Review article

# Glioblastoma – actual knowledge and future perspectives

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## ABSTRACT

Glioblastoma is the most severe IV-class glioma and therefore the prognosis for patients remains poor despite some improvement in the treatment area. The neurological or psychiatric symptoms especially fast-developing ones should be fully investigated. This article aims to summarize actual knowledge of glioblastoma and present future perspectives. The underlying causes are usually associated with mutations of *EGFR*, *PTEN*, *IDH1*, *p53* genes. The MRI scan, MGMT promoter methylation status, GFAP immunohistochemical detection and Karnofsky performance status are valuable diagnostic tools and some other potential biomarkers with high specificity are proposed. The standard of care is surgery and Stupp protocol which is the combination of radiotherapy and chemotherapy with temozolomide. Nevertheless, after remission the treatment possibilities are limited. Many efforts have been devoted to elaborate novel therapeutic strategies using e.g. CAR-T cells, nanoparticles, monoclonal antibodies, miRNA, siRNA or proteasome inhibitors.

**Key words:** glioblastoma multiforme, oncology, miRNA, pathogenesis

## INTRODUCTION

Glioblastoma multiforme (GBM) is the most prevalent primary malignant brain tumor [1]. While there are no known methods of prevention, and pre-symptomatic diagnosis is not accessible, a patient's life and wellbeing strongly rely on effective treatment. Nevertheless, much-needed progress in that area has not been made yet. With the current gold standard management (maximal safe resection, radiotherapy [RT], adjuvant chemotherapy with temozolomide [TMZ] [2]), the afflicted are very unlikely to survive the next 2 years after initial diagnosis (only 3–5% of them [3]). The majority of new promising therapeutic agents, successful at preclinical stages, do not show any considerable beneficial effects during clinical trials. On the other hand, a significant step forward in understanding the molecular mechanisms of GBM should allow conducting research in numerous directions. To prolong median overall survival there exists a need to establish a personalized therapy regimen. Obtaining genetic profiles of each patient's tumor can be of great importance for the design of specifically targeted agents. The main challenges are enabling drugs to sufficiently cross the blood–brain barrier (BBB) and creating combined targeted treatments of maximal efficacious potential [4].

It is thought, based on past trends, that GBM incidence will be rising. In the USA 12,970 cases are estimated for 2021 [5].

In this work we aim to look closer into constantly developing methods of treatment and provide basic information about management of GBM.

## PATHOGENESIS

Primary GBM (the most common clinical subtype – 95% of cases) develops *de novo*, within 3 to 6 months, usually in older patients. This subtype is characterized by amplified, mutated epidermal growth factor receptor (EGFR), an altered form of it is known as EGFRvIII. Commonly, it also has an amplified version of the *MDM2* gene (encoding an inhibitor of P53), phosphatase and tensin homolog (PTEN) mutations, and homozygous deletions of cyclin-dependent kinase inhibitor 2A (CDKN2A). Less than 5% of primary GBMs include isocitrate dehydrogenase 1 (IDH1) mutations. About 70–80% of primary tumors have TERT promoter mutation. 40% of this subtype present methylation of O-6-methylguanine-DNA methyltransferase (MGMT) promoter [6].

Secondary GBM develops as progressed low-grade astrocytoma (usually over 10–15 years) [7]. It demonstrates a greater prevalence of p53, *IDH1* mutations (more than 80% of tumors), amplified tyrosine-protein kinase Met gene (*MET*), and overexpression of platelet-derived growth factor receptor A (PDGFRA). A progression to GBM is correlated with an inactivation of the retinoblastoma gene (*RB1*) [8] and elevated activity levels of human double minute 2 (HDM2) [9].

Apart from clinical classification, there exists a molecular one. Based on molecular heterogeneity of GBM, 4 subclasses were distinguished: classical, mesenchymal, pro-neural, and neural [10]. The classical subtype is associated with amplified *EGFR* gene, astrocytic cell expression pattern and loss of chromosome 10, with *IDH1*, *TP53* or *NF1* mutations not being common. The mesenchymal subclass is associated with mesenchymal cell expression pattern, neurofibromin 1 gene (*NF1*), *PTEN* mutations, and lower *EGFR* levels than in other subclasses. The pro-neural type, which is almost always present in secondary GBM, is characterized by *IDH1* (prevalence of 30%), *TP53* mutations, and amplified PDGFRA. It usually presents at a younger age. Both neural and pro-neural subclasses present oligodendrocytic or astrocytic cell markers [11].

Certain mutations causing GBM can be passed with gametes, as around 5% of patients has diagnosed hereditary syndromes (e.g., Li-Fraumeni, Lynch syndromes, neurofibromatosis type 1 and 2) [12].

The malignant characteristics of GBM are originated and conditioned by proliferating, highly tumorigenic in *in vivo* trials GBM stem cells (GSCs), which are located in vascular niches in tumor tissue. Their molecular markers are promonin-1 (CD133) and L1 protein (L1CAM). These cells express a high level of vascular endothelial growth factor (VEGF) stromal-derived factor 1 (SDF-1 or CXCL12) which promotes proangiogenic activity in a tumoral site. It is thought that targeting GSCs is essential for a treatment to be effective [13].

## CLINICAL PRESENTATION

GBM is a fast-progressing disease [14]. The quick growth is accounted for a drastically poor overall survival. GBM is typically located in cerebral hemispheres, basal ganglia, commissural pathways with infiltrations developing along white matter tracts and perivascular spaces [15]. Around 25% of GBM patients develop seizures throughout the disease. The initial symptom of headaches is common and is correlated with a mass of neoplasm, size of oedema, their effect on surrounding structures (ventricular system, blood vessels), and increasing intracranial pressure [14].

Extracranial metastases are rare (affected are 0.4–0.5% of GBM patients). The short overall survival may be the main reason for such a low percentage [16].

## DIAGNOSIS

In case of a presence of GBM suggesting symptoms magnetic resonance imaging (MRI) is to be performed as a gold standard. When an MRI scan shows an intracranial tumor, the biopsy (surgical intervention) is next to be warranted in to distinguish the class of neoplasm [6]. Most of the symptomatic patients undergo computer tomography (CT) in the first step, before the initial presentation, to exclude hemorrhage. During the imaging tumor mass should be primarily identified. Advanced MRI techniques can play a crucial role in differentiation between primary GBM and solitary intracranial metastatic lesions [17].

According to National Comprehensive Cancer Network (2015) [18], biopsy and maximal safe resection are recommended before the following treatment [19].

There is also an undergoing pursuit of using liquid biomarkers from serum and CSF for diagnostic and prognostic purposes [20, 21].

## EPIDEMIOLOGY, PROGNOSIS AND RISK FACTORS

The most severe class IV glioblastoma has an incidence rate from 0.59 to 3.69 per 100,000 people depending on reporting country or organization [22]. Glioblastoma has a 5-year relative survival of approximately 5% with a survival median of 5–8 months because of low cure rate and high recurrence. The incidence is slightly higher in men than in women (1.6 : 1) and in Caucasians relative to other ethnicities [23]. There are many genetic aberrations associated with increased risk of glioma such as mutations in *NF1*, *NF2*, *TSC1*, *TSC2*, *MSH2*, *MLH1*, *MSH6*, *PMS2*, *TP53*, *IDH1/IDH2* genes [22].

## TREATMENT

Brain tissue is highly inaccessible for many therapeutic medicines because of the blood–brain barrier. Moreover, the brain presents also diminished ability to repair itself and therefore the treatment is challenging. The first line of glioblastoma treatment is surgery – more complete resection is correlated with better clinical outcomes. 5-aminolevulinic acid is used as a fluorescent dye to visualize glioma cells during surgery. It enables more complete resections and prolongation of progression-free survival (PFS) [24].

Since 2005, Stupp protocol [25] has been standard care for the treatment of glioblastoma (fig. 1). It consists of radiotherapy and chemotherapy with the alkylating agent – temozolomide. Recent studies proved that the addition of tumour-treating fields to maintenance temozolomide chemotherapy resulted in statistically significant improvement in survival. Tumour-treating fields consist of low-intensity, alternating electric fields delivered via transducer arrays applied to the scalp. It is the only OS-prolonging method since Stupp protocol was established [26]. Bevacizumab is the anti-VEGF monoclonal antibody which is approved by FDA as an anti-angiogenic therapy. However, such therapy does not significantly increase overall survival among patients

**Figure 1.** Description of Stupp protocol.

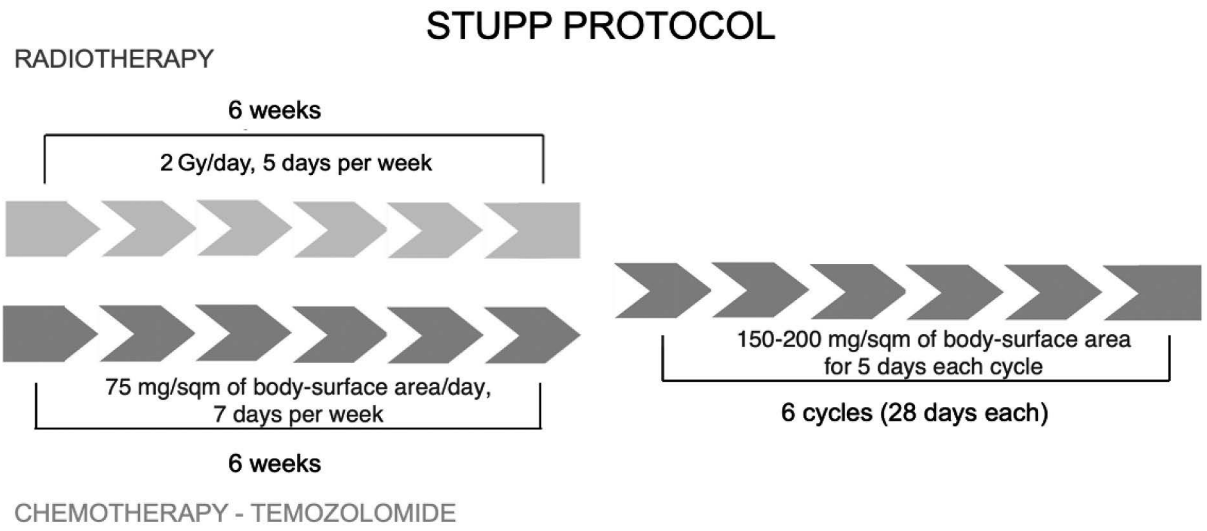

**Table 1.** LncRNAs and their role in tumorigenesis. All studies were performed on the patient-derived glioblastoma cell lines *in vitro* and in the murine model *in vivo*.

| NAME | **TARGETS** | **OUTCOMES** | **REF** |
|---|---|---|---|
| GAPLINC | miR-331-3p ↓ | KO – cells proliferation ↓, migration ↓, invasion ↓, apoptosis ↑ | [30] |
| HMMR-AS1 | ATM, RAD51, BMI1 | KO – cell migration ↓, invasion ↓, MES phenotypes ↓ radiosensitivity ↑ | [31] |
| HOTAIRM1 | HOXA gene methylation status ↑ | KO – cell proliferation ↓, migration ↓, invasion ↓ apoptosis ↑ | [32] |
| LINC01057 | NF-kB, promotion of MES differentiation | KO – proliferation ↓, invasion ↓ | [33] |
| MALAT1 | miR-199a ↓, ZHX1 ↑ | KO – apoptosis ↑, cell proliferation ↓, progression ↓ | [34, 35] |
| SNHG15 | miR-627-5p ↓ CDK6 ↑ | KO – tumorigenesis ↓, sensitivity to TMZ ↑ | [36] |
| SNHG7 | miR-5095 ↓, Wnt/b -catenin pathway ↑ | KO - proliferation ↓, migration ↓, invasion ↓, apoptosis ↑ | [37] |
| TP73-AS1 | ALDH1A1 (stem cell marker), TMZ resistance | KO – sensitivity to TMZ ↑ | [38] |

ALDH1A1 – aldehyde dehydrogenase 1 family member A1; AS – antisense RNA; ATM – ataxia telangiectasia mutated kinase; BMI1 – BMI1 proto-oncogene; Polycomb ring finger; CDK – cyclin-dependent kinase; CXCL14 – chemokine (C-X-C motif) ligand 14; GAPLINC – gastric adenocarcinoma associated, positive CD44 regulator, long intergenic non-coding RNA; HMMR – hyaluronan-mediated motility receptor; HOTAIRM1 – HOX antisense intergenic RNA myeloid 1; KO – knockout; MALAT1 – metastasis associated lung adenocarcinoma transcript 1; MES – mesenchymal; PFKFB2 – 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 2; RAD51 – RAD51 recombinase; SNHG – small nucleolar RNA host gene; TMZ – temozolomide; TP73 – p53-dependent apoptosis modulator; ZHX1 – zinc fingers and homeoboxes protein.

with newly diagnosed glioblastoma despite high vascularization of this neoplasm [27].

The essential part of treatment is symptomatic therapy with anticonvulsants [28] and corticosteroids to reduce peritumoral oedema.

## RESEARCH AND CLINICAL TRIALS

### LncRNA – long non-coding RNA

LncRNAs are a group of non-coding RNAs with more than 200 nucleotides. Their mode of action usually requires a miRNA to be inhibited (sponged) in order to elevate the expression of numerous genes involved in cell proliferation, invasion, migration, chemo- or radiosensitivity as well as apoptosis or transition to specific phenotypes. Thanks to the crucial role they can serve as the prognostic biomarker for the patient (as the elevated level of oncogenic lncRNAs usually correlates with the poor diagnosis) and as a future potential therapeutic target [29]. Table 1 presents lncRNA involved in the tumorigenesis of glioblastoma and the effects of the knockdown using siRNA or CRISPR.

### CAR-T

CAR-T therapies are successfully used in hematological malignancies thanks to high accessibility to neoplastic blood cells. Such therapies are broadly examined in the treatment of solid tumors; however, due to their immunosuppressive microenvironment and low penetrance, the results are not highly satisfactory in a clinical setting. Receptors characteristic for glioblastoma cells, such as EGFRvIII, IL13Rα2, are not expressed on all cells due to the heterogeneity. They are are usually downregulated after treatment with corresponding T cells. The upregulated genes, for instance, *PD-1*, *TIM-3*, *CTLA-4*, *TIGIT*, *KLGR-1* [39] have an inhibitory effect on T cells and their anti-tumor efficiency.

In the sphere of hypotheses are CAR-T cells containing tandem AND-gate which would require activation of both domains recognizing different receptors [40]. The process of manufacturing CAR-T can be also optimized by the incorporation of enhancements in CAR designs such as co-stimulatory domains or by using an enriched central memory T cell population [41]. Studies also revealed that neoantigen-targeting vaccines [42], as well as CAR-engineered natural killer (NK) cells, can have a great potential in glioblastoma treatment [43]. Novel immunotherapy targeting IL-13Ra2, EphA2 using SL-701 displayed in phase II trial the anti-tumor activity and promising survival curve [44].

Table 2 presents CAR-T therapies which were tested clinically or on the cell lines.

### miRNA

miRNA (microRNA), siRNA (small interfering RNA), circRNA (circular RNA) also can have a therapeutic effect. Up- or downregulation of certain RNAs in glioblastoma cells are connected with increased cell proliferation, invasiveness and decreased apoptosis [60]. Table 3 depicts some RNAs that can have prognostic and therapeutic properties.

**Table 2.** CAR-T clinical and preclinical trials.

| TARGET | OUTCOME | REF |
|---|---|---|
| BiTE-EGFRvIII | EGFR variant III is expressed by tumor cells in 30% of GBM patient tumors, genetically engineered macrophages secret EGRFvIII BiTE and IL-12 to induce T cell activation – tumor burden ↓ in murine model of GBM | [45] |
| CAIX | LB-100 inhibitor of protein phosphatase 2A enhances the anti-tumor activity and produces a synergistic anti-tumor effect with anti-CAIX CAR-T cell therapy – survival ↑ in GBM bearing mice | [46] |
| CD133 | CD133 mRNA into DCs – CD133+ GBM stem cell propagation ↓ and tumor growth ↓, T-cell activation ↑ CD4+ and CD8 in mice | [47] |
| CD70 | not detected in peripheral and brain normal tissues, expressed in GBM cells (78%), regression of the tumor in mice | [48] |
| CSPG4 | expressed in GBM neurospheres (71–99%), IFN-γ ↑, IL-2 ↑, tumor growth ↓ in the murine model, CAR-Ts encoding 4-1BB endodomain more efficient than those encoding CD28 or CD28-4-1BB | [49] |
| EGFFvIII + DGK KO | KO of DGK using CRISPR/Cas9 – immunosuppressive tumor environment ↓, anti-tumor efficacy ↑ in mice | [50] |
| EGFRvIII (human) | trafficking to the tumor was efficient, but regulatory T cells ↑, immunosuppressive tumor environment ↑ | [51] |
| EGFRvIII + PD-1 KO | KO of PD-1 using CRISPR/Cas9 – the growth of EGFRvIII-positive GBM cells *in vitro* ↓ without changing T-cell phenotype | [52] |
| EGFRvIII-triple KO | triple KO of the endogenous T-cell receptor (TRAC), B2M and PD-1 – survival ↑ in mice after i.c. but not i.v. infusion | [53] |
| EGFRvIII + IL-2 injection | IL-12 increased activity of anti-EGFRvIII-CAR T cells in the murine model, induction of remodeling of the tumor microenvironment, increase in long-term survival in a syngeneic mouse model | [54] |
| EGFRvIII + PD-1 antibody | blockade of PD-1 – the ability of CAR-T cells to infiltrate into solid tumors ↑, killing efficiency ↑, survival ↑ of tumor-bearing mice | [55] |
| HER2 | phase I trial – administration of HER2-CAR VSTs was feasible and safe, the clinical benefit for 8/17 patients | [56] |
| HER2 + SHP2 KO | KO of SHP2 using CRISPR/Cas9 increased elimination of GBM cell line *in vitro*, survival ↑ of mice *in vivo* | [57] |
| IL13Rα2 | a patient with recurrent multifocal GBM received multiple infusions of CAR-T cells intracranially, no toxic effects of grade ≥ 3, all intracranial and spinal tumors ↓, cytokines and immune cells in CSF ↑, clinical response for 7.5 mos | [58] |
| IL13Rα2 + TQM-13 | expressed in 75% of GBMs, conjugation of NPs to the surface of T cells expressing TQM-13 – efficient trafficking, DXR-loaded NPs – cytotoxic effect ↑ *in vitro,* pH-sensitive linkers – location specificity ↑ | [59] |

B2M – beta-2-microglobulin; BiTE – bi-specific T-cell engager; CAIX – carbonic anhydrase 9; CSF – cerebrospinal fluid; CSPG4 – chondroitin sulfate proteoglycan 4; DC – dendritic cell; DGK – diacylglycerol kinase; DXR – doxorubicin, i.c. – intracerebral; i.v. – intravenous; IL13Rα2 – IL-13-receptor-$\alpha_2$; KO – knockout; mos – months; NP – nanoparticle; PD-1 – programmed death cell protein 1; SHP2 – tyrosine-protein phosphatase non-receptor type 11; TQM-13 – targeted quadruple mutant-13; VST – virus-specific T-cell.

### Nanoparticles

Nanostructures have great efficiency in delivering not only RNAs to the glioblastoma cells but also other medicines such as temozolomide [61], doxorubicin [62] or paclitaxel [63].

The nanocomposite (LPLNP-PPT/TRAIL) for engineering and tracking of mesenchymal stem cells was created and showed induction of apoptosis in GBM cells both *in vitro* and *in vivo* [64].

### Proteasome inhibitors

Proteasome inhibitors are compounds that inhibit the enzymatic activity of proteasomes by stabilizing NFkB and tumor suppressor proteins and therefore lead to apoptosis [65]. Bortezomib is a proteasome inhibitor, approved for the treatment of multiple myeloma and mantle cell lymphoma. In glioblastoma cells interferes with MGMT expression, sensitizes them to TMZ and leads to prolongation of animal survival [66]. Another proteasome inhibitor – carfilzomib – reduces cell viability, migration, secretion and activation of MMP2 and cell invasion [66]. Marizomib has strong inhibitory properties against all enzymatic subunits of the proteasome and crosses BBB successfully, but its clinical effects have to be proven in further studies [65, 66].

### Monoclonal antibodies

Monoclonal antibodies can bind with receptors and other proteins to reduce their activity. Anti-PD-1 (anti-programmed cell death protein 1) antibody blocks PD-1 and alleviates the immunosuppressive effect of the tumor microenvironment. Moreover,

**Table 3.** miRNA, siRNA, shRNA preclinical trials.

| NAME | OUTCOME | REF |
|---|---|---|
| AON-DRR | AON against CD44 and EphA2 reduce DRR/FAM107A expression *in vitro*, tissue invasion ↓ cell metastasis ↓, less invasive phenotype | [71] |
| circ-PITX1 | downregulation of circ-PITX1 – cell proliferation ↓, apoptosis ↑ *in vitro*, circ-PITX1 is a sponge for miR-379-5p, the elevation of MAP3K2 expression | [72] |
| miR-128 | PHB-co-PEI nanoparticles loaded with miR-128 encoding plasmid increased apoptosis by 24,5% *in vitro* | [73] |
| miR-155 | when overexpressed – cell proliferation ↓, invasion ↓ and foci formation ↓, targets AGTR1/NF-κB/CXCR4 pathway | [74] |
| miR-7 | downregulation of miR-7 causes overexpression of TBX2 – migration ability ↑ of GBM cells *in vitro* | [75] |
| miRNA-181a PI3K/AKT ↓ | when overexpressed – sensitivity to carmustine ↑ via regulation of caspase-9, Bcl-2, SIRT1, migration ↓ via downregulation of MMP-2 and Bach1, G1 cell cycle arrest, apoptosis ↑ | [76] |
| shRNA-ARRB1 | delayed cell cycle progression and proliferation sensitivity ↑ to NK1R antagonists, G2/M transition arrest, downregulation of CDC25C/CDK1/cyclin B | [77] |
| shRNA-GDNFOS | GDNFOS1 interference – invasion ability ↓ and cell viability ↓ | [78] |
| shRNA-SLP2 | chitosan hydrogen contained irinotecan – cell apoptosis ↑ *in vitro*, shRNA reduced SLP2 protein expression – cell migration ↓, tumor size ↓ in a murine model | [79] |
| siRNA-ATM | RGD-PEG-ECO nanoparticles – efficient delivery, radiosensitivity ↑ *in vitro* | [80] |
| siRNA-CD73 | nasal administration in rats – cell apoptosis ↑, Treg ↓, microglia ↓ and macrophages ↓ in the tumor microenvironment; IL-6 ↑, CCL17 ↑, CCL22 ↑ | [81] |
| siRNA-Gal1 | chitosan nanoparticles administered intranasally – tumor cell motility ↓, Gal-1 expression ↓ | [82] |
| siRNA-GOLM1 | proliferation ↓, G1/S cell cycle arrest, tumor cell motility ↓, Wnt/β-catenin signaling ↓, tumor growth ↓ in a murine model | [83] |
| siRNA-Hsp27+resveratrol | silencing of Hsp27 *in vitro* and resveratrol have a synergistic effect on the induction of apoptosis | [84] |
| siRNA-OPN, shRNA-OPN | KO – the ability ↓ to recruit macrophages, T-cell effector activity ↑ in infiltrating the glioma *in vitro*, *in vivo* median survival time – by 68% in mice | [85] |
| siRNA-PLK1 and siRNA -VEGF2 | constructed nanoparticles which release siRNA after destabilization of the structure by ROS in the tumor microenvironment, enhancement with angiopep-2 peptide, *in vivo* survival time ↑ in mice | [86] |
| siRNA-RGD-PIK3CB | siRNA covalently conjugated to a molecule which specifically binds to integrin αvβ3 receptors, cell proliferation ↓, migration ↓, apoptosis ↑ on cell lines; *in vivo* – survival ↑ in mice | [87] |
| siRNA-STAT3 | nucleic acid aptamers carriers were used to specifically target siRNA-STAT3 to PDGFRb+ GBM cells – cell viability ↓, migration ↓ *in vitro*, tumor growth ↓ and angiogenesis ↓ in a murine model | [88] |
| siRNA-UCP2 | *in vitro* migration ↓, invasiveness ↓, clonogenicity ↓, proliferation ↓, cell apoptosis ↑, *in vivo* tumorigenicity ↓, downregulation of p38 MAPK pathway | [89] |
| siRNA-YAP | co-delivery of siRNA-YAP and paclitaxel in A hepatitis B core protein-virus-like-particle-based delivery system – apoptosis ↑, necrosis ↑, tumor invasion ↓, good BBB penetrance | [90] |

ACD – adrenocortical dysplasia; AGTR1 – angiotensin II receptor type 1; AKT – protein kinase; AON – antibody-antisense oligonucleotides; ARRB1 – arrestin β-1; ATM – ataxia telangiectasia mutated; AXL – AXL receptor tyrosine kinase; BBB – blood–brain barrier; Bcl-2 – B-cell lymphoma 2 – antiapoptotic protein; CCL – C-C motif chemokine ligand; CXCR4 – C-X-C chemokine receptor type 4 (CXCR-4); E2F6 – E2F transcription factor 6; ECO – 1-aminoethyl) iminobis[N-(oleicylcysteinyl-1-amino-ethyl)-propionamide; EphA2 – ephrin type-A receptor 2; FAM107A – family with sequence similarity 107 member A; Gal1 – galectin 1; GALE – UDP-galactose-4-epimerase; GAS6 – growth arrest – specific 6; GBM – glioblastoma; GDNFOS – GDNF-glial cell line-derived neurotrophic factor; GOLM1 – Golgi membrane protein; Hsp27 – heat shock protein 27; IL17RD – interleukin 17 receptor D; ITGA9 – integrin subunit alpha 9; KO – knockout; MAP3K2 – mitogen-activated protein kinase kinase kinase 2; MMP-2 – matrix metalloproteinase-2; NK1R – tachykinin-receptor neurokinin-1; NOVA1 – RNA-binding protein Nova-1; OPN – osteopontin; PAK4 – P21 activated kinases 4; PDCD4 – programmed cell death protein 4; PDGFRβ – platelet-derived growth factor receptor β; PEG – polyethylene glycol; PEI – polyethylenimine; PHB – polyhydroxy butyrate; PIK3CB – phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit beta isoform; PITX1 – paired-like homeodomain 1; PLK1 – polo-like kinase I; PTEN – phosphatase and tensin homolog; RGD – arginine-glycine-aspartic acid peptide; ROS – reactive oxygen species; SIRT1 – sirtuin 1; SLP2 – stomatin-like protein 2; SRC – SRC proto-oncogene, non-receptor tyrosine kinase; STAT3 – signal transducer and activator of transcription 3; TBX2 – T-box transcription factor 2; TMZ – temozolomide; TSPAN17 – tetraspanin 17; U87MG – Uppsala 87 malignant glioma; UCP2 – mitochondrial uncoupling protein 2; VEGF2 – vascular endothelial growth factor receptor – 2; YAP – yes1 associated transcriptional regulator – transcription co-activator of the Hippo Pathway; ZBTB20 – zinc finger and BTB domain containing 20.

**Table 4.** Other organic compounds.

| NAME | MODE OF ACTION | OUTCOME | REF |
|---|---|---|---|
| buparlisib | PI3K inhibitor | phase Ib/II study, buparlisib plus carboplatin or lomustine – insignificant anti-tumor activity | [91] |
| crizotinib | ALK/c-Met inhibitor | when combined with temozolomide – anti-tumor activity on FIG-ROS1-positive GBM cells *in vitro*, apoptosis – but not in FIG-ROS1-negative GBM cells | [92] |
| harmine | FAK/AKT inhibitor | extracted from perennial herbs – proliferation ↓, expression of MMP2 ↓, MMP9 ↓, VEGF ↓, tumor growth *in vivo* ↓ | [93] |
| lomustine | alkylating agent | combined with the TMZ trial showed an increase in OS among patients (with MGMT methylated promoter) who received lomustine + TMZ in comparison to TMZ only, no significant differences in neurocognitive abilities | [94] |
| loperamide, pimozide, | induction of autophagy | *in vitro* apoptosis ↑, dephosphorylation of mTORC1, induction of ATG5 ↑ and ATG7 ↑ dependent cell death in GBM cells | [95] |
| perillyl alcohol | Ras inhibitor | phase I/II clinical trial, intranasal administration, longer overall survival among patients with recurrent primary GBM, especially with tumor localised in deep regions of the brain | [96] |
| pimozide | ID1 inhibitor | sensitivity to TMZ – *in vitro* | [97] |
| ralimetinib | p38-MAPK inhibitor | phase I trial, combining with chemoradiation was feasible | [98] |
| regorafenib | VEGF inhibitor | case report, after 4 months of therapy significant reduction of lesion size | [99] |

ALK – anaplastic lymphoma kinase; c-Met – mesenchymal-epithelial transition factor kinase; SFN-Cys – sulforaphane-cysteine.

it upregulates T-cell- and interferon-γ-related gene expression [67]. Humanized anti-Chi3L1 antibody (chitinase 3-like 1) inhibits glioblastoma growth i*n vivo* in mice by more than 60% and reduces the mesenchymal "switch" mediated by Chi3L1 [68]. The phase III trial was conducted to assess the efficiency of nimotuzumab, the anti-EGFR antibody but the results showed no significant differences [69]. Antibody against immune-checkpoint inhibitor – LAG-3 also showed anti-tumor activity [67, 70].

### Other organic compounds

Organic compounds that are not peptides are also a useful tool in the process of treatment. Table 4 presents the positive impact of these substances on the overall survival, tumor growth reduction and increase in sensitivity to temozolomide among patients. Nevertheless, in preclinical studies, they expressed a strong cytotoxic effect as well both *in vitro* and *in vivo*.

## CONCLUSION

In the GBM diagnostic process the MRI scan, MGMT promoter methylation status, GFAP immunohistochemical detection and Karnofsky performance status are valuable diagnostic tools and some other potential biomarkers with high specificity are proposed. The standard of care is surgery and Stupp protocol which is the combination of radiotherapy and chemotherapy with temozolomide. Nevertheless, after remission the treatment possibilities are limited. As result, many efforts have been devoted to elaborate novel therapeutic strategies using e.g. CAR-T cells, nanoparticles, monoclonal antibodies, miRNA, siRNA or proteasome inhibitors.

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