

Viral and Non-Viral Gene Therapy for Glioblastoma: New Insights into the Treatment of Malignant Brain Tumors

Pedro M Costa^{1,2} and Maria C Pedrosa de Lima^{1,2*}

¹CNC - Center for Neuroscience and Cell Biology, University of Coimbra, 3004-517 Coimbra, Portugal

²Department of Life Sciences, Faculty of Science and Technology, University of Coimbra, 3001-401 Coimbra, Portugal

Abstract

Glioblastoma (GBM) is the most common and malignant type of primary brain tumor, due to its high heterogeneity and aggressive brain invasion patterns. Despite recent advances in imaging and surgical techniques, which allowed a more accurate diagnosis and enhanced tumoral resection while maintaining or even improving neurologic function, current therapeutic options for GBM lack effective long-term impact on disease control and patient survival remains around the year mark. Therefore, the development of new therapeutic strategies that provide highly specific tumor cell killing and effective/complete eradication of cancer is critical to improve the life expectancy of GBM patients.

Driven by the tremendous advances in molecular biology, gene therapy has emerged as a promising strategy that uses nucleic acid delivery for the treatment of different forms of cancer, including GBM. In this regard, numerous/a wide range of therapeutic approaches involving versatile delivery vehicles, including non-replicating and oncolytic viruses, lipid- and polymer-based nanoparticles and stem cells, have been developed and tested for therapeutic potential. In this report, we review the principles behind such approaches and underscore their application to GBM treatment, by summarizing recent results obtained both *in vitro* and pre-clinical studies including the authors', as well as those reported in patients. A brief discussion on the major achievements and the trends identified by these studies is intended to provide new prospects for future developments and further improvements of the existing approaches towards successful treatment of malignant brain tumors.

Keywords: Glioblastoma (GBM); Gene therapy; Non-replicating viruses; Oncolytic viruses; Liposomes; Polymer-based nanoparticles; Stem cells

Abbreviations: 5-FC: 5-Fluorocytosine; 5-FU: 5-Fluorouracil; Akt: Protein Kinase B; BBB: Blood-Brain Barrier; Bcl-2: B-cell Lymphoma 2; BCNU: Bis-Chloroethylnitrosourea; CD: Cytosine Deaminase; CED: Convection-Enhanced Delivery; CPP: Cell-Penetrating Peptide; CTX: Chlorotoxin; EGFR: Epidermal Growth Factor Receptor; EHCO: (1-Aminoethyl) Iminobis [N-(Oleicylcysteinylhistinyl)-1-Aminoethyl] Propionamide; FDA: Food And Drug Administration; GBM: Glioblastoma; GCV: Ganciclovir; GCV-Tp: Ganciclovir Triphosphate; GFP: Green Fluorescent Protein; HIF-1α: Hypoxia-Inducible Factor 1α; HSV-Tk: Herpes Simplex Virus Type 1 Thymidine Kinase; IDH1: Isocitrate Dehydrogenase (IDH1); IFN-β: Interferon-β; IL-12: Interleukin 12; IL-13: Interleukin-13; IL-2: Interleukin 2; MA: Myristic Acid; MGMT: O-6-Methylguanine-DNA Methyltransferase; MRI: Magnetic Resonance Imaging; Mscs: Mesenchymal Stem Cells; Mtor: Mammalian Target Of Rapamycin; NF1: Neurofibromin; NF-κB: Nuclear Factor κB; NK: Natural Killer; Nscs: Neural Stem Cells; Ovs: Oncolytic Viruses; PDCD4: Programmed Cell Death 4; PDGF: Platelet-Derived Growth Factor; PE: Pseudomonas Exotoxin; PEG: Polyethylene Glycol; PEI: Polyethylenimine; PFS: Progression-Free Survival; PI3K: Phosphoinositide-3 Kinase; PTEN: Phosphatase And Tensin Homolog; PU: Polyurethane; RB: Retinoblastoma; Rcvs: Replication-Competent Retroviruses; Rcvs: Replication-Competent Viruses; Rdvs: Replication-Defective Viruses; RTK: Receptor Tyrosine Kinase; Scs: Stem Cells; Shrnas: Short Hairpin Rnas; Sirnas: Small Interference Rnas; SLN: Solid Lipid Nanoparticles; SNALP: Stable Nucleic Acid Lipid Particle; S-TRAIL: Tumor Necrosis Factor-Related Apoptosis-Inducing Ligand; TCGA: The Cancer Genome Atlas; TGF-β2: Transforming Growth Factor-Beta 2; TMZ: Temozolomide; VEGF: Vascular Endothelial Growth Factor; VEGF-A: Vascular Endothelial Growth Factor A

Introduction

Glioblastoma (GBM), the most common and malignant type of a class of tumors arising from glia or glial precursors - glioma - is one of the deadliest forms of human cancer, with a median patient survival of 12 to 15 months after diagnosis [1,2]. It is a highly heterogeneous and aggressive type of tumor, characterized by hallmark features of uncontrolled cellular proliferation, diffuse infiltration throughout the brain parenchyma, extensive angiogenesis, resistance to apoptosis and development of necrosis [1]. Over the last decade, a concerted effort has been made to identify and characterize all the genomic and signaling abnormalities driving GBM tumorigenesis. In this regard, important findings from integrated genomic analysis of The Cancer Genome Atlas (TCGA) data [3,4] and targeted proteomic analysis [4] revealed not only the existence of molecular subtypes of GBM (classical, mesenchymal, proneural and neural), driven by different genetic alterations, but also of common pathways mutated in this disease. The majority of GBM tumors harbor abnormalities in p53, retinoblastoma (RB) and receptor tyrosine kinase (RTK) pathways, suggesting that such abnormalities constitute a core feature of GBM pathogenesis [3]. Chromosome 7 amplification and chromosome 10 deletion are also common chromosomal aberrations found in GBM, whereas alterations in the genes coding for the protein neurofibromin

***Corresponding author:** Maria C Pedrosa de Lima, Department of Life Sciences, University of Coimbra, Apartado 3046, 3001-401 Coimbra, Portugal, Tel: +351-239-104397; E-mail: mdelima@ci.uc.pt

Received July 02, 2013; **Accepted** July 20, 2013; **Published** July 22, 2013

Citation: Costa PM, de Lima MCP (2013) Viral and Non-Viral Gene Therapy for Glioblastoma: New Insights into the Treatment of Malignant Brain Tumors. J Genet Syndr Gene Ther 4: 161. doi:[10.4172/2157-7412.1000161](https://doi.org/10.4172/2157-7412.1000161)

Copyright: © 2013 Costa PM, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

1 (*NF1*) and the isocitrate dehydrogenase (*IDH1*) are associated with specific GBM subtypes (mesenchymal and proneural, respectively). As stated by Dunn and colleagues, the findings from the abovementioned studies depict GBM as a heterogeneous collection of distinct diseases with multiple dependencies both within and across each particular subtype [5].

Due to the highly infiltrative nature of GBM, cancer cells from the tumor core usually spread into the surrounding brain tissue. Complete surgical resection is, therefore, nearly impossible, although partial tumor removal decreases the symptoms associated with the presence of an intracranial mass, and allows tissue collecting for histopathological and genomic characterization.

Standard Treatment for Newly Diagnosed Patients and Alternative Therapeutic Approaches

The standard post-surgery treatment for GBM consists of concomitant administration of the alkylating drug temozolomide (TMZ) with fractionated radiotherapy, followed by up to six cycles of adjuvant TMZ [6]. Although this treatment regimen has shown to provide a slight increase in overall patient survival (14.6 months), when compared to that of radiotherapy *per se* (12.1 months) [7], tumor resistance to this drug usually occurs, due to the action of different DNA repair mechanisms that restore the structural integrity of the methylated DNA bases [5,8-10], and hence, GBM relapse is nearly universal [7]. However, as opposed to the newly diagnosed GBM, currently there is no standard treatment for recurrent GBM [11].

Therapeutic modalities that increase tumoral delivery of chemotherapeutic agents have been exploited for the treatment of GBM. The post-surgical implantation of biodegradable carmustine wafers, an approach that was approved by the US Food and Drug Administration (FDA) for the local treatment of patients with newly diagnosed and relapsed GBM [11,12], was shown to provide a small patient survival benefit (2 months) when applied either *per se* [13] or in combination with TMZ and radiation followed by rotational chemotherapy [14]. Nevertheless, such therapeutic approach has inherent limitations, including the delay in wound healing and the increased risk of edema [15], which restrict its widespread application. The local delivery of a therapeutic agent through a stereotactically inserted catheter, via pressure gradient-assisted diffusion (also known as convection-enhanced delivery, CED) [16], has also shown to lead to enhanced anti-tumoral activity for various agents, including anti-

neoplastic drugs and toxins [17,18], although its clinical application has not resulted in significant patient survival benefit [11].

Cytostatic drugs, such as cisretinoic acid, thalidomide and tamoxifen, anti-angiogenics and growth-factor inhibitors have also been tested for the treatment of GBM [19]. These compounds act by interfering with intracellular signaling pathways and the tumor microenvironment, thus preventing its growth and spread, rather than promoting direct tumor cell death. Although clinical studies involving anti-angiogenesis monotherapy for the treatment of GBM failed to demonstrate sufficient anti-tumor activity to be used in single-agent regimens [20,21], their therapeutic effect may be more effective when combined with cytotoxic therapy [22]. Phase II clinical trials involving administration of bevacizumab, a humanized monoclonal antibody that inhibits the highly expressed vascular endothelial growth factor A (VEGF-A), in combination with chemotherapy, revealed an increase in GBM patient progression-free survival (PFS) over those treated with the drug [23,24]. Furthermore, clinical trials involving targeted therapy to growth factor (RTK)-driven pathways, including the PI3K/Akt/mTOR pathway [25,26], resulted in modest non-durable responses, which appear to be highly dependent on the functional status of the tumor suppressor PTEN [27].

Gene Therapy Approaches for the Treatment of GBM

Gene therapy aims at delivering therapeutically important nucleic acids to either replace a defective gene or to express/modulate a specific gene in a target cell/tissue. The majority of gene therapy clinical trials have been addressed to cancer [28], among which fifty-seven were specifically, designed for the treatment of high grade gliomas, including GBM, as reported in the Wiley database (January 2013). The generated gene therapy approaches for GBM involved delivery of different therapeutic nucleic acids, either *per se* or mediated by vehicles, including viruses, lipid- and polymer-based nanoparticles and stem cells. Table 1 displays examples of clinical trials involving the application of these delivery systems to high grade gliomas.

The delivery of unconjugated nucleic acids has been tested for the treatment of high grade gliomas [39]. The most promising results have been obtained with a phosphorothioate-modified antisense oligonucleotide targeting the mRNA of the transforming growth factor-beta 2 (TGF- β 2) (trabedersen (AP12009), Antisense Pharma), a protein that is highly overexpressed in high grade gliomas and induces tumor cell proliferation, angiogenesis, invasion and metastasis [40]. Phase I/II clinical trials involving the CED of this agent in high grade

Vector		Therapeutic gene and clinical setting	Reference (s)
Viruses	naked nucleic acids	transforming growth factor β antisense inhibitor, phase III	[29]
	replication-defective (adenoviruses and retroviruses)	HSV-tk, phases II, III	[30-33]
	replication-competent (retroviruses)	bacterial CD, phase I/II	A
	oncolytic (herpes simplex G207)	phase 1b	[34]
	oncolytic (adenoviruses ONIX-015)	phase 1	[35]
Lipid-based nanoparticles	cationic liposomes	interferon- β , phase I/II	[36]
	cationic liposomes	HSV-tk, phase I/II	[37]
	cationic liposomes	interleukin-12 carried by RD Semliki Forest virus, phase I/II	[38]
Stem cells	neural stem cells	bacterial CD, phase I	B

Gene therapy-based clinical trials that were conducted (or are currently ongoing) for the treatment of high grade gliomas. The type of delivery vehicle, therapeutic gene and clinical trial stage are indicated. A: <http://clinicaltrials.gov/show/NCT01156584>; B: <http://clinicaltrials.gov/ct2/show/NCT01172964>. HSV-tk: Herpes simplex virus type 1 thymidine kinase; CD: Cytosine deaminase; RD: Replication- defective

Table 1: Representative gene therapy clinical trials involving viral and non-viral vectors towards high grade gliomas.

gliomas patients revealed that this approach is safe, well tolerated and significantly increases patient survival, when compared to that observed for patients treated with standard chemotherapy [29]. Importantly, phase III clinical trials are currently ongoing for the treatment of high grade glioma patients.

The development of vectors that can efficiently and specifically deliver nucleic acids that sensitize tumor cells or lead to tumor cell death promoted the generation of effective gene therapy approaches for the treatment of cancer. As viruses remain the most efficient vectors in transducing tumor cells, it is not surprising that the majority of gene therapy trials for high grade gliomas have involved these carriers [41]. Both replication-defective (RDVs) and replication-competent (RCVs) viruses have been employed in clinic trials for the treatment of GBM.

Replication-Defective and Replication-Competent Viruses

RDVs lack the genes that are involved in the viral replication and, consequently, cannot produce new virions and induce target cell death. RDVs have been, therefore, engineered to incorporate conditional or direct cytotoxic transgenes in their genome [42].

The most widely used conditional cytotoxic transgene is the Herpes simplex virus type 1 thymidine kinase (HSV-tk), which codes for the enzyme thymidine kinase that converts the prodrug ganciclovir into the metabolite deoxyguanosine monophosphate. This metabolite is further phosphorylated by cellular kinases to the corresponding nucleoside triphosphate, a highly toxic nucleotide analog that incorporates into the DNA strands causing early chain termination [43]. This enzyme/prodrug combination has the capacity to promote cell death, not only in the recipient cells but also in the neighboring cells, by the so-called "bystander effect". Such effect may occur via different mechanisms, including the passage of phosphorylated ganciclovir metabolites from transduced to untransduced cells through intercellular gap junctions [44] or apoptotic vesicles [45], thus compensating for the low efficacy of vectors in transferring genes into the tumor cells. Despite the encouraging results from pre-clinical studies, numerous clinical trials for GBM involving non-replicating adenoviral/retroviral-mediated HSV-tk delivery demonstrated, at best, a modest increase in patient survival, when compared to that observed with standard radiotherapy and chemotherapy [30-33]. The limited efficacy of this therapeutic approach may be explained by the difficulties for ganciclovir to cross the blood-brain barrier (BBB) and reach the tumor, resulting in inefficient local viral dispersion and low transduction of tumor cells [46].

Among the direct cytotoxic transgenes, those encoding human toxin-based chimeric proteins have shown potential to be used in the treatment of several human diseases, including cancer [47]. In this regard, an interesting therapeutic approach for GBM took advantage of the transduction efficiency of non-replicating adenoviral vectors to deliver a transgene encoding the highly toxic *Pseudomonas* exotoxin (PE), which inhibits protein translation in the target cell leading to cell death [48]. The authors constructed doxycycline-dependent adenoviral vectors encoding the PE fused with a mutated human interleukin-13 (IL-13), in order to target the chimeric protein to a variant of the IL-13 receptor (IL13Ra2), which is overexpressed in GBM. In pre-clinical experiments with human xenografts, intratumoral delivery of the adenoviral construct resulted in significant antitumoral activity and increased animal survival, in comparison to that observed for intratumoral administration of protein formulations [48]. Indeed, earlier phase I-III trials involving the protein formulations failed to provide significant survival benefits due to short half-life and poor local

distribution [49,50], which indicates that the adenoviral administration may confer an important advantage towards a therapeutic effect. Future clinical trials should clarify whether this approach is clinically safe and will provide significant therapeutic benefit.

RDVs were also shown to efficiently deliver non-cytotoxic genes, including siRNAs and shRNAs to glioma cells. Saydam and colleagues developed a HSV-construct expressing anti-epidermal growth factor receptor (anti-EGFR) siRNAs, which was shown to promote efficient knockdown of EGFR and significant decrease in proliferation of human GBM cells, both in culture and upon subcutaneous implantation of the transduced cells in athymic mice [51]. Similarly, Kock et al. developed lentivirally-based constructs that encode an anti-B-cell lymphoma 2 (anti-Bcl-2) shRNA to downregulate Bcl-2, and the secreted tumor necrosis factor-related apoptosis-inducing ligand (S-TRAIL) to induce apoptosis in glioma cells [52]. Upon transduction of cultured glioma cells, increased caspase 3/7 activation and apoptosis were observed in cells transduced with viruses encoding anti-Bcl-2 and S-TRAIL, when compared to that observed for cells transduced with viruses coding for anti-Bcl-2 or S-TRAIL *per se*. Moreover, following intracranial implantation of lentivirally-transduced cells, reduced tumor growth was detected in animals injected with cells expressing the anti-Bcl-2 shRNA and S-TRAIL, when compared to that observed in animals injected with cells expressing only S-TRAIL [52].

The limited efficacy of RDVs reported in several human clinical trials towards cancer, including GBM, prompted investigators to use RCVs as an alternative system. This type of carriers is capable of replicating in infected cancer cells and forming progeny that can spread throughout the tumor mass [41]. Among the RCVs, those exhibiting the capacity to lyse infected cells, which are also known as oncolytic viruses (OVs), do not need to carry transgenes to cause cytotoxicity.

Several types of OVs have been investigated as oncolytic agents for therapeutic application in GBM. One of the most widely studied is the conditionally replicating HSV G207, a mutated HSV that expresses the HSV-tk gene and is capable of infecting rapidly dividing cells, which allows the combination (of virus) with pro-drugs like ganciclovir to further increase the oncolytic effect [53]. A phase 1b trial demonstrated that multi-dose administration of G207 into GBM, before or after tumor resection, is clinically safe and provides limited antitumoral activity [34]. A different recombinant HSV engineered to target the human epidermal growth factor receptor 2 (HER2), frequently overexpressed in GBMs, was also shown to be safe and decrease tumor growth upon intratumoral administration in an animal model of GBM [54].

Viruses with capacity to infect both dividing and non-dividing cells, including adenoviruses, have also been tested in pre-clinical models and clinic trials for high grade gliomas. Pre-clinical studies in human malignant glioma xenografts involving ONYX-015, a modified oncolytic adenovirus capable of replication in p53-defective tumor cells [55] demonstrated that intratumoral viral delivery resulted in cell lysis and impaired tumor growth, although the response was independent of the p53 status. A phase I clinical trial for recurrent high grade glioma revealed that the viral administration into the tumor cavity after surgical resection is safe and does not cause significant inflammatory response. In addition, increased survival was observed in patients receiving the highest doses of ONYX-015 [35].

In contrast to replication-competent HSVs and adenoviruses, replication-competent retroviruses (RCRs) do not possess cytolytic activity and depend on the incorporation of cytotoxic genes into the vector genome to cause tumor cell death [56]. In this regard, an

interesting approach that has been successfully tested in pre-clinical studies involves a RCR vector engineered to efficiently deliver a modified cytosine deaminase (CD)-coding gene to glioma cells. The bacterial CD catalyzes the conversion of the pro-drug 5-fluorocytosine (5-FC) to the anticancer agent 5-fluorouracil (5-FU) within the tumor cells. Tai and colleagues reported a significant survival benefit in glioma-bearing animals injected with RCRs and further treated with single or multiple cycles of 5-FC, when compared to that observed for animals treated with saline vehicle control [57]. Importantly, phase I/II testing is currently undergoing in recurrent high-grade glioma patients (<http://clinicaltrials.gov/show/NCT01156584>).

Despite the potential of oncolytic virotherapy, the incapacity to reach distant tumor pockets after local delivery limits its efficacy, which may be related to several factors, including large viral particle size and cell-to-cell barriers [58]. In this regard, the antitumor activity of OV s has been reported to be significantly reduced by immune degradation as a result of tumor infiltration of natural killer (NK) cells, macrophages and microglia [46].

Over the last few years, viral vectors have also been employed in *ex vivo* approaches towards the generation of antigen-specific lymphocyte T cells for adoptive GBM immunotherapy. For this purpose, autologous T cells are genetically modified, via virally-mediated gene delivery, to express receptors targeting tumor-specific antigens, which are designated chimeric antigen receptors (CARs) [59]. Therapeutic approaches involving T cells expressing CARs for glioma-specific antigens, including IL-13R α 2, erythropoietin-producing hepatocellular carcinoma A2 (EphA2) and a truncated form of the EGFR (EGFRvIII) have shown potent antitumor activity in animal models [60-62], which should be confirmed in future clinical trials.

Lipid-based Nanoparticles

Synthetic non-viral vectors present several advantages over viral carriers, including their reduced immunogenicity and toxicity, as well as physicochemical versatility, which allows modification of their surface for targeting to specific tissues or cells [63,64].

Lipid-based carriers have been successfully applied for the delivery of therapeutic nucleic acids to high grade gliomas in pre-clinical models [65,66]. One of the most popular approaches involves the intratumoral liposome-mediated delivery of genes coding for immunostimulatory proteins, such as interferon- β (IFN- β), in order to stimulate an antitumoral immune response [67-69]. In this regard, studies from Yoshida and colleagues demonstrated that local administration of cationic liposomes containing the IFN- β gene in glioma-bearing mice induces NK and T-cell activation, resulting in a significant increase of animal survival [67,68]. Following these studies, phase I-II clinical trials involving five patients with recurrent malignant glioma [36] have shown that injection of complexes of cationic liposomes with the human IFN- β gene into the tumor cavity was well tolerated by all patients and a considerable decrease in tumor volume (50%) was observed in two of them. However, due to the reduced number of patients involved in this study, no significant conclusions could be drawn. Subsequent clinical trials involving a larger number of patients should clarify whether an "immunogene" therapy approach will be clinically beneficial for the treatment of GBM. Cationic liposomes encapsulating the HSV-tk gene (combined with systemic ganciclovir) or a replication-incompetent Semliki Forest virus vector carrying the human interleukin-12 (IL-12) gene have also shown some promise in clinical trials for GBM [37,38].

Cationic liposomes were also used for delivery of siRNAs to glioma/GBM cells. Liposome-mediated delivery of anti-O-6-

methylguanine-DNA methyltransferase (anti-MGMT) siRNAs enhanced the cytotoxicity of TMZ in cultured GBM cells and in both subcutaneous and intracranial GBM mouse models (when administered intratumorally) [66]. In a different study, liposome-mediated delivery of anti-H-ferritin siRNAs into subcutaneously implanted mouse gliomas was also shown to enhance the cytotoxic effect of the alkylating agent bis-chloroethylnitrosourea (BCNU) [70].

Modified liposomes have also been successfully applied for the delivery of therapeutic genes to glioma cells. Saw and colleagues generated polyplexes by complexing nuclear factor κ B (NF- κ B) decoy oligonucleotides with a cell-penetrating peptide (CPP) composed of nine arginines (R9), which were then entrapped within R9-modified anionic liposomes, yielding a liposomal gene carrier with R9 peptides both on the surface and in the core of the particles [71]. The resulting nanocarriers were shown to be efficiently internalized by cultured U87 GBM and to promote oligonucleotide-mediated blocking of NF- κ B transcription activity, which increased the cytotoxicity of the drug paclitaxel [71]. In our laboratory, we have recently developed stable nucleic acid lipid particles (SNALPs), which were targeted towards brain tumor cells by covalent coupling of the peptide chlorotoxin (CTX) to the liposomal surface [72]. Our studies demonstrated that targeted liposomes were able to efficiently and specifically deliver encapsulated anti-miR-21 oligonucleotides to cultured U87 GBM cells (Figures 1A-1D), which resulted in suppression of miR-21 expression, increased levels of the tumor suppressors PTEN and PDCD4 (Figure 1E), caspase 3/7 activation and increased cytotoxicity of the tyrosine kinase inhibitor sunitinib (Figure 1F). Importantly, *in vivo* studies revealed that CTX enhances particle internalization into established intracranial tumors (Figure 1G).

Advances in lipid chemistry also allowed the development of another type of lipid-based nanocarriers for delivering nucleic acids to glioma cells. In an interesting study by Jin and colleagues, cationic solid lipid nanoparticles (SLN) were generated by a modified emulsification-solvent evaporation protocol [73] and conjugated to pegylated anti-MET siRNAs. The authors have shown that incubation of cultured U87 GBM cells with these carriers resulted in a decrease in both MET expression and cell proliferation [73]. Importantly, in an orthotopic GBM xenograft tumor model, intravenous administration of the developed SLN nanoparticles led to specific intra-tumoral delivery of the siRNAs, which resulted in decreased tumor cell proliferation and tumorigenicity [73].

Polymer-Based Nanoparticles

Polymer-based nanoparticles have been extensively used in animal models of cancer, including GBM. Zhan et al. developed a polyethylene glycol-polyethylenimine (PEG-PEI) gene carrier targeted to cancer cells by coupling a cyclic RGD sequence (cyclic arginine-glycine-aspartic acid-D-tyrosine-lysine) [74]. This approach took advantage of the ability of the RGD peptide to bind to integrin α V β 3, a protein involved in invasion and angiogenesis that is overexpressed in GBM at the brain tumor border and vasculature. When complexed with a plasmid coding for TRAIL and combined with PEG-conjugated paclitaxel-loaded polylactic acid micelles, the resulting targeted nanoparticles were found to prolong survival in human intracranial GBM xenografts [74]. Similarly, Li et al. [75] conjugated PEI to the hydrophobic molecule myristic acid (MA) to deliver TRAIL-coding plasmids to glioblastoma cells. In addition to the capacity to cross the BBB, the generated nanoparticles were shown to increase the survival of nude GBM-bearing mice, when compared to that of animals injected with nanoparticles lacking MA or a saline solution [75].

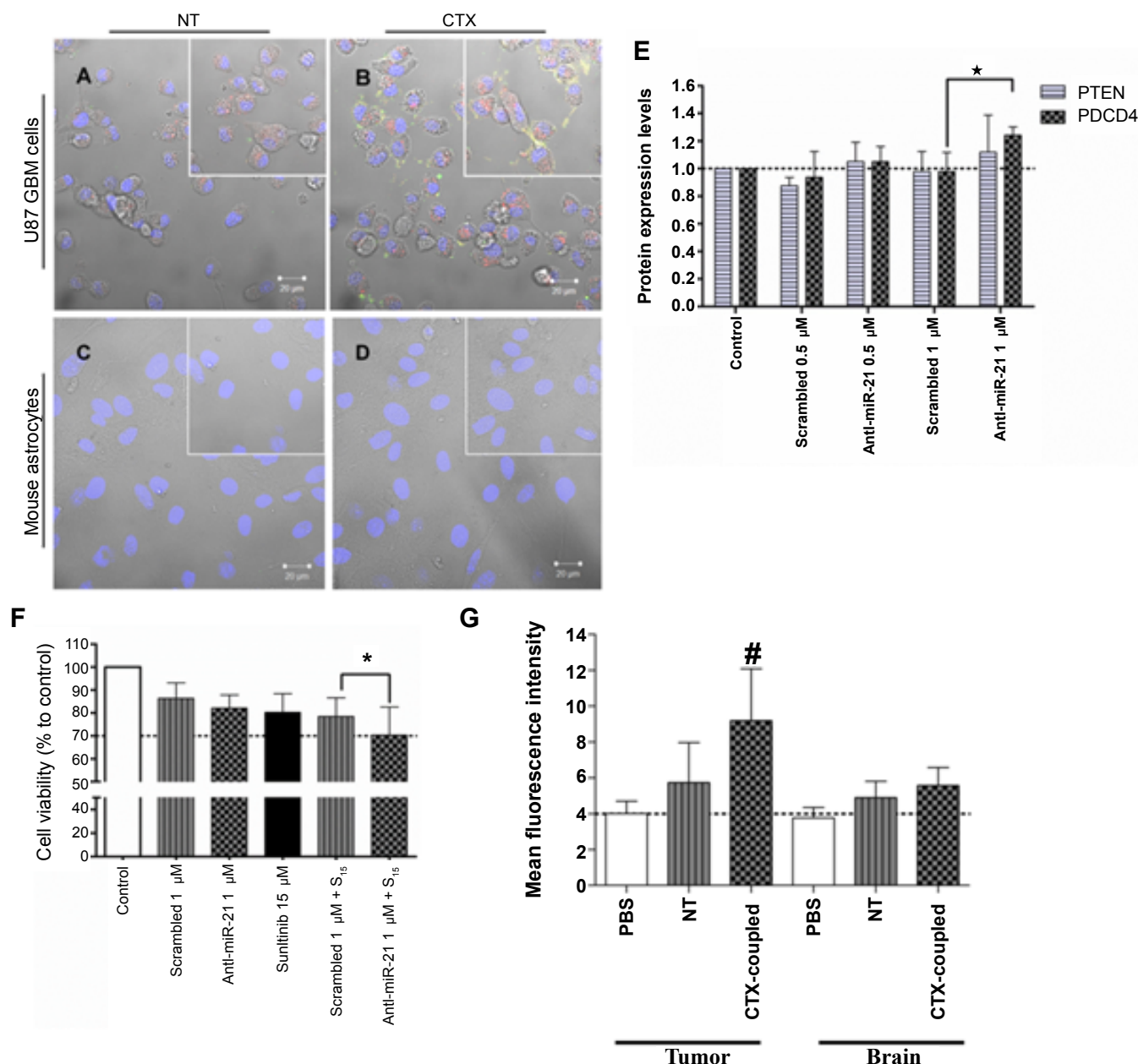


Figure 1: SNALP internalization in U87 human GBM cells and mouse astrocytes, PTEN/PDCD4 expression, tumor cell proliferation and biodistribution of systemically-administered SNALP-formulated FAM-labeled oligonucleotides. For evaluation of SNALP internalization, (A, B) U87 cells and (C, D) mouse astrocytes were incubated with rhodamine-labeled CTX-coupled (CTX) or nontargeted (NT) liposomes encapsulating FAM-labeled anti-miR-21 oligonucleotides (for 4 hours at 37°C), at a final oligonucleotide concentration of 1 μ M. Cells were then rinsed twice with PBS, stained with DNA-specific Hoechst 33342 (blue) and then observed by confocal microscopy. (E) PTEN and PDCD4 expression levels in U87 cells (corrected for individual α -tubulin signal intensity) 48 hours after cell incubation with CTX-coupled liposomes encapsulating anti-miR-21 or scrambled oligonucleotides, as determined by Western blot. Results are presented as PTEN and PDCD4 expression levels relative to control. * $p < 0.05$ to cells incubated with a similar amount of CTX-coupled liposomes encapsulating scrambled oligonucleotides. (F) For evaluation of tumor cell proliferation, U87 cells were incubated with CTX-coupled liposomes encapsulating anti-miR-21 or scrambled oligonucleotides for 4 hours, washed with PBS and further incubated for 24 hours with fresh medium. Cells were subsequently exposed to 15 μ M of sunitinib for 24 hours, rinsed with PBS, after which cell viability was evaluated by the Alamar Blue assay. Scrambled/anti-miR-21 1 μ M + S₁₅: cells transfected with scrambled or anti-miR-21 oligonucleotides and further incubated with 15 μ M sunitinib. * $p < 0.05$ compared to cells incubated with SNALP-formulated scrambled oligonucleotides and further treated with 15 μ M sunitinib. (G) Flow cytometry analysis (fluorescence intensity plots) of tumor and brain homogenates from animals injected intravenously with CTX-coupled and NT liposomes encapsulating FAM-labeled siRNAs or saline solution (PBS). # $p < 0.05$ compared to animals injected with a similar amount of NT SNALP-formulated siRNAs. Adapted from Costa et al. [68].

Polyurethanes (PU), which are conventionally used in tissue engineering and gene delivery due to their biocompatibility and physicochemical properties [76,77], have also been used for the

delivery of therapeutic genes to GBM. Using a combination of cationic PU with shortbranch PEI (PU-PEI) as a delivery vehicle, Yang and colleagues reported efficient delivery of miR-145 to CD133⁺ GBM

cells, which resulted in a considerable decrease in their tumorigenic potential and facilitated differentiation into CD133-negative cells [77]. When administered intratumorally, in combination with radiotherapy and TMZ, in an orthotopic GBM-CD133⁺ xenograft mouse model, nanoparticle-formulated miR145 significantly reduced tumorigenesis and improved animal survival, as compared to that observed in animals treated with PU-PEI *per se* [77].

Targeted delivery systems based on the multifunctional carrier (1-aminoethyl)iminobis[N-(oleicysteinylhistinyl)-1-aminoethyl] propionamide) (EHCO), a polymerizable surfactant with pH-sensitive amphiphilicity, demonstrated capacity to efficiently deliver siRNAs to target cells and promote endosomal release [78,79]. In this regard, Wang and colleagues developed a tumor-targeted delivery system by coupling the PEG-conjugated peptide bombesin to EHCO/siRNA nanoparticles. Upon systemic administration of targeted nanoparticle-formulated anti-hypoxia-inducible factor 1 α (anti-HIF-1 α) siRNAs, a significant decrease in tumor growth was observed in GBM-bearing mice xenografts, when compared to that observed for animals treated with nontargeted nanoparticles or naked siRNAs [80].

Magnetic nanoparticles modified with polymers have also been employed for the delivery of nucleic acids to glioma cells. Veisheh et al. developed a nanovector construct composed of a super paramagnetic iron oxide core coated with PEG-grafted chitosan and PEI, which was functionalized with the tumor-targeting peptide CTX, to improve tumor specificity and enhance cellular internalization [81]. The developed targeted nanovector was shown to specifically deliver anti-green fluorescent protein (anti-GFP) siRNAs to culture GFP-expressing C6 rat glioma cells and facilitate endosomal release, which resulted in efficient knockdown of GFP. Importantly, this carrier was reported to enhance magnetic resonance imaging (MRI) contrast *in vitro*, which, in combination with its capacity to target tumor cells, reveals potential to be used in the MRI monitoring of glioma treatment *in vivo* [81].

Despite the encouraging results obtained in pre-clinical studies, polymer-based nanocarriers have yet to be tested in clinical trials for GBM, to fully demonstrate their potential as a relevant tool towards application in a therapeutic context.

Neural and Mesenchymal Stem Cells

Stem cells (SCs) have been extensively used as vehicles for the delivery of therapeutic genes to brain tumors due to their remarkable capacity to target tumor cells, when injected either *in loco* [82] or intra-arterially [83,84]. Although the molecular basis of tumor tropism of SCs is not yet fully understood, several *in vitro* studies provided evidence that tumor-secreted cytokines and growth factors, including the vascular endothelial and platelet-derived growth factors (VEGF and PDGF, respectively) act as chemoattractants that promote SC migration towards tumor cells [85,86]. Two types of SCs from distinct origins – neural and mesenchymal – have been preferentially used in pre-clinical high grade glioma studies. Neural stem cells (NSCs) can be obtained from adult brains, fetal brains and embryonic SCs [87], while bone marrow and adipose tissue are excellent sources of mesenchymal stem cells (MSCs) [88,89].

Several *in vivo* studies demonstrated that SC-mediated gene delivery is able to induce tumor cell death and increase animal survival. Modified MSCs expressing immunostimulatory molecules, such as interleukin-2 (IL-2) and IFN- β , were shown to promote an antitumoral immune response and extend the survival of glioma-bearing rodents when administered intra-arterially [83,84]. Similarly, intratumoral MSC-based TRAIL gene delivery combined with the lipoxygenase

inhibitor MK886 showed a greater therapeutic efficacy in glioma xenografted mice, when compared to that observed with single-agent treatment [90]. A phase I clinical trial involving the post-resection local injection of genetically-modified NSCs expressing the bacterial CD combined with oral 5-FC is currently being tested in humans for the treatment of recurrent grade glioma (<http://clinicaltrials.gov/ct2/show/NCT01172964>).

SCs have also been suggested as potential cell-based carriers for delivery of OV, due to their exceptional migratory capacity and ability to act as immunosuppressors, which allows the therapeutic viruses to evade the host immune system [58,91]. Sonabend and colleagues demonstrated that MSCs were very effective in delivering oncolytic adenoviruses to glioma-bearing mice, even when administered at sites distant from the tumor mass [92]. Similarly, positive results have been reported using viral-loaded NSCs to treat intracranial tumor-bearing mice [93].

Nevertheless, a few concerns are associated with the clinical application of NSCs. The genetic modification of SCs to express therapeutic genes may result in insertion of the gene into a critical locus, dysregulating the normal cell function and inducing cancer cell phenotype [94,95]. Indeed, transformed NSCs have been suggested to be involved in the development of brain tumors [96]. Another limitation of SCs as nucleic acid delivery systems concerns the number of cells that can be obtained from each patient and the time involved in cell preparation to be successfully applied in the patient [95].

Conclusions and Future Perspectives

Considerable progress has been made over the last few years on the development and application of gene therapy approaches using a variety of delivery systems for treatment of GBM, as evidenced by the large number of pre-clinical and clinical trials reported for this disease. The demonstrated efficacy of such systems to mediate delivery of different types of nucleic acid molecules to target cells and the achieved therapeutic activity in anti-tumor strategies, both in animals models and patients, makes them highly promising in successful human gene therapies. Future clinical trials will allow to firmly determining whether the generated strategies will provide a significant therapeutic advantage, as complementary or alternative approaches, to the current treatment options.

Acknowledgements

This work was supported by the Portuguese Foundation for Science and Technology and FEDER/COMPETES (grants PTDC/DTP-FTO/0265/2012 and PEST-C/SAU/LA0001/20119). P. M. C. is recipient of a fellowship from the Portuguese Foundation for Science and Technology (SFRH/BD/45902/2008).

References

1. Furnari FB, Fenton T, Bachoo RM, Mukasa A, Stommel JM, et al. (2007) Malignant astrocytic glioma: genetics, biology, and paths to treatment. *Genes Dev* 21: 2683-2710.
2. Wen PY, Kesari S (2008) Malignant gliomas in adults. *N Engl J Med* 359: 492-507.
3. Verhaak RG, Hoadley KA, Purdom E, Wang V, Qi Y, et al. (2010) Integrated genomic analysis identifies clinically relevant subtypes of glioblastoma characterized by abnormalities in PDGFRA, IDH1, EGFR, and NF1. *Cancer Cell* 17: 98-110.
4. Brennan C, Momota H, Hambardzumyan D, Ozawa T, Tandon A, et al. (2009) Glioblastoma subclasses can be defined by activity among signal transduction pathways and associated genomic alterations. *PLoS One* 4: e7752.
5. Dunn GP, Rinne ML, Wykosky J, Genovese G, Quayle SN, et al. (2012) Emerging insights into the molecular and cellular basis of glioblastoma. *Genes Dev* 26: 756-784.

6. Stupp R, Mason WP, van den Bent MJ, Weller M, Fisher B, et al. (2005) Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. *N Engl J Med* 352: 987-996.
7. Stupp R, Hegi ME, Mason WP, van den Bent MJ, Taphoorn MJ, et al. (2009) Effects of radiotherapy with concomitant and adjuvant temozolomide versus radiotherapy alone on survival in glioblastoma in a randomised phase III study: 5-year analysis of the EORTC-NCIC trial. *Lancet Oncol* 10: 459-466.
8. Agnihotri S, Gajadhar AS, Ternamian C, Gorlia T, Diefes KL, et al. (2012) Alkylpurine-DNA-N-glycosylase confers resistance to temozolomide in xenograft models of glioblastoma multiforme and is associated with poor survival in patients. *J Clin Invest* 122: 253-266.
9. Cahill DP, Levine KK, Betensky RA, Codd PJ, Romany CA, et al. (2007) Loss of the mismatch repair protein MSH6 in human glioblastomas is associated with tumor progression during temozolomide treatment. *Clin Cancer Res* 13: 2038-2045.
10. Kaina B, Christmann M, Naumann S, Roos WP (2007) MGMT: key node in the battle against genotoxicity, carcinogenicity and apoptosis induced by alkylating agents. *DNA Repair (Amst)* 6: 1079-1099.
11. Anton K, Baehring JM, Mayer T (2012) Glioblastoma multiforme: overview of current treatment and future perspectives. *Hematol Oncol Clin North Am* 26: 825-853.
12. Buonerba C, Di Lorenzo G, Marinelli A, Federico P, Palmieri G, et al. (2011) A comprehensive outlook on intracerebral therapy of malignant gliomas. *Crit Rev Oncol Hematol* 80: 54-68.
13. Westphal M, Hilt DC, Bortey E, Delavault P, Olivares R, et al. (2003) A phase 3 trial of local chemotherapy with biodegradable carmustine (BCNU) wafers (Gliadel wafers) in patients with primary malignant glioma. *Neuro Oncol* 5: 79-88.
14. Affronti ML, Heery CR, Herndon JE, Rich JN, Reardon DA, et al. (2009) Overall survival of newly diagnosed glioblastoma patients receiving carmustine wafers followed by radiation and concurrent temozolomide plus rotational multiagent chemotherapy. *Cancer* 115: 3501-3511.
15. Sabel M, Giese A (2008) Safety profile of carmustine wafers in malignant glioma: a review of controlled trials and a decade of clinical experience. *Curr Med Res Opin* 24: 3239-3257.
16. Allard E, Passirani C, Benoit JP (2009) Convection-enhanced delivery of nanocarriers for the treatment of brain tumors. *Biomaterials* 30: 2302-2318.
17. Sampson JH, Akabani G, Archer GE, Berger MS, Coleman RE, et al. (2008) Intracerebral infusion of an EGFR-targeted toxin in recurrent malignant brain tumors. *Neuro Oncol* 10: 320-329.
18. Yamashita Y, Krauze MT, Kawaguchi T, Noble CO, Drummond DC, et al. (2007) Convection-enhanced delivery of a topoisomerase I inhibitor (nanoliposomal topotecan) and a topoisomerase II inhibitor (pegylated liposomal doxorubicin) in intracranial brain tumor xenografts. *Neuro Oncol* 9: 20-28.
19. Parney IF, Farr-Jones MA, Chang LJ, Petruk KC (2000) Human glioma immunobiology in vitro: implications for immunogene therapy. *Neurosurgery* 46: 1169-1177.
20. Batchelor TT, Sorensen AG, di Tomaso E, Zhang WT, Duda DG, et al. (2007) AZD2171, a pan-VEGF receptor tyrosine kinase inhibitor, normalizes tumor vasculature and alleviates edema in glioblastoma patients. *Cancer Cell* 11: 83-95.
21. Neyns B, Sadones J, Chaskis C, Dujardin M, Everaert H, et al. (2011) Phase II study of sunitinib malate in patients with recurrent high-grade glioma. *J Neurooncol* 103: 491-501.
22. Nieto-Sampedro M, Valle-Argos B, Gómez-Nicola D, Fernández-Mayoralas A, Nieto-Díaz M (2011) Inhibitors of Glioma Growth that Reveal the Tumour to the Immune System. *Clin Med Insights Oncol* 5: 265-314.
23. Desjardins A, Reardon DA, Coan A, Marcello J, Herndon JE 2nd, et al. (2012) Bevacizumab and daily temozolomide for recurrent glioblastoma. *Cancer* 118: 1302-1312.
24. Vredenburgh JJ, Desjardins A, Herndon JE 2nd, Dowell JM, Reardon DA, et al. (2007) Phase II trial of bevacizumab and irinotecan in recurrent malignant glioma. *Clin Cancer Res* 13: 1253-1259.
25. Chang SM, Wen P, Cloughesy T, Greenberg H, Schiff D, et al. (2005) Phase II study of CCI-779 in patients with recurrent glioblastoma multiforme. *Invest New Drugs* 23: 357-361.
26. Galanis E, Buckner JC, Maurer MJ, Kreisberg JI, Ballman K, et al. (2005) Phase II trial of temsirolimus (CCI-779) in recurrent glioblastoma multiforme: a North Central Cancer Treatment Group Study. *J Clin Oncol* 23: 5294-5304.
27. Abounader R (2009) Interactions between PTEN and receptor tyrosine kinase pathways and their implications for glioma therapy. *Expert Rev Anticancer Ther* 9: 235-245.
28. John Wiley and Sons Ltd. (2013) Gene therapy clinical trials worldwide. *Journal of Gene Medicine* 2013.
29. Hau P, Jachimczak P, Bogdahn U (2009) Treatment of malignant gliomas with TGF-beta2 antisense oligonucleotides. *Expert Rev Anticancer Ther* 9: 1663-1674.
30. Immonen A, Vapalahti M, Tyynelä K, Hurskainen H, Sandmair A, et al. (2004) AdvHSV-tk gene therapy with intravenous ganciclovir improves survival in human malignant glioma: a randomised, controlled study. *Mol Ther* 10: 967-972.
31. Mitchell P (2010) Ark's gene therapy stumbles at the finish line. *Nat Biotechnol* 28: 183-184.
32. Rainov NG (2000) A phase III clinical evaluation of herpes simplex virus type 1 thymidine kinase and ganciclovir gene therapy as an adjuvant to surgical resection and radiation in adults with previously untreated glioblastoma multiforme. *Hum Gene Ther* 11: 2389-2401.
33. Sandmair AM, Loimas S, Puranen P, Immonen A, Kossila M, et al. (2000) Thymidine kinase gene therapy for human malignant glioma, using replication-deficient retroviruses or adenoviruses. *Hum Gene Ther* 11: 2197-2205.
34. Markert JM, Liechty PG, Wang W, Gaston S, Braz E, et al. (2009) Phase Ib trial of mutant herpes simplex virus G207 inoculated pre-and post-tumor resection for recurrent GBM. *Mol Ther* 17: 199-207.
35. Chiocca EA, Abbed KM, Tatter S, Louis DN, Hochberg FH, et al. (2004) A phase I open-label, dose-escalation, multi-institutional trial of injection with an E1B-Attenuated adenovirus, ONYX-015, into the peritumoral region of recurrent malignant gliomas, in the adjuvant setting. *Mol Ther* 10: 958-966.
36. Yoshida J, Mizuno M, Fujii M, Kajita Y, Nakahara N, et al. (2004) Human gene therapy for malignant gliomas (glioblastoma multiforme and anaplastic astrocytoma) by in vivo transduction with human interferon beta gene using cationic liposomes. *Hum Gene Ther* 15: 77-86.
37. Voges J, Reszka R, Gossmann A, Dittmar C, Richter R, et al. (2003) Imaging-guided convection-enhanced delivery and gene therapy of glioblastoma. *Ann Neurol* 54: 479-487.
38. Ren H, Boulikas T, Lundstrom K, Soling A, Warnke PC, et al. (2003) Immunogene therapy of recurrent glioblastoma multiforme with a liposomally encapsulated replication-incompetent Semliki forest virus vector carrying the human interleukin-12 gene--a phase I/II clinical protocol. *J Neurooncol* 64: 147-154.
39. Caruso G, Caffo M, Raudino G, Alafaci C, Salpietro FM, et al. (2010) Antisense oligonucleotides as innovative therapeutic strategy in the treatment of high-grade gliomas. *Recent Pat CNS Drug Discov* 5: 53-69.
40. Schlingensiepen KH, Schlingensiepen R, Steinbrecher A, Hau P, Bogdahn U, et al. (2006) Targeted tumor therapy with the TGF-beta 2 antisense compound AP 12009. *Cytokine Growth Factor Rev* 17: 129-139.
41. Fulci G, Chiocca EA (2007) The status of gene therapy for brain tumors. *Expert Opin Biol Ther* 7: 197-208.
42. Aghi M, Chiocca EA (2006) Gene therapy for glioblastoma. *Neurosurg Focus* 20: E18.
43. Hamel W, Magnelli L, Chiarugi VP, Israel MA (1996) Herpes simplex virus thymidine kinase/ganciclovir-mediated apoptotic death of bystander cells. *Cancer Res* 56: 2697-2702.
44. Mesnil M, Yamasaki H (2000) Bystander effect in herpes simplex virus-thymidine kinase/ganciclovir cancer gene therapy: role of gap-junctional intercellular communication. *Cancer Res* 60: 3989-3999.
45. Colombo BM, Benedetti S, Ottolenghi S, Mora M, Pollo B, et al. (1995) The "bystander effect": association of U-87 cell death with ganciclovir-mediated apoptosis of nearby cells and lack of effect in athymic mice. *Hum Gene Ther* 6: 763-772.
46. Fulci G, Breyman L, Gianni D, Kurozumi K, Rhee SS, et al. (2006) Cyclophosphamide enhances glioma virotherapy by inhibiting innate immune responses. *Proc Natl Acad Sci U S A* 103: 12873-12878.

47. Lorberboum-Galski H (2011) Human toxin-based recombinant immunotoxins/chimeric proteins as a drug delivery system for targeted treatment of human diseases. *Expert Opin Drug Deliv* 8: 605-621.
48. Candolfi M, Xiong W, Yagiz K, Liu C, Muhammad AK, et al. (2010) Gene therapy-mediated delivery of targeted cytotoxins for glioma therapeutics. *Proc Natl Acad Sci U S A* 107: 20021-20026.
49. Assi H, Candolfi M, Baker G, Mineharu Y, Lowenstein PR, et al. (2012) Gene therapy for brain tumors: basic developments and clinical implementation. *Neurosci Lett* 527: 71-77.
50. Kunwar S, Prados MD, Chang SM, Berger MS, Lang FF, et al. (2007) Direct intracerebral delivery of cintredekin besudotox (IL13-PE38QQR) in recurrent malignant glioma: a report by the Cintredekin Besudotox Intraparenchymal Study Group. *J Clin Oncol* 25: 837-844.
51. Saydam O, Glauser DL, Heid I, Turkeri G, Hilbe M et al. (2005) Herpes simplex virus 1 amplicon vector-mediated siRNA targeting epidermal growth factor receptor inhibits growth of human glioma cells in vivo. *Mol Ther* 12: 803-812.
52. Kock N, Kasmieh R, Weissleder R, Shah K (2007) Tumor therapy mediated by lentiviral expression of shBcl-2 and S-TRAIL. *Neoplasia* 9: 435-442.
53. Hunter WD, Martuza RL, Feigenbaum F, Todo T, Mineta T, et al. (1999) Attenuated, replication-competent herpes simplex virus type 1 mutant G207: safety evaluation of intracerebral injection in nonhuman primates. *J Virol* 73: 6319-6326.
54. Gambini E, Reisoli E, Appoloni I, Gatta V, Campadelli-Fiume G, et al. (2012) Replication-competent herpes simplex virus retargeted to HER2 as therapy for high-grade glioma. *Mol Ther* 20: 994-1001.
55. Georger B, Grill J, Opolon P, Morizet J, Aubert G, et al. (2002) Oncolytic activity of the E1B-55 kDa-deleted adenovirus ONYX-015 is independent of cellular p53 status in human malignant glioma xenografts. *Cancer Res* 62: 764-772.
56. Kroeger KM, Muhammad AK, Baker GJ, Assi H, Wibowo MK, et al. (2010) Gene therapy and virotherapy: novel therapeutic approaches for brain tumors. *Discov Med* 10: 293-304.
57. Tai CK, Wang WJ, Chen TC, Kasahara N (2005) Single-shot, multicycle suicide gene therapy by replication-competent retrovirus vectors achieves long-term survival benefit in experimental glioma. *Mol Ther* 12: 842-851.
58. Ferguson SD, Ahmed AU, Thaci B, Mercer RW, Lesniak MS (2010) Crossing the boundaries: stem cells and gene therapy. *Discov Med* 9: 192-196.
59. Morgan RA, Dudley ME, Rosenberg SA (2010) Adoptive cell therapy: genetic modification to redirect effector cell specificity. *Cancer J* 16: 336-341.
60. Kong S, Sengupta S, Tyler B, Bais AJ, Ma Q, et al. (2012) Suppression of human glioma xenografts with second-generation IL13R-specific chimeric antigen receptor-modified T cells. *Clin Cancer Res* 18: 5949-5960.
61. Chow KK, Naik S, Kakarla S, Brawley VS, Shaffer DR, et al. (2013) T cells redirected to EphA2 for the immunotherapy of glioblastoma. *Mol Ther* 21: 629-637.
62. Bullain SS, Sahin A, Szentirmai O, Sanchez C, Lin N, et al. (2009) Genetically engineered T cells to target EGFRvIII expressing glioblastoma. *J Neurooncol* 94: 373-382.
63. Yoshida J, Mizuno M (2003) Clinical gene therapy for brain tumors. Liposomal delivery of anticancer molecule to glioma. *J Neurooncol* 65: 261-267.
64. Zhou J, Atsina KB, Himes BT, Strohbehn GW, Saltzman WM (2012) Novel delivery strategies for glioblastoma. *Cancer J* 18: 89-99.
65. Gupta B, Levchenko TS, Torchilin VP (2007) TAT peptide-modified liposomes provide enhanced gene delivery to intracranial human brain tumor xenografts in nude mice. *Oncol Res* 16: 351-359.
66. Kato T, Natsume A, Toda H, Iwamizu H, Sugita T, et al. (2010) Efficient delivery of liposome-mediated MGMT-siRNA reinforces the cytotoxicity of temozolomide in GBM-initiating cells. *Gene Ther* 17: 1363-1371.
67. Natsume A, Mizuno M, Ryuke Y, Yoshida J (1999) Antitumor effect and cellular immunity activation by murine interferon-beta gene transfer against intracerebral glioma in mouse. *Gene Ther* 6: 1626-1633.
68. Natsume A, Tsujimura K, Mizuno M, Takahashi T, Yoshida J (2000) IFN-beta gene therapy induces systemic antitumor immunity against malignant glioma. *J Neurooncol* 47: 117-124.
69. Yagi K, Hayashi Y, Ishida N, Ohbayashi M, Ohishi N, et al. (1994) Interferon-beta endogenously produced by intratumoral injection of cationic liposome-encapsulated gene: cytotoxic effect on glioma transplanted into nude mouse brain. *Biochem Mol Biol Int* 32: 167-171.
70. Liu X, Madhankumar AB, Slagle-Webb B, Sheehan JM, Surguladze N, et al. (2011) Heavy chain ferritin siRNA delivered by cationic liposomes increases sensitivity of cancer cells to chemotherapeutic agents. *Cancer Res* 71: 2240-2249.
71. Saw PE, Ko YT, Jon S (2010) Efficient Liposomal Nanocarrier-mediated Oligodeoxynucleotide Delivery Involving Dual Use of a Cell-Penetrating Peptide as a Packaging and Intracellular Delivery Agent. *Macromol Rapid Commun* 31: 1155-1162.
72. Costa PM, Cardoso AL, Mendonça LS, Serani A, Custódia C, et al. (2013) Tumor-targeted Chlorotoxin-coupled Nanoparticles for Nucleic Acid Delivery to Glioblastoma Cells: A Promising System for Glioblastoma Treatment. *Mol Ther Nucleic Acids* 2: e100.
73. Jin J, Bae KH, Yang H, Lee SJ, Kim H, et al. (2011) In vivo specific delivery of c-Met siRNA to glioblastoma using cationic solid lipid nanoparticles. *Bioconjug Chem* 22: 2568-2572.
74. Zhan C, Wei X, Qian J, Feng L, Zhu J, et al. (2012) Co-delivery of TRAIL gene enhances the anti-glioblastoma effect of paclitaxel in vitro and in vivo. *J Control Release* 160: 630-636.
75. Li J, Gu B, Meng Q, Yan Z, Gao H, et al. (2011) The use of myristic acid as a ligand of polyethylenimine/DNA nanoparticles for targeted gene therapy of glioblastoma. *Nanotechnology* 22: 435101.
76. Guan J, Fujimoto KL, Sacks MS, Wagner WR (2005) Preparation and characterization of highly porous, biodegradable polyurethane scaffolds for soft tissue applications. *Biomaterials* 26: 3961-3971.
77. Yang YP, Chien Y, Chiou GY, Cherng JY, Wang ML, et al. (2012) Inhibition of cancer stem cell-like properties and reduced chemoradioresistance of glioblastoma using microRNA145 with cationic polyurethane-short branch PEI. *Biomaterials* 33: 1462-1476.
78. Wang XL, Nguyen T, Gillespie D, Jensen R, Lu ZR (2008) A multifunctional and reversibly polymerizable carrier for efficient siRNA delivery. *Biomaterials* 29: 15-22.
79. Wang XL, Ramusovic S, Nguyen T, Lu ZR (2007) Novel polymerizable surfactants with pH-sensitive amphiphilicity and cell membrane disruption for efficient siRNA delivery. *Bioconjug Chem* 18: 2169-2177.
80. Wang XL, Xu R, Wu X, Gillespie D, Jensen R, et al. (2009) Targeted systemic delivery of a therapeutic siRNA with a multifunctional carrier controls tumor proliferation in mice. *Mol Pharm* 6: 738-746.
81. Veiseh O, Kievit FM, Fang C, Mu N, Jana S, et al. (2010) Chlorotoxin bound magnetic nanovector tailored for cancer cell targeting, imaging, and siRNA delivery. *Biomaterials* 31: 8032-8042.
82. Aboody KS, Brown A, Rainov NG, Bower KA, Liu S, et al. (2000) Neural stem cells display extensive tropism for pathology in adult brain: evidence from intracranial gliomas. *Proc Natl Acad Sci U S A* 97: 12846-12851.
83. Nakamizo A, Marini F, Amano T, Khan A, Studeny M, et al. (2005) Human bone marrow-derived mesenchymal stem cells in the treatment of gliomas. *Cancer Res* 65: 3307-3318.
84. Nakamura K, Ito Y, Kawano Y, Kurozumi K, Kobune M, et al. (2004) Antitumor effect of genetically engineered mesenchymal stem cells in a rat glioma model. *Gene Ther* 11: 1155-1164.
85. Cheng P, Gao ZQ, Liu YH, Xue YX (2009) Platelet-derived growth factor BB promotes the migration of bone marrow-derived mesenchymal stem cells towards C6 glioma and up-regulates the expression of intracellular adhesion molecule-1. *Neurosci Lett* 451: 52-56.
86. Schmidt NO, Przylecki W, Yang W, Ziu M, Teng Y, et al. (2005) Brain tumor tropism of transplanted human neural stem cells is induced by vascular endothelial growth factor. *Neoplasia* 7: 623-629.
87. Gottlieb DI (2002) Large-scale sources of neural stem cells. *Annu Rev Neurosci* 25: 381-407.
88. Tocci A, Forte L (2003) Mesenchymal stem cell: use and perspectives. *Hematol J* 4: 92-96.

-
89. Zuk PA, Zhu M, Ashjian P, De Ugarte DA, Huang JI, et al. (2002) Human adipose tissue is a source of multipotent stem cells. *Mol Biol Cell* 13: 4279-4295.
90. Kim SM, Woo JS, Jeong CH, Ryu CH, Lim JY, et al. (2012) Effective combination therapy for malignant glioma with TRAIL-secreting mesenchymal stem cells and lipoxigenase inhibitor MK886. *Cancer Res* 72: 4807-4817.
91. Dembinski JL, Spaeth EL, Fueyo J, Gomez-Manzano C, Studeny M, et al. (2010) Reduction of nontarget infection and systemic toxicity by targeted delivery of conditionally replicating viruses transported in mesenchymal stem cells. *Cancer Gene Ther* 17: 289-297.
92. Sonabend AM, Ulasov IV, Tyler MA, Rivera AA, Mathis JM, et al. (2008) Mesenchymal stem cells effectively deliver an oncolytic adenovirus to intracranial glioma. *Stem Cells* 26: 831-841.
93. Tyler MA, Ulasov IV, Sonabend AM, Nandi S, Han Y, et al. (2009) Neural stem cells target intracranial glioma to deliver an oncolytic adenovirus in vivo. *Gene Ther* 16: 262-278.
94. Aboody KS, Najbauer J, Danks MK (2008) Stem and progenitor cell-mediated tumor selective gene therapy. *Gene Ther* 15: 739-752.
95. Kosztowski T, Zaidi HA, Quiñones-Hinojosa A (2009) Applications of neural and mesenchymal stem cells in the treatment of gliomas. *Expert Rev Anticancer Ther* 9: 597-612.
96. Sanai N, Alvarez-Buylla A, Berger MS (2005) Neural stem cells and the origin of gliomas. *N Engl J Med* 353: 811-822.