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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.

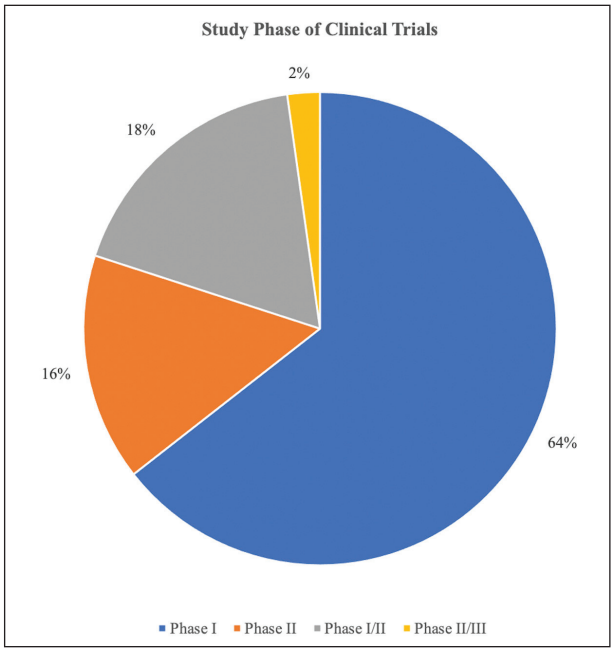
6 Clinical trials

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).

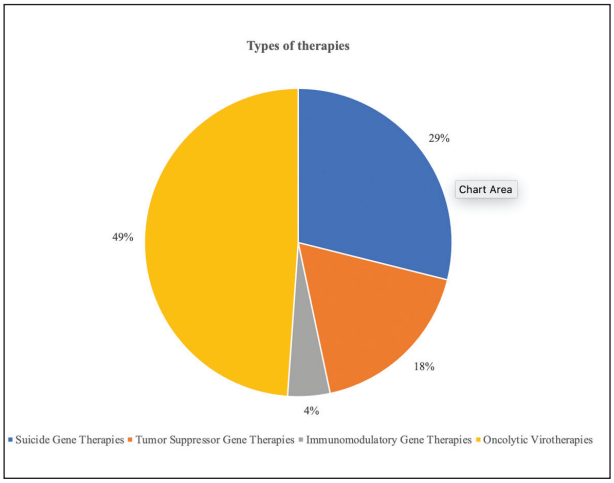
Table 3 summarizes the clinical trials on novel gene therapies for HGGs. (Table 3).

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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