Acta Biomed 2020; Vol. 91, Supplement 7: 5-17 DOI: 10.23750/abm.v91i7-S.9951

Original article

Innovative therapies for malignant brain tumors: the road to a tailored cure

Alice Giotta Lucifero[1], Sabino Luzzi[1,2], Ilaria Brambilla[3], Chiara Trabatti[3], Mario Mosconi[4], Salvatore Savasta[3], Thomas Foiadelli[3]

[1] Neurosurgery Unit, Department of Clinical-Surgical, Diagnostic and Pediatric Sciences, University of Pavia, Pavia, Italy; [2] Neurosurgery Unit, Department of Surgical Sciences, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy; [3] Pediatric Clinic, Department of Pediatrics, Fondazione IRCCS Policlinico San Matteo, University of Pavia, Pavia, Italy; [4] Orthopaedic and Traumatology Unit, Department of Clinical-Surgical, Diagnostic and Pediatric Sciences, University of Pavia, Pavia, Italy

Abstract. *Background:* Immune tolerance, immune escape, neoangiogenesis, phenotypic changes, and glioma stem cells are all responsible for the resistance of malignant brain tumors to current therapies and persistent recurrence. The present study provides a panoramic view of innovative therapies for malignant brain tumors, especially glioblastoma, aimed at achieving a tailored approach. *Methods:* PubMed/Medline and ClinicalTrials.gov were the main sources of an extensive literature review in which "Regenerative Medicine," "Cell-Based Therapy," "Chemotherapy," "Vaccine," "Cell Engineering," "Immunotherapy, Active," "Immunotherapy, Adoptive," "Stem Cells," "Gene Therapy," "Target Therapy," "Brain Cancer," "Glioblastoma," and "Malignant Brain Tumor" were the search terms. Only articles in English published in the last 5 years were included. A further selection was made according to the quality of the studies and level of evidence. *Results:* Cell-based and targeted therapies represent the newest frontiers of brain cancer treatment. Active and adoptive immunotherapies, stem cell therapies, and gene therapies represent a tremendous evolution in recent years due to many preclinical and clinical studies. Clinical trials have validated the effectiveness of antibody-based immunotherapies, including an in-depth study of bevacizumab, in combination with standard of care. Preclinical data highlights the role of vaccines, stem cells, and gene therapies to prevent recurrence. *Conclusion:* Monoclonal antibodies strengthen the first-line therapy for high grade gliomas. Vaccines, engineered cells, stem cells, and gene and targeted therapies are good candidates for second-line treatment of both newly diagnosed and recurrent gliomas. Further data are necessary to validate this tailored approach at the bedside. (www.actabiomedica.it)

Keywords: Cell-based Therapy; Glioblastoma; Immunotherapy Malignant Brain Tumor, Target Therapy.

Background

Treatment of malignant brain tumors remains one of the greatest challenges in oncology.

Glioblastoma (GBM) represents 60%–75% of primary malignant brain tumors[87] and has an annual incidence rate of 3–4 cases/100,000 people each year[18,56].

Despite primary multimodal management with gross total surgical resection followed by chemoradiotherapy, GBM still has a dismal prognosis with a median survival of 12–14 months and a 5-year overall survival rate of less than 10%[80,79].

The relative lack of success of treatment revealed the necessity for innovative techniques. GBM therapy resistance is attributable to high rates of cell growth and angiogenesis, intrinsic heterogeneity, the presence of glioma stem cells, and many molecular mechanisms associated with anomalous signaling pathways that recognize and adapt to ongoing threats[25,3,72].

Progress in genetic studies, identification of molecular abnormalities, and advances in regenerative

medicine offer new insights for the development of new therapeutic strategies tailored to specific molecular targets in different pediatric and adulthood central nervous system (CNS) pathologies[61,75,21,23,55,60,73].

Regenerative medicine is a broad field that encompasses a range of bioengineering approaches and advanced therapy medicinal products; among these, cell-based therapy is one of the most attractive therapeutic platforms[53,44].

The aim of this study was to summarize innovative therapies for malignant brain tumors. The most recent advances in chemotherapy (i.e., targeted molecular agents, virotherapy, engineered cells, and stem cell-based and gene therapies) are discussed in detail, also focusing on the future challenges of a tailored approach.

Methods

A comprehensive literature review was conducted using PubMed/Medline search engine with combinations of Medical Subject Heading (MeSH) terms and text words.

The MeSH terms "Regenerative Medicine," "Cell-Based Therapy," "Chemotherapy," "Vaccine," "Cell Engineering," "Immunotherapy, Active," "Immunotherapy, Adoptive," "Stem Cells," "Gene Therapy," and "Target Therapy" were used. They were combined with further MeSH terms: "Brain Cancer," "Glioblastoma," and "Malignant brain tumor."

Our research included articles for a historical review of CNS tumor therapy and then focused on articles on novel therapeutic approaches and emerging techniques. The results were further filtered based on their titles and abstracts to sort the most relevant articles, and a descriptive analysis was performed.

The limits used included a publication period of 2015–2020 and articles published in the English language or translated to English and pertinent to neuro-oncology.

Results

Cell-based therapies

Cell-based therapies represent a new frontier for the treatment of malignant CNS tumors. This new therapeutic approach has been tested in many clinical trials and has demonstrated its enormous validity in combination with conventional surgery and radiotherapy (RT). Advanced cell-based therapies are categorized according to the type of medicinal product involved. This technology-based classification for treatment of GBM includes the somatic cell, gene modification, and genome editing[53].

1 Somatic cell therapies

This approach involves propagated or differentiated human cells that were autologous, allogenic or xenogenic[45], purified, and administered for therapeutic purposes. Somatic cell technologies include two forms of treatment: immunotherapy and stem cell-based therapy[53].

1.1 Immunotherapies

The rationale for the use of immunotherapy to treat malignant brain tumors is supported by evidence of a better prognosis together with a high level of expression of tumor-infiltrating lymphocytes and CD8+ and CD4+ T helper and regulatory T cells (Tregs)[52]. Immunotherapy is active (checkpoint inhibitors and vaccines) or adoptive (engineered T or NK cells)[53].

1.1.1 Active immunotherapies

1.1.1.1 Checkpoint inhibitors

Checkpoint inhibitors are at the forefront of the immunotherapy revolution, with real survival benefits in multiple solid tumors. They are categorized as chemotherapy drugs, which carry out their function in specific stages of the cell cycle, or antibody-based therapies.

Alkylating agents

First-line treatment is based on temolozomide (TMZ, Temodar®), an oral alkylating agent with 100% bioavailability and the ability to cross the blood-brain barrier due to its lipophilic properties. TMZ modifies DNA or RNA via alkylation of guanine and adenine and causes mismatched base pair, G2 phase arrest, and cell apoptosis. The activity of TMZ closely depends on DNA

repair programs, such as O6-methylguanine-DNA methyltransferase (MGMT), a demethylating enzyme. MGMT expression influences the efficacy of TMZ, and methylation of the MGMT gene, which is located on chromosome 10q26, is a strong predictor of tumor sensitivity and better outcomes after treatment[93,59,32].

The major limit of TMZ is rapid in vivo hydrolytic degradation, which requires frequent and massive doses, increasing the risk of potential adverse effects. Several recent studies tested the possibility of combining conjugate TMZ with polymer scaffold molecules to prevent its rapid clearance and improve tumor uptake and antitumor activity. In 2015, Fang et al. demonstrated that the conjugation of TMZ with copolymers (a polyethylene glycol-chitosan graft) increased the TMZ half-life and incorporation into tumor-targeting cells[20].

For patients with evidence of tumor progression after first-line treatment, second-line treatment includes a TMZ re-challenge or PCV regimen, which consists of procarbazine, lomustine (1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea; CCNU), and vincristine. Despite the approval of these therapies for recurrence, there are insufficient clinical trials demonstrating sufficient therapeutic effectiveness[76].

Many phase III clinical trials have also demonstrated the efficacy of 1,3-bis(2-dichloroethyl)-1-nitrosourea (BCNU, carmustine, Gliadel®) wafers, a biodegradable polymer containing a chemotherapeutic agent, implanted during surgery at the tumor site to locally provide a therapeutic dose of BCNU. This technique, combined with RT and systemic TMZ, increases survival by 8 weeks[2,54].

Antibody-based therapies

Antibody-based therapies aim to overcome the ability of GBM to escape the immune response, remaining effective against the tumor.

The therapy is based on human monoclonal antibodies (MAbs) that directly target specific molecular ligands to interrupt aberrant cellular pathways and activate the antitumor immune cascade.

A milestone agent in this group is bevacizumab (Avastin®), a MAb that targets vascular endothelial growth factor A (VEGF-A), which blocks the action of VEGF and inhibits angiogenesis, counteracting tumor growth. Bevacizumab has been tested in some clinical trials, and it is currently approved in addition to RT and adjuvant TMZ for recurrent disease, showing significant improvements in survival[17,36] (https://www.clinicaltrials.gov, #NCT00501891).

Concurrent use of irinotecan, a chemotherapeutic agent that inhibits topoisomerase I, and bevacizumab has shown a synergistic effect in phase II trials with a 6-month increase in survival[29].

The best studied immuno-targets include programmed cell death protein 1 (PD-1) and its ligand, PD-L1, cytotoxic T-lymphocyte antigen 4 (CTLA-4), T cell immunoglobulin and mucin domain 3 (TIM-3), and indoleamine 2,3-dioxygenase-1 (IDO1).

The PD-1 receptor is expressed on activated immune cells, and their ligands, PD-L1 and PD-L2, are expressed on the surface of dendritic cells and macrophages. Physiologically, the PD-1/PD-L1 interaction promotes immune cell regulation, triggering the apoptosis of T cells and minimizing chronic autoimmune inflammation. PD-L1 overexpression on GBM cells with PD-1/PD-L1 upregulation is a system of immunity evasion in the tumor microenvironment as negative feedback for T cells to inhibit the activity of cytotoxic CD8+ lymphocytes[91,10].

Nivolumab, a human IgG4 subtype targeting PD-1, has been tested for its safety and efficacy in phase II and III clinical trials and was also combined with bevacizumab (https://www.clinicaltrials.gov, #NCT03890952). In addition, many anti-PD-1 antibodies (pembrolizumab and cemiplimab) and anti-PD-L1 agents (atezolizumab[43], avelumab, and durvalumab[4]) have also been approved for GBM.

CTLA-4 is an inhibitory receptor on the surface of T cells that binds the CD80 and CD86 ligands on antigen cells to downregulate T cell activity. Ipilimumab, a human monoclonal IgG1 antibody for CTLA-4, is the standard therapy for metastatic melanoma and is used in combination with PD-1 inhibitors and other immunotherapies for recurrent GBM[41,57].

Recent studies have investigated the development of antibodies against TIM-3[13,33], a surface receptor expressed on CD4+ and CD8+ T cells, and IDO1, an intracellular enzyme, which are both involved in T cell exhaustion in GBM[66,95] .

Several MAbs, such as anti-EGFR agents (cetuximab and nimotuzumab) remain under investigation[27,84].

Vaccines

The addition of standard anticancer vaccine therapy has greatly improved long-term survival in patients with GBM. Numerous phase I to phase III trials of vaccines against glioblastoma are being conducted.

The most relevant target is epidermal growth factor receptor variant III (EGFRvIII)[15]. The EGFRvIII peptide vaccine, rindopepimut (Rintega®), was tested in phase III clinical trials, which showed its effectiveness in combination with standard chemotherapy (https://www.clinicaltrials.gov, #NCT01480479).

A double-blind, randomized phase III trial tested the efficacy of rindopepimut for bevacizumab-resistant patients with recurrent GBM[86].

Another peptide vaccine was studied in a phase II clinical trial, which targeted human leukocyte antigen (HLA)-restricted Wilms tumor 1 (WT1) in patients with recurrent GBM[31].

A dendritic cell vaccine (DCVax-Brain) was approved in Switzerland for the treatment of GBM. It is composed of dendritic cells with purified tumor-specific antigens or tumor cell extracts[64,65]. Experimental studies on the administration of this vaccine for newly diagnosed and recurrent GBM remain ongoing, and some of these trials have demonstrated an increase in vaccine effectiveness if boosted with the tetanus/diphtheria toxoid vaccine[51].

Another ongoing phase II clinical trial is testing the Personalized Cellular Vaccine for Recurrent Glioblastoma (PerCellVac2), which employs autologous tumor cells combined with allogeneic peripheral blood mononuclear cells as antigens (https://www.clinicaltrials.gov, #NCT02808364)

Heat shock proteins (HSPs) were designed as vehicles to present tumor antigens for a personalized peptide polyvalent vaccine, which was obtained by purifying HSP-96 protein complexes from patients' tumors, showing promising results in recurrent GBM[8].

1.1.1 Adoptive immunotherapies

Adoptive immunotherapies are truly a cell-based strategy and consist of engineered T cells, natural killer (NK) cells, and natural killer T (NKT) cells.

1.1.1.1 Engineered T cells

Therapeutic application of engineered T cells includes chimeric antigen receptor (CAR) T cell and the T cell receptor (TCR) transgenic T cell therapies.

Autologous or allogenic CAR T cells are obtained from the blood, integrated with the CAR gene by retrovirus or lentivirus vectors, induced to replicate with interleukin 2, and then transplanted. These engineered CAR T cells expose the chimeric receptor, which selectively binds molecules expressed by neoplastic cells, promoting destruction[26]. CAR genes tested for glioblastoma therapy mainly target EGFRvIII[39,58], which is a growth signal for adjacent tumor cells; human epidermal growth factor receptor 2 (HER2) [1,28]; and erythropoietin-producing hepatocellular carcinoma A2 (EphA2)[11].

TCRs are expressed on the surface of human T cells and commonly bind the major histocompatibility complex (MHC), which has an antigenic function on infected human cells and, thus, allows activation of the immune system. The TCR is composed of an alpha (α) and a beta (β) chain, which are isolated, mutated, integrated into a viral genome for replication, and inserted into patients' T cells. Therefore, TCR transgenic T cells are potentially suitable for directly activating the immune response against tumor cells.

1.1.1.1 NK cells

NK cells have a small therapeutic role in GBM because of the excessive expression of MHC class I molecules and HLA ligands on cancer cells, which bind inhibitory NK cells and killer immunoglobulin-like receptors (KIRs), negating NK cell activity[35].

Several studies have reported the use of allogenic NK cells, which cannot be recognized or inactivated by the MHC I or HLA of tumor cells, and the use of antibodies for KIRs with the aim of increasing the effect of NK cells. Another effective therapy is the use of specific NK receptors, which cause tumor cell apoptosis when activated. Navarro et al. tested the transplantation of autologous NK cells expressing KIR2DS2 receptors as potent tumor killers[24]. In addition, Yvon et al. studied the role of TGF-β in the inhibition of expression of NK activating receptors, such as

NKG2D[94]. They investigated the dominant negative TGF-β receptor II (DNRII) on cord blood NK cells and evaluated their ability to kill glioblastoma cells via retroviral transduction[94].

In addition, a new type of CAR (CAR-KHYG-1) targeting EGFRvIII and capable of inhibiting cell-growth and apoptosis has been developed.

1.1.1.1 NKT cells

Invariant NKT cells are characterized by the co-expression of T and NK cell markers. The activation of these cells in culture with autologous mature DCs pulsed with a synthetic glycolipid α-galactosyl ceramide can be used to enhance NKT cell cytotoxic activity against GBM[16].

Several studies have reported the role of miR-92a in the development of cancer tolerance against NKT cells via the production of an immune tolerant IL-6+ IL-10+ NKT cell phenotype and inhibition of CD8+ T cells[81].

1.1.1.1 Hybrid NKT cell therapy

The Autologous Lymphoid Effector Cells Specific Against Tumor cells (ALECSAT) technology was proposed by CytoVac A/S (Hørsholm, Denmark) to treat many solid tumors. This treatment takes 26 days and involves the transplantation of autologous T and NK lymphocytes, which are activated ex vivo. Autologous lymphocytes and monocytes are isolated from the blood, and the latter are induced to differentiate into dendritic cells (DC). DCs and lymphocytes are cultured and generate activated T helper (Th) cells, which are treated with 5-aza-2'-deoxycytidine, a DNA-demethylation agent, to express cancer/testis antigens (CTAs). The CTA-expressing activated Th cells stimulate non-activated lymphocytes, and ultimately, CD8+ cytotoxic lymphocytes (CTLs) are obtained. Cancer cells that do not express the antigen are destroyed by activated NK cells[89].

1.1 Stem Cell–Based Therapies

Stem cells are immature undifferentiated cells, which are found in every human tissue, with self-renewal capacity and the ability to repair and control the tissue's functions.

In the nervous system, neural stem cells (h-NSCs) have been identified to be responsible for the regeneration and differentiation of neurons and glial cells, and they are involved in tumor responses[49,12].

In 2004, Staflin et al. reported a study on the antitumor activity of h-NSCs expressed by the intense production of TGF-β[77]. The h-NSCs can also be integrated via a viral genome, with genes codifying tumor necrosis factors or IL-12 and, due to their extreme migration capacity, can also be exploited as delivery vehicles to deliver materials to the tumor site. The extensive tumor tracking capability of NSCs and the tumoricidal potency of IL-12 are thought to render exceptional therapeutic benefits[50].

In the periphery surrounding GBM, there are glioma stem cells (GSCs), which have an enormous role in tumorigenicity and metastasis and high rates of recurrence after treatment as well as in the development of resistance to treatment.

GSCs express CD133 on their surface, and a novel therapeutic strategy is to selectively target this marker using lentiviral vectors (CD133-LV)[5].

The revolutionary technique of Cell-Systematic Evolution of Ligands by Exponential Enrichment (Cell-SELEX) leverages selective aptamers that bind to and are internalized by GSCs, leading to destruction of the GSCs[34].

Gene Therapies

Gene modification technology directly introduces genetic material carried by viral vectors into human cells, inducing in vivo infection. Ongoing phase I, II, and III trials employ adenoviruses, retroviruses, and lentiviruses as carriers to introduce vectors into human genes that codify therapeutic factors or enzymes.

The most useful technique exploits the insertion of the thymidine kinase (TK) gene via the herpes simplex virus (HSV) into the GBM cell genome. This action has an immediate consequence of superficial expression of HSV-TK, an optimal target for antiviral drugs (acyclovir, ganciclovir, and valacyclovir) (https://www.clinicaltrials.gov, #NCT00002824). The results of this

novel approach (i.e., suicide gene therapy) were shown by a randomized phase III trial with the application of adenovirus-mediated gene therapy and HSV-TK in patients with newly diagnosed glioblastoma after resection[7,30,82].

Adenovirus vectors are used to inject the p53 gene into GBM cells to replace the normal p53 pathway[40]. Another example of virotherapy is the use of poliovirus (PVSRIPO), as shown in a phase I clinical trial, which replicates and selectively destroys tumor cells and spares healthy tissue[42].

Genome Editing Therapies

This approach is based on wider DNA manipulation with the use of nucleases, which can modify and regulate genomic loci to achieve therapeutic effects. Meganucleases, zinc finger nucleases (ZFNs), and transcription activator-like effector nucleases (TALENs) are the most commonly adopted nucleases.

ZFNs and TALENs are enzymes with two domains: one destined for DNA-binding and the other for DNA-cleavage[92]. They can be delivered to tumor cells via plasmids or ex vivo, and selectively modify target genes and introduce exogenous DNA for therapeutic purposes.

One of the most advanced genome editing therapies adopted is the (CRISPR)/Cas9 system, which was originally identified in bacteria. The Cas9 nuclease protein functions as molecular scissors, cutting and altering the DNA itself, which induces specific genome changes. Cas9 programming is performed through specific guide RNAs to target specific genetic material represented by CRISPR sequences, with a much more specific and effective action than other endonucleases[19,74].

Target Therapies

The most avant-garde and revolutionary therapeutic route against malignant CNS tumors is target therapy. This therapeutic strategy focuses on GBM intrinsic targets and pathways involved in tumorigenesis and cell growth maintenance.

Tyrosine kinase (TK) inhibitors

The most involved pathway is that of TKs, which are enzymes that regulate cellular processes, proliferation, differentiation, and oncogenesis. TKs phosphorylate the tyrosine residues of some receptors and intracellular proteins, activating a cascade of second messengers involved in many cellular mechanisms.

EGFR is one of the most important targets, since it is overexpressed in 40–60% of GBMs, and the typical mutation is EGFRvIII, resulting in increased cell proliferation and invasiveness.

The available EGFR TK inhibitors are gefitinib and erlotinib, which are currently administered as monotherapy or combined with TMZ and provide minimal benefit for GBM treatment[70,37,63,68].

Platelet-derived growth factor receptors (PDGFR) are also aberrantly overexpressed and activated in GBM, stimulating tumor growth and angiogenesis. Imatinib is a TK inhibitor of the PDGFR that was tested in a phase II trial showing no significant benefits (https://www.clinicaltrials.gov, #NCT00049127).

Mammalian target of rapamycin (mTOR) is an intracellular protein kinase involved in cell growth signaling through the PI3K/AKT/mTOR pathway, normally implicated in the pathogenesis of high-grade gliomas. Many clinical trials on recurrent GBM tested mTOR inhibitors (sirolimus, temsirolimus, and everolimus) and a PI3K inhibitor (buparlisib) and demonstrated these agents to be inactive, with unfavorable toxicity and low tolerance in patients[68,90,88].

In addition, TK inhibitors directed against mesenchymal–epithelial transition (MET), the fibroblast growth factor receptor (FGFR), BRAF mutants (V600E), and the Ras–MAPK pathway, which are involved in glioma cell growth, spreading and apoptosis, are under consideration.

p53 Replacement

The p53/ARF/MDM2 pathway is aberrant in 84% of GBM cases. The mutation of p53 is a gain of function mutation that deregulates cell proliferation and apoptosis. A revolutionary strategy is PRIMA-1 (2, 2-bis(hydroxymethyl)-3-quinuclidinone), which is a small molecular weight compound capable of

restoring sequence-specific DNA binding to the active conformation of p53 proteins, the normal function of p53, and tumor cell apoptosis. The applicability of PRIMA-1 in clinical practice remains under investigation[85,9,62].

Discussion

GBM is the most aggressive CNS tumor and has a poor prognosis, high recurrence rate, and high mortality rate. The standard of care provides gross total surgical resection, followed by a regimen of concomitant/adjuvant TMZ combined with RT.

Surgery remains the mainstay of treatment; refinements in neurosurgical preoperative planning and intraoperative imaging, such as neuronavigation, and image-guided surgery, such as fluorescein- or 5-aminolevulinic acid (5-ALA)-based intraoperative magnetic resonance imaging (MRI), have helped to define tumor margins and maximize the extent of resection[78].

Several clinical trials demonstrated that maximum surgical resection (i.e., at least 95% of the contrast-enhancing tumor mass) improves progression-free survival at 6 months compared to subtotal resection[38,71].

In 2005, Stupp et al. designed a standard chemoradiotherapy protocol based on the results of a phase III study conducted in 85 centers with over 573 patients with GBM. They compared the results of treatment with only RT and RT plus 6 cycles of concurrent TMZ, and the 5-year survival rates were 1.9% and 9.8%, respectively. The current protocol, which was based on a revised study by Stupp et al. in 2009, includes surgery followed by RT within 6–7 weeks (total dose of 56–60 Gy in 30 fractions over 6 weeks) with concomitant TMZ at 75 mg/m^2 and maintenance with 6 cycles of TMZ for 28 days (150 and 200 mg/m^2, respectively) (15758009; 19269895).

Despite the aggressive combined approach, patients with GBM invariably relapse, with a median progression-free survival of 10 weeks and overall survival of 30 weeks.

Advances in genomic profiling, with the detection of molecular abnormalities underlying a malignant phenotype of GBM, and the biotechnological revolution in medicine, involving neuro-oncology and other fields[69,14,22,46,48,47], have paved the way to new therapeutic prospects, personalized treatments, and novel drugs that specifically target tumor cells.

Applications of biotechnology, and specifically cell-based therapy, have allowed the use of strategies based on somatic cells, immunotherapies, staminal cells, and genome manipulation technologies.

Immunotherapies have led to an essential breakthrough in the management of high-grade gliomas. The goal of this approach is to achieve synergy between the increase in the immune response and the simultaneous inhibition of the tumor's immunosuppressive mechanisms. Checkpoint inhibitors and MAbs are mainly administered together with RT, as this combination modulates the tumor microenvironment in favor of immune stimulation and recruitment of immune cells. In addition, vaccination strategies with the choice of an appropriate target, combined with immunomodulators, is a promising lead for more durable responses in patients with GBM.

Adoptive immunotherapy is part of a broad expansion in immuno-oncology. The administered engineered T and NK cells allow bypass of antigen presentation and stimulation of a primary immune response, directly targeting specific antigens through CARs. The focal point of therapy is the development of new CARs designed to bind selective and appropriate cell surface antigens.

Among somatic cell technologies, the stem cell-based approach is also used. This approach involves autologous cells, free from immunological risk, and their intrinsic homing property makes them specific and selective for the target tissue. In addition, agents that selectively target GSCs, responsible for tumor cell renewal and recurrence after initial treatment, can theoretically revolutionize GBM management, significantly increasing progression-free and overall survival.

The main limitations of somatic cell-based therapies are the loss of their biological activity[83] and the development of adaptive solutions by the tumor through mechanisms of immune tolerance and immunophenotypic adaptations.

Gene therapy allows modification of the tumor cell genome via viral vectors. The main challenges of

this approach are the identification of target gene promotors and the choice of the most suitable viral carrier, which should have transportation, diffusion, and replication capabilities.

Lastly, the concept of target therapy dramatically changed the approach to oncological diseases, providing agents that targeted tumor-specific features, such as altered cellular signaling pathways, aberrant vascularization, and the tumor microenvironment[67,6]. In the management of malignant CNS tumors, TK inhibitors are mostly being developed to interrupt intracellular expansion and proliferation signals.

A common limitation for all these therapeutic strategies is the blood-brain barrier, which reduces the effective penetration of drugs into the tumor site. The locoregional administration of antitumor agents and innovative strategies as nanostructures employed to carry drugs can concretely improve the administration route and make the therapy more effective.

Conclusion

MAbs, primarily bevacizumab, are pivotal first-line innovative immunotherapies for high grade gliomas.

Vaccines, engineered cells, and stem cell-based and gene therapies are potential valuable options to be adopted as second-line therapies for recurrence.

Genomic profiling is essential for choosing the most suitable approach and implementing tailored and target therapy.

The effectiveness of these personalized approaches is currently being validated in ongoing clinical trials.

Conflict of interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article

References

1. Ahmed N, Salsman VS, Kew Y, Shaffer D, Powell S, Zhang YJ, Grossman RG, Heslop HE, Gottschalk S (2010) HER2-specific T cells target primary glioblastoma stem cells and induce regression of autologous experimental tumors. Clin Cancer Res 16:474–485. doi:10.1158/1078-0432.CCR-09-1322
2. Attenello FJ, Mukherjee D, Datoo G, McGirt MJ, Bohan E, Weingart JD, Olivi A, Quinones-Hinojosa A, Brem H (2008) Use of Gliadel (BCNU) wafer in the surgical treatment of malignant glioma: a 10-year institutional experience. Ann Surg Oncol 15:2887–2893. doi:10.1245/s10434-008-0048-2
3. Auffinger B, Spencer D, Pytel P, Ahmed AU, Lesniak MS (2015) The role of glioma stem cells in chemotherapy resistance and glioblastoma multiforme recurrence. Expert Rev Neurother 15:741–752. doi:10.1586/14737175.2015.1051968
4. Baldini C, Romano PM, Varga A, Champiat S, Dumont S, Dhermain F, Louvel G, Marabelle A, Postel-Vinay S, Angevin E, Gazzah A, Ribrag V, Bahleda R, Michot JM, Hollebecque A, Soria JC, Massard C (2018). Bull Cancer 105 Suppl 1:S59-S67. doi:10.1016/S0007-4551(18)30391-6
5. Bayin NS, Modrek AS, Dietrich A, Lebowitz J, Abel T, Song HR, Schober M, Zagzag D, Buchholz CJ, Chao MV, Placantonakis DG (2014) Selective lentiviral gene delivery to CD133-expressing human glioblastoma stem cells. PLoS One 9:e116114. doi:10.1371/journal.pone.0116114
6. Bellantoni G, Guerrini F, Del Maestro M, Galzio R, Luzzi S (2019) Simple schwannomatosis or an incomplete Coffin-Siris? Report of a particular case. eNeurologicalSci 14: 31–33. doi:10.1016/j.ensci.2018.11.021
7. Black ME, Newcomb TG, Wilson HM, Loeb LA (1996) Creation of drug-specific herpes simplex virus type 1 thymidine kinase mutants for gene therapy. Proc Natl Acad Sci U S A 93:3525–3529. doi:10.1073/pnas.93.8.3525
8. Bloch O, Crane CA, Fuks Y, Kaur R, Aghi MK, Berger MS, Butowski NA, Chang SM, Clarke JL, McDermott MW, Prados MD, Sloan AE, Bruce JN, Parsa AT (2014) Heat-shock protein peptide complex-96 vaccination for recurrent glioblastoma: a phase II, single-arm trial. Neuro Oncol 16:274–279. doi:10.1093/neuonc/not203
9. Bykov VJ, Zache N, Stridh H, Westman J, Bergman J, Selivanova G, Wiman KG (2005) PRIMA-1(MET) synergizes with cisplatin to induce tumor cell apoptosis. Oncogene 24:3484–3491. doi:10.1038/sj.onc.1208419
10. Chen L, Han X (2015) Anti-PD-1/PD-L1 therapy of human cancer: past, present, and future. J Clin Invest 125:3384–3391. doi:10.1172/JCI80011
11. Chow KK, Naik S, Kakarla S, Brawley VS, Shaffer DR, Yi Z, Rainusso N, Wu MF, Liu H, Kew Y, Grossman RG, Powell S, Lee D, Ahmed N, Gottschalk S (2013) T cells redirected to EphA2 for the immunotherapy of glioblastoma. Mol Ther 21:629–637. doi:10.1038/mt.2012.210
12. Daniela F, Vescovi AL, Bottai D (2007) The stem cells as a potential treatment for neurodegeneration. Methods Mol Biol 399:199–213. doi:10.1007/978-1-59745-504-6_14
13. Das M, Zhu C, Kuchroo VK (2017) Tim-3 and its role in regulating anti-tumor immunity. Immunol Rev 276:97–111. doi:10.1111/imr.12520

14. Del Maestro M, Luzzi S, Gallieni M, Trovarelli D, Giordano AV, Gallucci M, Ricci A, Galzio R (2018) Surgical Treatment of Arteriovenous Malformations: Role of Preoperative Staged Embolization. Acta Neurochir Suppl 129:109–113. doi:10.1007/978-3-319-73739-3_16
15. Del Vecchio CA, Li G, Wong AJ (2012) Targeting EGF receptor variant III: tumor-specific peptide vaccination for malignant gliomas. Expert Rev Vaccines 11:133–144. doi:10.1586/erv.11.177
16. Dhodapkar KM, Cirignano B, Chamian F, Zagzag D, Miller DC, Finlay JL, Steinman RM (2004) Invariant natural killer T cells are preserved in patients with glioma and exhibit antitumor lytic activity following dendritic cell-mediated expansion. Int J Cancer 109:893–899. doi:10.1002/ijc.20050
17. Diaz RJ, Ali S, Qadir MG, De La Fuente MI, Ivan ME, Komotar RJ (2017) The role of bevacizumab in the treatment of glioblastoma. J Neurooncol 133:455–467. doi:10.1007/s11060-017-2477-x
18. Dolecek TA, Propp JM, Stroup NE, Kruchko C (2012) CBTRUS statistical report: primary brain and central nervous system tumors diagnosed in the United States in 2005-2009. Neuro Oncol 14 Suppl 5:v1-49. doi:10.1093/neuonc/nos218
19. Doudna JA, Charpentier E (2014) Genome editing. The new frontier of genome engineering with CRISPR-Cas9. Science 346:1258096. doi:10.1126/science.1258096
20. Fang C, Wang K, Stephen ZR, Mu Q, Kievit FM, Chiu DT, Press OW, Zhang M (2015) Temozolomide nanoparticles for targeted glioblastoma therapy. ACS Appl Mater Interfaces 7:6674–6682. doi:10.1021/am5092165
21. Foiadelli T, Piccorossi A, Sacchi L, De Amici M, Tucci M, Brambilla I, Marseglia GL, Savasta S, Verrotti A (2018) Clinical characteristics of headache in Italian adolescents aged 11-16 years: a cross-sectional questionnaire school-based study. Ital J Pediatr 44:44. doi:10.1186/s13052-018-0486-9
22. Gallieni M, Del Maestro M, Luzzi S, Trovarelli D, Ricci A, Galzio R (2018) Endoscope-Assisted Microneurosurgery for Intracranial Aneurysms: Operative Technique, Reliability, and Feasibility Based on 14 Years of Personal Experience. Acta Neurochir Suppl 129:19–24. doi:10.1007/978-3-319-73739-3_3
23. Garone G, Reale A, Vanacore N, Parisi P, Bondone C, Suppiej A, Brisca G, Calistri L, Cordelli DM, Savasta S, Grosso S, Midulla F, Falsaperla R, Verrotti A, Bozzola E, Vassia C, Da Dalt L, Maggiore R, Masi S, Maltoni L, Foiadelli T, Rossetti A, Greco C, Marino S, Di Paolantonio C, Papetti L, Urbino AF, Rossi R, Raucci U (2019) Acute ataxia in paediatric emergency departments: a multicentre Italian study. Arch Dis Child 104:768–774. doi:10.1136/archdischild-2018-315487
24. Gras Navarro A, Kmiecik J, Leiss L, Zelkowski M, Engelsen A, Bruserud O, Zimmer J, Enger PO, Chekenya M (2014) NK cells with KIR2DS2 immunogenotype have a functional activation advantage to efficiently kill glioblastoma and prolong animal survival. J Immunol 193:6192–6206. doi:10.4049/jimmunol.1400859
25. Hardee ME, Zagzag D (2012) Mechanisms of glioma-associated neovascularization. Am J Pathol 181:1126–1141. doi:10.1016/j.ajpath.2012.06.030
26. Hartmann J, Schussler-Lenz M, Bondanza A, Buchholz CJ (2017) Clinical development of CAR T cells-challenges and opportunities in translating innovative treatment concepts. EMBO Mol Med 9:1183–1197. doi:10.15252/emmm.201607485
27. Hasselbalch B, Lassen U, Hansen S, Holmberg M, Sorensen M, Kosteljanetz M, Broholm H, Stockhausen MT, Poulsen HS (2010) Cetuximab, bevacizumab, and irinotecan for patients with primary glioblastoma and progression after radiation therapy and temozolomide: a phase II trial. Neuro Oncol 12:508–516. doi:10.1093/neuonc/nop063
28. Hegde M, Corder A, Chow KK, Mukherjee M, Ashoori A, Kew Y, Zhang YJ, Baskin DS, Merchant FA, Brawley VS, Byrd TT, Krebs S, Wu MF, Liu H, Heslop HE, Gottschalk S, Yvon E, Ahmed N (2013) Combinational targeting offsets antigen escape and enhances effector functions of adoptively transferred T cells in glioblastoma. Mol Ther 21:2087–2101. doi:10.1038/mt.2013.185
29. Hofland KF, Hansen S, Sorensen M, Engelholm S, Schultz HP, Muhic A, Grunnet K, Ask A, Costa JC, Kristiansen C, Thomsen C, Poulsen HS, Lassen U (2014) Neoadjuvant bevacizumab and irinotecan versus bevacizumab and temozolomide followed by concomitant chemoradiotherapy in newly diagnosed glioblastoma multiforme: A randomized phase II study. Acta Oncol 53:939–944. doi:10.3109/0284186X.2013.879607
30. Izquierdo M, Martin V, de Felipe P, Izquierdo JM, Perez-Higueras A, Cortes ML, Paz JF, Isla A, Blazquez MG (1996) Human malignant brain tumor response to herpes simplex thymidine kinase (HSVtk)/ganciclovir gene therapy. Gene Ther 3:491–495
31. Izumoto S, Tsuboi A, Oka Y, Suzuki T, Hashiba T, Kagawa N, Hashimoto N, Maruno M, Elisseeva OA, Shirakata T, Kawakami M, Oji Y, Nishida S, Ohno S, Kawase I, Hatazawa J, Nakatsuka S, Aozasa K, Morita S, Sakamoto J, Sugiyama H, Yoshimine T (2008) Phase II clinical trial of Wilms tumor 1 peptide vaccination for patients with recurrent glioblastoma multiforme. J Neurosurg 108:963–971. doi:10.3171/JNS/2008/108/5/0963
32. Jiang X, Reardon DA, Desjardins A, Vredenburgh JJ, Quinn JA, Austin AD, Herndon JE, 2nd, McLendon RE, Friedman HS (2013) O6-methylguanine-DNA methyltransferase (MGMT) immunohistochemistry as a predictor of resistance to temozolomide in primary CNS lymphoma. J Neurooncol 114:135–140. doi:10.1007/s11060-013-1162-y
33. Kim JE, Patel MA, Mangraviti A, Kim ES, Theodros D, Velarde E, Liu A, Sankey EW, Tam A, Xu H, Mathios D, Jackson CM, Harris-Bookman S, Garzon-Muvdi T, Sheu M, Martin AM, Tyler BM, Tran PT, Ye X, Olivi A, Taube JM, Burger PC, Drake CG, Brem H, Pardoll DM, Lim M (2017) Combination Therapy with Anti-PD-1,

Anti-TIM-3, and Focal Radiation Results in Regression of Murine Gliomas. Clin Cancer Res 23:124–136. doi:10.1158/1078-0432.CCR-15-1535
34. Kim Y, Wu Q, Hamerlik P, Hitomi M, Sloan AE, Barnett GH, Weil RJ, Leahy P, Hjelmeland AB, Rich JN (2013) Aptamer identification of brain tumor-initiating cells. Cancer Res 73:4923–4936. doi:10.1158/0008-5472.CAN-12-4556
35. Kmiecik J, Poli A, Brons NH, Waha A, Eide GE, Enger PO, Zimmer J, Chekenya M (2013) Elevated CD3+ and CD8+ tumor-infiltrating immune cells correlate with prolonged survival in glioblastoma patients despite integrated immunosuppressive mechanisms in the tumor microenvironment and at the systemic level. J Neuroimmunol 264: 71–83. doi:10.1016/j.jneuroim.2013.08.013
36. Koukourakis GV (2015) Bevacizumab for Malignant Brain Gliomas. Which is the Current Evidence? Recent Pat Inflamm Allergy Drug Discov 9:136–143. doi:10.2174/1872213x09666150807110031
37. Kreisl TN, Lassman AB, Mischel PS, Rosen N, Scher HI, Teruya-Feldstein J, Shaffer D, Lis E, Abrey LE (2009) A pilot study of everolimus and gefitinib in the treatment of recurrent glioblastoma (GBM). J Neurooncol 92:99–105. doi:10.1007/s11060-008-9741-z
38. Kuhnt D, Becker A, Ganslandt O, Bauer M, Buchfelder M, Nimsky C (2011) Correlation of the extent of tumor volume resection and patient survival in surgery of glioblastoma multiforme with high-field intraoperative MRI guidance. Neuro Oncol 13:1339–1348. doi:10.1093/neuonc/nor133
39. Kwatra MM (2017) A Rational Approach to Target the Epidermal Growth Factor Receptor in Glioblastoma. Curr Cancer Drug Targets 17:290–296. doi:10.2174/1568009616666161227091522
40. Lang FF, Bruner JM, Fuller GN, Aldape K, Prados MD, Chang S, Berger MS, McDermott MW, Kunwar SM, Junck LR, Chandler W, Zwiebel JA, Kaplan RS, Yung WK (2003) Phase I trial of adenovirus-mediated p53 gene therapy for recurrent glioma: biological and clinical results. J Clin Oncol 21:2508–2518. doi:10.1200/JCO.2003.21.13.2508
41. Larkin J, Chiarion-Sileni V, Gonzalez R, Grob JJ, Cowey CL, Lao CD, Schadendorf D, Dummer R, Smylie M, Rutkowski P, Ferrucci PF, Hill A, Wagstaff J, Carlino MS, Haanen JB, Maio M, Marquez-Rodas I, McArthur GA, Ascierto PA, Long GV, Callahan MK, Postow MA, Grossmann K, Sznol M, Dreno B, Bastholt L, Yang A, Rollin LM, Horak C, Hodi FS, Wolchok JD (2015) Combined Nivolumab and Ipilimumab or Monotherapy in Untreated Melanoma. N Engl J Med 373:23–34. doi:10.1056/NEJMoa1504030
42. Longo DL, Baden LR (2018) Exploiting Viruses to Treat Diseases. N Engl J Med 379:194–196. doi:10.1056/NEJMe1807181
43. Lukas RV, Rodon J, Becker K, Wong ET, Shih K, Touat M, Fasso M, Osborne S, Molinero L, O'Hear C, Grossman W, Baehring J (2018) Clinical activity and safety of atezolizumab in patients with recurrent glioblastoma. J Neurooncol 140:317–328. doi:10.1007/s11060-018-2955-9
44. Luzzi S, Crovace AM, Del Maestro M, Giotta Lucifero A, Elbabaa SK, Cinque B, Palumbo P, Lombardi F, Cimini A, Cifone MG, Crovace A, Galzio R (2019) The cell-based approach in neurosurgery: ongoing trends and future perspectives. Heliyon 5:e02818. doi:10.1016/j.heliyon.2019.e02818
45. Luzzi S, Crovace AM, Lacitignola L, Valentini V, Francioso E, Rossi G, Invernici G, Galzio RJ, Crovace A (2018) Engraftment, neuroglial transdifferentiation and behavioral recovery after complete spinal cord transection in rats. Surg Neurol Int 9:19. doi:10.4103/sni.sni_369_17
46. Luzzi S, Del Maestro M, Bongetta D, Zoia C, Giordano AV, Trovarelli D, Raysi Dehcordi S, Galzio RJ (2018) Onyx Embolization Before the Surgical Treatment of Grade III Spetzler-Martin Brain Arteriovenous Malformations: Single-Center Experience and Technical Nuances. World Neurosurg 116:e340-e353. doi:10.1016/j.wneu.2018.04.203
47. Luzzi S, Del Maestro M, Elia A, Vincitorio F, Di Perna G, Zenga F, Garbossa D, Elbabaa S, Galzio R (2019) Morphometric and Radiomorphometric Study of the Correlation Between the Foramen Magnum Region and the Anterior and Posterolateral Approaches to Ventral Intradural Lesions. Turk Neurosurg. doi:10.5137/1019-5149.JTN.26052-19.2
48. Luzzi S, Gallieni M, Del Maestro M, Trovarelli D, Ricci A, Galzio R (2018) Giant and Very Large Intracranial Aneurysms: Surgical Strategies and Special Issues. Acta Neurochir Suppl 129:25–31. doi:10.1007/978-3-319-73739-3_4
49. McKay R (1997) Stem cells in the central nervous system. Science 276:66–71. doi:10.1126/science.276.5309.66
50. Mercapide J, Rappa G, Anzanello F, King J, Fodstad O, Lorico A (2010) Primary gene-engineered neural stem/progenitor cells demonstrate tumor-selective migration and antitumor effects in glioma. Int J Cancer 126:1206-1215. doi:10.1002/ijc.24809
51. Mitchell DA, Batich KA, Gunn MD, Huang MN, Sanchez-Perez L, Nair SK, Congdon KL, Reap EA, Archer GE, Desjardins A, Friedman AH, Friedman HS, Herndon JE, 2nd, Coan A, McLendon RE, Reardon DA, Vredenburgh JJ, Bigner DD, Sampson JH (2015) Tetanus toxoid and CCL3 improve dendritic cell vaccines in mice and glioblastoma patients. Nature 519:366–369. doi:10.1038/nature14320
52. Mohme M, Neidert MC (2020) Tumor-Specific T Cell Activation in Malignant Brain Tumors. Front Immunol 11:205. doi:10.3389/fimmu.2020.00205
53. Mount NM, Ward SJ, Kefalas P, Hyllner J (2015) Cell-based therapy technology classifications and translational challenges. Philos Trans R Soc Lond B Biol Sci 370:20150017. doi:10.1098/rstb.2015.0017
54. Nagpal S (2012) The role of BCNU polymer wafers (Gliadel) in the treatment of malignant glioma. Neurosurg Clin N Am 23:289–295, ix. doi:10.1016/j.nec.2012.01.004

55. Nosadini M, Granata T, Matricardi S, Freri E, Ragona F, Papetti L, Suppiej A, Valeriani M, Sartori S, Italian Working Group on Paediatric Anti Nm-DaRE (2019) Relapse risk factors in anti-N-methyl-D-aspartate receptor encephalitis. Dev Med Child Neurol 61:1101-1107. doi:10.1111/dmcn.14267
56. Omuro A, DeAngelis LM (2013) Glioblastoma and other malignant gliomas: a clinical review. JAMA 310:1842–1850. doi:10.1001/jama.2013.280319
57. Omuro A, Vlahovic G, Lim M, Sahebjam S, Baehring J, Cloughesy T, Voloschin A, Ramkissoon SH, Ligon KL, Latek R, Zwirtes R, Strauss L, Paliwal P, Harbison CT, Reardon DA, Sampson JH (2018) Nivolumab with or without ipilimumab in patients with recurrent glioblastoma: results from exploratory phase I cohorts of CheckMate 143. Neuro Oncol 20:674–686. doi:10.1093/neuonc/nox208
58. Padfield E, Ellis HP, Kurian KM (2015) Current Therapeutic Advances Targeting EGFR and EGFRvIII in Glioblastoma. Front Oncol 5:5. doi:10.3389/fonc.2015.00005
59. Pandith AA, Qasim I, Zahoor W, Shah P, Bhat AR, Sanadhya D, Shah ZA, Naikoo NA (2018) Concordant association validates MGMT methylation and protein expression as favorable prognostic factors in glioma patients on alkylating chemotherapy (Temozolomide). Sci Rep 8:6704. doi:10.1038/s41598-018-25169-2
60. Parisi P, Vanacore N, Belcastro V, Carotenuto M, Del Giudice E, Mariani R, Papetti L, Pavone P, Savasta S, Striano P, Toldo I, Tozzi E, Verrotti A, Raucci U, Pediatric Headache Commission" of Societa Italiana di Neurologia P (2014) Clinical guidelines in pediatric headache: evaluation of quality using the AGREE II instrument. J Headache Pain 15:57. doi:10.1186/1129-2377-15-57
61. Pascual-Castroviejo I, Lopez-Pereira P, Savasta S, Lopez-Gutierrez JC, Lago CM, Cisternino M (2008) Neurofibromatosis type 1 with external genitalia involvement presentation of 4 patients. J Pediatr Surg 43:1998-2003. doi:10.1016/j.jpedsurg.2008.01.074
62. Patyka M, Sharifi Z, Petrecca K, Mansure J, Jean-Claude B, Sabri S (2016) Sensitivity to PRIMA-1MET is associated with decreased MGMT in human glioblastoma cells and glioblastoma stem cells irrespective of p53 status. Oncotarget 7:60245–60269. doi:10.18632/oncotarget.11197
63. Peereboom DM, Ahluwalia MS, Ye X, Supko JG, Hilderbrand SL, Phuphanich S, Nabors LB, Rosenfeld MR, Mikkelsen T, Grossman SA, New Approaches to Brain Tumor Therapy C (2013) NABTT 0502: a phase II and pharmacokinetic study of erlotinib and sorafenib for patients with progressive or recurrent glioblastoma multiforme. Neuro Oncol 15:490–496. doi:10.1093/neuonc/nos322
64. Phuphanich S, Wheeler CJ, Rudnick JD, Mazer M, Wang H, Nuno MA, Richardson JE, Fan X, Ji J, Chu RM, Bender JG, Hawkins ES, Patil CG, Black KL, Yu JS (2013) Phase I trial of a multi-epitope-pulsed dendritic cell vaccine for patients with newly diagnosed glioblastoma. Cancer Immunol Immunother 62:125–135. doi:10.1007/s00262-012-1319-0
65. Polyzoidis S, Ashkan K (2014) DCVax(R)-L--developed by Northwest Biotherapeutics. Hum Vaccin Immunother 10:3139–3145. doi:10.4161/hv.29276
66. Prendergast GC, Malachowski WP, DuHadaway JB, Muller AJ (2017) Discovery of IDO1 Inhibitors: From Bench to Bedside. Cancer Res 77:6795–6811. doi:10.1158/0008-5472.CAN-17-2285
67. Raysi Dehcordi S, Ricci A, Di Vitantonio H, De Paulis D, Luzzi S, Palumbo P, Cinque B, Tempesta D, Coletti G, Cipolloni G, Cifone MG, Galzio R (2017) Stemness Marker Detection in the Periphery of Glioblastoma and Ability of Glioblastoma to Generate Glioma Stem Cells: Clinical Correlations. World Neurosurg 105:895–905. doi:10.1016/j.wneu.2017.05.099
68. Reardon DA, Desjardins A, Vredenburgh JJ, Gururangan S, Friedman AH, Herndon JE, 2nd, Marcello J, Norfleet JA, McLendon RE, Sampson JH, Friedman HS (2010) Phase 2 trial of erlotinib plus sirolimus in adults with recurrent glioblastoma. J Neurooncol 96:219–230. doi:10.1007/s11060-009-9950-0
69. Ricci A, Di Vitantonio H, De Paulis D, Del Maestro M, Raysi SD, Murrone D, Luzzi S, Galzio RJ (2017) Cortical aneurysms of the middle cerebral artery: A review of the literature. Surg Neurol Int 8:117. doi:10.4103/sni.sni_50_17
70. Rich JN, Reardon DA, Peery T, Dowell JM, Quinn JA, Penne KL, Wikstrand CJ, Van Duyn LB, Dancey JE, McLendon RE, Kao JC, Stenzel TT, Ahmed Rasheed BK, Tourt-Uhlig SE, Herndon JE, 2nd, Vredenburgh JJ, Sampson JH, Friedman AH, Bigner DD, Friedman HS (2004) Phase II trial of gefitinib in recurrent glioblastoma. J Clin Oncol 22:133–142. doi:10.1200/JCO.2004.08.110
71. Roder C, Bisdas S, Ebner FH, Honegger J, Naegele T, Ernemann U, Tatagiba M (2014) Maximizing the extent of resection and survival benefit of patients in glioblastoma surgery: high-field iMRI versus conventional and 5-ALA-assisted surgery. Eur J Surg Oncol 40:297–304. doi:10.1016/j.ejso.2013.11.022
72. Roos A, Ding Z, Loftus JC, Tran NL (2017) Molecular and Microenvironmental Determinants of Glioma Stem-Like Cell Survival and Invasion. Front Oncol 7:120. doi:10.3389/fonc.2017.00120
73. Salpietro V, Mankad K, Kinali M, Adams A, Valenzise M, Tortorella G, Gitto E, Polizzi A, Chirico V, Nicita F, David E, Romeo AC, Squeri CA, Savasta S, Marseglia GL, Arrigo T, Johanson CE, Ruggieri M (2014) Pediatric idiopathic intracranial hypertension and the underlying endocrine-metabolic dysfunction: a pilot study. J Pediatr Endocrinol Metab 27:107–115. doi:10.1515/jpem-2013-0156
74. Sander JD, Joung JK (2014) CRISPR-Cas systems for editing, regulating and targeting genomes. Nat Biotechnol 32:347–355. doi:10.1038/nbt.2842
75. Savasta S, Chiapedi S, Perrini S, Tognato E, Corsano L, Chiara A (2008) Pai syndrome: a further report of a case with bifid nose, lipoma, and agenesis of the corpus callosum. Childs Nerv Syst 24:773–776. doi:10.1007/s00381-008-0613-9

76. Schmidt F, Fischer J, Herrlinger U, Dietz K, Dichgans J, Weller M (2006) PCV chemotherapy for recurrent glioblastoma. Neurology 66:587–589. doi:10.1212/01.wnl.0000197792.73656.c2
77. Staflin K, Honeth G, Kalliomaki S, Kjellman C, Edvardsen K, Lindvall M (2004) Neural progenitor cell lines inhibit rat tumor growth in vivo. Cancer Res 64:5347–5354. doi:10.1158/0008-5472.CAN-03-1246
78. Stummer W, Pichlmeier U, Meinel T, Wiestler OD, Zanella F, Reulen HJ, Group AL-GS (2006) Fluorescence-guided surgery with 5-aminolevulinic acid for resection of malignant glioma: a randomised controlled multicentre phase III trial. Lancet Oncol 7:392–401. doi:10.1016/S1470-2045(06)70665-9
79. Stupp R, Hegi ME, Mason WP, van den Bent MJ, Taphoorn MJ, Janzer RC, Ludwin SK, Allgeier A, Fisher B, Belanger K, Hau P, Brandes AA, Gijtenbeek J, Marosi C, Vecht CJ, Mokhtari K, Wesseling P, Villa S, Eisenhauer E, Gorlia T, Weller M, Lacombe D, Cairncross JG, Mirimanoff RO, European Organisation for R, Treatment of Cancer Brain T, Radiation Oncology G, National Cancer Institute of Canada Clinical Trials G (2009) Effects of radiotherapy with concomitant and adjuvant temozolomide versus radiotherapy alone on survival in glioblastoma in a randomised phase III study: 5-year analysis of the EORTC-NCIC trial. Lancet Oncol 10:459–466. doi:10.1016/S1470-2045(09)70025-7
80. Stupp R, Mason WP, van den Bent MJ, Weller M, Fisher B, Taphoorn MJ, Belanger K, Brandes AA, Marosi C, Bogdahn U, Curschmann J, Janzer RC, Ludwin SK, Gorlia T, Allgeier A, Lacombe D, Cairncross JG, Eisenhauer E, Mirimanoff RO, European Organisation for R, Treatment of Cancer Brain T, Radiotherapy G, National Cancer Institute of Canada Clinical Trials G (2005) Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. N Engl J Med 352:987–996. doi:10.1056/NEJMoa043330
81. Tang B, Wu W, Wei X, Li Y, Ren G, Fan W (2014) Activation of glioma cells generates immune tolerant NKT cells. J Biol Chem 289:34595–34600. doi:10.1074/jbc.M114.614503
82. Trask TW, Trask RP, Aguilar-Cordova E, Shine HD, Wyde PR, Goodman JC, Hamilton WJ, Rojas-Martinez A, Chen SH, Woo SL, Grossman RG (2000) Phase I study of adenoviral delivery of the HSV-tk gene and ganciclovir administration in patients with current malignant brain tumors. Mol Ther 1:195–203. doi:10.1006/mthe.2000.0030
83. Villa A, Navarro-Galve B, Bueno C, Franco S, Blasco MA, Martinez-Serrano A (2004) Long-term molecular and cellular stability of human neural stem cell lines. Exp Cell Res 294:559–570. doi:10.1016/j.yexcr.2003.11.025
84. Wang Y, Pan L, Sheng XF, Chen S, Dai JZ (2016) Nimotuzumab, a humanized monoclonal antibody specific for the EGFR, in combination with temozolomide and radiation therapy for newly diagnosed glioblastoma multiforme: First results in Chinese patients. Asia Pac J Clin Oncol 12:e23–29. doi:10.1111/ajco.12166
85. Weinmann L, Wischhusen J, Demma MJ, Naumann U, Roth P, Dasmahapatra B, Weller M (2008) A novel p53 rescue compound induces p53-dependent growth arrest and sensitises glioma cells to Apo2L/TRAIL-induced apoptosis. Cell Death Differ 15:718–729. doi:10.1038/sj.cdd.4402301
86. Weller M, Butowski N, Tran DD, Recht LD, Lim M, Hirte H, Ashby L, Mechtler L, Goldlust SA, Iwamoto F, Drappatz J, O'Rourke DM, Wong M, Hamilton MG, Finocchiaro G, Perry J, Wick W, Green J, He Y, Turner CD, Yellin MJ, Keler T, Davis TA, Stupp R, Sampson JH, investigators AIt (2017) Rindopepimut with temozolomide for patients with newly diagnosed, EGFRvIII-expressing glioblastoma (ACT IV): a randomised, double-blind, international phase 3 trial. Lancet Oncol 18:1373–1385. doi:10.1016/S1470–2045(17)30517-X
87. Wen PY, Kesari S (2008) Malignant gliomas in adults. N Engl J Med 359:492–507. doi:10.1056/NEJMra0708126
88. Wen PY, Touat M, Alexander BM, Mellinghoff IK, Ramkissoon S, McCluskey CS, Pelton K, Haidar S, Basu SS, Gaffey SC, Brown LE, Martinez-Ledesma JE, Wu S, Kim J, Wei W, Park MA, Huse JT, Kuhn JG, Rinne ML, Colman H, Agar NYR, Omuro AM, DeAngelis LM, Gilbert MR, de Groot JF, Cloughesy TF, Chi AS, Roberts TM, Zhao JJ, Lee EQ, Nayak L, Heath JR, Horky LL, Batchelor TT, Beroukhim R, Chang SM, Ligon AH, Dunn IF, Koul D, Young GS, Prados MD, Reardon DA, Yung WKA, Ligon KL (2019) Buparlisib in Patients With Recurrent Glioblastoma Harboring Phosphatidylinositol 3-Kinase Pathway Activation: An Open-Label, Multicenter, Multi-Arm, Phase II Trial. J Clin Oncol 37:741–750. doi:10.1200/JCO.18.01207
89. Wenger A, Werlenius K, Hallner A, Thoren FB, Farahmand D, Tisell M, Smits A, Rydenhag B, Jakola AS, Caren H (2018) Determinants for Effective ALECSAT Immunotherapy Treatment on Autologous Patient-Derived Glioblastoma Stem Cells. Neoplasia 20:25–31. doi:10.1016/j.neo.2017.10.006
90. Wick W, Gorlia T, Bady P, Platten M, van den Bent MJ, Taphoorn MJ, Steuve J, Brandes AA, Hamou MF, Wick A, Kosch M, Weller M, Stupp R, Roth P, Golfinopoulos V, Frenel JS, Campone M, Ricard D, Marosi C, Villa S, Weyerbrock A, Hopkins K, Homicsko K, Lhermitte B, Pesce G, Hegi ME (2016) Phase II Study of Radiotherapy and Temsirolimus versus Radiochemotherapy with Temozolomide in Patients with Newly Diagnosed Glioblastoma without MGMT Promoter Hypermethylation (EORTC 26082). Clin Cancer Res 22:4797–4806. doi:10.1158/1078-0432.CCR-15–3153
91. Wintterle S, Schreiner B, Mitsdoerffer M, Schneider D, Chen L, Meyermann R, Weller M, Wiendl H (2003) Expression of the B7-related molecule B7-H1 by glioma cells: a potential mechanism of immune paralysis. Cancer Res 63:7462–7467
92. Wood AJ, Lo TW, Zeitler B, Pickle CS, Ralston EJ, Lee AH, Amora R, Miller JC, Leung E, Meng X, Zhang L, Rebar EJ, Gregory PD, Urnov FD, Meyer BJ (2011) Targeted

genome editing across species using ZFNs and TALENs. Science 333:307. doi:10.1126/science.1207773
93. Yu W, Zhang L, Wei Q, Shao A (2019) O(6)-Methylguanine-DNA Methyltransferase (MGMT): Challenges and New Opportunities in Glioma Chemotherapy. Front Oncol 9:1547. doi:10.3389/fonc.2019.01547
94. Yvon ES, Burga R, Powell A, Cruz CR, Fernandes R, Barese C, Nguyen T, Abdel-Baki MS, Bollard CM (2017) Cord blood natural killer cells expressing a dominant negative TGF-beta receptor: Implications for adoptive immunotherapy for glioblastoma. Cytotherapy 19:408–418. doi:10.1016/j.jcyt.2016.12.005
95. Zhai L, Ladomersky E, Lauing KL, Wu M, Genet M, Gritsina G, Gyorffy B, Brastianos PK, Binder DC, Sosman JA, Giles FJ, James CD, Horbinski C, Stupp R, Wainwright DA (2017) Infiltrating T Cells Increase IDO1 Expression in Glioblastoma and Contribute to Decreased Patient Survival. Clin Cancer Res 23:6650–6660. doi:10.1158/1078-0432.CCR-17-0120

Received: 10 May 2020
Accepted: 1 June 2020
Correspondence:
Sabino Luzzi M.D., Ph.D.
Neurosurgery Unit, Department of Clinical-Surgical, Diagnostic and Pediatric Sciences, University of Pavia
Polo Didattico “Cesare Brusotti”, Viale Brambilla, 74
27100 - Pavia (Italy)
E-mail: sabino.luzzi@unipv.it