1 (HIF-1) which is a transcription factor that promotes VEGF production and release [2,23]. The VEGF family proteins are associated with specific receptors, such as VEGFR-1, VEGFR-2, VEGFR-3, neuropilin-1 and neuropilin-2. VEGF activation promotes endothelial cells in new vessels to activate the angiogenesis pathway. This induces proliferation, growth and migration of endothelial cells and increases vascular permeability [22].

BEVACIZUMAB

Bevacizumab is a recombinant human monoclonal antibody that neutralizes VEGF-A activity and shows antiangiogenic action [24]. It has been approved by the FDA in 2009 as second-line therapy for recurrent glioblastoma multiforme based on two phase II clinical trials [25,26]. Patients with GBM recurrence after first-line therapy (surgery with adjuvant chemotherapy with temozolomide) received bevacizumab (10 mg/m²) every two weeks. After tumor progression, the antiangiogenic therapy was combined with chemotherapy using irino-tecan. The treatment response rate and 6-month progression-free survival (PFS) improved compared with a historical control group [25,26]. According to the MacDonald criteria, the radiological response to bevacizumab monotherapy reached 28–35%. When bevacizumab was ad-ded to treatment after tumor progression, the MRI response based on the MacDonald criteria was noted [25,26]. Other studies conducted to assess bevacizumab monotherapy in glioblastoma multiforme compared to historical controls, for example a prospective phase II trial and a retrospective analysis, confirmed its activity; the response rates were 25% and 42%, respectively, and six-month progression-free survival (PFS) reached 32% and 42%, respectively [27,28]. The rationale behind the use of the combination of chemotherapy with bevaci-zumab is the improvement of tumor vasculatu-re by regression of the pathological vascular
network. This could increase cytostatic penetration and cause a synergistic effect of bevacizumab with standard chemotherapy [29]. It has also been attempted to implement bevacizumab in first-line treatment along with chemoradiotherapy with temozolomide. However, overall survival (OS) did not improve, and the treat-ment induced more adverse effects, such as thromboembolic events, hypertension problems, bleeding and wound healing complications [30,31]. In both studies, PFS was longer for the bevacizumab group compared to the placebo group, for instance 10.6 months vs 6.2 months [30,31]. In a phase II randomized multicenter trial called GLARIUS, conducted in patients with newly diagnosed glioblastoma multiforme with unmethylated MGMT, PFS was longer for bevacizumab + irinotecan combination compa-red with temozolomide + radiotherapy. Median PFS for chemoradiotherapy with temozolomide and methotrexate reached 5.9 months, while for bevacizumab + irinotecan – 9.7 months. OS showed no statistically significant differences (p>0.05) [32]. It has also been reported that a VEGF level reduction might sensitize the vascular endothelium to radiotherapy [33]. Anti-VEGF antibodies may reduce edema around the tumor by eliminating cancer cell hypoxia, thereby increasing radiosensitivity of tumor cells [34]. Moreover, a combination of hypofractionated stereotactic radiotherapy with bevacizumab in the treatment of recurrent glioblastoma multiforme resulted in a complete response rate of 50%, PFS-6 of 65% and mean overall survival (mOS) of 12.5 months [35]. However, there are also studies that show no significant increase in OS of patients with recurrent glioblastoma multi-forme after bevacizumab implementation compared to chemotherapy with lomustine [36,37]. A phase II trial, BELOB, indicated improved OS for bevacizumab + lomustine compared with bevacizumab monotherapy in the treatment of recurrent glioblastoma multiforme. Nine-month overall survival reached 38% for patients treated with bevacizumab in monotherapy, 43% for patients receiving lomustine monotherapy, and 88% for the bevacizumab + lomustine combination [38]. It has been shown that bevacizumab may decrease the need for corticoste-roids and improve the quality of life in pa-tients with the recurrent disease [39]. It has also been attempted to combine bevacizumab with a different platelet-derived growth factor (PDGF), tandutinib, in a phase II trial enrolling patients with recurrent glioblastoma. However, the efficacy of this therapy was comparable to that of bevacizumab monotherapy, and the combination of the two angiogenesis inhibitors was linked with greater toxicity [40]. Bevacizumab failure in glioblastoma treatment is of multifaceted background. Animal tests have revealed that, over time, glioblastoma cells may travel along normal vascular network, thereby contributing to the occurrence of distant satel-lite tumors [41,42]. VEGF stimulates mainly normal angiogenesis, while the neoplastic one is promoted by a range of other factors, such as platelet-derived growth factor (PDGF), tumor necrosis factor alpha (TNF-alpha), fibroblast growth factor (FGF), hepatocyte growth factor (HGF), interleukin-8 (IL-8), interleukin-6 (IL-6), and transforming growth factor beta (TGF-beta). That is why, new targets for antiangiogenic therapy are being searched [7]. PDGF is not only involved in abnormal angiogenesis, but its receptor expression is found in glioblastoma cells and it contributes to tumor growth by an autocrine loop [43]. PDGF helps stabilize a newly emerged vessel and promotes migration of Tunica adventitia cells and vascular smooth muscle cells [44].

**Sunitinib** is an oral small-molecule kinase inhibitor targeted at VEGFR, platelet-derived growth factor receptor (PDGFR) and tyrosine kinase c-Kit, FMS-like tyrosine kinase-3 (FLT3), colony stimulating factor 1 receptor (CSF-1R) and neurotrophic factor receptor for glioblastoma multiforme (RET) [45]. In a preclinical trial, sunitinib, owing to its antiangiogenic properties, exhibited anti-proliferative effects and prolonged survival in mice with orthotopic glioblastoma multiforme (GBM) [46]. However, in one of the phase II trials, sunitinib was not found effective (dosage: 37.5 mg/m^2 daily until progression or unacceptable toxicity) in second-line treatment of 21 patients with grade III and IV glioblastoma. Sunitinib is not as potent as bevacizumab in selective inhibition of VEGF/VEGFR signaling, which might explain the predominant- ce of the latter drug in GBM treatment [47]. In another study conducted in patients with recur-rent glioblastoma multiforme or gliosarcoma after first-line treatment and bevacizumab therapy, sunitinib failed to significantly increase PFS. This does not confirm the assumption that angiogenesis inhibitors suppressing several signaling pathways that contribute to tumor cell proliferation might be more effective [45]. Moreover, a study enrolling patients with non-resectable glioblastoma multiforme treated with sunitinib before and during radiotherapy showed no PFS or OS benefits, either [48].
Pazopanib is yet another tyrosine kinase inhibitor with antiangiogenic properties. It is targeted against VEGFR, PDGFR-α and β, FGFR (fibroblast growth factor receptor) and stem cell factor receptor (c-KIT). In a phase II trial, pazopanib administered to patients with recurrent glioblastoma multiforme and gliosarcoma had no influence on PFS, but partial radiological response by the MacDonald criteria was noted [49]. Another phase II trial evaluating combined treatment using pazopanib and lapatinib in patients with recurrent glioblastoma and confirmed tumor PTEN/EGFRvIII mutations showed no anti-tumor efficacy of this combination in the form of no PFS benefit [50].

Imatinib is a tyrosine kinase inhibitor that, apart from suppressing tyrosine kinase Bcr-Abl, which is used in the treatment of chronic myeloid leukemia, also exhibits potent inhibitory effects towards PDGFR. There are phase II trials enrolling patients with recurrent glioblastoma multiforme treated with standard chemoradiotherapy where imatinib mesylate and hydroxy-urea demonstrated some minimal effects against tumor cells. One of the study’s endpoints was PFS-6 achieved in five of 31 GBM patients (16%). In some patients treated with imatinib, contrast enhancement in MRI was reduced despite deteriorated neurological condition. PDGFR is expressed on both endothelial cells and pericytes and therefore imatinib may lead to the normalization of abnormal vascular permeability with no genuine anti-tumor effect. The drug was well-tolerated by patients [51,52].

Cediranib is a potent inhibitor of all three endothelial growth factor receptors (VEGFR-1,-2,-3). One of the phase II trials reports quite encouraging treatment outcomes. Recurrent GBM patients treated with cediranib achieved partial radiological response in brain MRI with PFS-6 at the level of 25.8% [53]. Another study evaluating cediranib demonstrated a reduction of the vasogenic edema around the tumor and, as in the studies on bevacizumab, this enabled corticosteroid dose reduction in GBM patients [54]. However, in a phase III trials, treatment outcomes were not as encouraging any more. There was no benefit to PFS after monotherapy or combined treatment with temozolomide in patients with recurrent glioblastoma multiforme [55].

Cilengitide is a cyclic peptide targeted against integrins α, which are present in blood vessels and GBM cells, and take part in angiogenesis. Cilengitide blocks neoangiogenesis as well as suppresses tumor cell invasion and proliferation [56]. In a phase II trial (CORE), the activity of cilengitide in patients with newly diagnosed glioblastoma multiforme with unmethylated MGMT promoter showed activity in the form of slight overall survival and PFS improvement [57]. In a phase I/II trial, the addition of cilengitide to standard chemoradiotherapy with temozolomide in patients with glioblastoma multiforme with MGMT promoter methylation also yielded encouraging outcomes in the form of improved PFS and OS [58]. Unfortunately, in the phase III CENTRIC trial, OS and PFS did not improve upon the administration of temozolomide combined with cilengitide to patients with newly diagnosed glioblastoma multiforme with MGMT promoter methylation undergoing adjuvant radiotherapy. Integrins remain an interesting anti-cancer therapy target and they do require more investigation [59]. In antiangiogenic therapy, it has been attempted to use antisense oligonucleotides in order to inhibit TGF-β2 expression. A phase II trial in which TGF-β2 suppression was achieved by administering trabedersen (AP12009) to patients with grade III/IV glioblastoma showed no statistically significant survival benefit in AP12009 patients as compared with the standard chemotherapy group. This issue surely requires more studies [60,61]. Another interesting goal of antiangiogenic therapy is suppression of HIF-1-dependent pathways, such as m-TOR inhibitors. One of them is temsirolimus which, when administered in monotherapy to patients with recurrent glioblastoma multiforme in phase II trials, did not cause PFS or OS improvement [62,63]. The combination of temsirolimus with standard radiotherapy was evaluated in a phase I trial. However, the treatment occurred too toxic due to higher risk of bacterial infections [64]. Moreover, another phase II trial evaluating temsirolimus combined with radiotherapy in patients with glioblastoma multiforme with MGMT promoter methylation did not reveal any PFS or OS benefit as compared with standard chemoradiotherapy [65].


