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Novel therapeutic approaches for tumors of the central nervous system

Guest Editors: Salvatore Savasta, Sabino Luzzi

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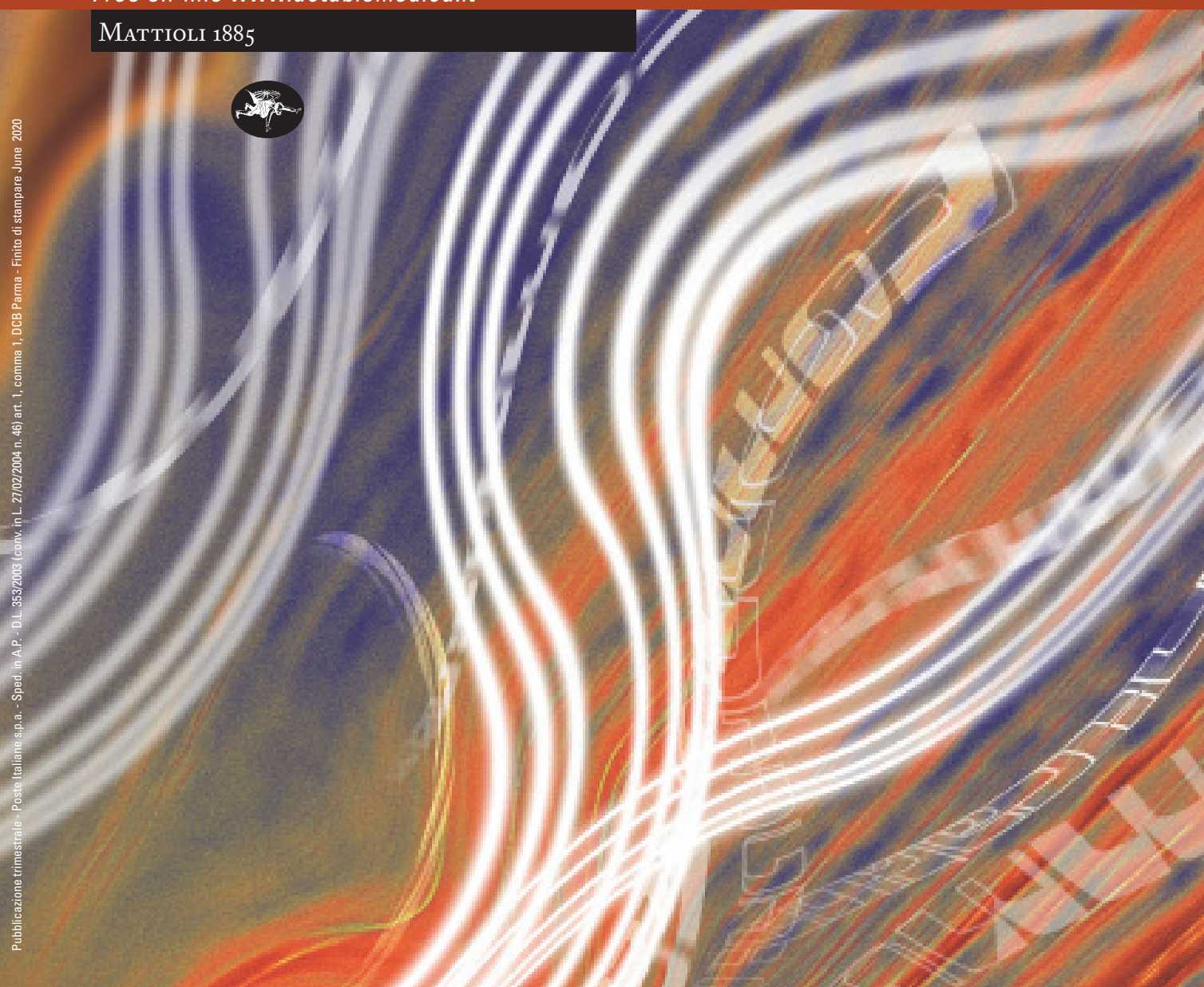

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Index

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Original article

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

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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

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Original article

Adoptive immunotherapies in neuro-oncology: classification, recent advances, and translational challenges

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Abstract. *Background:* Adoptive immunotherapies are among the pillars of ongoing biological breakthroughs in neuro-oncology, as their potential applications are tremendously wide. The present literature review comprehensively classified adoptive immunotherapies in neuro-oncology, provides an update, and overviews the main translational challenges of this approach. *Methods:* The PubMed/MEDLINE platform, Medical Subject Heading (MeSH) database, and ClinicalTrials.gov website were the sources. The MeSH terms "Immunotherapy, Adoptive," "Cell- and Tissue-Based Therapy," "Tissue Engineering," and "Cell Engineering" were combined with "Central Nervous System," and "Brain." "Brain tumors" and "adoptive immunotherapy" were used for a further unrestricted search. Only articles published in the last 5 years were selected and further sorted based on the best match and relevance. The search terms "Central Nervous System Tumor," "Malignant Brain Tumor," "Brain Cancer," "Brain Neoplasms," and "Brain Tumor" were used on the ClinicalTrials.gov website. *Results:* A total of 79 relevant articles and 16 trials were selected. T therapies include chimeric antigen receptor T (CAR T) cell therapy and T cell receptor (TCR) transgenic therapy. Natural killer (NK) cell-based therapies are another approach; combinations are also possible. Trials in phase 1 and 2 comprised 69% and 31% of the studies, respectively, 8 of which were concluded. CAR T cell therapy targeting epidermal growth factor receptor variant III (EGFRvIII) was demonstrated to reduce the recurrence rate of glioblastoma after standard-of-care treatment. *Conclusion:* Adoptive immunotherapies can be classified as T, NK, and NKT cell-based. CAR T cell therapy redirected against EGFRvIII has been shown to be the most promising treatment for glioblastoma. Overcoming immune tolerance and immune escape are the main translational challenges in the near future of neuro-oncology. (www.actabiomedica.it)

Key words: Adoptive Immunotherapies, CAR T Cell, Immunotherapy, Malignant Brain Tumor, NK Cell

Background

The rapid development of applied biotechnology in both diagnostics and therapeutics has led to a progressive but dramatic transition in neuro-oncology from an old era, which was purely based on the mechanical, physical or chemical features of conventional surgery, radiotherapy and chemotherapy, respectively, to a new era, which is considered purely biological due to its entirely molecular approach (1). Therefore, the World Health Organization (WHO) has profoundly revised the classification of central nervous system (CNS) tumors, which now involves biomolecular aspects that widely distinguish primitive neoplasms for diagnosis and prognosis of the disease and, especially, the responsiveness to therapy (2). Immunotherapies

are among the main pillars of a biological approach to malignant CNS tumors, with the rationale of enhancement of the neuroimmune response against neoplasms through selective immunomodulation. Immunotherapies of CNS malignancies involve three straightforward strategies: checkpoint inhibitors, vaccines, and adoptive cellular immunotherapies. In contrast to checkpoint inhibitors and vaccines, adoptive immunotherapies necessitate the injection, grafting, or implantation of a cellular product into the patient (3). Thus, adoptive immunotherapies are cell-based therapies, or cytotherapies, which are considered a part of the ongoing biotechnological revolution in neuro-oncology. The concomitant tremendous evolution of translational medicine and nanotechnologies, both propaedeutic to a clinical development in pediatric and adulthood population (4-7), has led to an improvement in bioengineering techniques, which have involved gene therapies more than immunotherapies in the last few years. The spectrum of the potential applications of adoptive immunotherapies is incredibly wide, is not yet thoroughly investigated, and offers a theoretically huge number of possible strategies against CNS and other tumors (8-19).

The aim of the present study was to comprehensively review the literature on the current role of adoptive immunotherapies in neuro-oncology. The future perspectives and challenges of this approach were analyzed in detail.

Methods

An online literature search was conducted with the PubMed/MEDLINE (https://pubmed.ncbi.nlm.nih.gov) platform and the ClinicalTrials.gov (https://clinicaltrials.gov) database, which reports privately and publicly funded clinical studies worldwide. For the MEDLINE search, the Medical Subject Heading (MeSH) database was used.

The MeSH terms "Immunotherapy, Adoptive," "Cell- and Tissue-Based Therapy," "Tissue Engineering," and "Cell Engineering" were selected. For each MeSH term, the search was restricted to specific subheadings (i.e., the classification criteria and clinical employment of adoptive cellular immunotherapies). The aforementioned main terms were combined with further MeSH terms: "Central Nervous System" and "Brain."

A further free text search was conducted using the combination of the terms "brain tumors" [text word] and "adoptive immunotherapy" [MeSH].

Only articles in English or articles translated to English published in the last 5 years and pertinent to neuro-oncology were selected. Review articles and editorials were included, whereas case reports were excluded. An additional sorting was conducted based on the best match and relevance inferred by the titles and abstracts.

On ClinicalTrials.gov, the search terms "Central Nervous System Tumor," "Malignant Brain Tumor," "Brain Cancer," "Brain Neoplasms," and "Brain Tumor" were used. No restrictions for drug name, country, recruitment status, or study phase were applied.

Based on the identifier, duplicated studies were excluded, and only trials regarding adoptive immunotherapies were selected according to the interventions. The retrieved trials were summarized, and the current phase of the studies was highlighted. A descriptive analysis of the most relevant studies from the overall literature search was reported.

Results

1. Literature Volume

The search retrieved 310 articles and 24 clinical trials. After the implementation of the exclusion criteria and removal of duplicates, 79 articles and 16 trials remained.

2. Classification of Adoptive Immunotherapies

Table 1 reports the classification of adoptive immunotherapies for malignant brain tumors.

2.1 Engineered and Activated T cells

Engineered T cell adoptive immunotherapies include chimeric antigen receptor (CAR) T cell therapy and T cell receptor (TCR) transgenic therapy.

Table 1 - Classification of adoptive immunotherapies for malignant brain tumors

Cell	Engineered Effector	
T	TCR transgenic T	
	CAR T (re-directed against)	EGFRIII
		IL-13Ra2
		CD133
		HER2
		EphA2
NK	Allogenic NK	
	Antibody-mediated blocking of KIR	
	Antibodies against EGFR (ADCC)	
	Transplantation of KIR2DS2+ genotype NKs	
	Immunoligands binding NKG2D receptor	
	Cord blood NK cells transduced with (TGF)-β receptor II (DNRII)	
	NKs' exosomes	
	CAR NK targeting EGFR variant III	
NKT	Autologous NKT expanded w/ autologous mature DC loaded with the NKT ligand α-galactosyl ceramide	
Hybrid	Autologous NK + CD8+ cytotoxic T lymphocytes (ALECSAT)	

T: T lymphocyte; NK: natural killer cells; NKT: T lymphocyte-natural killer cells; ALECSAT: Autologuos Lymphoid Effector Cells Specific Against Tumour; CAR T: chimeric antigen receptor; EGFRIII: epidermal growth factor receptor variant III; IL-13Ra2: interleukin-13 receptor α2; CD: cluster differentiation; HER2: human epidermal growth factor 2; EphA2: erythropoietin-producing hepatocellular carcinoma A2; EGFR: epidermal growth factor receptor; ADCC: antibody-dependent cellular cytotoxicity; KIR2DS2: killer cell immunoglobulin like receptor, two Ig domains and short cytoplasmic tail 2; TGF: transforming growth factor; DNRII: dominant-negative receptor II; CTL: cytotoxic T-lymphocytes.

2.1.1 CAR T Cells

CAR T cell therapy is based on an ex vivo expansion of leukocytes, and the engineering of these cells aims to form a chimeric receptor powered by selectivity for neoplastic targets, which is several orders of factors higher than its naïve form, and the autologous or allogenic transplant.

Interleukin 2 and anti-CD3 antibodies and gamma-retroviruses and lentiviruses are used for the activation and proliferation of T cells and transfection of CAR genes, respectively (20).

The oncolytic capability of these cells, as well as their proficiency to overcome immune tolerance, lies in the chimeric nature of CAR, which involves single receptor antigen-binding and T-cell activating properties. CAR T cells are redirected against a specific protein expressed on neoplastic cell membranes, and the neoplastic cells are thus selectively killed (21, 22). Consequently, the specificity of CAR T cells for a specific type of tumor largely depends on the types of transfected CAR genes. Adoptive immunotherapy for malignant brain tumors, and primarily glioblastoma, has tested several CAR genes, namely, epidermal growth factor receptor variant III (EGFRvIII) (23-25), interleukin-13 receptor a2 (IL-13Ra2) (26-29), CD133 (26), human epidermal growth factor receptor 2 (HER2) (30, 31), and erythropoietin-producing hepatocellular carcinoma A2 (EphA2) (32). Lymphodepletion prior to adoptive transfer of tumor-specific CAR T lymphocytes has been reported to be among the key factors enhancing the expansion and efficacy of the transplant, mainly by means of the abolishment of regulatory T cell activity and competing elements of the immune system (cytokine sinks) (33-35).

2.1.2 TCR Transgenic T Cells

TCR transgenic T cell therapy involves the isolation of the α and β chains of the TCR, with the latter binding the major histocompatibility complex (MHC) on the cellular membrane, their manipulation_aimed to enhance the selectivity and specificity for specific tumoral antigens, their insertion into retroviruses or lentiviruses, the amplification of the viral vectors and, patient infection (36-38).

2.2 Natural Killer (NK) Cells

The spectrum of the possible molecular mechanisms of NK cell-mediated adoptive immunotherapy is highly variable.

2.2.1 Allogenic NK Cell Transplant

The rationale of allogenic NK cell transplant lies in the inability of these cells to recognize MHC class I molecules and human leukocyte-antigen (HLA) type A ligands expressed by glioma cells, which ultimately enhances the oncolytic effect. Transplantation of the cells belonging to the immune system has been reported to be less affected by the risk of rejection than other allogenic transplants, and this concept is the backbone of allogenic immunotherapies (39).

2.2.2 NK Killer Immunoglobulin-Like Receptor (KIR) Antibody-Mediated Blocking

Antibody-mediated blocking of inhibitory cell KIRs has been associated with a dramatic increase in NK-mediated killing of neoplastic cells, mainly due to the inhibition of the well-known negative regulatory properties of this receptor of the NK cell function (40).

2.2.3 Antibody-Dependent Cellular Cytotoxicity (ADCC)

ADCC has been employed to treat glioblastoma and classically uses EGFR antibodies. The fragment crystallizable (FC) region of the antibody binds some activating receptors expressed by NK cells, ultimately leading to cancer cell apoptosis. CD16 (FcγIIIA), KIR two domains, short cytoplasmic tail, 2 (KIR2DS2), and NK Group 2D (NKG2D) are the most studied among these receptors. The KIR2DS2+ genotype has been reported to have the greatest cytotoxicity and non-negligible inhibition of angiogenesis in experimental models (41).

2.2.4 NK Cell Immunoligands

Immunoligands able to selectively bind NKG2D receptors have also been tested (41).

2.2.5 Retrovirally Transduced Cord Blood NK Cells

Yvon et al emphasized the properties of cord blood-derived NK cells retrovirally transduced to express a dominant negative form of transforming growth factor (TGF)-β receptor II (DNRII) specifically for gliobastoma (42). DNRII makes these cells immune to the detrimental effects of TGF-β produced by the microenvironment and causes immune escape of the glioma cells.

2.2.6 NK Cell Exosome Mimetics

Evidence of the efficacy of NK cell exosome mimetics against malignant brain tumors was derived from in vitro and in vivo studies. NK cell exosomes are endogenous nanocarriers that can enhance the biological activity of NK cells against tumors.

2.2.7 CAR NK Cells

The CAR NK cell line targeting EGFRvIII was produced according to the aforementioned mechanisms described for CAR T cells and has been successfully employed for glioblastoma (43).

Regardless of the type of approach used, NK cell adoptive immunotherapy for glioblastoma has been combined with the mAb9.2.27 antibody, which is able to inhibit angiogenesis through the secretion of interferon (IFN)-γ and tumor necrosis factor (TNF)-α (44, 45).

Figure 3 displays an overview of the main molecular mechanisms involved in NK cell-based immunotherapy for glioblastoma.

2.2.8 NKT Cells

CD1d-restricted NKT cells have been reported to have a fundamental role in both the innate and acquired immune responses against tumors. Differences do exist among CD1d-restricted NKT cells between type I and type II, which have invariant Valpha14 and heterogeneous non-Valpha14 receptors, respectively (46).

The immunological escape of malignant CNS tumors from NKT cells occurs through the high level of expression of microRNA-92a and an immune tolerant IL-6+ IL-10+ NKT cell phenotype (47-50). An approach aimed to overcome the immune tolerance of glioma cells includes the expansion in culture of NKT cells using autologous mature dendritic cells (DCs) loaded with the NKT ligand α-galactosyl ceramide, which effectively stimulates murine and human type I NKT cells (46, 51-53).

2.3 Hybrid Therapies

Autologous Lymphoid Effector Cells Specific Against Tumor cells (ALECSAT) therapy (Cytovac A/S, Hørsholm, Denmark) is an epigenetic, thus not involving DNA manipulation, cancer adoptive immunotherapy under investigation for glioblastoma and prostate and pancreatic cancer. The main steps of ALECSAT therapy entail the following distinct phases: isolation of lymphocytes and monocytes from the patient's peripheral blood sample; culture and differentiation of monocytes into DCs; co-culture of mature DCs and lymphocytes to create autologous activated T helper (Th) cells; induction of CD4+ Th cells with 5-aza-2′-deoxycytidine, a DNA-demethylation agent, to express cancer/testis antigens (CTA-Th); addiction of CTA-Th cells to non-activated lymphocytes to obtain activated and expanded CD8+ cytotoxic lymphocyte (CTL) effectors; and injection of autologous NK and CD8+ CTL effectors (54). Activated NK cells are directed against glioma cells that do not express the antigen. The ALECSAT immunization protocol lasts 26 days. Strengths of this approach are the population of secondary lymphoid organs for a long-lasting effect and the wide variety of tumor antigens.

3. Clinical Trials on Adoptive Immunotherapies

Out of 16 clinical trials, 69% were phase 1, and 31% were phase 2 (Figure 1). Most of them are still ongoing in the USA and China (56% and 25%, respectively) (Figure 2). Only 8 of these studies have been concluded. Three involved ALECSAT immunotherapy. The first two ALECSAT trials (NCT02799238 and NCT01588769) aimed to evaluate the tolerability and efficacy of this therapy, whereas the third trial (NCT02060955) compared its efficacy to bevacizumab plus irinotecan. To date, no results have been released for any of these trials. A phase 2 completed study on CAR T cell receptor immunotherapy targeting EGFRvIII for patients with malignant gliomas expressing EGFRvIII (NCT01454596) concluded that this approach effectively and safely reduces the recurrence rate of glioblastoma after standard-of-care treatment, specifically by means of the elimination of

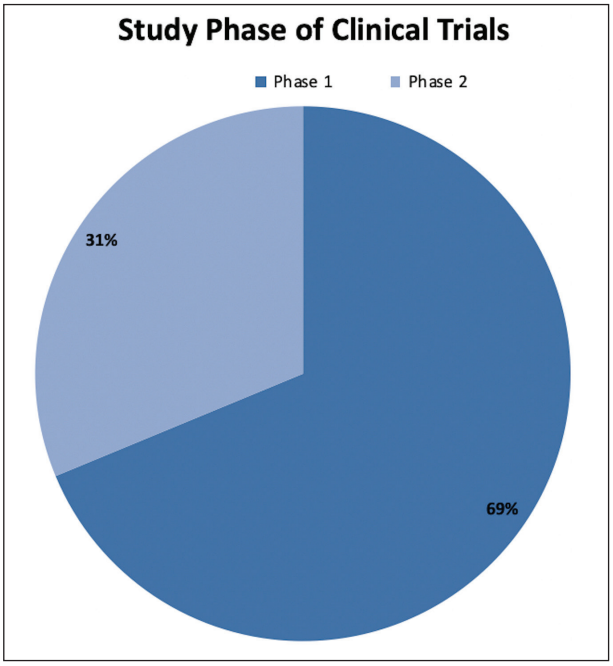

Figure 1. Pie graph showing the distribution of the clinical trials according to the study phase

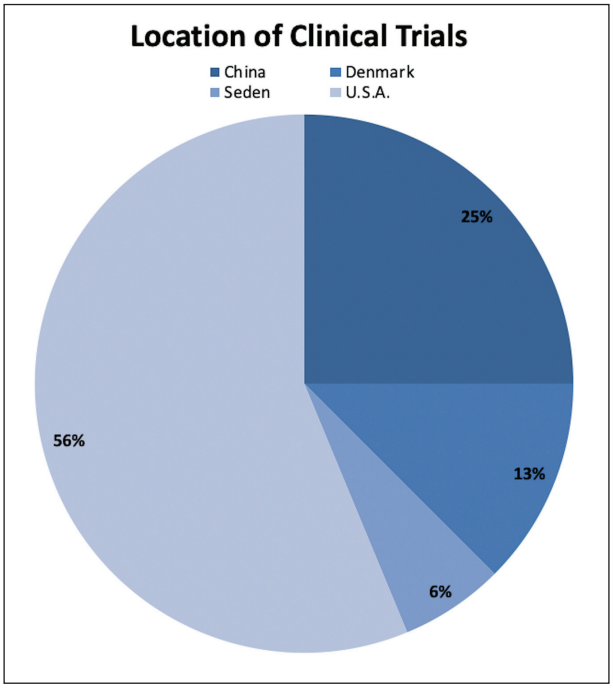

Figure 2. Pie graph showing the distribution of the selected clinical trials according to study location

glioma stem cells (55). The remaining completed trials tested the efficacy of alloreactive CD8+ cytotoxic T lymphocytes or the combination of adoptive T cell-based immunotherapy with other immunomodulators, such as aldesleukin, a lymphokine produced through recombinant DNA technology using a genetically engineered *E. coli* strain containing an analog of the human interleukin 2 gene (56-60). Positive results were reported for some of these combinations. Table 2 summarizes the clinical trials on adoptive immunotherapies for malignant gliomas.

Discussion

Recently, neuro-oncology has experienced a landmark transition from a mechanical era to a biological era (61-63).

A concrete aspect of this evolution is the last WHO classification of CNS tumors, which originated from the advances in genomic profiling and proteomics (64) and led to an improvement in their overall management in terms of diagnosis, prognosis, and, especially, adjuvant therapy.

Despite the refinements in neurosurgical techniques in neuro-oncology and other fields (65-73), the progression free survival and the overall survival for patients with high grade gliomas remain dismal. This aspect has justified the compulsive search of adjunctive biological therapies based on the new insights in neuroimmunology.

Theoretical application of adoptive immunotherapies and implementation of clinical trials have been possible due to the tremendous advances in somatic cell biotechnologies (74). These technologies involve manipulation of the allogenic or xenogeneic immunological cells to obtain a cellular product that is transplanted as a living drug. A straightforward and practical classification of adoptive immunotherapy is shown in Table 1 and is essentially based on the immunophenotype of the cellular product. A classification scheme like this has a strength mainly in pursuing a modular approach of biological immunotherapy, often involving the combination of different immunophenotypes with a subsequent potential synergic effect. The overall level of evidence of the efficacy of adoptive immunotherapies in neuro-oncology is remarkably promising but remains insufficient to be considered immediately applicable in daily clinical practice. Most of the trials are in phase 1, and most of those in phase 2 remain ongoing or incomplete. CAR T cell therapy has a valuable rationale for brain cancer, and this rationale is likely the main reason why this approach has fostered much attention. An addition reason is the tremendously positive results of this approach in hematology and other fields, where CAR T cell therapy accounts for more than 25 years of cumulative experience (75-77). In glioblastoma adjuvant therapy, CAR T cells redirected against EGFRvIII have especially shown positive results (23-25, 55). ALECSAT immunotherapy also has received much attention, even though no consistent data have been reported apart from a good safety profile (54).

Most adoptive immunotherapies involve therapeutic depletion of regulatory T cells (Tregs), as an immunomodulatory approach, based upon the assumption that both thymus-derived and inducible therapies that play a role are involved in the immune tolerance of glioblastoma (78, 79).

Adoptive immunotherapies for malignant brain tumors face a non-negligible number of translational challenges, almost all of which converge toward the need to overcome the immunological tolerance of glioma and the immune escape of glioma stem cells. Several factors are responsible for the immune tolerance of glioma cells, which are primarily the lack of tumor antigen expression and the subsequent loss of the tumor immunological phenotype. This aspect is deleterious for the success of both T-cell based and vaccine immunotherapy. Thus, aberrant nitric oxide synthase 2 is gaining more interest as a further potential therapeutic target. For TCR therapy, the main limiting factor is the mispairing between endogenous α/β and transgenic α/β TCR chains, and no clinical trials have been established for malignant brain tumors (38, 80). An NK cell-based approach recognizes that the lack of the representativeness of these cells within the tumor microenviroment is its main limitation (81). The main reason for this limitation seems to be the high representativeness of the MHC class I molecules and of the HLA ligand type A on glioma cells. Both of these molecules can interact with inhibitory NK cells

Table 2. Clinical Trials on Adoptive Immunotherapies for Malignant Brain Gliomas

#	ClinicalTrials.gov Identifier	Condition or Disease	# of Pts. Estimated Enrollment	Intervention/Treatment	Study Arms	Study Phase	Recruitment Status	Locations
1	NCT03392545	High Grade Glioma; Glioblastoma Glioma of Brainstem Glioma, Malignant	30	Combined immune adjuvants and radiation	Combination of Immunization and Radiotherapy for Recurrent GBM (InSituVac1)	1	Recruiting	CH
2	NCT03389230	Glioblastoma HER2/Neu Positive Malignant Glioma Recurrent Glioma Refractory Glioma WHO Grade III Glioma	42	HER2(EQ)BBζ/CD19t+ Tcm	Memory-Enriched T Cells in Treating Patients with Recurrent or Refractory Grade III-IV Glioma	1	Recruiting	U.S.
3	NCT03347097	Glioblastoma Multiforme	40	TIL	Tumor-infiltrating T Lymphocyte (TIL) Adoptive Therapy for Patients with Glioblastoma Multiforme	1	Recruiting	CH
4	NCT03344250	Glioblastoma Glioblastoma Multiforme	18	EGFR BATs with SOC RT and TMZ	Phase I EGFR BATs in Newly Diagnosed Glioblastoma	1	Recruiting	U.S.
5	NCT03170141	Glioblastoma Multiforme of Brain Glioblastoma Multiforme	20	Antigen-specific IgT cells	Immunogene-modified T (IgT) Cells Against Glioblastoma Multiforme	1	Enrolling by invitation	CH
6	NCT02937844	Glioblastoma Multiforme	20	Anti-PD-L1 CSR T cells	Pilot Study of Autologous Chimeric Switch Receptor Modified T Cells in Recurrent Glioblastoma Multiforme	1	Recruiting	CH

(continued on next page)

Table 2. Clinical Trials on Adoptive Immunotherapies for Malignant Brain Gliomas

#	ClinicalTrials.gov Identifier	Condition or Disease	# of Pts. Estimated Enrollment	Intervention/Treatment	Study Arms	Study Phase	Recruitment Status	Locations
7	NCT02799238	Glioblastoma	62	ALECSAT	Autologuos Lymphoid Effector Cells Specific Against Tumour (ALECSAT) as Add on to Standard of Care in Patients with Glioblastoma	2	Active, not recruiting	SW
8	NCT02208362	Malignant Glioma Refractory Brain Neoplasm Recurrent Brain Neoplasm Glioblastoma	92	IL13Rα2-specific, hinge-optimized, 41BB-costimulatory CAR/truncated CD19-expressing Autologous T lymphocytes, Vaccine Therapy	Genetically Modified T-cells in Treating Patients with Recurrent or Refractory Malignant Glioma	1	Recruiting	U.S.
9	NCT02060955	Glioblastoma Multiforme	25	ALECSAT	Randomized Phase 2 Study to Investigate Efficacy of ALECSAT in Patients with GBM Measured Compared to Avastin/Irinotecan	2	Completed	DE
10	NCT01588769	Glioblastoma Multiforme	23	Anti-EGFRvIII CAR transduced PBL	A Phase I Study to Investigate Tolerability and Efficacy of ALECSAT Administered to Glioblastoma Multiforme Patients (ALECSAT-GBM)	1	Completed	DE
11	NCT01454596	Malignant Glioma Glioblastoma Brain Cancer Gliosarcoma	18	Anti-EGFRvIII CAR Transduced PBL	CAR T Cell Receptor Immunotherapy Targeting EGFRvIII for Patients with Malignant Gliomas Expressing EGFRvIII	2	Completed	U.S.

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Table 2. Clinical Trials on Adoptive Immunotherapies for Malignant Brain Gliomas

#	ClinicalTrials.gov Identifier	Condition or Disease	# of Pts. Estimated Enrollment	Intervention/Treatment	Study Arms	Study Phase	Recruitment Status	Locations
12	NCT01144247	Gliomas Anaplastic Astrocytoma Anaplastic Oligodendroglioma Anaplastic Mixed Glioma Glioblastoma Multiforme Malignant Meningioma	10	Alloreactive CTL	Cellular Immunotherapy Study for Brain Cancer	1	Completed	U.S.
13	NCT01082926	Anaplastic Astrocytoma Anaplastic Ependymoma Anaplastic Meningioma Anaplastic Oligodendroglioma Brain Stem Glioma Ependymoblastoma Giant Cell Glioblastoma Glioblastoma Gliosarcoma Grade III Meningioma Meningeal Hemangiopericytoma Mixed Glioma Pineal Gland Astrocytoma Brain Tumor	6	Therapeutic allogeneic lymphocytes - aldesleukin	Phase I Study of Cellular Immunotherapy for Recurrent/Refractory Malignant Glioma Using Intratumoral Infusions of GRm13Z40-2, An Allogeneic CD8+ Cytolitic T-Cell Line Genetically Modified to Express the IL 13-Zetakine and HyTK and to be Resistant to Glucocorticoids, in Combination with Interleukin-2	1	Completed	U.S.

(continued on next page)

Table 2. Clinical Trials on Adoptive Immunotherapies for Malignant Brain Gliomas

#	ClinicalTrials.gov Identifier	Condition or Disease	# of Pts. Estimated Enrollment	Intervention/Treatment	Study Arms	Study Phase	Recruitment Status	Locations
14	NCT00730613	Brain and Central Nervous System Tumors	3	Biological: therapeutic autologous lymphocytes	Cellular Adoptive Immunotherapy Using Genetically Modified T-Lymphocytes in Treating Patients with Recurrent or Refractory High-Grade Malignant Glioma	1	Completed	U.S.
15	NCT00331526	Brain and Central Nervous System Tumors	83	Aldesleukin	Cellular Adoptive Immunotherapy in Treating Patients with Glioblastoma Multiforme	2	Completed	U.S.
16	NCT00004024	Brain and Central Nervous System Tumors	60	Aldesleukin, autologous tumor cell vaccine, muromonab-CD3, sargramostim, therapeutic autologous lymphocytes	Biological Therapy Following Surgery and Radiation Therapy in Treating Patients with Primary or Recurrent Astrocytoma or Oligodendroglioma	2	Completed	U.S.

HER2(EQ)BBζ/CD19t+ Tcm: preparation of genetically modified autologous central memory enriched T-cells (Tcm) expressing a chimeric antigen receptor consisting of an anti-human epidermal growth factor 2 (HER2) variable fragment that is linked to the signaling domain of the T-cell antigen receptor complex zeta chain (BBζ), and truncated cluster of differentiation (CD)19; **TIL**: Tumor-infiltrating T-Lymphocyte; **EGFR**: epidermal growth factor; **EGFRvIII:** epidermal growth factor receptor variant III; **EGFR BATs:** EGFR Bi-armed Activated T-cells; **RT:** radiotherapy**; TMZ**: temozolomide; **PD-L1 CSR**: programmed death Ligand 1 chimeric switch receptor; **IL-13Rα2**: interleukin-13 receptor α2; **ALECSAT**: Autologuos Lymphoid Effector Cells Specific Against Tumour; **PBL**: peripheral blood lymphocytes; **CTL**: cytotoxic T-lymphocytes; **GBM**: glioblastoma; **CH:** China; **U.S.**: United States; **SW:** Sweden; **DE:** Denmark.

and KIRs, ultimately inhibiting the functions of NK cells (40).

Similar challenges are related to adoptive immunotherapies for other solid tumors (82).

A further consideration for adoptive immunotherapies, which are somatic cell-based therapies, is their susceptibility to genetic and phenotypic modifications with a subsequent dramatic decrease in their biological activity as a consequence of extensive tissue culture expansion (83).

Conclusion

Adoptive immunotherapies can be classified based on the immunophenotype of the cellular product. They involve treatments based on T, NK, and NKT cells, along with hybrid approaches from their combination.

CAR T cells redirected against EGFRvIII have shown positive results in the treatment of recurrent glioblastoma. Different NK cell-based approaches are also being considered, ranging from allogenic transplant to exosomes mimetics, each with different potential.

The comprehensive level of evidence for the efficacy and safety of adoptive immunotherapies in neuro-oncology is non-negligible but remains insufficient to consider these therapies as a standard of care.

Constant immune tolerance and immune escape by high grade gliomas are the main limiting factors of these therapies, and they are among the most important translational challenges for the near future of neuro-oncology.

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Original article

Gene therapies for high-grade gliomas: from the bench to the bedside

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Abstract. *Background:* Gene therapy is the most attractive therapeutic approach against high-grade gliomas (HGGs). This is because of its theoretical capability to rework gene makeup in order to yield oncolytic effects. However, some factors still limit the upgrade of these therapies at a clinical level of evidence. We report an overview of glioblastoma gene therapies, mainly focused on the rationale, classification, advances and translational challenges. *Methods:* An extensive review of the online literature on gene therapy for HGGs was carried out. The PubMed/MEDLINE and ClinicalTrials.gov websites were the main sources. Articles in English published in the last five years were sorted according to the best match with the multiple relevant keywords chosen. A descriptive analysis of the clinical trials was also reported. *Results:* A total of 85 articles and 45 clinical trials were selected. The main types of gene therapies are the suicide gene, tumor suppressor gene, immunomodulatory gene and oncolytic therapies (virotherapies). The transfer of genetic material entails replication-deficient and replication-competent oncolytic viruses and nanoparticles, such as liposomes and cationic polymers, each of them having advantages and drawbacks. Forty-eight clinical trials were collected, mostly phase I/II. *Conclusion:* Gene therapies constitute a promising approach against HGGs. The selection of new and more effective target genes, the implementation of gene-delivery vectors capable of greater and safer spreading capacity, and the optimization of the administration routes constitute the main translational challenges of this approach. (www.actabiomedica.it)

Key words: Gene Therapy; Glioblastoma; High Grade Glioma; Suicide Gene Therapies; Virotherapy.

Background

High-grade gliomas (HGGs) are by far the deadliest primary brain neoplasms.[1,2] Despite the evolution of the different therapies, prognosis of these tumors remains poor, with a median survival ranging between 12 and 15 months, and less than 10% of the patients surviving at 5 years.[3-5] In line with the urgent need for new and more effective approaches, the increased understanding of the glioma genetic landscape, together with the tremendous advances in biotechnologies, led to the development of new and more sophisticated treatment options.[6-12] Gene therapy is among the most attractive therapeutic approach for malignant brain tumors, primarily glioblastoma (GBM). The rationale of the gene therapies lies in reworking the gene makeup in order to yield therapeutic effects. These types of therapies propose transferring and manipulating target genes, resulting in ceasing the progression of cancer and contextually enhancing the antitumoral immune response.[13-16] The engineering of delivery agents, including viral vectors, oncolytic viruses and non-viral

nanoparticles, constitutes an essential aspect of the gene therapies.[17-19]

The literature review herein reported is an overview of the gene therapies for the treatment of high-grade gliomas. The rationale, classification, advances, limitations, challenges, evidence from the clinical trials and future prospects of gene therapies in the neuro-oncological field are also discussed.

Methods

An online search of the literature was conducted on the PubMed/MEDLINE (https://pubmed.ncbi.nlm.nih.gov) and ClinicalTrials.gov (https://clinicaltrials.gov) websites.

On the PubMed/MEDLINE search the MeSH (Medical Subject Headings) database and free mode search used with the terms "Gene Therapy", "Genetic Strategies", "Gene Modification Technologies", "Genome Editing Technologies", "Immunomodulation therapies", "Suicide Gene Therapy", "Tumor Suppression Gene Therapy", "Oncolytic Viral Therapy", "Nanotechnology-Based Gene Therapy", "Viral Delivery Strategies" and "Virotherapy", with the following keywords: "High-grade gliomas", "Malignant brain tumor" and "Glioblastoma". Only articles in English or translated into English, published in the last five years were preferred, sorted according to the best match and relevance.

On the ClinicalTrials.gov website the text words were "Central Nervous System Tumor", "Malignant Brain Tumor", "Brain Cancer", "High-grade gliomas" and "Brain Tumor", used in the field "condition/disease", without restrictions for drug name, study phase and recruitment status. A descriptive analysis of the retrieved trials was reported.

Results

1 Volume of the literature

The search returned a total of 120 articles and 56 clinical trials. After the implementation of the exclusion criteria and removal of duplicates, 85 relevant articles and 45 clinical trials were collected.

2 General Aspects

A common aspect of the gene therapies lies in the need to introduce the genetic material into the target cells. This is achieved by means of specific biological or manufactured carriers differentiated by size, tumor tropism, transduction efficacy, oncolytic effect, pathogenicity and immunological potential.[20-23] Viral and non-viral carriers are the methods commonly used, each of them having advantages and drawbacks. Among non-viral carriers, nanoparticles and liposomes have been tested. Table 1 reports an overview of the vectors tested[24] (Table 1).

3 Classification of Gene Therapies

A proposed classification of the gene therapies involves the distinction between the suicide gene, tumor suppressor gene, immunomodulatory gene and oncolytic therapies (virotherapies).

Table 2 summarizes the proposed classification of gene therapies (Table 2).

3.1 Suicide Gene Therapies

The suicide gene strategy is based on the introduction of a transgene into the tumor cells and the concomitant systemic delivery of a prodrug. The transgene, namely the "suicide gene", codifies for one or more enzymes capable of converting the administered inactive prodrug into its oncolytic equivalent.[25] Herpes Simplex Virus Thymidine Kinase (HSV-TK), Cytosine Deaminase (CD) and E. coli-derived Purine Nucleoside Phosphorylase (PNP) have been the most studied suicide genes in GBM therapy. A further amplification of the therapeutic effect of suicide gene therapy comes from the so-called "bystander effect", consisting in the possibility that the encoded gene and the apoptotic signal also affect the neighboring non-transduced cells through the gap-junctions and further complex molecular mechanisms.

3.1.1 HSV-TK

The HSV-TK enzyme is involved in DNA replication and catalyzes the phosphorylation of some

Table 1. Comparison between viral and non-viral vectors

Vectors	Viral				Non-viral
	AV	HSV	RT	AAV	Liposomes
Size (nm)	100-200	120-300	100	20	20-200
Cargo	dsDNA	dsDNA	RNA	ssDNA	dsDNA/RNA
Transport Capacity (kB)	> 5	30-50	10-15	< 5	+/-
Transduction Efficacy	+	++	+/-	-	+
Oncolytic Effect	Yes/No	Yes/No	No	No	No
Immunogenic Potential	++	++	+/-	+/-	--
Risk of Mutagenesis	No	No	Yes	No	No

AAV: Adeno Associated Virus; AD: Adenovirus; HSV: Herpes Simplex Virus; RT: Retrovirus
"++": very high; "+": high; "+/-": medium; "-": low; "- -": very low.

Table 2. Classification of Gene Therapies for Malignant Brain Tumors

Strategies	Suicide Gene Therapies	Tumor Suppressor Gene Therapies	Immunomodulatory Gene Therapies	Oncolytic Virotherapies		Genome Editing Therapies	
Mechanism	Gene encoding a prodrug activating enzyme	Restoration of antitumoral genes function through their replacement	Enhancing antitumoral immune response throughout genes encoding immunostimulating factors	Replication-competent virus capable of infect and replicate in tumor cells		DNA editing and rearrangement throughout specific nucleases	
Genes	HSV-TK	p53	IFN-β	Oncolytic viruses	HSVs	Nucleases	ZFNs
	CD	p16			CRAds		TALENs
	PNP	PTEN	IL-2, IL-4, IL-12		MV		(CRISPR)/ Cas9 system
					PVS-RIPO		

CD: Cytosine Deaminase; CRAds: Conditionally Replicating Adenovirus; HSV-TK: Herpes Simplex Virus Thymidine Kinase; IFN-β: Human Interferon β; IL: Interleukine; MV: Measles Paramyxovirus; PNP: Purine Nucleoside Phosphorylase; PTEN: Phosphatase and Tensin Homologue; PVS-RIPO: Recombinant Nonpathogenic Polio-Rhinovirus; TALENs: Transcription Activator-Like Effector Nucleases; ZFNs: Zinc-Finger Nucleases.

nucleoside analogue antiviral prodrugs, such as ganciclovir (GCV), acyclovir and valacyclovir. The introduction of the HSV-TK gene into the tumor cells, via a non-replicating herpesvirus or adenovirus, makes them susceptible to antiviral drugs, finally halting the cell division.

The prodrug is activated by the HSV-TK and incorporated into the DNA of the tumor cells, where it causes damage to the genome and tumor apoptosis.[26, 27]

Since 1991, multiple phase I and II clinical trials tested the HSVTK/Nucleoside-analogue system in GBM treatment, conveyed by replication-defective retroviruses and adenoviruses.[28-34] Cerepro® (Ark Therapeutics; UK and Finland) and adenoviral vector-based HSV-TK/valacyclovir were studied in some preclinical and phase I/II clinical trials (www.clinicaltrials.gov, #NCT03603405, #NCT03596086), where they proved to increase the patients' overall survival, also with a good safety profile.

3.1.2 CD

CD converts 5-fluorocytosine (5-FC) into 5-fluorouracil (5-FU), which exerts its antitumor effect,

irreversibly inhibiting the synthesis of DNA.[35, 36] Several preclinical and phase I-III clinical trials tested the efficacy and safety profile of CD/5-FC for high grade gliomas (#NCT01985256, #NCT01156584, #NCT01470794).[37] A further enhancement of the cytotoxicity comes from the combination of CD/5-FC with Uracil Phosphoribosyl Transferase (UPRT). The synergic antitumoral activity of both these enzymes has been reported to also potentiate the effect of conventional radiotherapy of GBM in the animal model.[38] In 2012, Tocagen Inc. (San Diego, CA, USA) tested a new non-lytic retroviral replicating vector encoding CD, called Toca 511, for recurrent HGGs.[39] In combination with standard chemotherapy, Toca 511 showed a 6-month survival rate of 59% (#NCT01156584, #NCT01470794).[40]

3.1.3 PNP

PNP converts fludarabine, an adenosine ribonucleoside, into toxic 2-fluoroadenine, the latter able to inhibit RNA replication. Several studies proved the long-term benefits of PNP gene therapy. Through the antibiotic-based suppression of the intestinal flora, which limits the conversion of the prodrug, it is theoretically possible to enhance the efficacy of PNP gene therapy.[41, 42]

3.2 Tumor Suppressor Gene Therapies

Tumor suppressor gene therapies aim at the restoration of the suppressed function of the antitumoral genes through their substitution with functional equivalents. p53, p16 and Phosphatase and Tensin Homologue (PTEN) pathways are frequently mutated in high-grade gliomas, consequently resulting in the loss of both DNA repair and the regulation of cell proliferation.[43]

3.2.1 p53

Playing a pivotal role in DNA repair and cycle-cell arrest is p53. It is found to be inactivated in 25-30% of primary GBMs, and 60-70% of recurrent ones.[44, 45]

Tumor suppressor gene strategies involve a non-replicating adenovirus, combined with the cytomegalovirus promoter (CMV), in which the E1 gene is replaced by the p53 gene (AD5CMV-P53).[46-48] Adenovirus-mediated p53 gene transfer showed an oncolytic effect against recurrent GBMs in many phase I trials, where it was administered by stereotactic injection, resulting in a median progression-free survival of 13 weeks and an overall survival of 43 weeks (#NCT00004041, #NCT00004080).

3.2.2 p16

Regulating the cell cycle at the G1-S transition is p16.[49] The adenovirus-mediated restoration of its function proved to reduce cancer growth, but also to counteract the spreading of GBM cells through the inhibition of the matrix metalloprotease 2 activity within the tumor microenvironment.[50]

3.2.3 PTEN

PTEN suppression is found in about 40% of high-grade gliomas, resulting in a dysregulation of the downstream signaling pathways.[51]

Some studies proved the efficacy of the restoration of the PTEN function, via adenoviral vectors, in inducing tumor cell apoptosis and modification of the tumor microenvironment.

Furthermore, adenoviral-PTEN strategies showed an anti-angiogenic response in preclinical surveys.[52, 53]

3.3 Immunomodulatory gene therapies

High-grade gliomas acquire a high resistance to the standard treatments thanks to immunosuppression mechanisms.

Immunomodulatory gene therapies are aimed at boosting the antitumoral immune response, throughout engineered viruses which deliver immunostimulating cytokines.[16, 54, 55]

Many cytokines have been selected because of their capability of recruiting immune effectors. Adenoviral-mediated delivery of the human interferon β (IFN-β) gene was tested in some clinical studies.[56-58]

In a phase I trial, IFN-β was stereotactically introduced in the tumor microenvironment before its

resection, resulting in increased cytotoxic T and NK cell activity (#NCT00031083).

Another immunomodulatory strategy used the recombinant parvoviruses as a vehicle of IFN-gamma-inducible protein 10 (CXCL10) and TNF-alpha, showing a synergic effect against GBM cells in the mouse model.[59]

Non-replicating adenoviral-associated virus (AAV) and HSV were used to carry the interleukine-12 (IL-12) gene in experimental models, resulting in a local antitumor effect.

In 2005, Colombo et al. tested the efficacy of the local injection of HSV-TK/GCV and IL-2 for recurrent malignant gliomas. It resulted in a 12-month progression-free survival and overall survival of 14% and 25%, respectively.[60]

Okada et al. also investigated the synergic effect of a retrovirally transduced IL-4 and HSV-TK gene in glioma models, obtaining positive results.[61]

As a rule, the near totality of immunomodulatory therapies demonstrated better results when administered in combination with conventional chemotherapy.

3.4 Oncolytic virotherapies

Oncolytic virotherapies are based on the activity of specific replication-competent oncolytic viruses (OVs). They are able to, first, infect the tumor cells, second, lyse them, and third, evoke a strong immune response.[62, 63]

OVs act as a biologic anti-tumor complex, which is independent from the transfer of genetic material. Oncolytic HSV, conditionally replicating adenovirus (CRAd), Measles Paramyxovirus (MV) and recombinant nonpathogenic polio-rhinovirus (PVS-RIPO) have been used in this form of anticancer therapy.

3.4.1 Oncolytic HSVs

HSV G207 and HSV1716 are the main engineered HSVs used in the treatment of malignant gliomas. HSV G207, deleted for the γ34.5 gene, selectively targets replicating cells.[64, 65] In many phase I/II clinical trials, HSV G207 was locally administered, with limited evidence of anti-tumor activity (#NCT00157703, #NCT00028158).[66]

HSV 1716, deleted in both copies of the γ34.5 gene, was tested, in combination with standard surgery and intravenous dexamethasone, in a phase II clinical trial for childhood and adult HGGs (#NCT02031965).

Recently, a new oncolytic mutant HSV (rQNestin34.5) was engineered to express the infected cell protein 34.5 (ICP34.5). rQNestin34.5 showed strong oncolytic activity against high-grade glioma in a phase I clinical trial, with a good safety profile (#NCT03152318).[67]

3.4.2 CRAds

ONYX-015 and Ad5-Delta24 are CRAds modified to selectively target glioma cells.

ONYX-015, deleted in the E1B 55K gene, is able to replicate in p53-deficient cells. It was tested in a phase I clinical study, where it was directly injected into the tumor cavity after surgical resection (#NCT00006106).[68, 69]

Ad5-Delta24, deleted in the E1A protein, replicates selectively in Rb-deficient tumor cells.[70-72] It was studied in a phase I trial for HGGs (#NCT03896568). In another phase I trial, it was engineered to express an integrin-binding RGD domain (#NCT00805376).[73]

3.4.3 MV

This approach involves a modification of the attenuated oncolytic MV, derived from the Edmonston vaccine lineage, targeted to making it capable of selectively binding the EGFR vIII expressed on the surface of tumor cells.

Two phase I clinical trials tested the effectiveness of MV in recurrent GBMs (#NCT00390299, #NCT0296216). Carcinogenic embryonic antigen (MV-CEA) and the human thyroidal sodium iodide symporter gene (MV-NIS) were added to enhance its antitumoral action.[74, 75]

3.4.4 PVS-RIPO

Oncolytic PVS-RIPO is an attenuated type 1 Sabin poliovirus in which the internal ribosomal entry site (IRES) has been replaced with the IRES of human rhinovirus type 2.[76, 77] PVS-RIPO targets and destroys

glioma cells with a classic oncolytic mechanism.[78] Data collected from the PVS-RIPO clinical trials confirmed the antitumoral activity, however, limited by low tolerability (#NCT02986178; #NCT01491893).

4 Carriers

The carriers of genetic material used in gene therapies are viruses and nanoparticles.

4.1 Viruses

Many viruses have proven to hold a specific neurotropism, which makes them perfect vehicles for targeting the glioma cells, transferring gene copies, codifying antitumor factors and, ultimately, fulfilling the therapeutic action.[79] Gene modification strategies have also involved engineered and replication-defective viruses. These are capable of delivering specific transgenes, reprogramming genetic expression and selectively lysing the tumor cells. Basically, two viral types have been progressively selected, namely, replication-deficient and replication-competent oncolytic viruses, the former being by far the most widely tested. Replication-deficient viruses are characterized by the removal of viral replication genes, and their replacement with transduced therapeutic genes. Conversely, replication-competent oncolytic viruses normally infect the cancer cells and replicate until causing the death of the tumor cells.

4.2 Nanoparticles

Nanoparticles are non-viral vehicles coming from the tremendous evolution of the nanotechnologies, which are able to carry some genetic material directly into the tumor cells.[80] Liposomes and cationic polymers, loaded with plasmid DNA and RNA, have been investigated as candidates for gene delivery.[81, 82] Nevertheless, these strategies ought to be considered as still largely experimental.

4.2.1 Liposomes

Synthetic lipid-based particles, also called as liposomes, are the gene carriers to have achieved the best level of evidence for HGGs.[83] Liposomes have been used mainly for carrying the IFN-β encoding gene. With the aim of facilitating the transport through the blood-brain barrier, some molecules have been added to the liposomes. Angiopeptide is an example. The combination of IFN-β and standard chemotherapy resulted in a more favorable outcome.[84] A recent study tested the efficacy of the combination between the liposome-angiopeptide-vector, associated with the TNF-related apoptosis-inducing ligand (TRAIL) gene, and the paclitaxel.[85]

4.2.2 Polymers

Polymers are macromolecules capable of binding DNA through electrostatic interactions.

Polyethylenimine (PEI) is a linear polymer, added with poly-ethileneglycol (PEG) in order to improve penetration into the tumor, used for the delivering of a TRAIL gene into glioma cells in mice.[86, 87]

The PEG-PEI polymer was further improved by introducing the integrin-binding RGD domain.[88]

The poly-amidoamine polymer (PAMAM) was conjugated with nanoparticles and viral Tat-peptide, and was used to deliver anti-EGFR and IFN- β. These polymers resulted in a reduction of tumor progression both in vitro and in vivo.[89-91]

5 Genome editing therapies

In the field of genome engineering, the genome editing technologies provide for a wider scale of DNA manipulation, which is performed throughout specific nucleases.

Nucleases are able to rearrange the genome as well as correct or silence some gene functions, thus explaining their therapeutic effects.

Zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs) and the novel CRISPR-Cas9 have been the most frequently examined.[92] ZFNs are enzymes consisting in a zinc finger DNA-binding domain which selectively binds and edits a target gene within complex genomes. Similarly, the TALENs can be delivered by plasmids and used for site-specific genome cleavage.[93]

The most advanced strategy includes the bacterial (CRISPR)/Cas9 system.

Cas9 protein is able to cut and modify a selected gene, under control of CRISPR sequences, resulting in a more exclusive genome reprogramming.[94, 95]

Overall, this is a very promising field that is likely to foster the next generation of CNS gene therapy.

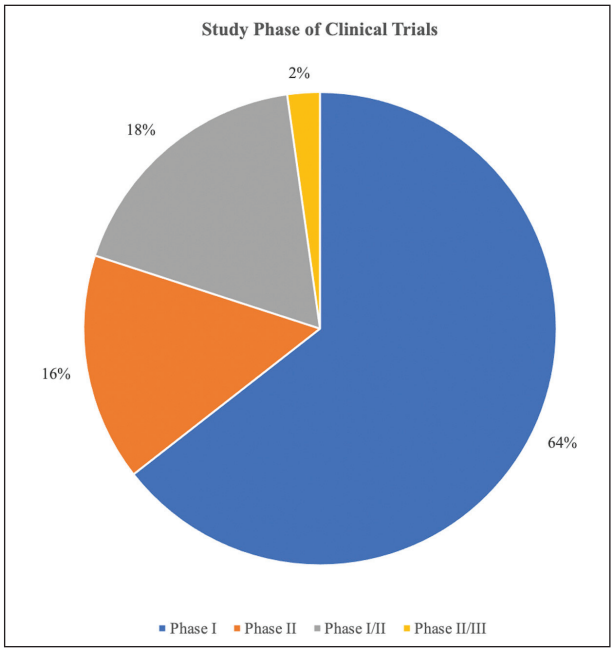
6 Clinical trials

Out of 45 clinical trials, 64 % were phase I, 18% phase I/II, 16% phase II and 2% phase II/III respectively (Graph 1). Oncolytic virotherapy, suicide gene therapy, tumor suppressor gene therapy and immunomodulatory gene therapy and were tested in 49%, 29%, 18% and 4% of them, respectively (Graph 2).

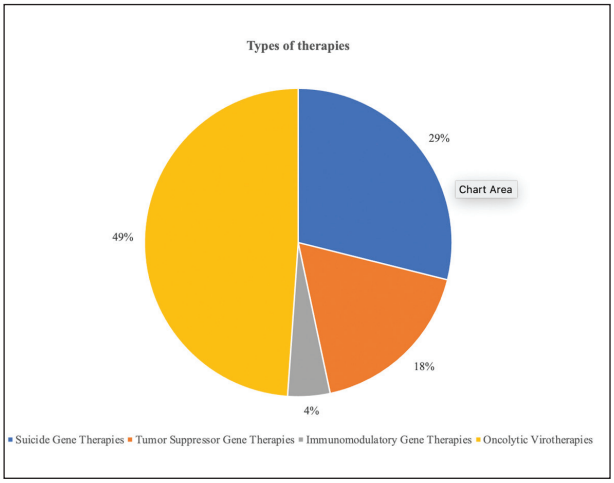
Table 3 summarizes the clinical trials on novel gene therapies for HGGs. (Table 3).

Discussion

The current biotechnological revolution, the progress made in translational medicine and the advances in neurology and neurosurgery have resulted in the development of revolutionary therapeutic approaches for a wide range of neuro-vascular and neuro-oncological pathologies.[96-99]



Graph 1. Pie graph showing the distribution of the clinical trials according to the study phase.



Graph 2. Pie graph showing the distribution of the clinical trials according to the type of gene therapy.

The identification of those mutations which are mainly responsible for the malignant behavior of HGGs has been the starting point for new and tailored therapies.[54, 100]

Gene therapies are designed for delivering and/or editing specific genes directly in the tumor genome. They ultimately destroy cancer cells, also enhancing the antitumoral immune response.

Translational Challenges

The selection process of the target genes to be transduced or replaced is greatly limited by an intrinsic genetic heterogeneity of the GBMs, but also by the progressive accumulation of mutations during the malignant progression. The major translational challenges of the gene therapies may be summarized in the widening of the spectrum of target genes within the tumor genome, improvement of the transduction efficiency of the carriers, and optimization of the administration routes. The major weakness of all the virus-based gene therapies lies in their immunogenic and inflammatory potential, which can be limited through the tailoring of their dosages.[101, 102] The risk of insertional

Table 3. Clinical trials on Gene therapies for high-grade gliomas

#	ClinicalTrials.gov Identifier	Title	Status	Study Phase	Conditions	Interventions	# of Patients Enrollment	Locations
1	NCT00870181	ADV-TK Improves Outcome of Recurrent High-Grade Glioma	Completed	II	Malignant Glioma of Brain Glioblastoma	ADV-TK/GCV, Surgery, Systemic chemotherapy	47	CHN
2	NCT00002824	Gene Therapy in Treating Patients With Primary Brain Tumors	Completed	I	Brain and Central Nervous System Tumors	Gene therapy, Chemotherapy, Ganciclovir, Surgery	NA	USA
3	NCT00751270	Phase 1b Study of AdV-tk + Valacyclovir Combined With Radiation Therapy for Malignant Gliomas	Completed	I	Malignant Glioma Glioblastoma Multiforme Anaplastic Astrocytoma	ADV/HSV-tk, Valacyclovir	15	USA
4	NCT03596086	HSV-tk + Valacyclovir + SBRT + Chemotherapy for Recurrent GBM	Recruiting	I/ II	Glioblastoma Multiforme Astrocytoma, Grade III	ADV/HSV-tk	62	USA
5	NCT00634231	A Phase I Study of AdV-tk + Prodrug Therapy in Combination With Radiation Therapy for Pediatric Brain Tumors	Active, not Recruiting	I	Malignant Glioma Recurrent Ependymoma	ADV/HSV-tk, Valacyclovir, Radiation	12	USA
6	NCT00589875	Phase 2a Study of AdV-tk With Standard Radiation Therapy for Malignant Glioma (BrTK02)	Completed	II	Malignant Glioma Glioblastoma Multiforme Anaplastic Astrocytoma	ADV/HSV-tk, Valacyclovir	52	USA
7	NCT00001328	Gene Therapy for the Treatment of Brain Tumors	Completed	I	Brain Neoplasm Neoplasm Metastasis	Ganciclovir, G1TKS-VNa.53 Producer Cell Line	15	USA
8	NCT03603405	HSV-tk and XRT and Chemotherapy for Newly Diagnosed GBM	Recruiting	I/ II	Glioblastoma Anaplastic Astrocytoma	ADV/HSV-tk	62	USA
9	NCT03576612	GMCI, Nivolumab, and Radiation Therapy in Treating Patients With Newly Diagnosed High-Grade Gliomas	Recruiting	I	Malignant Glioma	ADV/HSV-tk, Valacyclovir, Radiation, Temozolomide, Nivolumab	36	USA
10	NCT01985256	Study of a Retroviral Replicating Vector Given Intravenously to Patients Undergoing Surgery for Recurrent Brain Tumor	Completed	I	Glioblastoma Multiforme Anaplastic Astrocytoma Anaplastic Oligodendroglioma Anaplastic Oligoastrocytoma	Toca 511, Toca FC	17	USA

#	ClinicalTrials.gov Identifier	Title	Status	Study Phase	Conditions	Interventions	# of Patients Enrollment	Locations
11	NCT01156584	A Study of a Retroviral Replicating Vector Combined With a Prodrug Administered to Patients With Recurrent Malignant Glioma	Completed	I	Glioblastoma Anaplastic Astrocytoma Anaplastic Oligodendroglioma Anaplastic Oligoastrocytoma	Toca 511, Toca FC	54	USA
12	NCT01470794	Study of a Retroviral Replicating Vector Combined With a Prodrug to Treat Patients Undergoing Surgery for a Recurrent Malignant Brain Tumor	Completed	I	Glioblastoma Multiforme Anaplastic Astrocytoma Anaplastic Oligodendroglioma Anaplastic Oligoastrocytoma	Toca 511, Toca FC	58	USA
13	NCT02414165	The Toca 5 Trial: Toca 511 & Toca FC Versus Standard of Care in Patients With Recurrent High Grade Glioma	Terminated	II/ III	Glioblastoma Multiforme Anaplastic Astrocytoma	Toca 511, Toca FC, Lomustine, Temozolomide, Bevacizumab	403	USA
14	NCT01811992	Combined Cytotoxic and Immune-Stimulatory Therapy for Glioma	Active, not Recruiting	I	Malignant Glioma Glioblastoma Multiforme	Dose Escalation of Ad-hCMV-TK, Ad-hCMV-Flt3L	19	USA
15	NCT03544723	Safety and Efficacy of Ad-p53 Combined With Checkpoint Inhibitor in Head and Neck Cancer	Recruiting	II	Recurrent Head and Neck Cancer	Ad-P53	20	USA
16	NCT02842125	Safety and Efficacy of Intra-Arterial and Intra-Tumoral Ad-p53 With Capecitabine (Xeloda) or Anti-PD-1 in Liver Metastases of Solid Tumors and Recurrent Head and Neck Squamous Cell Cancer	Recruiting	I/ II	Metastatic Solid Tumor Cancer Recurrent Head and Neck Cancer	Ad-P53, Xeloda, Keytruda, Opdivo	24	USA
17	NCT00017173	S0011, Gene Therapy & Surgery Followed by Chemo & RT in Newly Diagnosed Cancer of the Mouth or Throat	Terminated	II	Head and Neck Cancer	Ad5CMV-p53 gene, Cisplatin, Surgery, Radiation therapy	13	USA
18	NCT00003257	Gene Therapy in Treating Patients With Recurrent Head and Neck Cancer	Unknown	II	Head and Neck Cancer	Ad5CMV-p53 gene	39	USA

#	ClinicalTrials.gov Identifier	Title	Status	Study Phase	Conditions	Interventions	# of Patients Enrollment	Locations
19	NCT00004041	Gene Therapy in Treating Patients With Recurrent Malignant Gliomas	Completed	I	Brain and Central Nervous System Tumors	Ad5CMV-p53 gene, Surgery	NA	USA
20	NCT00004080	Gene Therapy in Treating Patients With Recurrent or Progressive Brain Tumors	Completed	I	Brain and Central Nervous System Tumors	Recombinant adenovirus-p53 SCH-58500, Surgery	NA	NA
21	NCT02031965	Oncolytic HSV-1716 in Treating Younger Patients With Refractory or Recurrent High Grade Glioma That Can Be Removed By Surgery	Terminated	I	Recurrent Childhood Anaplastic Astrocytoma Recurrent Childhood Anaplastic Oligoastrocytoma Recurrent Childhood Anaplastic Oligodendroglioma Recurrent Childhood Giant Cell Glioblastoma Recurrent Childhood Glioblastoma Recurrent Childhood Gliomatosis Cerebri Recurrent Childhood Gliosarcoma	Oncolytic HSV-1716, Dexamethasone, Surgery	2	USA
22	NCT00031083	Dose Escalation Study to Determine the Safety of IFN-Beta Gene Transfer in the Treatment of Grade III & Grade IV Gliomas"	Suspended	I	Glioblastoma Multiforme Anaplastic Astrocytoma Oligoastrocytoma Gliosarcoma	Interferon-beta	35	USA
23	NCT02026271	A Study of Ad-RTS-hIL-12 With Veledimex in Subjects With Glioblastoma or Malignant Glioma	Active, not Recruiting	I	Glioblastoma Multiforme Anaplastic Oligoastrocytoma	Ad-RTS-hIL-12, Veledimex	48	USA
24	NCT02062827	Genetically Engineered HSV-1 Phase 1 Study for the Treatment of Recurrent Malignant Glioma	Recruiting	I	Recurrent Glioblastoma Multiforme Progressive Glioblastoma Multiforme Anaplastic Astrocytoma or Gliosarcoma	M032 (NSC 733972)	36	USA

#	ClinicalTrials.gov Identifier	Title	Status	Study Phase	Conditions	Interventions	# of Patients Enrollment	Locations
25	NCT03911388	HSV G207 in Children With Recurrent or Refractory Cerebellar Brain Tumors	Recruiting	I	Brain and Central Nervous System Tumors Glioblastoma Multiforme Astrocytoma Neuroectodermal Tumors Primitive Cerebellar PNET Childhood Brain Neoplasms Malignant Cerebellar Neoplasm Medulloblastoma Recurrent Virus, HSV	G207	15	USA
26	NCT02457845	HSV G207 Alone or With a Single Radiation Dose in Children With Progressive or Recurrent Supratentorial Brain Tumors	Recruiting	I	Supratentorial Malignant Neoplasms Malignant Glioma Glioblastoma Anaplastic Astrocytoma PNET Cerebral Primitive Neuroectodermal Tumor Embryonal Tumor	G207	18	USA
27	NCT00028158	Safety and Effectiveness Study of G207, a Tumor-Killing Virus, in Patients With Recurrent Brain Cancer	Completed	I/ II	Glioma Astrocytoma Glioblastoma	G207	65	NA
28	NCT00157703	G207 Followed by Radiation Therapy in Malignant Glioma	Completed	I	Malignant Glioma	G207	9	USA

#	ClinicalTrials.gov Identifier	Title	Status	Study Phase	Conditions	Interventions	# of Patients Enrollment	Locations
29	NCT02031965	Oncolytic HSV-1716 in Treating Younger Patients With Refractory or Recurrent High Grade Glioma That Can Be Removed By Surgery	Terminated	I	Recurrent Childhood Anaplastic Astrocytoma Recurrent Childhood Anaplastic Oligoastrocytoma Recurrent Childhood Anaplastic Oligodendroglioma Recurrent Childhood Giant Cell Glioblastoma Recurrent Childhood Glioblastoma Recurrent Childhood Gliomatosis Cerebri Recurrent Childhood Gliosarcoma	HSV-1716, Dexamethasone, Surgery	2	USA
30	NCT03152318	A Study of the Treatment of Recurrent Malignant Glioma With rQNestin34.5v.2	Recruiting	I	Malignant Glioma Malignant Astrocytoma Oligodendroglioma Anaplastic Ependymoma Ganglioglioma Pylocytic/Pylomyxoid Astrocytoma Glioblastoma Multiforme	rQNestin, Cyclophosphamide, Stereotactic biopsy	108	USA
31	NCT02197169	DNX-2401 With Interferon Gamma (IFN-γ) for Recurrent Glioblastoma or Gliosarcoma Brain Tumors	Completed	I	Glioblastoma or Gliosarcoma	Single intratumoral injection of DNX-2401, Interferon-gamma	37	USA
32	NCT00006106	ONYX-015 With Cisplatin and Fluorouracil in Treating Patients With Advanced Head and Neck Cancer	Withdrawn	I	Lip and Oral Cavity Cancer Head and Neck Cancer Oropharyngeal Cancer	Cisplatin, Fluorouracile, ONYX-015	0	USA
33	NCT00805376	DNX-2401 (Formerly Known as Delta-24-RGD-4C) for Recurrent Malignant Gliomas	Completed	I	Brain Cancer Central Nervous System Diseases	DNX-2401, Tumor Removal	37	USA

#	ClinicalTrials.gov Identifier	Title	Status	Study Phase	Conditions	Interventions	# of Patients Enrollment	Locations
34	NCT03896568	Oncolytic Adenovirus DNX-2401 in Treating Patients With Recurrent High-Grade Glioma	Recruiting	I	Recurrent Anaplastic Astrocytoma Recurrent Glioblastoma/ Gliosarcoma Recurrent Malignant Glioma	Oncolytic Adenovirus Ad5-DNX-2401, Therapeutic Conventional Surgery	36	USA
35	NCT01956734	Virus DNX2401 and Temozolomide in Recurrent Glioblastoma	Completed	I	Glioblastoma Multiforme Recurrent Tumor	DNX2401, Temozolomide	31	ES
36	NCT01301430	Parvovirus H-1 (ParvOryx) in Patients With Progressive Primary or Recurrent Glioblastoma Multiforme.	Completed	I/ II	Glioblastoma Multiforme	H-1PV	18	DE
37	NCT01582516	Safety Study of Replication-competent Adenovirus (Delta-24-rgd) in Patients With Recurrent Glioblastoma	Completed	I/ II	Brain Tumor Recurring Glioblastoma	delta-24-RGD adenovirus	20	NL
38	NCT02962167	Modified Measles Virus (MV-NIS) for Children and Young Adults With Recurrent Medulloblastoma or Recurrent ATRT	Recruiting	I	Medulloblastoma, Childhood, Recurrent Atypical Teratoid/Rhabdoid Tumor Medulloblastoma Recurrent	Modified Measles Virus, Modified Measles Virus Lumbar Puncture	46	USA
39	NCT00390299	Viral Therapy in Treating Patients With Recurrent Glioblastoma Multiforme	Completed	I	Anaplastic Astrocytoma Anaplastic Oligodendroglioma Mixed Glioma Recurrent Glioblastoma	Carcinoembryonic Antigen-Expressing Measles Virus, Therapeutic Conventional Surgery	23	USA
40	NCT01491893	PVSRIPO for Recurrent Glioblastoma (GBM)	Active, not recruiting	I	Glioblastoma Glioma Malignant Glioma	Recombinant nonpathogenic polio-rhinovirus chimera (PVSRIPO)	61	USA
41	NCT02986178	PVSRIPO in Recurrent Malignant Glioma	Recruiting	II	Malignant Glioma	PVSRIPO	122	USA

#	ClinicalTrials.gov Identifier	Title	Status	Study Phase	Conditions	Interventions	# of Patients Enrollment	Locations
42	NCT03973879	Combination of PVSRIPO and Atezolizumab for Adults With Recurrent Malignant Glioma	Withdrawn	I/ II	Malignant Glioma	PVSRIPO, Atezolizumab	0	NA
43	NCT03043391	Phase 1b Study PVSRIPO for Recurrent Malignant Glioma in Children	Recruiting	I	Malignant Glioma Anaplastic Astrocytoma/ Oligoastrocytoma/ Oligodendroglioma Glioblastoma/ Gliosarcoma Atypical Teratoid/Rhabdoid Tumor of Brain Medulloblastoma Ependymoma Pleomorphic Xanthoastrocytoma of Brain Embryonal Tumor of Brain	Polio/Rhinovirus Recombinant (PVSRIPO)	12	USA
44	NCT01174537	New Castle Disease Virus (NDV) in Glioblastoma Multiforme (GBM), Sarcoma and Neuroblastoma	Withdrawn	I/ II	Glioblastoma Sarcoma Neuroblastoma	New Castle Disease Virus	0	IL
45	NCT02340156	Phase II Study of Combined Temozolomide and SGT-53 for Treatment of Recurrent Glioblastoma	Terminated	II	Recurrent Glioblastoma	SGT-53, Temozolomide	1	USA, TW

CHN: Cina; DE: Germany; ES: Spain; IL: Israel; NL: Netherlands; TW: Taiwan; USA: United States of America.

mutagenesis is a further major hurdle. The viral genotoxicity, namely the potential activation of oncogenes due to an incorrect transduction, can be decreased by manufacturing self-inactivating vectors without their own promoter.[103, 104] The route of administration of these drugs is also a concern. Since most viral vehicles are characterized by a too rapid systemic clearance, stereotactic or endoscopic minimally invasive administration routes have been proposed, with the same advantage already reported for other pathologies.[105, 106]

Ongoing Trends and Future Prosepects

One of the most promising genetic approaches is the restoration of the physiologic antitumor function of oncosuppressor genes or interleukins, such as p53 and IFN. Similarly, the encouraging results of the suicide gene and oncolytic virotherapies justify their increasingly large role. It must be stressed, however, that to date none of these therapies have proven their effect as a monotherapy. The near future should also focus on the engineering of better carriers, capable of leading the therapeutic effect due to their smaller size, lower toxicity and immunologic potential, as well as improved cell penetrance compared to viral vectors. Nanotechnologies came into aid with biocompatible nanoparticles, liposomes primarily, whose known advantages have been reported.[107, 108] The ideal carriers should be capable of a wider tissue distribution. The advances in genetic engineering will make it possible to personalize the treatments, according to patient and tumor genetics.

The development of new administration routes improved therapeutic protocols and concomitant immune-boosting strategies will optimize the gene therapies.

Conclusion

Gene therapy is the newest approach among the tailored therapies for malignant brain tumors.

The suicide gene, tumor suppressor gene, immunomodulatory gene, and oncolytic therapies have been most widely tested in clinical trials, although the totality of evidence about their effectiveness is still at an experimental level.

The transfer and manipulation of the target genes involved biological carriers such as adenoviruses, HSVs, retroviruses and AAVs. The advances of nanotechnology have led to the recent introduction of liposomes and polymers.

The future of gene therapies is represented by the selection of new and more effective target genes, along with the engineering and manufacturing of non-viral gene-delivery vectors, given that they are capable of a greater and safer spreading capacity.

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Original article

The impact of stem cells in neuro-oncology: applications, evidence, limitations and challenges

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Abstract. *Background:* Stem cells (SCs) represent a recent and attractive therapeutic option for neuro-oncology, as well as for treating degenerative, ischemic and traumatic pathologies of the central nervous system. This is mainly because of their homing capacity, which makes them capable of reaching the inaccessible SC niches of the tumor, therefore, acting as living drugs. The target of the study is a comprehensive overview of the SC-based therapies in neuro-oncology, also highlighting the current translational challenges of this type of approach. *Methods:* An online search of the literature was carried out on the PubMed/MEDLINE and ClinicalTrials.gov websites, restricting it to the most pertinent keywords regarding the systematization of the SCs and their therapeutic use for malignant brain tumors. A large part of the search was dedicated to clinical trials. Only preclinical and clinical data belonging to the last 5 years were shortlisted. A further sorting was implemented based on the best match and relevance. *Results:* The results consisted in 96 relevant articles and 31 trials. Systematization involves a distinction between human embryonic, fetal and adult, but also totipotent, pluripotent or multipotent SCs. Mesenchymal and neuronal SCs were the most studied for neuro-oncological illnesses. 30% and 50% of the trials were phase I and II, respectively. *Conclusion:* Mesenchymal and neuronal SCs are ideal candidates for SCs-based therapy of malignant brain tumors. The spectrum of their possible applications is vast and is mainly based on the homing capacity toward the tumor microenvironment. Availability, delivery route, oncogenicity and ethical issues are the main translational challenges concerning the use of SCs in neuro-oncology. (www.actabiomedica.it)

Key words: Cell-Based Therapy, High-Grade Glioma, Neuro-Oncology, Somatic Cell Therapy, Stem Cells

Background

A large part of modern neurology rests on the seminal work of Santiago Ramón y Cajal, which in 1913, demonstrated for the first time in the history of medicine that neurons can regenerate equally to other tissues (1-3). Since that time, this along with other pivotal points, has led to several steps forward in a better understanding of the pathophysiology of several illnesses affecting the central nervous system (CNS) (4-10). More recently, in the CNS as in other systems and tissues, the regenerative property was clarified as being attributable to the existence of 'stem cells' which, by definition, are immature undifferentiated cells having a capacity of self-renewal. The self-renewal capacity practically consists in the fact that one of the two daughters arising from the progenitor cell can differentiate into any other specialized cell of a given tissue, with the remaining one instead maintaining the tissue-specific stem cell heritage. The possibility of growth,

regeneration and repair of a given tissue is entirely attributable to the subsistence of this cellular population, which seems to hold and play regulative functions, while also being subject to a functional control within its specific microenvironment, also referred to as 'niche' (11-22). Currently, no field of medicine can be thought as immune to the enthusiasm coming from the potential applications of stem-cell therapy, which can currently be considered the fully-fledged backbone of regenerative medicine.

The neuro-oncological field has been among the first to be interested in the stem cell revolution, mainly because of the kinetic and qualitative aspects which this specific cellular population has in common with tumors, namely, the high replicative rate, lack of contact inhibition, as well as capability to origin teratocarcinomas in mice, to cite just a few. However, in recent years, the explosive volume of the literature about the use of stem cells in any field of neuroscience, on one hand, and the dramatic increase in the qualitative and quantitative spectrum related to the stem cells on the other, have unavoidably led to confusion, especially regarding the line between the preclinical and clinical level of evidence.

This study is aimed at an updated and comprehensive overview of the theoretical and practical impact of the stem cell-based therapies in neuro-oncology, along with the assessment of their clinical level of evidence, limitations and future challenges.

Methods

An online search of the literature was carried out on the PubMed/MEDLINE (https://pubmed.ncbi.nlm.nih.gov) and ClinicalTrials.gov websites (https://clinicaltrials.gov). On PubMed/MEDLINE, both the MeSH (Medical Subject Headings) database and free mode search were used to carry out a search of the literature combining the following keywords: "Stem Cells" [MeSH], "Cell- and Tissue-Based Therapy" [MeSH], "Regenerative Medicine" [MeSH], "Cell Engineering" [MeSH], "Genetic Therapy" [MeSH], "Gene Transfer Techniques" [MeSH], "Central Nervous System" [MeSH], "Brain" [MeSH], "brain tumors" [text word] and "Stem Cells" [text word]. "Classification criteria", "clinical employment" and "therapeutic use" were the subheadings of the MeSH database search. Only articles in English or translated into English, published in the last five years, and regarding the field of neuro-oncology were selected. Based on the best match and relevance inferred by the titles and summaries, a further sorting was carried out.

On the ClinicalTrials.gov finder, the search terms "Brain tumors" and "Stem Cells" were used in the "condition/disease" and "other terms" fields, respectively. No restriction for country, recruitment status and study phase were applied. A brief summary of the retrieved trials was reported focusing on the status and phase, separately from the results.

Results

1. Volume of the Literature

The search returned a total of 1,802 articles and 81 clinical trials. After the implantation of the exclusion criteria and removal of duplicates, 96 relevant articles and 31 trials were sorted.

2. Overview and Systematization of the Stem Cells

2.1 Origin

Based on their origin, stem cells may be classified as embryonic, fetal or adult.

Human embryonic stem cells (h-ESCs) originate from a blastocyst inner cell mass. They hold atypical cell cycle regulation, which explains their unlimited potential of propagation in culture, specific set of markers, lack of contact inhibition and maximal potential of differentiation (14, 23-27). Typically, they are known to form teratocarcinomas in nude mice (23, 28-30).

Fetal stem cells come from fetal blood and fetal tissues and form blood cells, tissues and organs. Umbilical cord blood, veins and matrix are sources of fetal stem cells, along with the amnion and placenta. Umbilical cord fetal stem cells have yielded great interest because they are readily available, inexpensive, multipotent and immune from ethical issues (31-36).

Adult stem cells are present in all differentiated tissue (37-39). They were isolated for the first time in the hematopoietic system, but subsequently also in the adult CNS (40-44). Adult stem cells have been reported to have tremendous plasticity and an equally extensive regenerative capability. The main strength of this type of stem cell lies, first, in its theoretically high availability for autologous transplantation, and second, in its absence of immunological complications (45, 46).

2.2 Plasticity

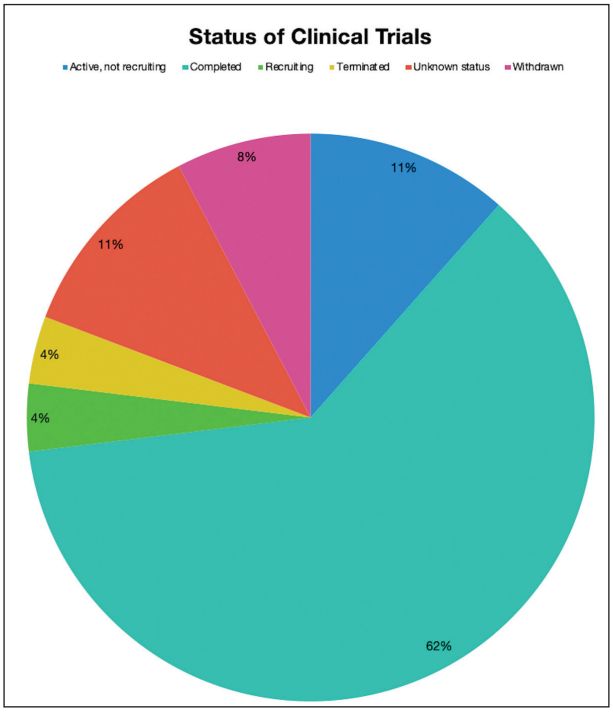
Stem cells may also be classified according to their plasticity. This systematization entails the distinction between totipotent, pluripotent or multipotent cells.

In principle, the sole and unique totipotent cell is the zygote along with its progeny (47). Every somatic cell, embryonic and extra-embryonic tissue included, comes from the totipotent progenitor cell. In contrast, the pluripotent cell, also referred to as h-ESCs, since it originates from the blastocyst inner cell mass, may stem from all three of the germ layers, giving birth to ectodermal, mesodermal and endodermal tissues, but it does not stem from embryonic or extra-embryonic tissue (22, 48). Multipotent cells, belonging to the three germ layers even in the embryonic stage, are capable of giving birth to a vast amount of cell lineage which, in the past, was thought to generate lines belonging exclusively to the same tissue where they reside. Nevertheless, this assumption has been recently questioned (49). Being present also in the adult age, multipotent cells sustain auto-regeneration and allows tissues to repair themselves after damage. There are four known main types of human multipotent cells, namely, mesenchymal stem cells (h-MSCs), neural stem cells (h-NSCs), bone marrow stromal cells, and olfactory ensheathing cells. Within the CNS, h-NSCs have been isolated from the three sites capable of a neuronal turnover *par excellence*: the adult ventricular-subventricular zone, the olfactory bulb and the hippocampus (50, 51). At these sites, h-NSCs have been proven to differentiate into neurons, astrocytes and oligodendrocytes, as well as being responsible for the maintenance of the homeostatic and regenerative processes (52, 53). The h-NSCs hold a restricted neural differentiation capability, which is practically committed to specific subpopulation lineages (54-60). Both adult h-NSCs and h-ESCs are related to specific biomarkers of embryogenesis and adult neurogenesis (61). A further, more recent class of stem cells is represented by the human-induced pluripotent staminal cells (h-iPSCs). They derive from genetically reprogrammed adult somatic cells, thus making them theoretically unlimited in number. They also have proven to have the same potential of pluripotent cells (62-64). Both of these aspects account for the reasons why h-iPSCs have aroused the maximum interest among all stem cells, being that there is a theoretically inexhaustible source of pluripotent cells.

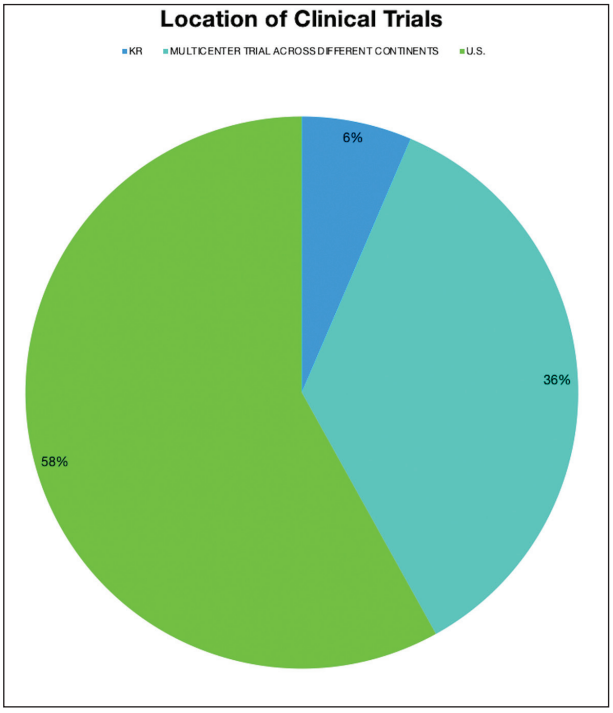
3. Evidence on the Effectiveness and Safety in Neuro-oncology

The highest clinical level of evidence about the effectiveness of stem cell-based therapy consisted in 31 clinical trials, for a total of 1,103 patients recruited, summarized in Table 1 (Suppl Table). Of these, 30% were phase 1, 50% phase 2 and 7% phase 3 (Graph 1). Most of the trials were executed in the U.S. (60%), whereas 32% were multicentric (Graph 2). To date, only 64% were completed (Graph 3). In 24 trials (77.4%), peripheral blood stem cells, namely hematopoietic cells, were involved, with the aim of assessing their effectiveness in counteracting the myeloablative effects of the chemotherapy against malignant brain tumors. In 4 trials (12.9%), h-NSCs were tested basically as carriers for oncolytic viruses (3.2%), or also as drugs in a genetically modified form (9.6%). In 2 further trials, tumor-derived stem cells were used for a vaccine (Graph 4). In all cases, stem cells were used in association with a defined chemo-radiotherapy protocol considered as standard of care. Only 2 trials have tabular results available. Both of them studied the effectiveness of radiation therapy in achieving a significant increase of progression-free survival and overall survival of glioblastoma, secondary to the inclusion of tumor peripheral margin encompassing the tumor stem cells. Both were able to prove that this strategy adds benefits and has a good safety profile.

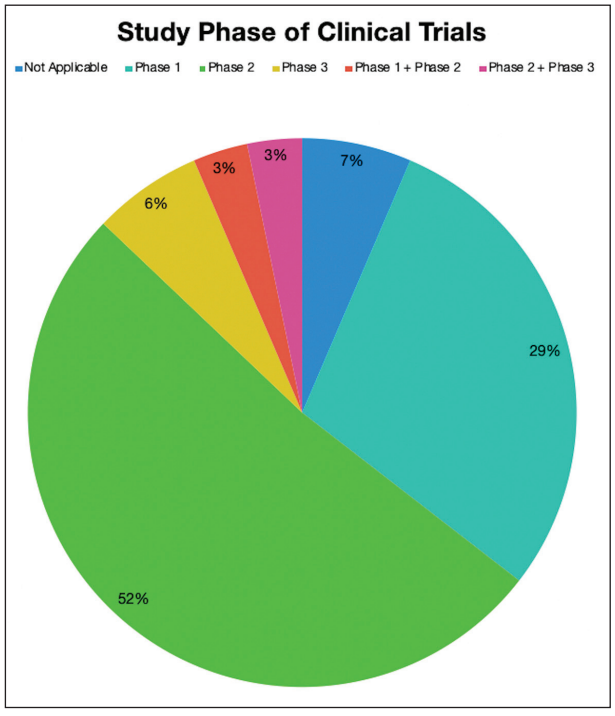
Most of the evidence about the effectiveness of the h-NSCs-based therapy, however, belongs to a preclinical level (65-74). Apart from h-NSCs, h-MSCs also have been widely tested for their potential use in



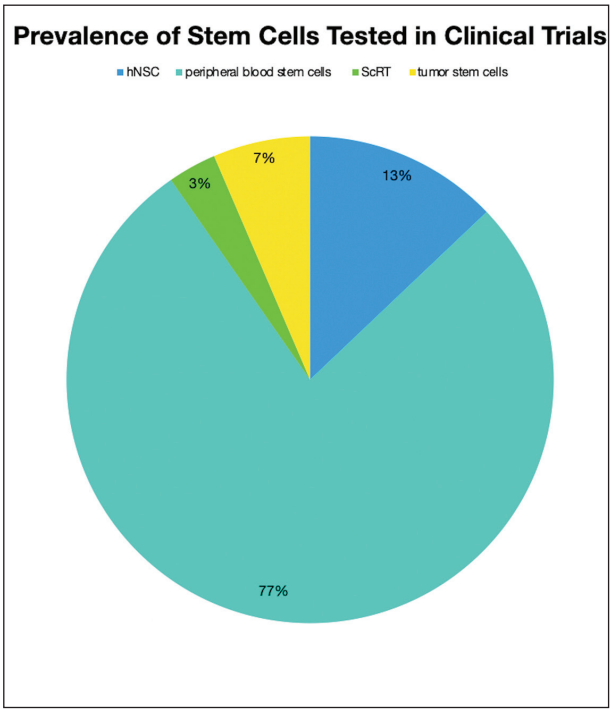
Graph 1. Pie graph showing the distribution of the clinical trials according to the status



Graph 3. Pie graph showing the distribution of the clinical trials according to the location



Graph 2. Pie graph showing the distribution of the clinical trials according to the study phase



Graph 4. Pie graph showing the distribution of the type of stem cell tested

the treatment of CNS malignancies, often with positive results being obtained in animals (75, 76).

Discussion

The rationale at the base of the use of stem cells for treating malignant CNS tumors lies in various aspects. These cells are theoretically capable of: surrounding the glioblastoma and inhibiting the spreading of the tumor (77, 78); being selective deliverers of drugs (79); transferring retrovirus-mediated transgene against tumors (80); delivering adenovirus-mediated tumor necrosis factor genes inducing apoptosis (79, 81); carrying oncolytic herpes simplex viruses (82), and so forth.

The aspect common to all the aforementioned potential mechanisms is the intrinsic homing property of specific types of stem cells toward the neural tissue (83). The homing also involves the great aptitude of these cells to migrate into the 'niches' of the tumor, which are the sites where the tumor stem cells reside, giving rise to recurrences both in malignant gliomas and in other CNS tumors (22, 84-86). The homing property regards particularly the h-NSCs and h-MSCs, which have been, not by chance, the most studied lineages in this sense. From a molecular standpoint, the most known pathway at the base of stem cell homing is the complex CXCR4 receptor-stromal cell-derived factor 1 ligand (CXCL12), which is coupled with a G-protein (87). Typically, this complex is expressed at a high level at sites known for their neurogenesis, namely, the subventricular zone, olfactory bulb and the hippocampus. In the mouse brain, the pattern of migration of the therapeutic stem cells toward the tumor site has been reported to be similar to that of h-NSCs (77, 88). Further mediators of cellular migration, through the interaction with specific receptors, are the stem cell factor, the platelet-derived growth factor BB, and the vascular endothelial growth factor (VEGF) (89). In particular, quantitative and qualitative variations of the VEGF and interactions with chemotactic factors Ang2 and GROα have been associated with the tropism of h-NSCs, but also affect a wide range of vascular pathologies of the CNS (90-92). In regard to h-MSCs, the complex macrophage migration inhibitory factor-CXCR4 has been recently reported to be among the main pathways in migration and homing in this specific population of stem cells (93). Even h-iPSCs are thought to hold chemotactic properties toward the glioma cells, although with mechanisms that are still largely unknown (94). For all of these types of cells, the migration property is significantly conditioned by the tumoral microenvironment (95). The selectivity of the stem cells, acting as organic delivery vehicles toward the tumor, is paramount for overcoming the immune tolerance and immune escape of conventional chemotherapy, and has even been brought into play for pathologies other than CNS tumors (96-98). Once inside the tumor, stem cells can deliver toxins, anti-proliferative drugs, pro-apoptotic, anti-angiogenetic and immunomodulating agents, prodrug activators, nanoparticles and also viral vectors, the last two with the goal of infecting and killing the neoplastic cells (99). These approaches may also be combined with one another or used with conventional chemotherapy in order to enhance the overall effectiveness of the stem-cell therapies. The route of administration of the therapeutic stem cell is a concern in the management of these therapies. In localized brain tumors that underwent surgical gross total resection, the residual tumor cavity may be considered as an elective site for direct release of these drugs. Conversely, diffused, bilateral or advanced CNS tumors present more challenges in their treatment, and the possible routes of administration can be stereotactic or endoscopic. Endoscopy in particular is the means by which the stem cell is delivered into the ventricular cavity, with this technique being moreover considered as something new in addition to the known advantages coming from this minimal invasive approach for other neurological and neurosurgical pathologies (100, 101).

The results of the present study have highlighted, however, that the near totality of the evidence arises from in-vitro or in-vivo data on animals, therefore, they have to be considered as being part of a still preclinical phase. None of the reported trials have been, at the current time, conclusive about the effectiveness and safety of the stem cell-based neoadjuvant therapy for brain tumors. Even today, several factors limit the use of stem cells in the current therapeutic protocol of CNS tumors, with these aspects representing, at the

same time, the major challenges of the stem cell-based therapies. A primary factor to be considered is their availability, which is undoubtedly higher for h-MSCs and h-iPSCs, when compared to h-NSCs, for the reason that a precious source of h-iPSCs is the adipose tissue. The same concepts can be extended also to the numerous ethical issues affecting mainly the h-NSCs, and affecting the h-MSCs and h-iPSCs to a lesser extent. The theoretical possibility of a xenogeneic source of stem cells should be considered as a further possible solution to most of these issues in the future . With the advent of the i-PSC, a large part of the problem regarding the use of stem cells has been partially solved, and significant steps forward have been taken in the context of the translational field. Nevertheless, it must be stressed that the therapeutic capability of this specific cell population is still uncertain.

A further issue of no less importance is that of the oncogenicity related to the grafted stem cell, about which several shadows still do exist. Not surprisingly, non-immortalization techniques are generally considered safer than immortalization ones, even though also this assumption requires further evidence.

Conclusion

The current approach related to the implementation of the stem cell-based therapies in neuro-oncology mainly involves the use of multipotent stem cells, having the h-iPSCs has, however, aroused interest because of their theoretically unlimited availability.

There has been much more testing on h-MSCs and h-NSCs compared to other types of cells, due to a high tropism toward malignant CNS tumor niches.

The possible approaches to CNS malignancies involving the stem cells are numerous, ranging between the inhibition of the spreading of the neoplastic cells and the carrying of oncolytic viruses.

Almost the entire volume of evidence about the effectiveness and safety of the stem cell therapies in neuro-oncology is still at a preclinical level.

The availability, delivery route and oncogenicity, along with the ethical issues, constitute the main challenges related to the use of stem cells in neuro-oncology.

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Disclosure - 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.

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Original article

Potential roads for reaching the summit: an overview on target therapies for high-grade gliomas

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Abstract. *Background:* The tailored targeting of specific oncogenes represents a new frontier in the treatment of high-grade glioma in the pursuit of innovative and personalized approaches. The present study consists in a wide-ranging overview of the target therapies and related translational challenges in neuro-oncology. *Methods:* A review of the literature on PubMed/MEDLINE on recent advances concerning the target therapies for treatment of central nervous system malignancies was carried out. In the Medical Subject Headings, the terms "Target Therapy", "Target drug" and "Tailored Therapy" were combined with the terms "High-grade gliomas", "Malignant brain tumor" and "Glioblastoma". Articles published in the last five years were further sorted, based on the best match and relevance. The ClinicalTrials.gov website was used as a source of the main trials, where the search terms were "Central Nervous System Tumor", "Malignant Brain Tumor", "Brain Cancer", "Brain Neoplasms" and "High-grade gliomas". *Results:* A total of 137 relevant articles and 79 trials were selected. Target therapies entailed inhibitors of tyrosine kinases, PI3K/AKT/mTOR pathway, farnesyl transferase enzymes, p53 and pRB proteins, isocitrate dehydrogenases, histone deacetylases, integrins and proteasome complexes. The clinical trials mostly involved combined approaches. They were phase I, II, I/II and III in 33%, 42%, 16%, and 9% of the cases, respectively. *Conclusion:* Tyrosine kinase and angiogenesis inhibitors, in combination with standard of care, have shown most evidence of the effectiveness in glioblastoma. Resistance remains an issue. A deeper understanding of the molecular pathways involved in gliomagenesis is the key aspect on which the translational research is focusing, in order to optimize the target therapies of newly diagnosed and recurrent brain gliomas. (www.actabiomedica.it)

Key words: Glioblastoma; Malignant Brain Tumors; Neuro-Oncology; Target Therapy; Tyrosine Kinase Inhibitors.

Background

High-grade gliomas, with glioblastoma (GBM) being the progenitor, are the most lethal primary brain tumors of all because of the certainty of recurrence and mortality.[1-4] As a matter of fact, the median overall survival is no longer than 15 months, despite current multimodality treatment including surgery, radiotherapy and chemotherapy.[5,6]

The significant resistance of GBM to therapy is related to the heterogeneous genetic landscape of the tumor. High-grade gliomas harbor recurrent molecular abnormalities which are involved in the maintenance of the cell's cycle and growth, the tumor

microenvironment, pathological angiogenesis, DNA repair and apoptosis.[7-10]

Advances in genetics and the studies of epigenetics in many pathologies affecting the central nervous system (CNS) have allowed the molecular characterization, as well as the identification of the anomalies in the cellular signaling pathways[11-14]. The same insights have been of utmost importance also in neuro-oncological field, GBM first, where they led to a better understanding of tumor progression and cancer drug escape.[15-20] A deeper understanding of the malignant GBM phenotype has recently improved the knowledge about the biology of cancer, which is the starting point for identifying specific biomarkers and for developing new agents for targeting specific steps in the transduction pathways of glioma cells.[21] Novel tailored therapies include drugs aimed at counteracting the effects of the neoplastic genetic deregulation, pathological angiogenesis and growth factor receptors; the latter with their downstream signaling pathways.

An overview of the target therapeutic strategies and challenges in developing effective agents is reported as follows.

Methods

The search of the literature was performed on the PubMed/MEDLINE (https://pubmed.ncbi.nlm.nih.gov) search engine, with combinations of Medical Subject Headings (MeSH) terms and text words, and on the ClinicalTrials.gov website (https://clinicaltrials.gov). The MeSH terms "Target Therapy", "Target drug" and "Tailored Therapy" were combined with the MeSH terms "High-grade gliomas", "Malignant brain tumor" and "Glioblastoma". In addition to original articles, our research involved reviews and editorials. The sorting of articles was carried out focusing on the most relevant studies chosen according to titles and abstracts.

On the ClinicalTrials.gov database the texts words "Central Nervous System Tumor", "Malignant Brain Tumor", "Brain Cancer", "High-grade gliomas" and "Brain Tumor" were used for the field "condition/disease". Only trials regarding target therapies, without restrictions for localization, study phase and recruitment status were selected. Filtering included articles published in the last five years, in English or translated into English. A descriptive analysis was provided.

Results

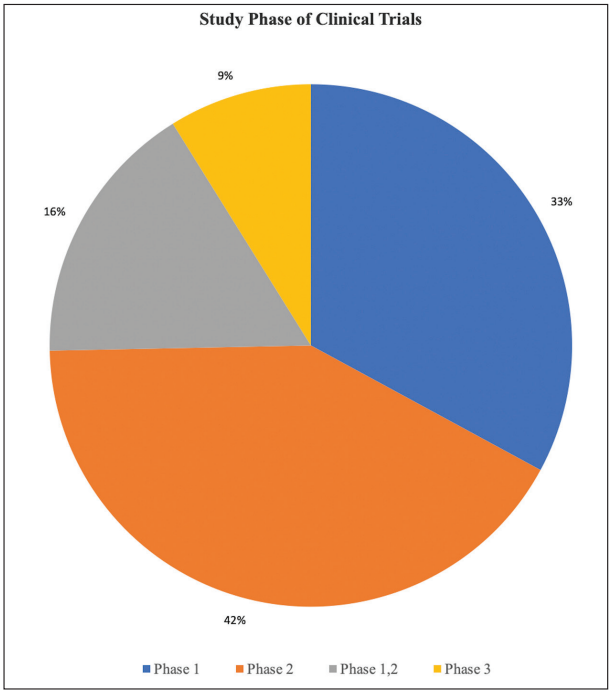
1. *Volume of the Literature*

The search retrieved a total of 178 articles and 148 clinical trials. After the implementation of the exclusion criteria and removal of duplicates, 137 articles and 79 randomized and non-randomized clinical trials were collected.

About the clinical trials, 33% were phase I, 42% phase II, 16% phase I/II and 9% phase III (Graph 1). Table 1 summarizes the most relevant clinical trials on target therapies for high-grade gliomas (Table 1).

2. *Classification of The Target Therapies*

The target therapies are mostly categorized according to the targets, which, in their turn, include molecular alterations and oncogenic signaling. The



Graph 1. Pie graph showing the distribution of the selected clinical trials according to the study phase.

Table 1. Clinical Trials on Target Therapies for High-Grade Gliomas.

#	ClinicalTrials.gov Identifier	Conditions	# of Patients Enrollment	Interventions	Study Phase	Status	Locations
1	NCT00025675	Brain and Central Nervous System Tumors	105	Gefitinib	2	Completed	USA
2	NCT00016991	Brain and Central Nervous System Tumors	53	Gefitinib	2	Completed	USA
3	NCT00238797	Glioblastoma Multiforme	36	Gefitinib	2	Completed	SW
4	NCT00027625	Brain and Central Nervous System Tumors	n/a	Gefitinib, Temozolomide	1	Completed	USA
5	NCT00418327	Malignant Brain Tumor	48	Erlotinib	1	Completed	FR
6	NCT00301418	Glioblastoma Multiforme Anaplastic Astrocytoma	11	Erlotinib	1, 2	Completed	USA
7	NCT00086879	Brain and Central Nervous System Tumors	110	Carmustine, Erlotinib, Temozolomide	2	Completed	BE, FR, IT, NL, UK
8	NCT01591577	Newly Diagnosed Glioblastoma Multiforme	50	Lapatinib, Temozolomide, Radiotherapy	2	Completed	USA
9	NCT00099060	Brain and Central Nervous System Tumors	24	Lapatinib	1, 2	Completed	CN
10	NCT02423525	Brain Cancer	24	Afatinib	1	Completed	USA
11	NCT00977431	Glioblastoma Multiforme	36	Afatinib, Temozolomide, Radiotherapy	1	Completed	UK
12	NCT01520870	Glioblastoma Multiforme Brain Tumor, Recurrent	49	Dacomitinib	2	Completed	ES
13	NCT01112527	Glioblastoma Multiforme	58	Dacomitinib	2	Completed	USA
14	NCT00463073	Malignant Gliomas	32	Cetuximab, Bevacizumab, Irinotecan	2	Completed	DK
15	NCT01800695	Glioblastoma Multiforme	202	Depatuxizumab mafodotin (ABT-414) , Temozolomide, Whole Brain Radiation	1	Completed	AU
16	NCT02573324	Glioblastoma Multiforme	691	Depatuxizumab mafodotin (ABT-414) , Temozolomide	3	Active, not recruiting	USA
17	NCT04083976	Advanced Solid Tumor	280	Erdafitinib	2	Recruiting	USA
18	NCT00049127	Recurrent Adult Brain Neoplasm	64	Imatinib	1, 2	Completed	USA
19	NCT00613054	Glioblastoma Multiforme	27	Imatinib, Hydroxyurea	1	Completed	USA
20	NCT01331291	Glioblastoma Multiforme	36	Bosutinib	2	Completed	USA
21	NCT00601614	Glioblastoma Multiforme Gliosarcoma	119	Temozolomide, Vandetanib	1.2	Completed	USA

#	ClinicalTrials.gov Identifier	Conditions	# of Patients Enrollment	Interventions	Study Phase	Status	Locations
22	NCT00427440	Advanced Malignant Glioma	61	AMG 102	2	Completed	USA
23	NCT01632228	Glioblastoma Multiforme	135	Onartuzumab, Bevacizumab	2	Completed	CN, FR, DE, IT, ES, SW, UK , USA
24	NCT01113398	Glioblastoma Multiforme Gliosarcoma	36	AMG 102, Bevacizumab	2	Completed	USA
25	NCT01632228	Glioblastoma Multiforme	135	Bevacizumab, Onartuzumab	2	Completed	USA
26	NCT00606879	Advanced Cancer	46	SGX523	1	Terminated	USA
27	NCT00607399	Advanced Cancer	46	SGX523	1	Terminated	USA
28	NCT00784914	Brain and Central Nervous System Tumors	12	Temsirolimus	1	Completed	USA
29	NCT00016328	Adult Glioblastoma Multiforme Adult Gliosarcoma Recurrent Adult Brain Tumor	33	Temsirolimus	2	Completed	USA
30	NCT00047073	Brain and Central Nervous System Tumors	13	Sirolimus, Surgery	1, 2	Completed	USA
31	NCT00672243	Glioblastoma Multiforme Gliosarcoma	32	Erlotinib, Sirolimus	2	Completed	USA
32	NCT00553150	Brain and Central Nervous System Tumors	122	Everolimus, Temozolomide, Radiotherapy	1.2	Completed	USA
33	NCT00085566	Brain and Central Nervous System Tumors Prostate Cancer	61	Everolimus, Gefitinib	1.2	Completed	USA
34	NCT01339052	Glioblastoma Multiforme	65	Buparlisib, Surgery	2	Completed	USA
35	NCT01473901	Glioblastoma Multiforme	38	Buparlisib, Temozolomide, Radiotherapy	1	Completed	USA
36	NCT01349660	Glioblastoma Multiforme	88	Buparlisib, Bevacizumab	1, 2	Active, not recruiting	USA
37	NCT00590954	Malignant Gliomas Brain Cancer	32	Perifosine	2	Completed	USA
38	NCT00005859	Brain and Central Nervous System Tumors	136	Tipifarnib	1.2	Completed	USA
39	NCT00049387	Adult Giant Cell Glioblastoma Adult Glioblastoma Adult Gliosarcoma	19	Tipifarnib, Temozolomide, Radiotherapy	1	Completed	USA
40	NCT00015899	Brain and Central Nervous System Tumors	53	Lonafarnib	1	Completed	USA

#	ClinicalTrials.gov Identifier	Conditions	# of Patients Enrollment	Interventions	Study Phase	Status	Locations
41	NCT00038493	Glioblastoma Multiforme	23	Temozolomide, Lonafarnib	2	Completed	USA
42	NCT01748149	Pediatric BRAFV600E-mutant Gliomas	40	Vemurafenib	1	Active, not recruiting	USA
43	NCT02345824	Glioblastoma Glioma	3	Ribociclib	1	Active, not recruiting	USA
44	NCT02896335	Metastatic Malignant Brain Tumors	30	Palbociclib	2	Recruiting	USA
45	NCT03834740	Glioblastoma Multiforme Brain Gliomas	24	Ribociclib, Everolimus	1	Recruiting	USA
46	NCT03224104	Astrocytoma, Grade III Glioblastoma	81	Zotiraciclib, Temozolomide, Radiotherapy	1	Recruiting	SW
47	NCT02942264	Brain Tumors Astrocytoma, Astroglioma Glioblastoma Gliosarcoma	152	Zotiraciclib, Temozolomide	1, 2	Recruiting	USA
48	NCT02073994	Cholangiocarcinoma Chondrosarcoma Glioma Other Advanced Solid Tumors	170	Ivosidenib	1	Active, not recruiting	USA, FR
49	NCT02481154	Glioma	150	Vorasidenib	1	Active, not recruiting	USA
50	NCT00884741	Glioblastoma Multiforme Gliosarcoma Supratentorial Glioblastoma	637	Bevacizumab, Temozolomide, Radiotherapy	3	Completed	USA
51	NCT00731731	Adult Glioblastoma	125	Temozolomide, Vorinostat	1, 2	Active, not recruiting	USA
52	NCT00128700	Brain and Central Nervous System Tumors	20	Temozolomide, Vatalanib, Radiotherapy	1, 2	Completed	BE, DE, IT, NL, SW
53	NCT00108056	Glioma	26	Enzastaurin	1	Terminated	USA
54	NCT00190723	Malignant Glioma	120	Enzastaurin	2	Completed	USA
55	NCT00503724	Brain and Central Nervous System Tumors Neuroblastoma	32	Enzastaurin	1	Completed	USA
56	NCT00006247	Brain and Central Nervous System Tumors	33	Semaxanib	1	Terminated	USA
57	NCT01229644	Glioma	10	Crenolanib	2	Terminated	USA
58	NCT01393912	Diffuse Intrinsic Pontine Glioma Progressive or Refractory High-Grade Glioma	55	Crenolanib	1	Completed	USA

#	ClinicalTrials.gov Identifier	Conditions	# of Patients Enrollment	Interventions	Study Phase	Status	Locations
59	NCT00305656	Adult Giant Cell Glioblastoma Adult Glioblastoma Adult Gliosarcoma Recurrent Adult Brain Tumor	31	Cediranib	2	Completed	USA
60	NCT00326664	Recurrent Glioblastoma	55	Cediranib	1	Completed	USA
61	NCT00503204	Brain Tumor	20	Cediranib, Lomustine	1	Completed	USA, UK
62	NCT00704288	Glioblastoma Multiforme	222	Cabozantinib	2	Completed	USA
63	NCT00960492	Glioblastoma Multiforme Gliosarcoma	26	Cabozantinib, Temozolomide, Radiotherapy	1	Completed	USA
64	NCT00337207	Brain and Central Nervous System Tumors	55	Bevacizumab	2	Completed	USA
65	NCT01740258	Malignant Glioma Grade IV Malignant Glioma Glioblastoma Gliosarcoma	69	Bevacizumab, Temozolomide, Radiotherapy	2	Completed	USA
66	NCT00271609	Recurrent High-Grade Gliomas Malignant Gliomas	88	Bevacizumab	2	Completed	USA
67	NCT01290939	Glioblastoma Multiforme Cognition Disorders Disability Evaluation	433	Bevacizumab, Lomustine	3	Unknown	USA
68	NCT01860638	Glioblastoma Multiforme	296	Bevacizumab, Lomustine	2	Completed	AU
69	NCT00884741	Glioblastoma Multiforme GliosarcomaSupratentorial	637	Bevacizumab, Chemiotherapy, Radiotherapy	3	Completed	USA
70	NCT00943826	Glioblastoma Multiforme	921	Bevacizumab, Temozolomide, Radiotherapy	3	Completed	USA
71	NCT00895180	Adult Glioblastoma Multiforme	80	Olaratumab, Ramucirumab	2	Completed	USA
72	NCT00369590	Adult Anaplastic Astrocytoma Adult Anaplastic Oligodendroglioma Adult Giant Cell Glioblastoma Adult Gliosarcoma Recurrent Adult Brain Tumor	58	Aflibercept	2	Completed	USA
73	NCT00093964	Glioblastoma Multiforme	81	Cilengitide	2	Completed	USA

#	ClinicalTrials.gov Identifier	Conditions	# of Patients Enrollment	Interventions	Study Phase	Status	Locations
74	NCT00085254	Adult Giant Cell Glioblastoma Adult Glioblastoma Adult Gliosarcoma	112	Cilengitide, Temozolomide, Radiotherapy	1, 2	Completed	USA
75	NCT00689221	Glioblastoma Multiforme	545	Cilengitide, Temozolomide, Radiotherapy	3	Completed	USA, DE
76	NCT00165477	Glioblastoma Multiforme Gliosarcoma Malignant Gliomas	23	Lenalidomide, Radiotherapy	2	Completed	USA
77	NCT03345095	Newly Diagnosed Glioblastoma	750	Marizomib, Temozolomide, Radiotherapy	3	Recruiting	AU, BE
78	NCT00006773	Adult Anaplastic Astrocytoma Adult Anaplastic Oligodendroglioma Adult Giant Cell Glioblastom Adult Glioblastoma Adult Gliosarcoma Recurrent Adult Brain Tumor	42	Bortezomib	1	Terminated	USA
79	NCT00998010	Brain and Central Nervous System Tumors	25	Bortezomib, Temozolomide, Radiotherapy	2	Completed	USA

AU: Austria; BE: Belgium; CA: Canada; DE: Germany; DK: Denmark; ES: Spain; FR: France; IT: Italy; NL: Netherlands; SW: Switzerland; UK: United Kingdom; USA: United States of America

majority of approaches are directed against signaling pathways related to cell proliferation and glioma invasion, angiogenesis and inhibition of apoptosis.[22-25] Table 2 reports the classification of the target therapies used for malignant brain tumors (Table 2).

2.1. Tyrosine Kinase Inhibitors

Tyrosine kinase receptors consist in an extracellular ligand-binding and a transmembrane tyrosine kinase domain containing sites for autophosphorylation. Upon the binding of its ligand, the receptors undergo dimerization and phosphorylation of specific tyrosines, those become binding sites, recruit proteins and activate downstream intracellular pathways, ultimately resulting in tumor maintenance and proliferation.[26-28] The most widely studied tyrosine kinase receptors are the epidermal growth factor receptor (EGFR), the platelet-derived growth receptor (PDGFR), the fibroblast growth factor receptor (FGFR) and the hepatocyte growth factor receptor (HGFR). All of them are constantly overexpressed or mutated in GBMs. Tyrosine kinase inhibitors (TKIs) are molecules which bind the aforementioned receptors, blocking their downstream signals.

2.1.1 EGFR

The EGFR gene is amplified or overexpressed in 40% to 60% of the primary GBMs, whereas loss of exons 2 to 7 (EGFRvIII) is present in 40-50% of the cases.[29-31]

Table 2. Classification of Target Therapies for Malignant Brain Tumors

Target Therapy		
Candidate Drugs	Target	Biological Role in GBM
TKIs	EGFRvIII	Proliferation, migration, invasion, and resistance to apoptosis
	PDGFR	
	FGFR	
	HGFR	
PI3K/AKT/ mTOR Is	PI3K	Growth, metabolism, proliferation, migration
	AKT	
	mTORC1	
FTIs	RAS/MAPK	Cell cycle maintenance and proliferation
	BRAF V600E	
p53Is	MDM2/ MDM4	Cell cycle progression and resistance to apoptosis
pRBIs	CDK4/CDK6	
IDHIs	IDH1	Metabolism, proliferation, invasion, angiogenesis
HDACIs	Histones	Dysregulation DNA transcription, expansion of gene mutations
AIs	VEGF-A	Blood vessel formation, proliferation, therapeutic resistance
	VEGFR1	
	PKC	Tumor microenvironment maintenance
IIs	Integrins	Cell adhesion, migration, metastasis
PIs	Proteasome complex	Homeostasis, growth and resistance to apoptosis

AIs Angiogenesis Inhibitors, EGFR: Epidermal Growth Factor Receptor; FGFR: Fibroblast Growth Factor Receptor FTIs: Farnesyl Transferase Inhibitors; HDACIs: Histone Deacetylases Inhibitors; HGFR: Hepatocyte Growth Factor Receptor; IDH1: Isocitrate Dehydrogenase 1; IDHIs: Isocitrate Dehydrogenase Inhibitors; IIs: Integrin Inhibitors; mTOR: Mammalian Target of Rapamycin; mTORC1: Mammalian Target of Rapamycin Complex 1; PDGFR: Platelet- Derived Growth Receptor; PI3K: Phosphatidylinositol 4,5-Bisphosphate–3; PIs: Proteasome Inhibitors; PKC: Protein Kinase C; TKIs: Tyrosine Kinase Inhibitors; VEGF-A: Vascular Endothelial Growth Factor A; VEGFR1: Vascular Endothelial Growth Factor Receptor 1

EGFRvIII mutation leads to a ligand-independent kinase activity and, accordingly, an EGFR-pathway overactivation, resulting in increased cell proliferation, invasiveness and resistance to chemotherapeutic agents.[32, 33] Gefinitib (Iressa®) and erlotinib (Tarceva®) are approved TKIs directed against EGFRvI-II. Three phase II clinical trials (#NCT00025675, #NCT00238797, #NCT00016991) highlighted the efficacy of gefinitib, pointing out a progression-free survival at 6 months (PFS-6) of 13%.[34] Erlotinib lacked success as a monotherapy, but enhanced the efficacy of chemo-radiotherapy, especially if associated with temozolomide (TMZ) and carmustine at a dose of 150 or 300 mg/daily.[35, 36] Similar results have been reported for lapatinib, afatinib and dacomitinib.[37]

In addition, two monoclonal antibodies (MAbs) are under observation. Cetuximab, a chimeric murine-human IgG1 Mab that binds the extracellular EGFR domain inducing tumor apoptosis.[38] As a monotherapy, it demonstrated a PFS-6 of 9.2% and an increased overall survival (OS) of 5 months. In combination with bevacizumab and irinotecan cetuximab, it showed a PFS-6 of 30% and a median OS of 7.2 months.[39] ABT-414, an EGFR-directed MAb conjugated to an anti-microtubulin agent, had a PFS-6 of 28.3% in monotherapy or when combined with standard temozolomide chemoradiotherapy (#NCT02573324).[40]

2.1.2. PDGFR

PDGFR gene amplification is found in nearly 15% of GBMs, and the receptor's overexpression, which leads to tumor growth and angiogenesis, is frequently associated with transition from low- to high-grade glioma.[30] Imatinib is the most famous PDGFR inhibitor, used in many hematological tumors for its activity against the mast/stem cell growth factor receptor (c-KIT), and oncogene fusion protein BCR-ABL.

Many phase II clinical trials have proven that imatinib monotherapy failed to improve PFS-6 or OS in patients with GBM,[41] but resulted in a good response in combination with hydroxyurea.[42]

Sorafenib, vandetanib, dasatinib and bosutinib are other PDGFR inhibitors. However, many clinical trials have failed to demonstrate the efficacy of dasatinib,

both as monotherapy and combined with radiotherapy, TMZ and lomustine.[43, 44]

2.1.3. FGFR

Erdafitinib, a selective FGFR TKI, showed promising results in patients with GBM harboring oncogenic FGFR-TACC fusion.[45, 46]

2.1.4. HGFR/c-MET

HGFR, also known as c-Met, amplification/mutation has a role in promoting gliomagenesis and drug resistance.[47, 48] Crizotinib, specifically designed against c-Met, has given some results in combination with dasatinib.[49, 50] Analogous results have been reported for SGX523[51, 52] (#NCT00606879, #NCT00607399). Conversely, onartuzumab and rilotumumab (AMG102) basically demonstrated no clinical benefits.[53, 54] Two phase II clinical trials have been completed, one with AMG102 as monotherapy (#NCT00427440), and the other with AMG102 plus bevacizumab (#NCT01113398), both for patients with recurrent high-grade gliomas.

2.2. PI3K/AKT/mTOR Inhibitors

The Cancer Genome Atlas analysis highlighted the presence of PI3K/AKT/ mTOR signaling pathway dysregulation in 50-60% of GBMs.[55, 56] The activation of phosphatidylinositol 4.5-bisphosphate-3 (PI3K) regulates the activity of many kinase proteins, such as AKT. It transduces the signals to many downstream intracellular effectors, like the mammalian target of rapamycin (mTOR). A fundamental intracellular protein is mTOR, involved in cell growth signaling and tumorigenesis. It is composed of two subunits, mTORC1-2, with different roles, and mTORC1, particularly involved in the transition of the cell cycle from G1 to S. The Food and Drug Administration (FDA) approved three mTORC1 inhibitors: sirolimus (Rapamycin, Rapamune®), everolimus® and temsirolimus®.

Temsirolimus has been evaluated in some significant clinical trials; one of these was a phase II study involving 65 patients with recurrent GBM. It demonstrated a radiographic improvement in 36% of the patients, a PFS-6 of 7.8% and median OS of 4.4 months.[57]

Sirolimus has been tested in combination with surgery (#NCT00047073), gefitinib in 34 recurrent glioma patients, and erlotinib (#NCT00672243), demonstrating moderate effectiveness.[58]

Everolimus was studied in combination with gefitinib (#NCT00085566), bevacizumab or chemioradiotherapy. A phase II clinical trial tested the combination of everolimus, TMZ and radiotherapy versus conventional standard of care (#NCT00553150).

However, mTOR inhibitors have not demonstrated significant clinical activity, if not in combination with other treatments. This is due to their selectivity for mTORC1 and not mTORC2, ensuring only a partial blocking of the mTOR function.

In fact, two novel ATP-competitive mTORC2 inhibitors (CC214-1 and CC214-2) are under investigation, in order to overcome the resistance of mTOR inhibitors.[59]

Other promising strategies involve the selective PI3K inhibitor, buparlisib, which has an antitumor activity, especially when associated with bevacizumab in patients with recurrent GBM.[59]

Perifosine is a novel selective AKT inhibitor, currently tested in some ongoing trials. A phase II study investigated perifosine as a monotherapy for recurrent malignant gliomas[60] (#NCT00590954).

2.3. Farnesyl Transferase Inhibitors

Following the activation of TK receptors, the intracellular RAS protein family undergoes post-translational modifications and triggers multiple effector pathways, including the RAF and MAP kinases (MAPK) involved in cell proliferation, differentiation and survival.

However, translocation of RAS to the cell membrane requires a post-translational alteration catalyzed by the farnesyl transferase enzyme.[30, 61]

Farnesylation is the limiting step in RAS activities and the specific farnesyl transferase inhibitors (FTIs) lock all its functions upstream, and consequently the intracellular RAS-RAF-MEK-MAPK pathway.[62]

Among these, tipifarnib (Zarnestra®), exhibited in a phase II trial, had modest efficacy as a monotherapy or after radiotherapy, in patients with newly diagnosed and recurrent malignant gliomas.[63, 64]

Lonafarnib, an FTI, was tested in a phase I clinical trial in combination with TMZ and radiotherapy, with promising results[65] (#NCT00049387).

2.3.1. BRAF V600E

RAF kinases, also triggered by the RAS system, are involved in intracellular growth pathways and stimulation.

Several studies reported the presence of BRAF V600E mutation, especially in infant gliomas.[66] Vemurafenib, a BRAF inhibitor, is under investigation in a phase I ongoing trial, for children with recurrent BRAFV600E-Mutant gliomas[67] (#NCT01748149).

2.4. MDM2/MDM4/p53 inhibitors

The dysregulation of p53 signaling pathways is found in more than 80% of high-grade gliomas. The p53 is fundamental in cell-cycle arrest and apoptosis; mutation results in clonal expansion of tumor cells and genetic instability.[68, 69]

In 20% of the patients, the p53 inactivity is due to the MDM2 or MDM4 overexpression. MDM2/MDM4 inactivates p53 and consequently leads to loss of cancer suppression.[30, 70]

Therefore, an effective strategy rationale is to restore the p53 activity, by molecules targeting MDM2 or MDM4. Preclinical studies demonstrated the successful suppression of GBM growth with several MDM2 inhibitors, including RG7112,[71] RG7388 and AMG232 as well as many others in progress (#NCT03107780).

2.5. CDK4/CDK6/pRB inhibitors

The altered function of retinoblastoma protein (pRB) contributes to gliomagenesis in 78% of the cases and the overexpression of CDK4/CDK6 plays a fundamental role in the modulation of this pathway, involved in cell growth.[72-74]

Novel agents directed to CDK4 and CDK6 demonstrated strong antitumor efficacy in RB1-wild-type GBM, such as ribociclib and palbociclib.

Ribociclib was tested in a phase I trial for recurrent glioblastoma or anaplastic glioma[75] (#NCT02345824); palbociclib was employed as a monotherapy for brain metastases[76] (#NCT02896335).

Zotiraclib, a multi-CDK inhibitor, has been explored in clinical trials for newly diagnosed or recurrent gliomas (#NCT02942264, #NCT03224104).

2.6. Isocitrate dehydrogenase-1 inhibitors

Isocitrate dehydrogenase-1 (IDH1) mutation is one of the most frequent abnormalities found in high-grade gliomas, and according to the World Health Organization, is a new classification of brain tumors also having predictive value of treatment response. This mutation consists in the gain-of-function with the production of D-2-hydroxyglutarate, which interferes with cellular metabolism [77, 78]. Ivosidenib, an IDH1 inhibitor, is being evaluated in a phase I ongoing trial, as a monotherapy, for advanced solid tumors including IDH-mutated gliomas (#NCT02073994).

2.7. Histone deacetylases inhibitors

Histone deacetylases (HDAC) are enzymes involved in the regulation of histones, which are proteins that organize the DNA structure and regulate gene transcription.

HDAC inhibitors have an emerging role in the treatment of GBMs, potentially promoting the apoptosis of the cancer cells.[79]

Vorinostat, an oral quinolone HDAC inhibitor, is being studied in phase I/II clinical trials, as a monotherapy in recurrent GBM,[80] and in combination with TMZ, showing good tolerance and giving promising results[81] (#NCT00731731).

Panobinostat, Romidepsin and other HDAC inhibitors are still under evaluation.

2.8. Angiogenesis inhibitors

The tumor's microenvironment, together with pathological angiogenesis and neovascularization, play

a fundamental role in the development and progression of high-grade gliomas.

Acting as managers for the angiogenesis process, as well as for a wide range of CNS vascular pathologies, they are mainly vascular growth factors of all the vascular endothelial growth factor-A (VEGF-A) and its receptors, VEGFR1 and VEGFR2, found on the glioma's endothelial cells.[82-85]

Efforts to downregulate this pathway have been pursued through the development of agents directed to VEGF/VEGFR, which not only block neoangiogenesis, but also have an effect on the vascular phenotype.

The inhibition of VEGF signaling also changes the vessels' diameter, permeability and tortuosity, decreasing tumor hypoxia and consequently disrupting the survival mechanism in glioma cells as well as increasing chemotherapy delivery and radiosensitivity.[83-85]

2.8.1. VEGFR

Several studies evaluated VEGFR inhibitors for patients with newly diagnosed, as well as recurrent GBM.

Vatalanib has been tested in phase I/II studies in combination with TMZ and radiotherapy (#NCT00128700). Cediranib demonstrated no clinical benefits in a phase II clinical trial as a monotherapy (#NCT00305656), yet there was greater benefit together with lomustine in a randomized phase III study[86] (#NCT00503204).

Cabozantinib is a promising agent against VEGFR and MET signaling, evaluated in two phase II studies involving newly diagnosed (#NCT00960492) and recurrent GBM (#NCT00704288). Ramucirumab and icrucumab are new MAbs under evaluation, directed to VEGFR-2 and VEGFR-1, respectively.[87]

2.8.2. VEGF

The most relevant of the VEGF inhibitors is bevacizumab, a humanized IgG1 monoclonal antibody against VEGF-A, which in 2009 received FDA-approval for the treatment of recurrent GBM, after the high radiographic response rates (ranging from 28% to 59%) achieved in two clinical trials.[88, 89]

The significant antitumor potential of bevacizumab has been proven in many studies, using it as a monotherapy or in combination with lomustine (#NCT01290939) and radiochemiotherapy.[90, 91]

Combinations of bevacizumab with the standard of care were examined in two phase III clinical trials, AVAglio[92] (#NCT00943826) and RTOG- 0825[93] (#NCT00884741), and although both demonstrated encouraging results in PFS survival benefit, bevacizumab remains only an alternative treatment in the recurrent setting.

Another promising agent is aflibercept, known as VEGF-trap, a recombinant product fusion protein which has been studied in phase II trials with a PFS-6 of 7.7% and median OS of 3 months.[94, 95]

2.8.3. Protein kinase C

Protein kinase C (PKC) is implicated in activation of the angiogenesis process, cell proliferation and constitution of the microenvironment, therefore, it is a potentially attractive therapeutic target.

Enzastaurin, a potent PKC inhibitor, demonstrated in a phase I/II trial a 25% radiographic response and a PFS-6 of 7% in GBM.[96]

Tamoxifen, a modulator of the estrogen receptor, has been described as a PKC inhibitor and was tested in GBM therapy with a median OS of 9.7 months.[97, 98]

2.9. Integrin inhibitors

The integrins are transmembrane proteins which bind multiple extracellular ligands and mediate cell adhesion and migration. They are expressed at a high level in malignant glioma cells and play a central role in the angiogenesis, development, invasion and metastasis of the tumor.[99, 100] Integrin inhibitors are being investigated as a means of reducing this mechanism.

Cilengitide, which competitively inhibits integrin ligand binding,[101] has been evaluated in a phase I/II study stand-alone;[102] or in a phase III trial, associated to TMZ and radiotherapy, resulting in a good improvement of PFS-6[103] (#NCT00689221).

Thalidomide and lenalidomide, which interfere with the expression of integrin receptors and have an

antiangiogenic effect, are being studied for GBM therapy, with results that are still unsatisfactory.[104-106]

2.10. Proteasome inhibitors

Proteasomes are proteins with enzymatic activities involved in the regulation of homeostasis, cell growth and apoptosis.

Bortezomib (Velcade®), the most used proteasome inhibitor in the oncological field, has also been tested for GBM therapy in combination with chemioradiotherapy[107] (#NCT00006773).

The pan-proteasome inhibitor, Marizomib, is currently undergoing phase III evaluation in newly diagnosed GBMs[108] (#NCT03345095).

Discussion

The present literature review highlights the current role of a series of target therapies, especially tyrosine kinase and angiogenesis inhibitors, in the treatment of malignant CNS tumors.

Several steps forward have been done in the recent years toward a deep understanding of complex pathophysiologic pathways associated with a wide spectrum of neurological and neuro-oncological pathologies of adulthood and pediatric age. [109-111] Nevertheless, the lack of success of the standard of care and the still largely dismal prognosis of patients affected by high-grade gliomas dictate the urgent need of new and more effective therapeutic approaches.

In this scenario, the improved understanding of genome mutations underlying the GBM phenotype has led to greater insight into the biology of the tumor, at the same time providing the opportunity for designing novel and personalized treatment strategies.[82, 112, 113]

Data from the Cancer Genome Atlas project [55] revealed the complicated genetic profile of GBMs and recognized the core signaling and transduction pathways commonly involved in the growth, proliferation, angiogenesis and spreading of the tumor.[114]

A further tangible aspect of these advances is the latest World Health Organization's classification of brain tumors, which integrates data from traditional histological analysis with biomolecular connotation obtained by specific genetic analysis and characterizations.[115]

Accordingly, the target therapies developed on the basis of the above have detected molecular abnormalities, and have made use of pharmacological agents tailored to specific mutations, specific to tumor subtypes.

Typical genetic alterations of GBMs are the overexpression of the tyrosine kinase receptors, especially the EGFR, PDGFR, FGFR and HGFR, dysregulation of PI3K/AKT/mTOR and RAS/MAPK pathways, as well as p53 or pRB mutations.[30, 116, 117]

TKIs have long been investigated in several clinical trials with disappointing results. Despite the extreme specificity of these agents, they were not efficacious as a monotherapy, thus the current approach consists in the combination of multiple molecular agents within the same targets or between separate pathways.[33, 118, 119]

PI3K/AKT/mTOR pathway and farnesyltransferase inhibitors show low tolerability and safe profiles during clinical studies, but have a synergistic effect only in combination with standard of care.[58, 120]

Likewise, agents directed at restoring p53 and pRB activity gave encouraging results in association with chemotherapy and whole brain radiotherapy.[76, 121] The newly discovered alterations in metabolic pathways, including IDH1 and HDAC enzymes, seem to be up-and-coming targets. Currently, anti-angiogenetic drugs are among the most promising. They focused on the blocking of VEGF/VEGFR,[122, 123] along with components of the tumor microenvironment, such as protein kinase C, integrins and proteasome complexes.[89, 124, 125]

Despite the rationale of the target therapies, the vast intratumoral heterogeneity and GBM cell plasticity have caused a rapid shift toward resistant tumor phenotypes, the latter responsible for the failure of the therapy.[126-128]

Additionally, the route of drug administration still presents a limitation for the efficacy of these therapies. Recent progress has been made through the use of stereotactic or endoscopic techniques for the intrathecal administration of pharmacological agents directly into the tumor site, also benefiting from the minimal invasiveness of these approaches, well evident also for other neurosurgical pathologies.[129-131]

Last but not least, the immunological tumor microenvironment, composed of glia cells and lymphocytes, consistently modulates tumor sensitivity to treatment.[132-134]

Conclusion

The improved knowledge of the biology of tumors has recently made it possible to transform the molecular alterations at the base of the high malignancy of GBM, into different treatment strategies.

Good results came from tyrosine kinase inhibitors, primarily erlotinib and gefinitinb. Similarly, PI3K/AKT/mTOR inhibitors and p53 restoring agents proved their efficacy in several clinical trials. Bevacizumab, in association with TMZ and radiotherapy, has been approved for recurrent GBMs.

An in-depth identification of driver molecular alterations may make it possible to appropriately select those patients who are candidates for a target therapy.

The greatest challenge of the near future consists in overcoming the issue of escape of GBM that is present in all of these therapies.

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Original article

Targeting the medulloblastoma: a molecular-based approach

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Abstract. *Background:* The lack of success of standard therapies for medulloblastoma has highlighted the need to plan a new therapeutic approach. The purpose of this article is to provide an overview of the novel treatment strategies based on the molecular characterization and risk categories of the medulloblastoma, also focusing on up-to-date relevant clinical trials and the challenges in translating tailored approaches into clinical practice. *Methods:* An online search of the literature was carried out on the PubMed/MEDLINE and ClinicalTrials.gov websites about molecular classification of medulloblastomas, ongoing clinical trials and new treatment strategies. Only articles in the English language and published in the last five years were selected. The research was refined based on the best match and relevance. *Results:* A total 58 articles and 51 clinical trials were analyzed. Trials were of phase I, II, and I/II in 55%, 33% and 12% of the cases, respectively. Target and adoptive immunotherapies were the treatment strategies for newly diagnosed and recurrent medulloblastoma in 71% and 29% of the cases, respectively. *Conclusion:* Efforts are focused on the fine-tuning of target therapies and immunotherapies, including agents directed to specific pathways, engineered T-cells and oncoviruses. The blood-brain barrier, chemoresistance, the tumor microenvironment and cancer stem cells are the main translational challenges to be overcome in order to optimize medulloblastoma treatment, reduce the long-term morbidity and increase the overall survival. (www.actabiomedica.it)

Key words: Adoptive Immunotherapies; Medulloblastoma; Sonic Hedgehog Medulloblastoma; Target Therapy; Wingless Medulloblastoma.

Background

Medulloblastoma (MB) is the most common malignant pediatric tumor, accounting for 15-20% of childhood brain neoplasms.[1] MB usually occurs in the posterior fossa and has a high risk for early leptomeningeal spread at first diagnosis.

Current multimodal therapies, including surgery and radiochemotherapy, lengthens the long-term survival to 60-80%, but 33% of children diagnosed die in five years, the median survival for recurrent MBs being less than twelve months. Treatment also leads to severe and debilitating long-term complications.[2-5]

The persistence of high mortality rates and severe side effects of standard treatments has highlighted the need for more effective and sophisticated therapeutic strategies.

Advanced molecular research and whole-genome sequence analysis in many neurological and neurooncological pediatric central nervous system (CNS) pathologies[6-9] has made it possible to deepen the understanding of the heterogeneity and genome make-ups of MBs, resulting in the novel classification underpinned on different molecular features.[10-15]

The subgroups have substantial biological differences, express specific markers of prognosis leading to a more accurate risk stratification, and underly distinct deregulated signaling pathways, exploitable as potential therapeutic targets.[4, 16-18]

Breakthroughs of risk-adapted interventions based on molecular characteristics, including target agents, immunotherapies and stem-cell strategies, have made it possible to plan an effective personalized approach and have reduced long-term morbidity.

In this article, we outline the molecular landscape of MB subtypes, along with prognostic markers, and examine the ongoing transition toward the innovative molecularly targeted strategies; focusing on the therapeutic options currently available, most relevant clinical trials, and future challenges in the management of newly diagnosed and recurrent MBs.

Methods

An online search of the literature was conducted on the PubMed/MEDLINE (https://pubmed.ncbi.nlm.nih.gov) platform and the ClinicalTrials.gov website (https://clinicaltrials.gov). For the PubMed/MEDLINE search the MeSH (Medical Subject Headings) database has been used and the terms "Target Therapy", "Molecular Classification", "Adoptive Immunotherapy", "Cell-Based Therapy", "Stem Cell Therapy" and "Tailored Therapy" have been chosen; combined with the following keywords: "Pediatric Brain Tumors", "Pediatric Central Nervous System tumors", "Brain tumors in childhood" and "Medulloblastoma".

Only articles in English or translated into English, published in the last five years, and concerning neuro-oncology were selected and then sorted based on the best match and relevance.

On the ClinicalTrials.gov website, the search terms were "Medulloblastoma", "Pediatric Malignant Brain Tumor", "Pediatric Brain Cancer" and "Pediatric central nervous system Neoplasms". No restrictions for drug name, study phase and recruitment status country have been applied.

A descriptive analysis has been reported about the most relevant studies of the overall research.

Results

1 Molecular classification of MBs

Based on histopathological characteristics, the World Health Organization (WHO) classified MBs in classic, desmoplastic-nodular, with extensive nodularity, anaplastic, and large cell types.[19]

Several cytogenetic studies and the increased understanding of the pathophysiology of several CNS pediatric pathologies [20-22] and, within this context, the biological heterogeneity of MBs have been translated into classification refinements.

The four subtypes, based on genome sequencing, DNA analysis and phenotypic profiles, are as it follows: wingless (WNT), sonic hedgehog (SHH), Group 3 and Group 4.[16, 23-25]

This novel molecular subgrouping has potential prognostic implications, so the current risk stratification divides the MBs in "low", "standard", "high", and "very high risk", based on age, presence of metastases, histologic phenotype, prognostic molecular markers, and especially, molecular subtype.[16-18] Table 1 and 2 report the molecular and prognostic classification of medulloblastoma (Table 1 and 2).

1.1 WNT-MBs

WNT proteins play a central role in cell growth, proliferation, motility and homeostasis. The pathway is triggered by β-catenin protein and various kinases as transduction enhancers.

In 85-90% of the cases, the WNT-MB subgroup harbors a point mutation in exon 3 of the CTNNB1 gene

Table 1. Molecular Classification of Medulloblastoma[16-18, 23-25, 32, 82]

Molecular Subtype	WNT	SHH	Group 3	Group 4
Proportion of MBs	10-15%	25%	25%	35%
Age Distribution	10-12 years old	Bimodal, < 5-> 16 years old	< 3 years old	Children
Male/Female Ratio	1:1	1:1	2:1	3:1
Location	Midline, Fourth Ventricle	Cerebellar Hemispheres, Vermis	Midline, Fourth Ventricle	Midline, Fourth Ventricle
Histology	Classic, rarely LCA	DN, Classic, LCA	Classic, rarely LCA	Classic, rarely LCA
Metastasis	5-10%	15-20%	45%	30-40%
Recurrence	Rare	Local	Metastatic	Metastatic
Driver Genes	o CTNNB1 (90%)-WNT o DDX3X (50 %) o SMARCA4 (25%) o TP53 (12.5 %)	o TERT (83%) o PTCH1 (45%) -SHH o TP53 (13%) o SUFU (10 %) o SMO (9%) o MYCN (8%) o GLI2 (5%)	o GFI1/GFI1B (30 %) o MYC (10-20 %) o PVT1 (12 %) o SMARCA4 (11%) o OTX2 (10 %)	o KDM6A (13 %) o SNCAIP (10%) o MYCN (6%) o CDK6 (5%) o GFI1/GFI1B (5-10 %)
Chromosome Aberration	Monosomy 6 (> 80%)	Loss 9q (PTCH1 locus)	Isochromosome 17q	Isochromosome 17q
MYC status	+	+	+++	-
5-year Survival	> 90%	70%	40-60%	75%

DN: Desmoplastic-Nodular; LCA: Large Cell/Anaplastic; SHH: sonic hedgehog; WNT: wingless

Table 2. Prognostic Classification for Medulloblastoma[82]

Risk Categories	Molecular Profile	5-year overall survival
Low Risk	Non-metastatic WNT-MBs	>90%
	Localized Group 4-MBs, with loss of chromosome 11 and gain of chromosome 17	
Standard Risk	Non-metastatic SHH-MBs without p53 mutation	76-90%
	Group 3 non-MYC amplified	
	Group 4 without p53 mutation and loss of chromosome 11	
High Risk	Metastatic SHH-MBs MYC amplified	50-75%
	Metastatic Group 4	
Very High Risk	Metastatic Group 3	< 50%
	SHH-MBs MYC amplified with p53 mutation	

MBs: Medulloblastomas; SHH: Sonic Hedgehog; WNT: Wingless

which renders the β-catenin resistant to degradation and leads to an upregulation of the WNT pathway.[18, 26-29]

In 70-80% of the cases, the monosomy/diploidy of chromosome 6 and the overexpression of MYC e MYCN proteins, markers of worse prognosis, results in the activation of the WNT signalings.[30, 31]

Less frequent driving genetic alterations concern the DDX3X, SMARCA4 and p53 genes, with a frequency of 50%, 26% and 13%, respectively.[32]

WNT-MBs are the least common, accounting for 10%, with a peak incidence in 10-12 years, and almost equal male/female ratio.[32] More than 90% have a

classic histology, location in the midline of the fourth ventricle and relatively rare metastasis (5-10%)[33].

This group has the better prognosis, with more than 90% of 5-year event-free survival.[33]

1.2 SHH-MBs

The hedgehog (HH) signaling pathway is involved in the proliferation of neuronal precursor cells and is fundamental for tissue maintenance and regeneration.

HH ligands bind the receptor protein patched homolog 1 (PTCH1) and activate the intracellular cascade of smoothened (SMO) proteins.

Among mammalian homologs of the hedgehog, the aberrant upregulation of the SHH signaling pathway promotes tumor formation in about 30% of MBs.[18, 23, 24, 34]

The typical activating mutations for the SHH subtype include the TERT in 83%, PTCH1 in almost 45%, the modulator suppressor of fused homolog (SUFU) in 10%, and SMO in 9% of the cases.[35-37]

In the SHH signaling pathway, SMO activates the downstream target gene FOXM1, a GLI transcription factor, which activates genes for mitosis, including PLK1 and MYCN.

The expression at a high level of FOXM1/PLK1, MYCN and GLI 1 and 2 are also prognostic markers and potential therapeutic targets.[29, 38, 39]

Other molecular characterizations typical of SHH-MBs are in genes coding for ErbB family proteins, such as EGFR and ERBB3, deregulation of the p53 and PI3K/AKT/mTOR pathway and the deletion of chromosome 9q (PTCH1 locus), which modifies the transcription of CDKN2A/2B, known as tumor suppressor factors.

In many cases, these mutations suggest the concomitant presence of a hereditary genetic disease such as Gorlin syndrome, associated with mutations affecting the PTCH1 and SUFU genes. The SHH subgroup, 25% of all cases, has a bimodal age distribution, less than 3 and more than 16 years, with equivalent sex ratio and the majority has nodular/desmoplastic histology.[16, 40] They are frequently located in cerebellar hemispheres and vermis, and metastasis are not common.[16, 34]

SHH-MBs have an intermediate prognosis with 5-year overall survival of 70% after standard treatment.[16]

1.3 Group 3

Group 3 MBs represent 25% of all cases and are mostly characterized by amplification of various proto-oncogenes: GFI1/GFI1B (30%), MYC (16.7%), PVT1 (12%), SMARCA4 (11%) and OTX2 (10%).[18]

Additionally, fibroblast growth factor, tyrosine kinase receptors, and their consequent downstream signaling pathways, such as PI3K/AKT and MAPK/ERK, are frequently deregulated. Isochromosome 17q is present in 25% of SHH cases and among those with MYC amplification (10%-17%), are strong indicators of poor prognosis.

Group 3 is limited to children (3-5 years old), with male predominance and classic, anaplastic or large-cell histology.[18]

This is the group with the worst prognosis, as metastases are present in 45% of the cases.[16, 23]

1.4 Group 4

Group 4 is the most common, accounting for 35% of MBs, with no age prevalence and high male predominance. Isochromosome 17q occurs in 80% of the cases and the mutation of the KDM6A gene is frequently detached (13%).[41] The KDM6A encodes for a histone demethylase enzyme and is located on the X-chromosome, explaining the male predominance of Group 4.

Additionally, MYCN, cyclin dependent kinase 6 (CDK6) and NOTCH1, 2, 3 are commonly amplified. The expression of the NOTCH network is directly linked to therapy resistance, because it regulates the tumor's immune response and maintains the tumor microenvironment. The overexpression of cytokine receptors and their downstream signaling, such as the JAK-STAT pathway, estrogen-related receptor γ, and Fc receptors are found in this varied genomic landscape, not yet fully explored.

However, this subtype has an intermediate prognosis, like the SHH-MBs. However, leptomeningeal spread occurs more frequently (30-40%).[16, 18, 23]

2 Target Therapy

2.1 HH inhibitors

The most investigated target approach concerns the inhibitors of the HH pathway and the first one discovered was cyclopamine. It binds the transmembrane domain of SMO and definitively suppresses the growth and proliferation of the tumor's cells.[16, 42]

Although having excellent premises, cyclopamine did not show efficacy when applied in vivo, but led to the development of many molecules with the same drug-like properties. They were vismodegib, saridegib, sonidegib and erismodegib, all having improved pharmacokinetics and lower toxicities.[43, 44] Vismodagib, an SMO antagonist, was approved by the FDA and tested in some phase I and II clinical trials. Many of these are ongoing and are evaluating the efficacy of vismodegib combined with standard chemotherapy in children and adults diagnosed with recurrent or refractory MBs (#NCT01601184, #NCT01878617). A phase II study on vismodegib, conducted in 2005, enrolled 43 patients (12 affected by SHH-MBs) and showed a 6-month progression-free survival in 41% of the SHH patients (#NCT01239316).

Sonidegib and ZSP1602, orally bioavailable drugs inhibiting the SMO pathway, are under clinical evaluation.

2.2 Bromodomain inhibitors (BET)

A recent therapeutic strategy involves the bromodomain proteins, which bind histones and modulate gene transcription. BET inhibitors, such as JQ1 and BMS-986158, have been tested in many clinical trials in order to evaluate their safety and tolerability profiles[45] (#NCT03936465). In the BET family, the BRD4 protein is being evaluated as potential therapeutics target against advanced MYC-amplified MBs.[46, 47]

2.3 Tyrosine kinase inhibitors (TKIs)

Tyrosine kinases enzymes catalyze the phosphorylation of tyrosine residues on specific receptors, activating the intracellular transduction pathways. TKIs target oncogene growth factor receptors, including the epidermal growth factor (EGFR), the platelet-derived growth factor (PDGFR), the fibroblast growth factor (FGFR) and the hepatocyte growth factor (HGFR) receptors, which are involved in the cell's maintenance, differentiation and metastasis.

MB TKIs therapy involves imatinib, gefitinib, lapatinib, dasatinib, sorafenib, sunitinib and erlotinib.

Imatinib, a PDGFR blocker, prevents the migration and invasion of MB cells; it has been investigated in several clinical trials, showing good ability for overcoming the blood-brain barrier (BBB).

Erlotinib has been proved in two clinical trials, combined with chemoradiotherapy, especially for recurrent MBs (#NCT00077454, #NCT00360854).

A phase I study demonstrated the efficacy of savolitinib, inhibitor of HGFR, in primary brain tumors, including recurrent MBs (#NCT03598244).

Many phase II clinical trials are focusing on patients carrying FGFR mutations by administering erdafitinib, an oral pan-FGFR inhibitor with promising results (#NCT03210714).

2.4 PI3K/AKT/mTOR inhibitors

The PI3K/AKT/mTOR pathway controls cell growth and dissemination. Target agents directed against PI3K have given satisfactory results.

The PI3K and mTOR signaling pathways inhibitors, such as fimepinostat (#NCT03893487) and samotolisib (#NCT03213678) are tested for pediatric CNS tumors.

Wojtalla et al. reported the antitumoral potential of combination therapy involving the humanized anti-IGF-1R antibody, R1507, with PIK75, a class IA PI3K inhibitor, in recurrent MBs and neuroblastomas.[48]

2.5 CDK4/CDK6/pRB inhibitors

The pRB plays a fundamental role in cell-cycle arrest and apoptosis. The dysregulation of the pRB signaling pathways is found in many MBs, resulting in clonal cell expansion. The pRB inactivity is caused by the overexpression of CDK4/CDK6 suppressing agents.

The restoration of pRB activity is an effective rational strategy.

Novel agents directed against CDK4/CDK6, such as ribociclib and palbociclib, proved to have strong antitumor efficacy, also in combination with the SMO inhibitor, sonidegib (#NCT03434262).

Palbociclib is evaluated in a phase I clinical trial in combination with irinotecan and temozolomide for children with central nervous system (CNS) tumors (#NCT03709680).

Ribociclib and everolimus is tested in children affected by recurrent and refractory MBs (#NCT03387020).

2.6 MDM2/MDM4/p53 inhibitors

p53 is a fundamental protein regulating the cell cycle and inducing cell apoptosis. It is mutated in almost 40% of MBs, facilitating the proliferation and spread of the tumor. p53 dysregulation is found in the WNT and SHH groups, resulting in a 40% reduction of 5-year survival and is considered one of the leading causes of treatment failure. MDM2/4, which induce p53 degradation and negatively regulate its activity, are also promising therapeutic strategies.[49]

Nutlin-3 selectively binds MDM2, inhibiting p53 degradation. In 2012, Annette et al. proved in vitro and in vivo the antitumor activity of nutlin-3 against MBs.[50]

2.7 Chemokines inhibitors

Chemokines are pivotal in tumor growth and in sustaining the tumor-related microenvironment.

CXCL12 chemokine and its CXCR4 receptor are overexpressed in many CNS tumors, and significantly higher in MBs.

In 2012, Sengupta et al. demonstrated the presence of CXCR4 in WNT and SHH-MBs, but only SHH subtype harbors the CXCR4 overexpression.[51]

AMD3100 (Plerixafor), a CXCR4 antagonist, has been tested in one phase I/II clinical trial, combined with chemoradiotherapy, for several CNS tumors (#NCT01977677).

2.8 Anti-Angiogenesis agents

MBs are characterized by a thriving pathological angiogenesis and, consequently, potential downstream targets are the VEGF/VEGFR, copiously expressed in WNT and SHH-MBs. Anti-angiogenic therapies applied for MB involve bevacizumab, a humanized IgG1 monoclonal antibody directed against VEGF-A, in combination with conventional chemotherapies[52, 53] (#NCT00381797, #NCT01217437).

2.9 Topoisomerase inhibitors

Topoisomerase I and II are enzymes involved in DNA replication, cellular senescence and apoptosis. Irinotecan, topotecan and camptothecan are directed against these enzymes.

Topotecan and irinotecan have the same pharmacodynamics, but different pharmacokinetics; topotecan easily crosses the BBB, demonstrating, in many stand-alone clinical trials, (#NCT00112619, #NCT00005811) or also in combination with chemotherapy (#NCT02684071), increased survival.[54-56]

Indimitecan and Indotecan (LMP 400), both topoisomerase inhibitors, are still under evaluation.

3 Adoptive Immunotherapies

3.1 Checkpoint inhibitors (CPIs)

The success of CPIs to augment the immunological response against many solid tumors has generated interest in the applicability also for MBs, especially in the advanced stages.

Two anti-PD-1 agents, pembrolizumab and nivolumab, are under evaluation for pediatric tumors. An ongoing phase I clinical trial is assessing the safety and efficacy of pembrolizumab in progressive and recurrent tumors, including MBs (#NCT02359565); another phase II trial is evaluating the efficacy of nivolumab in pediatric brain tumors (#NCT03173950).

The B7 homolog 3 (B7-H3), an antibody immune checkpoint inhibitor directed against T-cells, has been tested in a phase I trial in combination with radiotherapy, and for advanced metastatic MBs (#NCT00089245).

APX005M, an IgG monoclonal antibody directed at CD40, has been designed to stimulate the anti-tumor immune response. It has been tested in a

phase I pediatric trial (#NCT03389802) in patients with recurrent and refractory primary malignant brain tumors and has also shown an excellent success rate in combination with nivolumab.

Indoleamine 2.3-dioxygenase (IDO) is an enzyme, overexpressed in many tumors, which regulates the tumor microenvironment and enhances immune escape decreasing T-reg activity. Indoximod, an IDO inhibitor, has been studied in two different phase I/ II pediatric trials with concomitant use of temozolomide (#NCT02502708, #NCT04049669).

3.2 Engineered CAR-T and NK cells

Engineered T-cells expressing artificial chimeric antigen receptors (CAR-T) are largely employed in neuro-oncology, posing challenges in finding tumor-associated antigens.

HER2 is usually overexpressed in MBs, and preclinical studies are testing the efficacy of HER2-CAR T-cells in mouse models[54].

At the Seattle Children's Hospital the BrainChild-01 phase I trial was conducted, which tested autologous CD4+/CD8+ T-cells lentivirally transduced to express HER2 and EGFRt (truncated form of EGFR) CARs, delivered by catheter in the tumor resection cavity or ventricular system, for recurrent or refractory HER2+ CNS tumors (#NCT03500991).

Another phase I trial proved the EGFR806 and EGFRt CAR T-cells for patients with recurrent/refractory EGFR+CNS tumors (#NCT03638167).

NK cells are fundamental in immune response, recognizing tumor cells without specific antigens. In an ongoing phase I clinical trial, propagated ex vivo with artificial antigen-presenting cells, NK cells have been administered directly into the ventricles in recurrent and refractory malignant posterior fossa tumors (#NCT02271711).

3.3 Oncolytic viruses

The main advantages of oncolytic viruses (OVs)-based immunotherapy consist in the selective replication within the tumor cells, inducing lysis of tumor cells and releasing neoantigens to the tumor microenvironment, thus activating the immune cascade.

For pediatric brain tumors, several types of OVs have been investigated.

Genetically engineered herpes simplex viruses (HSV), rRp450, G207 and M002 revealed antitumor activity and prolonged survival in mice xenografts of aggressive MBs cells.[57]

A recruiting phase I trial evaluated the engineered HSV G207 for children with refractory cerebellar brain tumors[58] (#NCT03911388).

The measles virus expressing thyroidal sodium iodide symporter (MV-NIS) has been engineered in an ongoing phase I study testing its efficacy in pediatric recurrent MBs. MV-NIS is administered intrathecally[59] (#NCT02962167).

The highly attenuated recombinant polio/rhinovirus (PVSRIPO) recognizes the CD155 receptor expressed in the MBs tumor cell microenvironment. It is used in a phase I pediatric trial, administered by the intracerebral catheter for WHO grade III and IV malignant brain tumors (#NCT03043391).

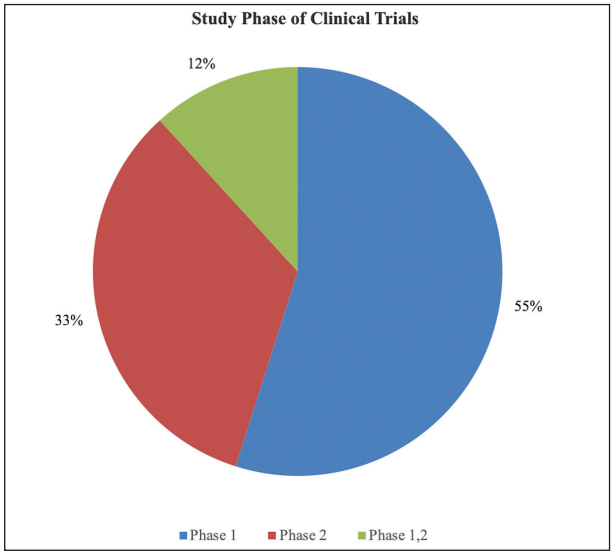
Phase I of the PRiME clinical trial evaluates a two-component cytomegalovirus specific multiepitope peptide vaccine (PEP-CMV), administered after temozolomide, in pediatric patients with recurrent MBs and high-grade gliomas (#NCT03299309).

4 Clinical Trials on MB Therapies

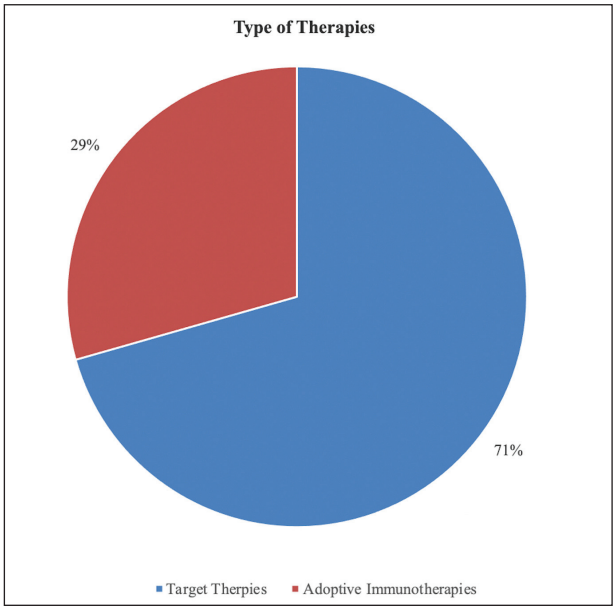
Out of 51 clinical trials, 55% were phase I, 33% phase II and 12% phase I/II (Graph 1). Target therapies and adoptive immunotherapies were tested in 71% and 29% of them, respectively (Graph 2). Table 3 summarizes the clinical trials on new therapeutic strategies for MBs (Table 3).

Discussion

Despite the refinements in neurosurgical techniques, concerning both neuro-oncology and other fields, present standard of care for MBs, including maximal surgical resection followed by adjuvant radio and chemotherapy protocols, fails to recognize heterogeneity within MB subtypes, resulting in low efficacy, high recurrence rate and risk of long-term toxicity.[60, 61]



Graph 1: Pie graph showing the distribution of the clinical trials according to the study phase.



Graph 2: Pie graph showing the distribution of the clinical trials according to types of therapy.

Challenges come from the need for distinguishing molecular subgroups and identifying patients for whom a personalized treatment approach would be recommended.

1 Molecular Subgroup-Based Tailored Strategies

1.1 WNT-MBs

WNT signaling was the first identified. However, no drugs directed against this pathway have been approved as an alternative to standard therapy.

Only two molecules have been tested, namely norcantharidin, which blocks the WNT pathway, and lithium chloride, which stabilizes β-catenin and reduces MB progression.[62-65]

The reason for the lack of success in inhibiting the WNT pathway lies in the fact that it seems to be involved in vascular dysfunction and BBB disrupting, therefore increasing the penetration of drugs. Further issues are the various developmental processes, including physiological tissue regeneration and bone growth.[66, 67] As a matter of fact, inhibition would result not only in reduced chemosensitivity, but also would have long-term complications. No further targeted therapies have been developed, and clinical trials have focused especially on decreasing the doses of radiochemotherapy for low- or standard-risk WNT-MBs (#NCT01878617, #NCT02724579).

1.2 SHH-MBs

Among the target therapies, agents directed against the SHH pathway gave the most promising results. Most SHH-MB patients harbor PTCH1 or SMO mutations. SMO inhibitors, primarily vismodegib, demonstrated their efficacy in several trials.[68]

Mutations of the SMO downstream pathway, such as SUFU or GLI1, make the SMO inhibitors ineffective. Several clinical trials increased the development of drugs directed against BET, SUFU, c-MET, CDK4/ 6 (ribociclib) and MET (foretinib) inhibitors, used in combination for overcoming therapeutic resistance.

In SMO-mutated MBs, PI3K signaling is usually increased, and the combined use of SHH-inhibitors with PI3K blockers also has a rationale.[69, 70] Finally, planning tailored therapies, made with a combination of HH inhibitors and TKIs, proteasome and chemokine inhibitors, may present a future opportunity in the management of this tumor group.

Table 3. Clinical trials on new therapeutic strategies for MBs

#	ClinicalTrials.gov Identifier	Title	Status	Study Phase	Conditions	Interventions	# of Patients Enrollment	Locations
1	NCT00822458	GDC-0449 in Treating Young Patients With Medulloblastoma That is Recurrent or Did Not Respond to Previous Treatment	Completed	I	Recurrent Childhood Medulloblastoma	Vismodegib	34	USA
2	NCT03434262	SJDAWN: St. Jude Children's Research Hospital Phase 1 Study Evaluating Molecularly-Driven Doublet Therapies for Children and Young Adults With Recurrent Brain Tumors	Recruiting	I	Central Nervous System Tumors	Gemcitabina, Ribociclib, Sonidegib, Trametinib, Filgrastim	108	USA
3	NCT01878617	A Clinical and Molecular Risk-Directed Therapy for Newly Diagnosed Medulloblastoma	Recruiting	II	Medulloblastoma	Vismodegib, Chemiotherapy, Radiation	625	USA
4	NCT01601184	Study of Vismodegib in Combination With Temozolomide Versus Temozolomide Alone in Patients With Medulloblastomas With an Activation of the Sonic Hedgehog Pathway	Terminated	I, II	"Medulloblastoma Activation of the Sonic Hedgehog (SHH) Pathway"	Vismodegib, Temozolomide	24	UK, SW, IT, FR
5	NCT00939484	Vismodegib in Treating Patients With Recurrent or Refractory Medulloblastoma	Completed	II	Adult Medulloblastoma	Vismodegib	31	USA
6	NCT01239316	Vismodegib in Treating Younger Patients With Recurrent or Refractory Medulloblastoma	Completed	II	Recurrent Childhood Medulloblastoma	Vismodegib	12	USA
7	NCT03734913	A Phase 1 Study of ZSP1602 in Participants With Advanced Solid Tumors	Recruiting	I	Basal Cell Carcinoma Medulloblastoma Adenocarcinoma of Esophagogastric Junction Small Cell Lung Cancer Neuroendocrine Neoplasm Glioblastoma	ZSP1602	65	CN
8	NCT01708174	A Phase II Study of Oral LDE225 in Patients With Hedge-Hog (Hh)-Pathway Activated Relapsed Medulloblastoma (MB)	Completed	II	Medulloblastoma	Sonidegib, Temozolomide	22	USA

#	ClinicalTrials.gov Identifier	Title	Status	Study Phase	Conditions	Interventions	# of Patients Enrollment	Locations
9	NCT01208831	An East Asian Study of LDE225 (Sonidegib)	Completed	I	Advanced Solid Tumor Cancers Medulloblastoma Basal Cell Carcinoma	Sonidegib	45	TW
10	NCT00880308	Dose Finding and Safety of Oral LDE225 in Patients With Advanced Solid Tumors	Completed	I	S Medulloblastoma Basal Cell Carcinoma	Sonidegib	103	UK, ES
11	NCT01125800	A Phase I Dose Finding and Safety Study of Oral LDE225 in Children and a Phase II Portion to Assess Preliminary Efficacy in Recurrent or Refractory MB	Completed	I, II	Medulloblastoma Rhabdomyosarcoma Neuroblastoma Hepatoblastoma Glioma Astrocytoma	Sonidegib	76	USA
12	NCT03936465	Study of the Bromodomain (BRD) and Extra-Terminal Domain (BET) Inhibitor BMS-986158 in Pediatric Cancer	Recruiting	I	Solid Tumor, Childhood Lymphoma Brain Tumor, Pediatric	BMS-986158	34	USA
13	NCT00095940	Lapatinib in Treating Young Patients With Recurrent or Refractory Central Nervous System Tumors	Completed	I, II	Central Nervous System Tumors	Lapatinib, Surgery	52	USA
14	NCT00788125	Dasatinib, Ifosfamide, Carboplatin, and Etoposide in Treating Young Patients With Metastatic or Recurrent Malignant Solid Tumors	Active, not recruiting	I, II	Central Nervous System Tumors	Carboplatin, Dasatinib, Etoposide, Ifosfamide	143	USA
15	NCT00077454	Erlotinib and Temozolomide in Treating Young Patients With Recurrent or Refractory Solid Tumors	Completed	I	Childhood Central Nervous System Tumors	Erlotinib, Temozolomide	95	USA
16	NCT00360854	Erlotinib Alone or in Combination With Radiation Therapy in Treating Young Patients With Refractory or Relapsed Malignant Brain Tumors or Newly Diagnosed Brain Stem Glioma	Unknown	I	Brain and Central Nervous System Tumors	Erlotinib, Radiotherapy	48	UK, IE

#	ClinicalTrials.gov Identifier	Title	Status	Study Phase	Conditions	Interventions	# of Patients Enrollment	Locations
17	NCT03598244	Volitinib in Treating Participants With Recurrent or Refractory Primary CNS Tumors	Recruiting	I	Primary Central Nervous System Neoplasm Recurrent/Refractory Diffuse Intrinsic Pontine Glioma Recurrent/Refractory Malignant Glioma Recurrent/Refractory Medulloblastoma	Savolitinib	36	USA
18	NCT03210714	Erdafitinib in Treating Patients With Relapsed or Refractory Advanced Solid Tumors, Non-Hodgkin Lymphoma, or Histiocytic Disorders With FGFR Mutations (A Pediatric MATCH Treatment Trial)	Recruiting	II	Advanced Malignant Solid Neoplasm Childhood Central Nervous System Tumors Childhood Hematologic Neoplasms	Erdafitinib	49	USA
19	NCT03893487	Fimepinostat in Treating Brain Tumors in Children and Young Adults	Recruiting	I	Diffuse Intrinsic Pontine Glioma Recurrent Anaplastic Astrocytoma Recurrent Glioblastoma Recurrent Malignant Glioma Recurrent Medulloblastoma	Fimepinostat, Surgery	30	USA
20	NCT03213678	PI3K/mTOR Inhibitor LY3023414 in Treating Patients With Relapsed or Refractory Advanced Solid Tumors, Non-Hodgkin Lymphoma, or Histiocytic Disorders With TSC or PI3K/MTOR Mutations (A Pediatric MATCH Treatment Trial)	Recruiting	II	Childhood Central Nervous System Tumors Childhood Hematologic Neoplasms	Samotolisib	144	USA

#	ClinicalTrials.gov Identifier	Title	Status	Study Phase	Conditions	Interventions	# of Patients Enrollment	Locations
21	NCT03155620	Targeted Therapy Directed by Genetic Testing in Treating Pediatric Patients With Relapsed or Refractory Advanced Solid Tumors, Non-Hodgkin Lymphomas, or Histiocytic Disorders (The Pediatric MATCH Screening Trial)"	Recruiting	II	Childhood Central Nervous System Tumors Childhood Hematologic Neoplasms	Ensartinib, Erdafitinib, Larotrectinib, Olaparib, Palbociclib, Samotolisib, Selpercatinib, Selumetinib Sulfate, Tazemetostat, Tipifarnib, Ulixertinib, Vemurafenib	1500	USA
22	NCT03526250	Palbociclib in Treating Patients With Relapsed or Refractory Rb Positive Advanced Solid Tumors, Non-Hodgkin Lymphoma, or Histiocytic Disorders With Activating Alterations in Cell Cycle Genes (A Pediatric MATCH Treatment Trial)	Recruiting	II	Advanced Malignant Solid Neoplasm Childhood Neoplasms	Palbociclib	49	USA
23	NCT03709680	Study Of Palbociclib Combined With Chemotherapy In Pediatric Patients With Recurrent/Refractory Solid Tumors	Recruiting	I	Ewing Sarcoma Rhabdoid Tumor, Rhabdomyosarcoma Neuroblastoma Medulloblastoma Diffuse Intrinsic Pontine Glioma	Palbociclib, Temozolomide, Irinotecan	100	USA
24	NCT02255461	Palbociclib Isethionate in Treating Younger Patients With Recurrent, Progressive, or Refractory Central Nervous System Tumors	Terminated	I	Childhood Central Nervous System Tumors	Palbociclib	35	USA

#	ClinicalTrials.gov Identifier	Title	Status	Study Phase	Conditions	Interventions	# of Patients Enrollment	Locations
25	NCT03387020	Ribociclib and Everolimus in Treating Children With Recurrent or Refractory Malignant Brain Tumors	Recruiting	I	Central Nervous System Embryonal Tumors Malignant Glioma Recurrent Atypical Teratoid/Rhabdoid Tumor Recurrent Childhood Ependymoma Recurrent/Refractory Diffuse Intrinsic Pontine Glioma Recurrent Medulloblastoma	Ribociclib, Everolimus	45	USA
26	NCT01977677	Plerixafor After Radiation Therapy and Temozolomide in Treating Patients With Newly Diagnosed High Grade Glioma	Completed	I, II	Adult Ependymoblastoma Adult Giant Cell Glioblastoma Adult Glioma, Glioblastoma, Gliosarcoma Adult Pineoblastoma Adult Medulloblastoma Adult Supratentorial Primitive Neuroectodermal Tumor (PNET) Adult Oligodendroglial Tumors	Temozolomide, Plerixafor, Radiotherapy	30	USA
27	NCT00381797	Bevacizumab and Irinotecan in Treating Young Patients With Recurrent, Progressive, or Refractory Glioma, Medulloblastoma, Ependymoma, or Low Grade Glioma	Completed	II	Childhood Cerebral Anaplastic Astrocytoma Childhood Oligodendroglioma Childhood Spinal Cord Neoplasm Recurrent Childhood Brain Stem Glioma Recurrent Childhood EpendymomaRecurrent Childhood Medulloblastoma Recurrent Childhood Ependymoma Recurrent Childhood Medulloblastoma	Bevacizumab, Fludeoxyglucose F-18, Irinotecan Hydrochloride	97	USA

#	ClinicalTrials.gov Identifier	Title	Status	Study Phase	Conditions	Interventions	# of Patients Enrollment	Locations
28	NCT01217437	Temozolomide and Irinotecan Hydrochloride With or Without Bevacizumab in Treating Young Patients With Recurrent or Refractory Medulloblastoma or CNS Primitive Neuroectodermal Tumors	Active, not recruiting	II	Recurrent Childhood Medulloblastoma Recurrent Childhood Pineoblastoma Recurrent Childhood Supratentorial Embryonal Tumor, Not Otherwise Specified	Bevacizumab, Temozolomide, Irinotecan Hydrochloride	108	USA
29	NCT00601003	Study of Nifurtimox to Treat Refractory or Relapsed Neuroblastoma or Medulloblastoma	Active, not recruiting	II	Neuroblastoma Medulloblastoma	Nifurtimox, Cyclophosphamide, Topotecan	112	USA
30	NCT02684071	Phase II Study of Intraventricular Methotrexate in Children With Recurrent or Progressive Malignant Brain Tumors"	Terminated	II	Recurrent Childhood Medulloblastoma Recurrent Childhood Ependymoma Childhood Atypical Teratoid/Rhabdoid Tumor Embryonal Tumors Metastatic Malignant Brain Neoplasm	Intrathecal Methotrexate, Topotecan, Cyclophosphamide	3	USA
31	NCT00005811	Topotecan Hydrochloride in Treating Children With Meningeal Cancer That Has Not Responded to Previous Treatment"	Completed	II	Childhood Central Nervous System Tumors Childhood Hematologic Neoplasms	Topotecan	77	USA
32	NCT00112619	Topotecan in Treating Young Patients With Neoplastic Meningitis Due to Leukemia, Lymphoma, or Solid Tumors	Terminated	I	Brain and Central Nervous System Tumors Primary Leukemia, Lymphoma Unspecified Childhood Solid Tumor	Topotecan	19	USA
33	NCT00404495	Combination of Irinotecan and Temozolomide in Children With Brain Tumors.	Completed	II	Glioma Medulloblastoma	Irinotecan, Temozolomide	83	AU

#	ClinicalTrials.gov Identifier	Title	Status	Study Phase	Conditions	Interventions	# of Patients Enrollment	Locations
34	NCT02095132	Adavosertib and Irinotecan Hydrochloride in Treating Younger Patients With Relapsed or Refractory Solid Tumors	Recruiting	I, II	Childhood Central Nervous System Tumors	Adavosertib, Irinotecan	154	USA
35	NCT00004078	Irinotecan in Treating Children With Refractory Solid Tumors	Completed	II	Childhood Central Nervous System Tumors	Irinotecan	181	USA
36	NCT00138216	Temozolomide, Vincristine, and Irinotecan in Treating Young Patients With Refractory Solid Tumors"	Completed	I	I Brain and Central Nervous System Tumors Unspecified Childhood Solid Tumor, Protocol Specific	Irinotecan, Temozolomide, Vincristine	42	USA
37	NCT02359565	Pembrolizumab in Treating Younger Patients With Recurrent, Progressive, or Refractory High-Grade Gliomas, Diffuse Intrinsic Pontine Gliomas, Hypermutated Brain Tumors, Ependymoma or Medulloblastoma	Recruiting	I	Constitutional Mismatch Repair Deficiency Syndrome Lynch Syndrome Malignant Glioma Recurrent Brain Neoplasm Recurrent/Refractory Childhood Ependymoma and Diffuse Intrinsic Pontine Glioma Medulloblastoma Recurrent/Refractory Medulloblastoma	Pembrolizumab	110	USA
38	NCT03173950	Immune Checkpoint Inhibitor Nivolumab in People With Select Rare CNS Cancers	Recruiting	II	Medulloblastoma Ependymoma Pineal RegionTumors Choroid Plexus Tumors Atypical/Malignant Meningioma	Nivolumab	180	USA
39	NCT00089245	Radiolabeled Monoclonal Antibody Therapy in Treating Patients With Refractory, Recurrent, or Advanced CNS or Leptomeningeal Cancer	Unknown	I	Brain and Central Nervous System Tumors Sarcoma Neuroblastoma	Iodine I 131 monoclonal antibody 8H9	120	USA
40	NCT03389802	Phase I Study of APX005M in Pediatric CNS Tumors	Recruiting	I	Brain and Central Nervous System Tumors	APX005M	45	USA

#	ClinicalTrials.gov Identifier	Title	Status	Study Phase	Conditions	Interventions	# of Patients Enrollment	Locations
41	NCT02502708	Study of the IDO Pathway Inhibitor, Indoximod, and Temozolomide for Pediatric Patients With Progressive Primary Malignant Brain Tumors	Active, not recruiting	I	Glioma, Glioblastoma, Gliosarcoma Malignant Brain Tumor Ependymoma Medulloblastoma Diffuse Intrinsic Pontine Glioma Primary CNS Tumor	Indoximod, Temozolomide, Radiotherapy, Cyclophosphamide, Etoposide	81	USA
42	NCT04049669	Pediatric Trial of Indoximod With Chemotherapy and Radiation for Relapsed Brain Tumors or Newly Diagnosed DIPG"	Recruiting	II	Glioblastoma Ependymoma Medulloblastoma Diffuse Intrinsic Pontine Glioma	Indoximod, Radiotherapy, Temozolomide, Cyclophosphamide, Etoposide, Lomustine	140	USA
43	NCT03500991	HER2-specific CAR T Cell Locoregional Immunotherapy for HER2-positive Recurrent/Refractory Pediatric CNS Tumors	Recruiting	I	Central Nervous System Tumor, Pediatric	HER2-specific chimeric antigen receptor (CAR) T cell	36	USA
44	NCT03638167	EGFR806-specific CAR T Cell Locoregional Immunotherapy for EGFR-positive Recurrent or Refractory Pediatric CNS Tumors	Recruiting	I	Central Nervous System Tumor, Pediatric	EGFR806-specific chimeric antigen receptor (CAR) T cell	36	USA
45	NCT02271711	Expanded Natural Killer Cell Infusion in Treating Younger Patients With Recurrent/Refractory Brain Tumors	Active, not recruiting	I	Recurrent Medulloblastoma Recurrent Ependymoma	Natural Killer Cell Therapy	12	USA
46	NCT04270461	NKG2D-based CAR T-cells Immunotherapy for Patient With r/r NKG2DL+ Solid Tumors	Not yet Recruiting	I	Hepatocellular Carcinoma Colon Cancer Glioblastoma Medulloblastoma	NKG2D-based CAR T-cells	10	China
47	NCT04185038	Study of B7-H3-Specific CAR T Cell Locoregional Immunotherapy for Diffuse Intrinsic Pontine Glioma/Diffuse Midline Glioma and Recurrent or Refractory Pediatric Central Nervous System Tumors	Recruiting	I	Brain and Central Nervous System Tumors	SCRI-CAR-B7H3(s); B7H3-specific chimeric antigen receptor (CAR) T cell	70	USA

#	ClinicalTrials.gov Identifier	Title	Status	Study Phase	Conditions	Interventions	# of Patients Enrollment	Locations
48	NCT03911388	HSV G207 in Children With Recurrent or Refractory Cerebellar Brain Tumors	Recruiting	I	Brain and Central Nervous System Tumors	HSV G207	15	USA
49	NCT02962167	Modified Measles Virus (MV-NIS) for Children and Young Adults With Recurrent Medulloblastoma or Recurrent ATRT	Recruiting	I	Medulloblastoma, Childhood, Recurrent Atypical Teratoid/Rhabdoid Tumor Medulloblastoma Recurrent	Modified Measles Virus	46	USA
50	NCT03043391	Phase 1b Study PVSRIPO for Recurrent Malignant Glioma in Children	Recruiting	I	Malignant Glioma, Glioblastoma, Gliosarcoma Anaplastic Astrocytoma, Oligoastrocytoma, Oligodendroglioma Atypical Teratoid/Rhabdoid Tumor Medulloblastoma Ependymoma Pleomorphic Xanthoastrocytoma Embryonal Tumor of Brain	Polio/ Rhinovirus Recombinant (PVSRIPO)	12	USA
51	NCT03299309	PEP-CMV in Recurrent MEdulloblastoma/Malignant Glioma	Recruiting	I	Recurrent Medulloblastoma Recurrent Brain Tumor Childhood Malignant Glioma	PEP-CMV	30	USA

AU: Australia; IE: Ireland; ES: Spain; CN: Cina; FR: France; IT: Italy; SW: Switzerland; TW: Taiwan; UK: United Kingdom; USA: United States of America

1.3 Group 3

The dismal prognosis occurring in Group 3 MBs, made an urgent development of targeted therapies necessary.[53] The increased expression level of the MYC gene, found in about 10-20 % of Group 3 patients, confers a very poor outcome. FDA approved pemetrexed and gemcitabine along with standard chemotherapy for this category.

Many clinical trials also demonstrated the efficacy of palbociclib, CDK4/6 inhibitor, PI3K inhibitor, BRD4 inhibitor and anti-vascularization therapies in monotherapy or in association with standard treatment for MBs of Group 3.

Alternative strategies, applicable to this subtype, include immunotherapies, mainly those that exploit engineered T and NK cells.

1.4 Group 4

The genomic heterogeneity of Group 4 is not clearly understood, and this constitutes the major limit in the development of target therapies.

It has been mainly immunotherapies, with CPIs, engineered T and NK cells and OVs that have been tested, with results that are still quite limited.

For those patients with relative activation of NOTCH signaling, a novel therapeutic opportunity is the administration of MK-0752 and RO4929097, both inhibitors of transcription of the NOTCH genes.[71]

2 Ongoing Challenges and Future Prospects

The main limitations in the development of an effective MB tailored approach are primarily the overcoming of the BBB, the tumor microenvironment and the tumor stem cell response.

The route of drug administration is still an issue in the management of these therapies.

In 2016, Phoenix et al. highlighted that the gene expression patterns applied to tumor subtypes determines the configuration of the BBB, which avoids drug penetration and reduces chemoresponsiveness.[64] WBT-MBs seem to have a better prognosis because of the presence of fenestrated vessels facilitating the penetration of drugs.[64]

Concerning strategies aimed at overcoming the BBB, possible routes of administration are intrathecal, stereotactic or endoscopic. These routes make it possible to deliver drugs directly into the tumor cavity, and as for other neurological and neurosurgical pathologies, they have the advantage of minimal invasiveness.[72, 73]

A valuable alternative comes from nanotechnology, which uses polymeric nanomedicines that are able to easily cross the BBB.[74, 75]

In addition, several studies have highlighted the presence of cancer stem cells (CSCs) in malignant brain tumors, which have self-renewing capabilities. The high incidence of dissemination and recurrence associated with MB is mainly attributable to the presence of CSCs. They have been reported to also be responsible for therapeutic resistance.[76,77] A further ongoing therapeutic approach targets the MB-CSCs, with agents directed at targeting specific pathways, such as CD133, SHH, PI3K/AKT, Stat3, and NOTCH.[78-80]

Yu et al. tested the Seneca Valley virus-001 (SVV-001) which can infect and destroy the CSCs, express CD133, and results in increased survival.[81]

However, the current amount of knowledge on MB-CSCs is still not sufficient for bedside application.

Conclusion

Advanced genetic studies resulted in the identification of prognostic factors of MBs, which have been translated into a risk stratification and an updated classification. The new genetic subgrouping provides the possibility for refining MB treatment strategies and developing novel molecular-guided clinical interventions.

Target agents directed against SHH, PI3K/AKT/mTOR and TKIs have been tested with favorable results, especially in SHH-MBs, whereas adoptive immunotherapies have been proposed for recurrent or refractory MBs.

The high genetic heterogeneity, especially of Group 3 and 4 MBs, the presence of CSCs and the BBB, are all responsible for chemoresistance.

Tailored therapies and combined chemotherapy approaches need to be further validated.

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Original article

Advanced pharmacological therapies for neurofibromatosis type 1-related tumors

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Abstract. Neurofibromatosis Type 1 (NF1) is an autosomal dominant tumor-predisposition disorder that is caused by a heterozygous loss of function variant in the NF1 gene, which encodes a protein called *neurofibromin*. The absence of neurofibromin causes increased activity in the Rat sarcoma protein (RAS) signalling pathway, which results in an increased growth and cell proliferation. As a result, both oncological and non-oncological comorbidities contribute to a high morbidity and mortality in these patients. Optic pathways gliomas, plexiform neurofibromas and malignant peripheral nerve sheath tumor (MPNST) are the most frequent NF1-associated tumors. The treatment of these complications is often challenging, since surgery may not be feasible due to the location, size, and infiltrative nature of these tumors, and standard chemotherapy or radiotherapy are burdened by significant toxicity and risk for secondary malignancies. For these reasons, following the novel discoveries of the pathophysiological mechanisms that lead to cell proliferation and tumorigenesis in NF1 patients, emerging drugs targeting specific signalling pathways (i.e. the MEK/ERK cascade), have been developed with promising results. (www.actabiomedica.it)

Key words: NF1, Malignant Peripheral Nerve Sheath Tumor, MPNST, Optic Pathway Glioma, Plexiform Neurofibroma, Selumetinib, Mtor Inhibitors

Background

Neurofibromatosis Type 1 (NF1) is an autosomal dominant tumor-predisposition disorder that is caused by a heterozygous loss of function variant in the tumor suppressor gene NF1. The average global prevalence is 33/100,000 individuals, varying among different countries from 12.8/100,000 in Russia to 104/100,000 in Israel (1–3).

NF1 was first described as a multisystemic disease by Friedrich Von Recklinghausen, in 1882. Nearly one century later, the National Institution of Health (NIH) Consensus Development Conference identified the diagnostic criteria (1987) (Table 1), which are still in use nowadays (4,5). The clinical hallmarks of NF1 are highly heterogeneous, and encompass non-malignant and malignant features. The former comprise pigmentary abnormalities (multiple café-au-lait macules, axillary and inguinal freckling, Lisch nodules), neurofibromas, skeletal deformities, hypertension and neurocognitive deficits. Risk of cancer in NF1 patients is 2 to 5 times higher than in the general population (6,7). Malignancies can develop within or without the nervous system. Nervous system tumours include: op-

Table 1. International Diagnostic criteria for Neurofibromatosis type 1 (4)

NIH Consensus Development Conference Diagnostic Criteria for NF1	
The diagnostic criteria for NF1 are met in an individual if two or more of the following are found:	1. 6 cafe au lait macules over 5 mm in greatest diameter in prepubertal individuals and over 15 mm in greatest diameter in postpubertal individuals
	2. 2 neurofibromas of any type or one plexiform neurofibromas
	3. Freckling in the axillary or inguinal regions
	4. Optic pathways glioma
	5. ≥ 2 Lisch nodules
	6. A distinctive osseous lesion such as sphenoid dysplasia or thinning of long bone cortex, with or without pseudarthrosis
	7. A first-degree relative (parent, sibling, or offspring) with NF-1 by the above criteria

tic pathway and brainstem glioma, glioblastoma, and malignant peripheral nerve sheath tumor (MPNST). Patients with NF1 also show an increased risk of tumours developing outside the nervous system, like gastrointestinal stromal tumor (GIST), breast cancer, leukaemia, phaeochromocytoma, duodenal carcinoid and rhabdomyosarcoma (8). Altogether, these clinical manifestations heavily affect life expectancy, which is in average 8-21 years shorter compared to the general population (6,9,10).

Resective surgery is the first line therapeutic option for most of the NF1-associated oncological complications. However, satisfactory results are not always achieved due to local extension and invasion of vital areas, tumor size, and risk of postoperative regrowth. On the other hand, the use of chemotherapy and radiotherapy is limited by high toxicity rates in NF1 patients. Furthermore, chemo- and radiotherapy should be strictly reserved to highly selected patients, when other therapeutic options (including watchful waiting) are not possible, and discussed with both patients and caregivers for the significant risk of developing secondary dysplasias and tumors later in life, due to the intrinsic tumor-predisposition of this syndrome. For these reasons, in the era of precision medicine, novel targeted therapies are highly demanded. In this review, we will briefly summarize the recent advances in the pathophysiological understanding of NF1-associated tumors and the available evidence for new emerging drugs.

Genetics and pathophysiology of NF1

The NF1 gene is located on chromosome 17q11.2 and encodes a 250 kDa cytoplasmatic protein called *neurofibromin*. About half of the cases are sporadic and due to *de novo* mutations. The germline mutation rate of NF1 is some 10-fold higher than that observed for most other inherited disease genes. Currently, over 2600 different inherited mutations in NF1 have been reported in the Human Gene Mutation Database (HGMD®) as a cause of NF1 (11–16).

Neurofibromin is a large multi-domain protein that acts as tumor suppressor. Neurofibromin includes a guanosine triphosphatase (GTPase)–activating protein (GAP) domain. GAP stimulates a GTPase activity intrinsic to RAS to inactivate the signal transduction pathway by converting RAS–guanosine triphosphate (GTP) to RAS–guanosine diphosphate (GDP) (17). This negative regulation of RAS reduces cell prolif-

eration and differentiation by forestalling activation of the downstream signalling pathways phosphatidylinositol 3-kinase/protein kinase B/mammalian target of rapamycin (PI3K/AKT/mTOR) and rapidly accelerated fibrosarcoma/mitogen activated protein kinase / extracellular signal regulated kinase (RAF/MEK/ERK) (7,18–21). Neurofibromin also regulates adenylyl cyclase and lowers the levels of intracellular cyclic adenosine monophosphate (cAMP) via RAS-dependent activation of atypical protein kinase C zeta (22). This protein is widely expressed in different organs and tissues, with high levels in the nervous system, and especially in neurons, astrocytes, oligodendrocytes, microglia, and Schwann cells (23,24).

From mutational analysis, the majority of germline NF1 mutations are predicted to be inactivating, resulting in almost complete absence of transcript or protein (25). Pathogenic mutations have been identified in most of its 61 exons, and include complete gene deletions, gene-disrupting chromosome rearrangements, smaller deletion or insertions, nonsense mutations, amino acid substitutions and splicing mutations (26). As a result, loss of neurofibromin expression leads to increased RAS activity and cell growth (27,28).

Some manifestations associated with NF1, such as cognitive problems, result from haploinsufficiency of NF1. Other clinical features require an additional somatic mutation, resulting in biallelic NF1 inactivation, as seen in the development of café-au-lait macules (CALMs), neurofibromas, GIST, glomus tumors, juvenile myelomonocytic leukemia (JMML), bone dysplasia and pheochromocytoma (25). Furthermore, mouse models of MPNST have shown that biallelic inactivation of the NF1 gene may not be sufficient for tumour formation and that additional genetic alterations such as mutation of TP53, CDKN2A or SUZ12, are required for the progression of MPNST (7,29,30).

Clinical evolution of the oncological complications in NF1

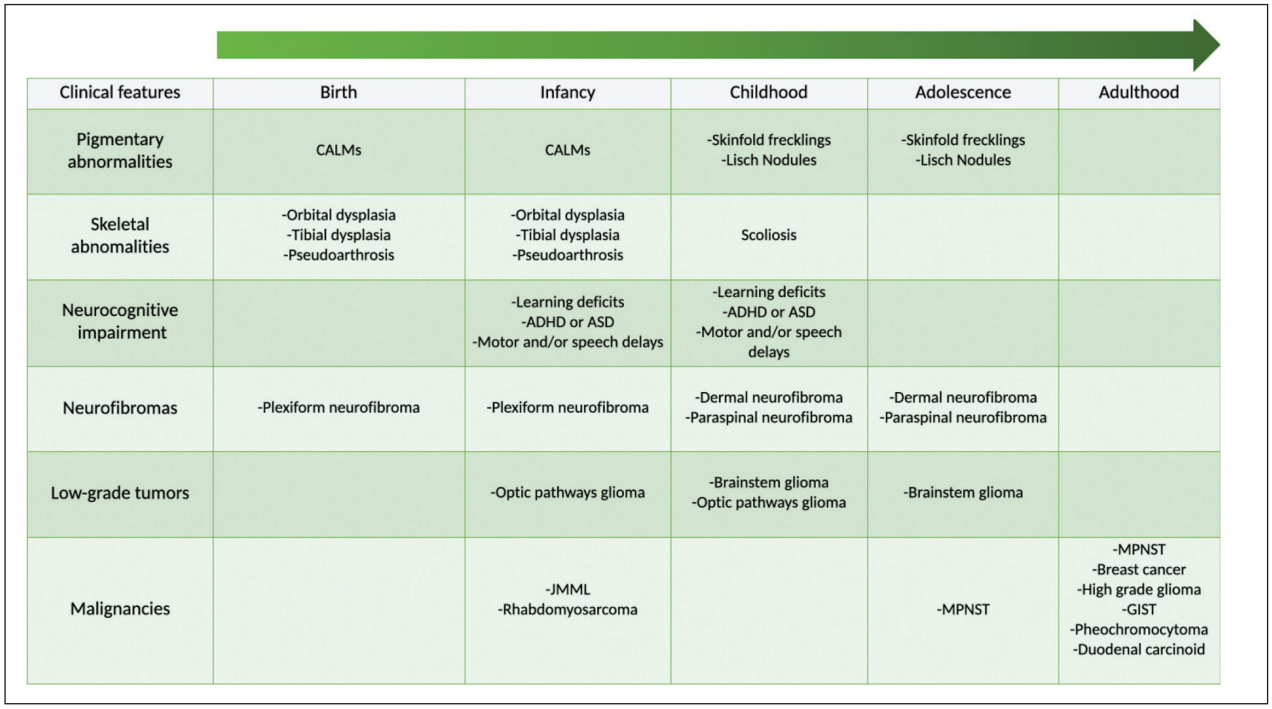
Most of the signs and symptoms of NF1 develop progressively from childhood to adulthood, and are rarely seen at birth. About 46% of the patients with sporadic NF1 do not meet the diagnostic criteria by the age of 1-year. When NF1 is suspected, annual monitoring until late childhood is necessary because 97% of the children with at least one feature of NF1 will eventually meet the diagnostic criteria by the age of eight (31). Skeletal deformities are frequently detected during infancy, while CALMs and axillary/inguinal freckling usually appear in childhood, and other typical signs and symptoms, including neurofibromas and lish nodules, only develop after puberty (Figure 1). Of note, also cognitive impairment and learning, memory, or attention deficits, are diagnosed lately during childhood (32–37). Early diagnosis is thus crucial to appropriately manage the neurocognitive and psycho-social issues and to reduce morbidity and mortality with preventive and therapeutic strategies.

Development and severity of clinical features of NF1 can vary between individuals, but usually follow a common timeline. Café-au-lait spots can be detected early during infancy, while skinfold freckling develops later in childhood. Cognitive dysfunction has a high impact on NF1 children, since school-age children with NF1 have higher rates of developmental delay and cognitive impairment than their pairs, and many of them carry a concurrent diagnosis of attention deficit/hyperactivity disorder. Moreover, one-third of all children with NF1 have a mild to severe autism spectrum disorder. Typical signs and symptoms of NF1 as neurofibromas and Lisch nodules usually develop only after puberty (5). Plexiform neurofibromas are detected on clinical examination in approximately 27% of individuals with NF1. However, these tumors do not always cause symptoms and may be clinically silent, especially when they reside deep within the body (5). About 15-20% of the patients will develop a glioma. Patients with NF1 are also at risk to develop other malignancies in adulthood, like gastrointestinal stromal tumors, pheochromocytoma, duodenal carcinoid, high grade glioma and breast cancer.

Low grade tumors

1. Glioma

About 15-20% of children with NF1 will develop a glioma, with a median age at diagnosis of 4.9 years.



Clinical features	Birth	Infancy	Childhood	Adolescence	Adulthood
Pigmentary abnormalities	CALMs	CALMs	-Skinfold frecklings -Lisch Nodules	-Skinfold frecklings -Lisch Nodules	
Skeletal abnomalities	-Orbital dysplasia -Tibial dysplasia -Pseudoarthrosis	-Orbital dysplasia -Tibial dysplasia -Pseudoarthrosis	Scoliosis		
Neurocognitive impairment		-Learning deficits -ADHD or ASD -Motor and/or speech delays	-Learning deficits -ADHD or ASD -Motor and/or speech delays		
Neurofibromas	-Plexiform neurofibroma	-Plexiform neurofibroma	-Dermal neurofibroma -Paraspinal neurofibroma	-Dermal neurofibroma -Paraspinal neurofibroma	
Low-grade tumors		-Optic pathways glioma	-Brainstem glioma -Optic pathways glioma	-Brainstem glioma	
Malignancies		-JMML -Rhabdomyosarcoma		-MPNST	-MPNST -Breast cancer -High grade glioma -GIST -Pheochromocytoma -Duodenal carcinoid

Figure 1. Clinical evolution in patients with neurofibromatosis type 1 (NF1).
ADHD: attention deficit/hyperactivity disorder; ASD: autism spectrum disorders; CALMS: café-au-lait macules; GIST: gastroIntestinal stromal tumors; JMML: juvenile myelomonocytic leukemia; MPNST: malignant peripheral nerve sheath tumour.

Optic pathways gliomas (OPGs) are pylocitic astrocitomas arising from the optic nerve, they can be unilateral or bilateral, and are the most frequent form (66%) of NF1-related gliomas (38,39).

OPGs can involve every part of the optic nerve from the papilla to the optic radiations, with different symptoms according to the location. NF1-related OPGs are usually asymptomatic, slowly growing and non-aggressive. However, symptoms of tumor progression may include decreased visual acuity, abnormal pupillary function, decreased colour vision, optic nerve atrophy, proptosis or other complications due to compression of the surrounding structures (i.e. between 12 and 40% children with chiasmal OPG develop precocious puberty) (39,40). Postchiasmatic OPGs presenting before the age of 2 years or after the age of 8 years tend to be more aggressive and should therefore be carefully followed up. Although the 5-year overall survival for patients with low grade glioma is 85%, progression-free survival for those with unresectable/residual disease requiring treatment is significantly lower (40%) (41).

The second most frequent CNS tumor in NF1 patients is brainstem glioma, which represent about 17% of all tumors in children with NF1 (42). Other gliomas are rarer, typically develop later in adulthood, and can involve all areas of the brain (37,43–48).

2. Neurofibroma

Neurofibromas are benign peripheral nerve sheath tumors composed of neoplastic Schwann cells, fibroblast, blood vessels and mast cells. According to their location, they can be divided in four types: cutaneous, subcutaneous, spinal and plexiform. Cutaneous and subcutaneous neurofibromas develop during childhood or early adolescence. They are benign tumors, and have no malignant potential (8). Spinal neurofibromas develop from the spinal foramina and can cause nerve roots compression or spinal deformities (i.e. scoliosis, kyphoscoliosis and vertebral body anomalies). When symptomatic, they can cause both motor and sensitive neuropathy and should be surgically treated (8,49). Plexiform neurofibromas (PNF) are

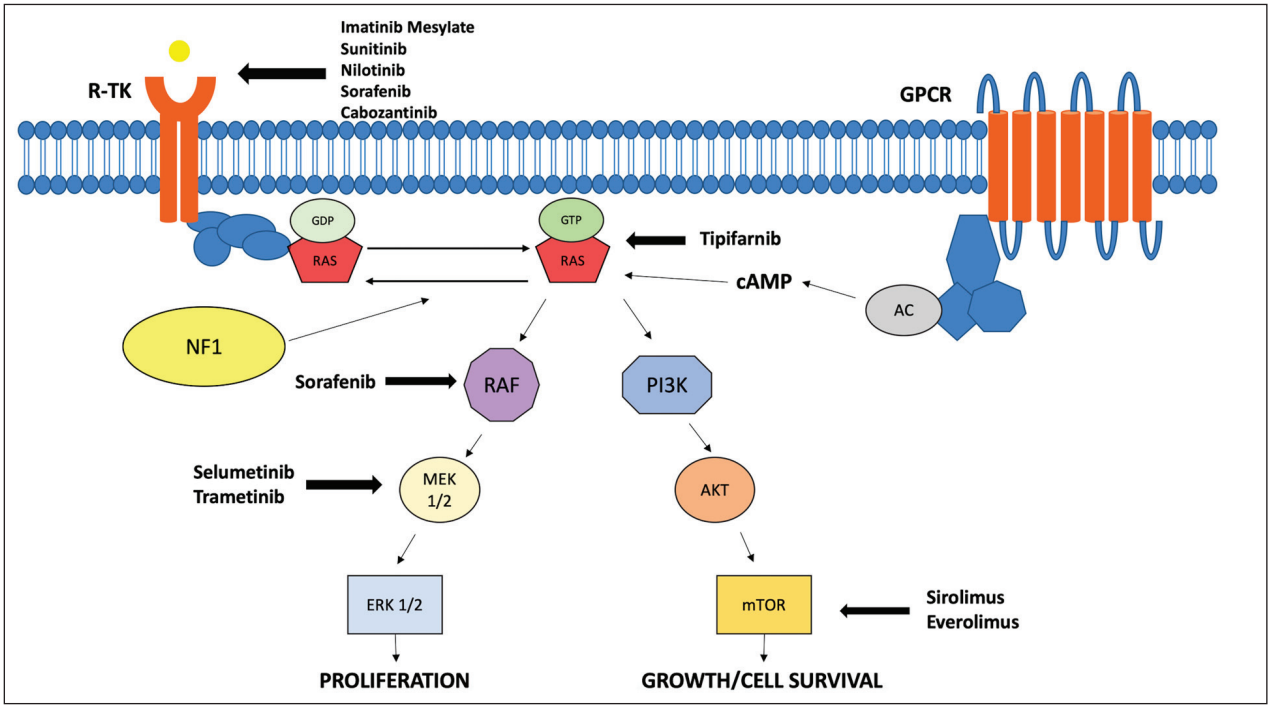


Figure 2. Signaling pathways and drug targets.
AC: Adenyl cyclase; cAMP: Cyclic adenosine monophosphate; AKT: Protein kinase B; GPCR: G-protein coupled receptor; GTP: Guanosine Triphosphate; MEK: Mitogen-activated protein kinase; ERK: extracellular signal-regulated kinase; mTOR: Mammalian target of rapamycine; PI3K: Phosphoinositide 3-kinase; RAF: Rapidly Accelerated Fibrosarcoma; RAS: Rat Sarcoma protein; R-TK: Receptor Tirosyne Kinase

benign nerve sheath tumors that can be found in 30-50% of NF1 patients (50). They are mostly congenital, and arise from the deep peripheral nervous plexuses. As all neurofibromas, they are slowly growing, but can often become large and bulky, developing in complex and infiltrative shapes (51). PNF can occur anywhere throughout the body, but particularly in extremities, thoracic and pelvic region, and tend to surround and invade nearby tissues and structures (i.e. bones) causing pain, disfigurement, neurologic impairment and motor dysfunction (51,52). Furthermore, PNF have a 8-15% lifetime risk of malignant transformation in MPNST (53–55). Therefore, surgery should be considered early and all patients should undergo a careful presurgical evaluations, especially when PNF become symptomatic. Unfortunately surgical outcomes are often dissatisfactory, especially when only partial resection is attainable (56), with post-operative re-growth rates that can reach 44% (57).

Malignancies

1. Malignant peripheral nerve sheath tumor

Malignant peripheral nerve sheath tumors (MPNST) are rare, biologically aggressive soft tissue sarcomas derived from Schwann cells or pluripotent cells of the neural crest. About 22-50% of all cases are associated with NF1. The median age at diagnosis is between 20 and 40 years (10-20 years earlier compared to the sporadic cases). MPNST usually arise from a pre-existing PNF. MPNST most commonly develop in the limbs (45%), the trunk (34%) and the head or neck (19%) (51,58). The clinical presentation is usually characterized by a rapid enlargement causing mass effect and neuropathic symptoms, such as paraesthesia, motor weakness or radicular pain. The prognosis for NF1-related MPNST is poor, with a 50% of early metastatic involvement at diagnosis (mainly

in the lungs) and a 5-year overall survival of 35-50% (59–61). As for other soft-tissue sarcomas, the best curative option is complete surgical resection, which is often not feasible due to location, size, and presence of metastasis. Furthermore, relapse rate is high and there is a lack of alternative therapeutic options (59). Adjuvant radiotherapy might be used to reduce local recurrence but needs a thorough risk-benefit evaluation for the heightened risk of secondary malignancies (62). Standard chemotherapy remains a treatment option in locally advanced or metastatic MPNST patients. It usually includes a combination of doxorubicin, ifosfamide, and etoposide, but response rate is usually poor compared to sporadic MPNST (17.9% vs 44.4%) (63).

2. Gastrointestinal Stromal tumor

Gastrointestinal stromal tumour (GIST) is a mesenchymal tumour that primarily arises in the gut mucosal wall. Unlike sporadic GISTs, those associated with NF1 usually lack somatic mutations of CD117 (c-KIT) or PDGFR-A (platelet-derived growth factor receptor A) (64,65). Instead, biallelic inactivation of the NF1 gene results in constitutive RAS activation, increasing the downstream mitogenic signalling through the MAP kinase cascade. Interestingly, gain-of-function mutations of c-KIT also activate many downstream signalling pathways including the RAS–MAP kinase cascade, suggesting a common pathogenetic mechanism in both sporadic and NF1-associated GISTs. As other tumors, NF1-associated GISTs have unique clinical features, compared to sporadic forms: they occur in younger patients (mean age at presentation 52.8 years), are multiple (60%) or develop in multiples sites, are smaller in size and with low mitotic activity, and occur mostly in the duodenum or small bowel. They are usually asymptomatic and incidentally detected during routine investigations. Surgery is the only modality that can offer a permanent cure of GIST, and complete surgical resection avoiding tumor rupture and injuries to the pseudocapsule is the initial treatment for primary and localized GISTs when the risk of morbidity and death from surgery is acceptable. The aims of surgery include complete resection with macroscopic and microscopic negative margins and functional preservation by wedge resection, when applicable. Unfortunately NF1 related GISTs show a variable but generally incomplete response to the tyrosine kinase inhibitor Imatinib treatment (64–69).

3. Pheochromocytoma

Pheochromocytomas are neuroendocrine catecholamine-secreting tumors, and occur in 2-2.9% of patients with NF1. Median age at presentation is 43 years (range 14–61 years) (70). This tumor is usually solitary, benign and localized in the adrenal glands, bilateral in 17% of the cases and metastatic or recurrent in 7.3%. Adrenalectomy remains the primary treatment of pheochromocytoma, with the entire gland being surgically removed in order to achieve cure. No differences have been described in the treatment and outcome of NF1-related pheochromocytoma compared to sporadic or other genetically determined forms of phaeochromocytoma (i.e. Multiple Endocrine Neoplasia type 2, Von Hippel Lindau syndrome, Hereditary paraganglioma–pheochromocytoma syndrome, Carney's triad) (70–75).

4. Breast Cancer

Although rare in patients with NF-1, few studies have shown that women with NF1 are at a higher risk of developing early onset breast cancer with aggressive behaviour and a poorer prognosis, compared to the general population. Cancer management is not well defined in this population, these lesions are usually treated with a combination of surgery, chemotherapy, and radiation in relation to the stage at diagnosis, although risks of secondary fibrosarcomas may be increased by radiotherapy in this vulnerable population group (76–79).

5. Duodenal carcinoid

Carcinoid tumors of the gastrointestinal tract are neuroendocrine tumors (NETs). Most of the cases of carcinoids are sporadic, but approximately 26% of all carcinoid tumors occur in patients with NF1, with the most common site being the periampullary region. Mean age at presentation is 47.9 years, with a 59% female preponderance (80). Clinical symptoms

are multiple, and vary depending on the tumor size, compression and dissemination. The most common presenting symptoms are jaundice (65%) and abdominal pain (31%). Biologically, the most common type of peri-ampullary NET in NF1 patients is somatostatinoma (40%). Surgical treatment is recommended: pancreaticoduodenectomy is the first choice approach for well-differentiated ampullary carcinoid >2 cm and for ampullary neuroendocrine carcinomas, while local tumor excision can be considered for carcinoids <2 cm. In patients who are not eligible for surgery, chemotherapy may be considered. Options for management of grade I and II tumors include octreotide, lanreotide, mTOR inhibitors (everolimus), and peptide-receptor radiotherapy (80–84).

6. Rhabdomyosarcoma

Rhabdomyosarcoma (RMS) is the most frequent soft-tissue sarcoma in children, and can be distinguished in alveolar and embryonal subtypes. Less than 1% of patient with NF1 develop RMS, and all have a embryonal histology (due to the known role of RAS activation in the pathogenesis of embryonal-type RMS). The median age at diagnosis is 2.9 years, significantly earlier compared to sporadic RMS (5 years). Frequent locations are pelvic and orbital. These patients tend to develop early non-metastatic RMS, most often in the pelvic sites, that appear to be genetically similar to sporadic cases. Complete resection is the best curative option and treatment does not differ from sporadic cases (85–90).

7. Juvenile myelomonocytic leukaemia

Juvenile myelomonocytic leukaemia (JMML) is a unique, aggressive hematopoietic disorder of infancy/early childhood caused by excessive proliferation of cells of monocytic and granulocytic lineages. Although JMML is an uncommon complication of NF1, it is estimated that patient with NF1 have a 200-350 fold increased risk of developing JMML, compared to the general population. Moreover, this association may be underestimated because patients with JMML may die at an age at which children do not manifest sufficient clinical signs to make the diagnosis of NF1. Allogeneic hematopoietic stem cell transplantation remains the therapy of choice for most patients with JMML, and should be recommended to any child with *NF1*-mutated JMML (91–93).

Emerging treatments for NF1-related tumors

Standard chemotherapy regimens are weighed by the toll of toxic effect that sometimes may lead to a discontinuation of therapy. Precision medicine is an approach that takes account for the characteristics of NF1 related tumors. Below an analysis of the current standard therapy and new, emerging drug for glioma, plexiform neurofibroma and malignant peripheral nerve sheath tumors. Table 2 illustrates novel target therapies that has been used or are currently under investigation.

1. Glioma

Despite the behaviour of this tumor is usually not aggressive, specific treatment might be necessary in case of tumor progression and clinical symptoms. The mainstay treatment is chemotherapy. Indications for radiation therapy and surgery are less frequent in NF1-associated gliomas. On one hand, radiotherapy it's not recommended because of the heightened risk of secondary tumors and moyamoya syndrome (94-95). On the other hand, most of the times this tumors are not surgically approachable for a complete resection, although a palliative debulking might be needed under specific circumstances (e.g. vision loss, corneal exposure due to proptosis, or pituitary localization) (95,96). Carboplatin and vincristine are the recommended first line chemotherapy for OPG (97-98), and the treatment protocol should always be handled by a specialist oncologist. Second line drugs include vinblastine, vinorelbine and temozolomide (99-100). Other options combine TPCV (thioguanine, procarbazine, lomustine, and vincristine) and weekly vinblastine (98). Recently, a phase II study of bevacizumab plus irinotecan was conducted in children with recurrent low-grade glioma, NF-1 related or not, to measure sustained response and/or stable disease lasting ≥6 months and progression-free survival, the results of

that study show that this therapeutic strategy could be useful (101).

All cited regimens seem to be effective but classic chemotherapy exposes children to toxic effects such as myelosuppression, allergic reactions, peripheral neuropathy, constipation, secondary malignancies, and infertility. Although effective, radiotherapy increases the risk of secondary malignancy, ototoxicity, endocrinopathies, and neurocognitive decline (102,103).

Among new emerging drugs, Selumetinib has shown promising results in the treatment of NF1-associated OPG. Selumetinib is an oral selective inhibitor of MEK 1 and 2. This inhibitor locks MEK1/2 into an inactive conformation that enables the binding of ATP and substrate but disrupts both the molecular interactions required for catalysis and the proper access to the ERK activation loop (104). First evidences of efficacy for selective MEK inhibition came from mouse models of NF1-deficient acute myeloid leukaemia, where it induced tumor regression (105-106). In 2017, the Pediatric Brain Tumor Consortium completed a phase I trial of Selumetinib in 38 children with recurrent, refractory, or progressive paediatric low-grade glioma, establishing the recommended phase II dose as 25 mg/m^2 twice daily. Five of 25 patients treated at the recommended phase 2 dose achieved a partial response (41). Simultaneously, in a phase I trial, 17/24 (71%) patients with NF1-associated PNF showed partial response after treatment with Selumetinib (107). Both trials showed tolerable toxicities and equal recommended treatment doses. A recent phase II multicentre trial (108) with Selumetinib has shown at least a partial response (≥50% tumour reduction on MRI) in 40% of the patients with NF1-associated low grade glioma. These preliminary results suggest a comparable efficacy to conventional chemotherapy, with a higher tolerability, manageability and safety profile (109).

2. Plexiform neurofibroma

At present, the only curative option for PNF is resective surgery, and it should therefore be considered as soon as possible, whenever applicable. However, due to their infiltrative nature, eventually involving vital structures, and tendency for regrowth, surgery might not always be performed. Unfortunately, as a matter of fact, the medical treatment of PNF hasn't found its keystone yet. As for many NF1-associated malignancies, radiotherapy is not recommended because of the risk of secondary malignancies (including radiation-induced MPNST, which typically have an even worse prognosis).Similarly, chemotherapeutic agents are not used because of their mutagenic nature and all drugs that have been used until now have shown little evidence of efficacy (56,62,110).

Among alternative treatments, interferon (INF) therapy has been reported in various studies (111,112) as an effective tumor-stabilizer. Jakacki and colleagues (111) eventually reported a 15-20% volume decrease in 29% of the patient. INF is safe and tolerable, and may be useful to reduce neuropathic pain. For this reason, a therapeutic trial of at least 6 months might be recommended, even if it will rarely be resolutive. The efficacy of Thalidomide (113) is less clear, as in a single study on 12 patients it showed a minor response in only 33%.

Since neurofibromin controls cell growth by negatively regulating the mTOR pathway activity, it seems reasonable to use mTOR inhibitors to manage NF1-related tumors (18). Sirolimus is a safe and well tolerated mTOR inhibitor that has been used to lengthen time to progression with fair success (mean increased time to progression: 4 months), but unfortunately failed in achieving a significant response in tumor shrinking or pain relief (114).

Sunitinib malate is a powerful, highly selective Tyrosine Kinase receptor inhibitor with activity against c-Kit, PDGFR, and vascular endothelial growth factor receptor (VEGFR), which are all implicated in the pathogenesis of MPNSTs. Preclinical studies showed that Sunitinib can induce reduction in PNF number and size, decreased mast cell infiltration, diminished fibroblast collagen deposition, and reduced metabolic activity (115). A phase II trial with Sunitinib was prematurely terminated because one patient died for uncertain (but possibly drug-related) causes (NCT01402817).

Meanwhile, other protein kinase inhibitors have undergone clinical trials for the treatment of PNF. A phase I trial (116) with Sorafenib, a protein kinase inhibitor with activity against RAF, PDGFRb, c-KIT and VEGFR-2, showed scarce tolerability at

substantially lower doses than the MTD, in children with refractory PNF. On the contrary, Pirfenidone, an oral anti-fibrotic and anti-inflammatory agent, demonstrated good tolerability in a phase II study (117), although it did not demonstrate clinical effectiveness and was not warranted further evaluation in children with progressive PN. Similarly, Tipirfanib, which selectively inhibits HRAS, did not offer significant efficacy compared to placebo (118,119). Imatinib Mesylate, a tyrosine kinase inhibitor with antineoplastic activity, targets c-KIT ligands secreted by biallelic NF1-inacrivated Schwann cells and is able to decrease the volume of PNF in mouse models (120). A phase II trial with Imatinib reported a 17% response with a ≥20% tumor reduction, although a few study limitations (i.e. relatively small sample size and significant heterogeneity of the selected population) may have underestimated its therapeutic effect (120).

Among emerging drugs for NF1, so far Selumetinib seems the most promising for the treatment of PNF. In a recent clinical trial on 24 patients with PNF (107), 71% showed partial tumor regression after a median follow up of 18 months, which is significantly high if compared to the response rates of imatinib (17%) (120) and interferon-alpha-2b (29%) (111). Moreover, all patients showed evidence of some degree of tumor reduction, with a response that remained stable without disease progression in 15/17. The most frequent toxic effects involved mainly the skin and the gastrointestinal tract, with a side-effect profile similar to adults (121), or an asymptomatic increase of the creatin kinase (107). Very recently, a phase II trial with Selumetinib in 50 children with inoperable PNF evidenced a 74% rate of partial response (defined as a ≥20% volume decrease), with a stable response in 56% after approximately one year (12 therapy cycles). In this study only a few children showed disease progression, and most of them (5/6) had experienced a dose reduction before progression. Notably, in addition to tumor shrinkage, 68% experienced improvements in neurofibroma-related complications such as pain or functional limitations. Toxic effects were similar to those evidenced in phase I, and always reversible. Taken together, these results identify Selumetinib as the most promising drug for the treatment of PNF, since its high tolerability and low toxicity profile may allow early prolonged treatments (122).

Finally, there are several ongoing trials with selective tyrosine kinase inhibitors like Nilotinib (NCT01275586), Trametinib (NCT03363217) (123), or Cabozantinib (NCT02101736), and mTOR pathway inhibitors like Everolimus (NCT01365468).

3. MPNST

The recent understandings in the pathogenesis of MPNST have led to the development of preclinical mouse models for the study of targeted agents and precision medicine. Unfortunately, most of these trials have been inconclusive, but several other are still ongoing. In a recent phase I/II study (124) Sirolimus was used in combination with Ganetespib, a novel injectable small molecule inhibitor of Hsp90, to treat MPNST. Despite the promising preclinical rationale and tolerability of the combination therapy, no significant responses were observed. Alike, several other mechanisms of actions are currently under investigation. These include the use of small molecules, like PLX3397 (an inhibitor of CSF1 and KIT) used in combination with mTor pathway inhibitors (NCT02584647) (125), or modified BET inhibitors to overcome resistance in MPNST (126). Knowing that many MPNST arise from previous PNF, however, the best approach would be to prevent malignant degeneration in high risk patients. In the future, the identification of risk factors, early biomarkers and eventually disease modifying drugs (like the promising Selumetinib) may radically change the natural history of these aggressive tumors.

Neurofibromin 1 (NF1) accelerates the conversion from active Guanosine Triphosphate bound RAS to inactive Guanosine Diphosphate bound RAS. RAS signalling transduces extracellular signals from ligand-activated receptors (Receptor Tirosyne Kinase and G-protein coupled receptor). Loss of neurofibromin results in elevated RAS signalling. GTP-RAS activates a multitude of effectors protein, including the RAF and the MEK/ERK signalling cascades, which promote proliferation, and the PI3K/mTOR pathway, which promotes growth and cell survival.

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

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