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.

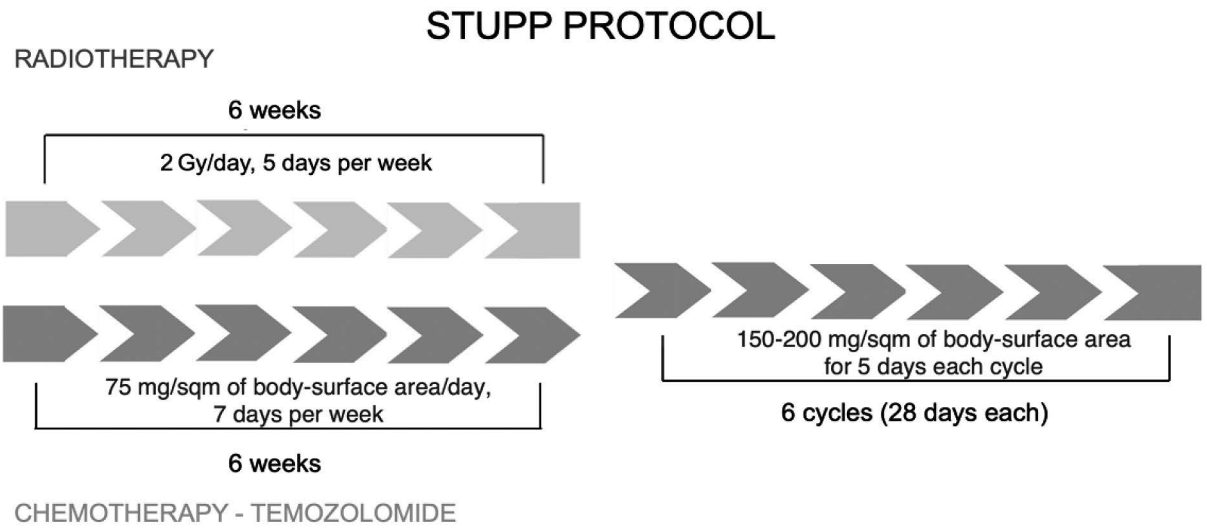

Table 1. LncRNAs and their role in tumorigenesis. All studies were performed on the patient-derived glioblastoma cell lines *in vitro* and in the murine model *in vivo*.

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-α_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.

References

1. Piccirillo SGM, Colman S, Potter NE et al. Genetic and functional diversity of propagating cells in glioblastoma. Stem Cell Reports. 2015; 4 (1): 7-15.
2. Gallego O. Nonsurgical treatment of recurrent glioblastoma. Curr Oncol. 2015; 22(4): e273-281.
3. Adamson C, Kanu OO, Mehta AI et al. Glioblastoma multiforme: a review of where we have been and where we are going. Expert Opin Investig Drugs. 2009; 18(8): 1061-83.
4. Taylor OG, Brzozowski JS, Skelding KA. Glioblastoma Multiforme: An Overview of Emerging Therapeutic Targets. Front Oncol. 2019; 9: 963.
5. Ostrom QT, Patil N, Cioffi G et al. CBTRUS Statistical Report: Primary Brain and Other Central Nervous System Tumors Diagnosed in the United States in 2013-2017. Neuro Oncol. 2020; 22(12 suppl 2): iv1-iv96.
6. Batash R, Asna N, Schaffer P et al. Glioblastoma Multiforme, Diagnosis and Treatment; Recent Literature Review. Curr Med Chem. 2017; 24 (27): 3002-9.
7. Ohgaki H, Kleihues P. The definition of primary and secondary glioblastoma. Clin Cancer Res. 2013; 19(4): 764-72.
8. Ludwig K, Kornblum HI. Molecular markers in glioma. J Neurooncol. 2017; 134(3): 505-12.
9. Kim CK, Nguyen TL, Joo KM et al. Negative regulation of p53 by the long isoform of ErbB3 binding protein Ebp1 in brain tumors. Cancer Res. 2010; 70(23): 9730-41.
10. Soomro SH, Ting LR, Qing YY et al. Molecular biology of glioblastoma: Classification and mutational locations. J Pak Med Assoc. 2017; 67(9): 1410-4.
11. Verhaak RG, Hoadley KA, Purdom E et al. Integrated genomic analysis identifies clinically relevant subtypes of glioblastoma characterized by abnormalities in PDGFRA, IDH1, EGFR, and NF1. Cancer Cell. 2010; 17(1): 98-110.
12. Alifieris C, Trafalis DT. Glioblastoma multiforme: Pathogenesis and treatment. Pharmacol Ther. 2015; 152: 63-82.
13. Huang Z, Cheng L, Guryanova OA et al. Cancer stem cells in glioblastoma-molecular signaling and therapeutic targeting. Protein Cell. 2010; 1(7): 638--55.
14. Alexander BM, Cloughesy TF. Adult Glioblastoma. J Clin Oncol. 2017; 35 (21): 2402-9.
15. Khandwala K, Mubarak F, Minhas K. The many faces of glioblastoma: Pictorial review of atypical imaging features. Neuroradiol J. 2021; 34(1): 33-41.
16. Cunha M, Maldaun MVC. Metastasis from glioblastoma multiforme: a meta-analysis. Rev Assoc Med Bras. 2019; 65(3): 424-33.
17. Lee EJ, Ahn KJ, Lee EK et al. Potential role of advanced MRI techniques for the peritumoural region in differentiating glioblastoma multiforme and solitary metastatic lesions. Clin Radiol. 2013; 68 (12): e689-697.
18. Nabors LB, Portnow J, Ammirati M et al. Central Nervous System Cancers, Version 1.2015. J Natl Compr Canc Netw. 2015; 13(10): 1191-202.
19. Staller A. Presumed Glioblastoma Multiforme: A Case for Biopsy Prior to Treatment. Clin J Oncol Nurs. 2016; 20(1): 95-7.
20. Figueroa JM, Carter BS. Detection of glioblastoma in biofluids. J Neurosurg. 2018; 129 (2): 334-40.
21. Quddusi A, Shamim MS. Serum biomarkers for glioblastoma multiforme. J Pak Med Assoc. 2019; 69(6): 913-4.
22. Ostrom QT, Bauchet L, Davis FG et al. The epidemiology of glioma in adults: a "state of the science" review. Neuro-Oncology. 2014; 16(7): 896-913.
23. Ellor SV, Pagano-Young TA, Avgeropoulos NG. Glioblastoma: background, standard treatment paradigms, and supportive care considerations. J Law Med Ethics. 2014; 42(2): 171-82.
24. Stummer W, Pichlmeier U, Meinel T et al. Fluorescence-guided surgery with 5-aminolevulinic acid for resection of malignant glioma: a randomised controlled multicentre phase III trial. Lancet Oncol. 2006; 7(5): 392-401.
25. Stupp R, Mason WP, van den Bent MJ et al. Radiotherapy plus Concomitant and Adjuvant Temozolomide for Glioblastoma. N Engl J Med. 2005; 352 (10): 987-96.
26. Stupp R, Taillibert S, Kanner A et al. Effect of Tumor-Treating Fields Plus Maintenance Temozolomide vs Maintenance Temozolomide Alone on Survival in Patients With Glioblastoma: A Randomized Clinical Trial. JAMA. 2017; 318(23): 2306-16.
27. Ameratunga M, Pavlakis N, Wheeler H et al. Anti-angiogenic therapy for high-grade glioma. Cochrane Database Syst Rev. 2018; 11(11): CD008218-CD008218.
28. Stevens GH. Antiepileptic therapy in patients with central nervous system malignancies. Curr Neurol Neurosci Rep. 2006; 6(4): 311-8.
29. Shao M, Liu W, Wang Y. Differentially expressed LncRNAs as potential prognostic biomarkers for glioblastoma. Cancer Genetics. 2018; 226-7: 23-9.
30. Chen HH, Zong J, Wang SJ. LncRNA GAPLINC promotes the growth and metastasis of glioblastoma by sponging miR-331-3p. Eur Rev Med Pharmacol Sci. 2019; 23(1): 262-70.
31. Li J, Ji X, Wang H. Targeting Long Noncoding RNA HMMR-AS1 Suppresses and Radiosensitizes Glioblastoma. Neoplasia. 2018; 20(5): 456-66.
32. Li Q, Dong C, Cui J et al. Over-expressed lncRNA HOTAIRM1 promotes tumor growth and invasion through up-regulating HOXA1 and sequestering G9a/EZH2/Dnmts away from the HOXA1 gene in glioblastoma multiforme. J Exp Clin Cancer Res. 2018; 37(1): 265.
33. Tang G, Luo L, Zhang J et al. lncRNA LINC01057 promotes mesenchymal differentiation by activating NF-κB signaling in glioblastoma. Cancer Letters. 2021; 498: 152-64.
34. Baspinar Y, Elmaci I, Ozpinar A et al. Long non-coding RNA MALAT1 as a key target in pathogenesis of glioblastoma. Janus faces or Achilles' heal? Gene. 2020; 739: 144518.
35. Liao K, Lin Y, Gao W et al. Blocking lncRNA MALAT1/miR-199a/ZHX1 Axis Inhibits Glioblastoma Proliferation and Progression. Molecular Therapy – Nucleic Acids. 2019; 18: 388-99.
36. Li Z, Zhang J, Zheng H et al. Modulating lncRNA SNHG15/CDK6/miR-627 circuit by palbociclib, overcomes temozolomide resistance and reduces M2-polarization of glioma associated microglia in glioblastoma multiforme. J Exp Clin Cancer Res. 2019; 38(1): 380.
37. Ren J, Yang Y, Xue J et al. Long noncoding RNA SNHG7 promotes the progression and growth of glioblastoma via inhibition of miR-5095. Biochem Biophys Res Commun. 2018; 496(2): 712-8.
38. Mazor G, Levin L, Picard D et al. The lncRNA TP73-AS1 is linked to aggressiveness in glioblastoma and promotes temozolomide resistance in glioblastoma cancer stem cells. Cell Death Dis. 2019; 10(3): 246.
39. Andrews LP, Yano H, Vignali DAA. Inhibitory receptors and ligands beyond PD-1, PD-L1 and CTLA-4: breakthroughs or backups. Nat Immunol. 2019; 20(11): 1425-34.
40. Sabahi M, Jabbari P, Alizadeh Haghighi M et al. Proposing a tandem AND-gate CAR T cell targeting glioblastoma multiforme. Med Hypotheses. 2020; 137: 109559.

41. Aguilar B, Sarkissian A, Brito A et al. 275. Optimization of IL13Rα2-Specific CAR T Cells for Clinical Development Using Orthotopic Human Glioblastoma Models in NSG Mice. Mol Ther. 2016; 24: S109.
42. Keskin DB, Anandappa AJ, Sun J et al. Neoantigen vaccine generates intratumoral T cell responses in phase Ib glioblastoma trial. Nature. 2019; 565(7738): 234-9.
43. Burger MC, Zhang C, Harter PN et al. CAR-Engineered NK Cells for the Treatment of Glioblastoma: Turning Innate Effectors Into Precision Tools for Cancer Immunotherapy. Front Immunol. 2019; 10: 2683.
44. Peereboom D, Nabors LB, Kumthekar P et al. 373O – Results of phase II trial of SL-701, a novel immunotherapy targeting IL-13Ra2, EphA2, and survivin, in adults with second-line recurrent glioblastoma (GBM). Ann Oncol. 2018; 29: viii122-viii123.
45. Gardell JL, Matsumoto LR, Chinn H et al. Human macrophages engineered to secrete a bispecific T cell engager support antigen-dependent T cell responses to glioblastoma. J Immunother Cancer. 2020; 8(2): e001202.
46. Cui J, Wang H, Medina R et al. Inhibition of PP2A with LB-100 Enhances Efficacy of CAR-T Cell Therapy Against Glioblastoma. Cancers (Basel). 2020; 12(1):139.
47. Do AS-MS, Amano T, Edwards LA et al. CD133 mRNA-Loaded Dendritic Cell Vaccination Abrogates Glioma Stem Cell Propagation in Humanized Glioblastoma Mouse Model. Mol Ther – Oncolytics. 2020; 18: 295-303.
48. Jin L, Ge H, Long Y et al. CD70, a novel target of CAR T-cell therapy for gliomas. Neuro Oncol. 2018; 20(1): 55-65.
49. Pellegatta S, Savoldo B, Su C et al. 507. Chondroitin Sulfate Proteoglycan 4 (CSPG4)-Redirected T Cells Eliminate Glioblastoma-Derived Neurospheres. Mol Ther. 2016; 24: S202.
50. Jung IY, Kim YY, Yu HS et al. CRISPR/Cas9-Mediated Knockout of DGK Improves Antitumor Activities of Human T Cells. Cancer Res. 2018; 78(16): 4692--703.
51. O'Rourke DM, Nasrallah MP, Desai A et al. A single dose of peripherally infused EGFRvIII-directed CAR T cells mediates antigen loss and induces adaptive resistance in patients with recurrent glioblastoma. Sci Transl Med. 2017; 9(399): eaaa0984
52. Nakazawa T, Natsume A, Nishimura F et al. Effect of CRISPR/Cas9-Mediated PD-1-Disrupted Primary Human Third-Generation CAR-T Cells Targeting EGFRvIII on In Vitro Human Glioblastoma Cell Growth. Cells. 2020; 9(4): 998.
53. Choi BD, Yu X, Castano AP et al. CRISPR-Cas9 disruption of PD-1 enhances activity of universal EGFRvIII CAR T cells in a preclinical model of human glioblastoma. J Immunother Cancer. 2019; 7(1): 304.
54. Liuzzi AR, Agliardi G, Becher B et al. 106P – A Single Dose of Local IL-12 Promotes Anti-Tumor Effect of Anti-EGFRvIII-CAR-T Cells in a Syngeneic Murine Model of Glioblastoma. Ann Oncol. 2019; 30: xi40.
55. Song Y, Liu Q, Zuo T et al. Combined antitumor effects of anti-EGFR variant III CAR-T cell therapy and PD-1 checkpoint blockade on glioblastoma in mouse model. Cell Immunol. 2020; 352: 104112.
56. Ahmed N, Brawley V, Hegde M et al. HER2-Specific Chimeric Antigen Receptor-Modified Virus-Specific T Cells for Progressive Glioblastoma: A Phase 1 Dose-Escalation Trial. JAMA Oncol. 2017; 3(8): 1094-101.
57. Sanber K, Nawas Z, Salsman V et al. Modulation of inhibitory receptor signaling pathways improves CAR T cell activity against glioblastoma. Cytotherapy. 2020; 22(5 suppl): S19-S20.
58. Brown CE, Alizadeh D, Starr R et al. Regression of Glioblastoma after Chimeric Antigen Receptor T-Cell Therapy. N Engl J Med. 2016; 375(26): 2561-9.
59. Kim GB, Aragon-Sanabria V, Randolph L et al. High-affinity mutant Interleukin-13 targeted CAR T cells enhance delivery of clickable biodegradable fluorescent nanoparticles to glioblastoma. Bioact Mater. 2020; 5(3): 624-35.
60. Møller HG, Rasmussen AP, Andersen HH et al. A Systematic Review of MicroRNA in Glioblastoma Multiforme: Micro-modulators in the Mesenchymal Mode of Migration and Invasion. Mol Neurobiol. 2013; 47(1): 131-44.
61. Marino A, Almici E, Migliorin S et al. Piezoelectric barium titanate nanostimulators for the treatment of glioblastoma multiforme. J Colloid Interface Sci. 2019; 538: 449-61.
62. Li T-F, Li K, Zhang Q et al. Dendritic cell-mediated delivery of doxorubicin-polyglycerol-nanodiamond composites elicits enhanced anti-cancer immune response in glioblastoma. Biomaterials. 2018; 181: 35-52.
63. Banerjee I, De K, Mukherjee D et al. Paclitaxel-loaded solid lipid nanoparticles modified with Tyr-3-octreotide for enhanced anti-angiogenic and anti-glioma therapy. Acta Biomater. 2016; 38: 69-81.
64. Wu S-Q, Yang C-X, Yan X-P. A Dual-Functional Persistently Luminescent Nanocomposite Enables Engineering of Mesenchymal Stem Cells for Homing and Gene Therapy of Glioblastoma. Adv Funct Mater. 2017; 27(11): 1604992.
65. Roth P, Mason WP, Richardson PG et al. Proteasome inhibition for the treatment of glioblastoma. Expert Opin Investig Drugs. 2020; 29(10): 1133-41.
66. Rahman MA, Gras Navarro A, Brekke J et al. Bortezomib administered prior to temozolomide depletes MGMT, chemosensitizes glioblastoma with unmethylated MGMT promoter and prolongs animal survival. Br J Cancer. 2019; 121(7): 545-55.
67. Cloughesy TF, Mochizuki AY, Orpilla JR et al. Neoadjuvant anti-PD-1 immunotherapy promotes a survival benefit with intratumoral and systemic immune responses in recurrent glioblastoma. Nat Med. 2019; 25(3): 477-86.
68. Guetta-Terrier C, Akosmam B, Chen JS et al. 185 Poster – Antibody blockade resets Chi3L1-induced glioma stem cell phenotypic transitions and reduces glioblastoma tumor burden. Eur J Cancer. 2020; 138: S52.
69. Westphal M, Heese O, Steinbach JP et al. A randomised, open label phase III trial with nimotuzumab, an anti-epidermal growth factor receptor monoclonal antibody in the treatment of newly diagnosed adult glioblastoma. Eur J Cancer. 2015; 51(4): 522-32.
70. Harris-Bookman S, Mathios D, Martin AM et al. Expression of LAG-3 and efficacy of combination treatment with anti-LAG-3 and anti-PD-1 monoclonal antibodies in glioblastoma. Int J Cancer. 2018; 143(12): 3201-8.
71. Arnold AE, Malek-Adamian E, Le PU et al. Antibody-Antisense Oligonucleotide Conjugate Downregulates a Key Gene in Glioblastoma Stem Cells. Mol Ther Nucleic Acids. 2018; 11: 518-27.
72. Lv X, Wang M, Qiang J et al. Circular RNA circ-PITX1 promotes the progression of glioblastoma by acting as a competing endogenous RNA to regulate miR-379–5p/MAP3K2 axis. Eur J Pharmacol. 2019; 863: 172643.
73. Memari E, Maghsoudi A, Yazdian F et al. Synthesis of PHB-co-PEI nanoparticles as gene carriers for miR-128-encoding plasmid delivery to U87 glioblastoma cells. Colloids Surf A: Physicochem Eng Asp. 2020; 599: 124898.
74. Singh A, Srivastava N, Yadav A et al. Targeting AGTR1/NF-κB/CXCR4 axis by miR-155 attenuates oncogenesis in glioblastoma. Neoplasia. 2020; 22(10): 497-510.

75. Pan C-M, Chan K-H, Chen C-H et al. MicroRNA-7 targets T-Box 2 to inhibit epithelial-mesenchymal transition and invasiveness in glioblastoma multiforme. Cancer Lett. 2020; 493: 133-42.
76. Rezaei T, Hejazi M, Mansoori B et al. microRNA-181a mediates the chemo-sensitivity of glioblastoma to carmustine and regulates cell proliferation, migration, and apoptosis. Eur J Pharmacol. 2020; 888: 173483.
77. Zhang Y-X, Li X-F, Yuan G-Q et al. β-Arrestin 1 has an essential role in neurokinin-1 receptor-mediated glioblastoma cell proliferation and G2/M phase transition. J Biol Chem. 2017; 292(21): 8933-47.
78. Wang S, Fan Y, Xu Y et al. GDNFOS1 knockdown decreases the invasion and viability of glioblastoma cells. Exp Ther Med. 2019; 18(2): 1315-22.
79. Lu YJ, Lan YH, Chuang CC et al. Injectable Thermo-Sensitive Chitosan Hydrogel Containing CPT-11-Loaded EGFR-Targeted Graphene Oxide and SLP2 shRNA for Localized Drug/Gene Delivery in Glioblastoma Therapy. Int J Mol Sci. 2020; 21(19): 7111.
80. Lee JA, Ayat N, Sun Z et al. Improving Radiation Response in Glioblastoma Using ECO Nanoparticle Delivery of siRNA Targeting DNA Damage Repair. Int J Radiat Oncol Biol Phys. 2017; 99(2 Suppl): E604.
81. Azambuja JH, Schuh RS, Michels LR et al. Blockade of CD73 delays glioblastoma growth by modulating the immune environment. Cancer Immunol Immunother. 2020; 69(9): 1801-12.
82. Danhier F, Messaoudi K, Lemaire L et al. Combined anti-Galectin-1 and anti-EGFR siRNA-loaded chitosan-lipid nanocapsules decrease temozolomide resistance in glioblastoma: In vivo evaluation. Int J Pharm. 2015; 481(1): 154-61.
83. Ding X, Deng G, Liu J et al. GOLM1 silencing inhibits the proliferation and motility of human glioblastoma cells via the Wnt/β-catenin signaling pathway. Brain Res. 2019; 1717: 117-26.
84. Önay Uçar E, Şengelen A. Resveratrol and siRNA in combination reduces Hsp27 expression and induces caspase-3 activity in human glioblastoma cells. Cell Stress Chaperones. 2019; 24(4): 763-75.
85. Wei J, Marisetty A, Schrand B et al. Osteopontin mediates glioblastoma-associated macrophage infiltration and is a potential therapeutic target. J Clin Invest. 2019; 129(1): 137-49.
86. Zheng M, Liu Y, Wang Y et al. ROS-Responsive Polymeric siRNA Nanomedicine Stabilized by Triple Interactions for the Robust Glioblastoma Combinational RNAi Therapy. Adv Mater. 2019; 31(37): e1903277.
87. Cen B, Wei Y, Huang W et al. An Efficient Bivalent Cyclic RGD-PIK3CB siRNA Conjugate for Specific Targeted Therapy against Glioblastoma In Vitro and In Vivo. Mol Ther Nucleic Acids. 2018; 13: 220-32.
88. Esposito CL, Nuzzo S, Catuogno S et al. STAT3 Gene Silencing by Aptamer-siRNA Chimera as Selective Therapeutic for Glioblastoma. Mol Ther Nucleic Acids. 2018; 10: 398-411.
89. Wu S, Luo C, Hameed NUF et al. UCP2 silencing in glioblastoma reduces cell proliferation and invasiveness by inhibiting p38 MAPK pathway. Exp Cell Res. 2020; 394(1): 112110.
90. Yang J, Zhang Q, Liu Y et al. Nanoparticle-based co-delivery of siRNA and paclitaxel for dual-targeting of glioblastoma. Nanomedicine (Lond). 2020; 15(14): 1391-409.
91. Rosenthal M, Clement PM, Campone M et al. Buparlisib plus carboplatin or lomustine in patients with recurrent glioblastoma: a phase Ib/II, open-label, multicentre, randomised study. ESMO Open. 2020; 5(4): e000672.
92. Das A, Cheng RR, Hilbert ML et al. Synergistic Effects of Crizotinib and Temozolomide in Experimental FIG-ROS1 Fusion-Positive Glioblastoma. Cancer Growth Metastasis. 2015; 8: 51-60.
93. Zhu Y-G, Lv Y-X, Guo C-Y et al. Harmine inhibits the proliferation and migration of glioblastoma cells via the FAK/AKT pathway. Life Sci. 2021; 270: 119112.
94. Weller J, Tzaridis T, Mack F et al. Health-related quality of life and neurocognitive functioning with lomustine–temozolomide versus temozolomide in patients with newly diagnosed, MGMT-methylated glioblastoma (CeTeG/NOA-09): a randomised, multicentre, open-label, phase 3 trial. Lancet Oncol. 2019; 20(10): 1444-53.
95. Zielke S, Meyer N, Mari M et al. Loperamide, pimozide, and STF-62247 trigger autophagy-dependent cell death in glioblastoma cells. Cell Death Dis. 2018; 9(10): 994.
96. da Fonseca CO, Simão M, Lins IR et al. Efficacy of monoterpene perillyl alcohol upon survival rate of patients with recurrent glioblastoma. J Cancer Res Clin Oncol. 2011; 137(2): 287-93.
97. Sachdeva R, Wu M, Smiljanic S et al. ID1 Is Critical for Tumorigenesis and Regulates Chemoresistance in Glioblastoma. Cancer Res. 2019; 79(16): 4057-71.
98. Biau J, Thivat E, Chautard E et al. Phase 1 trial of ralimetinib (LY2228820) with radiotherapy plus concomitant temozolomide in the treatment of newly diagnosed glioblastoma. Radiother Oncol. 2021; 154: 227-34.
99. Detti B, Scoccianti S, Lucidi S et al. Regorafenib in glioblastoma recurrence: A case report. Cancer Treat Res Commun. 2021; 26: 100263.

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