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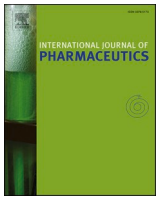

Review

Advances in Nanoparticle Systems for Targeted Therapy against Glioblastoma Multiforme

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ARTICLE INFO

This work is dedicated to Professor Giuseppe Tripodo, a lovely husband and father, a faithful and loyal friend, a sincere and honest person, and an extremely focussed and outstanding researcher. To his optimism, his love for live, his curiosity and creativity, his incredible strength, his smile and love for others, who were the inspiration for those who knew him. Dear Giuseppe, we all thank you for having been in our lives and our hearts, where you will live forever.

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Glioblastoma Multiforme
Blood-Brain Barrier
Blood-Brain Tumor Barrier
Dual-Targeting Polymeric-based Nanoparticles

ABSTRACT

Innovative treatment options for Gliobastoma Multiforme (GBM) are urgently needed due to poor patient prognosis. This Review provides new perspectives on nanotechnology systems designed as GBM treatments given their potential for translation. The first part of this work gives an overview of the complex disease that is GBM and its current therapies, whilst we describe barriers that must be overcome to enable translation and better therapy outcomes. A broad range of nanoparticle architectures are described, which we break down and analyze in terms of feasibility for translation and efficacy. A particular focus is dedicated to the Dual-Targeting Systems (DTSs), which are designed with one or more target ligands able to transport the system beyond the Blood–Brain Barrier (BBB) and, at the same time, direct them specifically to GBM cells. We also provide summaries of the challenging aspects that need to be addressed in multitargeted systems to support their translation to clinical testing. This Review summarizes the future perspectives of the most promising nanotechnologies and supports deeper investigations into the molecular mechanisms underlying GBM therapeutic failures in order to design better informed nanotechnologies as GBM treatment candidates.

Abbreviations: AMT, Adsorption-Mediated Transcytosis; ANG, Angiopep-2; ApoE, Apolipoprotein E; ART, Artesunate; ARTPC, Artesunate-phosphatidylcholine; BBB, Blood-Brain Barrier; BBTB, Blood-Brain Tumor Barrier; BCNU, Carmustine; BTMLPMS, Brain-Targeted anti-MiRNA-21 Lipid Polymer Micelle System; BTZ, Bortezomib; BW, Beeswax; CBSA, Cationic Bovine Serum Albumin; Chks, Checkpoint kinases; cIAP-1/2, Cellular inhibitor of apoptosis protein; *CPPs*, Cell Penetrating Peptides; CRISPR/Cas9, Clustered Regularly Interspaced Short Palindromic Repeats; DDS, Drug Delivery Systems; DOX, Doxorubicin; DTC, Dithiolane Trimethylene Carbonate; DTSs, Dual-Targeted Systems; DTX, Docetaxel; EGFR, Epidermal Growth Factor Receptor; EPR, Enhanced Permeability and Retention; Erlo, Erlotinib; ETO, Etoposide; FA, Folic acid; FDA, Food and Drug Administration; FUS, Focused ultrasound; GBM, Glioblastoma Multiforme; GICs, Glioma Initiating Cells; GLUT-1, Glucose Transporter 1; GMS, Glyceryl monostearate; Gp, GlycoProtein; HBMEC, Human Brain Microvascular Endothelial Cells; HIF-1, Hypoxia Inducible Factor 1; HSs, Hybrid Systems; IAPs, Inhibitors of Apoptosis Proteins; LBNPs, Lipid-Based Nanoparticles; LDLR, Low Density Lipoprotein Receptors; LfR, Lactoferrin receptor; MBs, Microbubbles; MDM2, Murine Double Minute-2; MGMT, O (6)-MethylGuanine-DNA MethylTransferase; MHC, Major Histocompatibility Complex; MMR, MisMatch Repair; MPEG, Methoxy Poly(ethylene glycol); nAChR, Acetylcholine receptor; NLCs, Nanostructured Lipid Carrier; NPs, Nanoparticles; NSCs, Neural Stem Cells; NVU, NeoVascular Unit; OPCs, Oligodendrocyte Precursor Cells; PA, Palmitic Acid; PAMAM, Polyamide amine; PARP, Poly-ADP Ribose Polymerase; PCL, Poly (ε- CaproLactone); PEG-P(CL-DTC)-MA, PEG-b-poly(PCL-co-dithiolane trimethylene carbonate) -mefenamate copolymer; PEG, Polyethylene Glycol; PLGA, Poly Lactic-co-Glycolic Acid; PNs, Polymeric-based Nanoparticles; PTX, Paclitaxel; P(VDF-TrFE), Polyvinylidene fluoride-trifluoroethylene; RGD, arginine–glycine–aspartic acid; RMT, Receptor-Mediated Transcytosis; ROS, Reactive Oxygen Species; RVG, Rabies Virus Glycoprotein; RES, Reticulo-Endothelial System; SA, Stearic Acid; SF, Sorafenib; SHH, Sonic Hedgehog; SLNs, Solid Lipid Nanoparticles; SMVT, Sodium Dependent Multivitamin Transporter; SPARC, Secreted Protein Acidic and Rich in Cysteine; SPC, Soybean PhosphatidylCholine; TAMs, Macrophages associated with cancer; Tf, Transferrin; TfR, Transferrin receptor; TMZ, Temozolomide; VEGF, Vascular Endothelial Growth Factor; WGA, Wheat Germ Agglutinin; WHO, World Health Organisation; XIAP, X-linked Inhibitor of Apoptosis Protein.

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1. Introduction

The World Health Organisation classifies Glioblastoma multiforme (GBM) as a Grade IV astrocytoma (Louis et al., 2007). The incidence of GBM is higher in men than in women, as well as in Caucasians than in other ethnicities (Grochans et al., 2022). Prevalence of GBM increases with age: 70 % of the cases are reported in patients between 45 and 70 years old (Iturrioz-Rodríguez et al., 2021), and it is considered the most malignant and aggressive human brain tumor and one of the most common forms in adults (Wu et al., 2021). GBM is also one of the most lethal human cancers with a life expectancy following diagnosis of only 12–18 months with just 8.7 % of the patients surviving more than two years post-diagnosis (Mittal et al., 2021).

GBM derives from alteration of Neural Stem Cells (NSCs), NSC-derived astrocytes, and Oligodendrocyte Precursor Cells (OPCs) (Wu et al., 2021). In particular, most glioblastomas (~90 %) originate from normal glial cells, which are referred as primary glioblastomas while, whilst a secondary glioblastoma is a tumor developed from low-grade gliomas. Primary and secondary glioblastomas are different in both their oncogenic ontogeny and in their growth, with the primary glioblastomas growing more aggressively than secondary glioblastomas. Despite these features, they show similar morphological characteristics and lead to similar clinical symptoms, the most common of which are headaches, vomiting, focal or progressive neurologic deficits, vision disturbances and syncope. These symptoms are influenced by and depend on the increase in intracranial pressure and, therefore, also on the size and localization of the tumor. In most cases, GBM is found in the cerebral hemispheres of the brain, such as the frontal and temporal lobes; however, in some cases, GBM has been found in the cerebellum, brainstem, and spinal cord (Wiwatchaitawee et al., 2021; Grochans et al., 2022).

Glioblastoma multiforme is characterized by various features that make its treatment difficult. In particular, the main characteristics are:

1. the high infiltration of GBM cells in the healthy tissue that makes impossible to completely resect the tumor;
2. the tumor heterogeneity which can originate from multiple and different DNA abnormalities;
3. the immunosuppressive microenvironment: GBM has been referred to as a "cold tumor" because of lack of tumor antigens, defects in antigen presentation, and high accumulation of immunosuppressive cells;
4. the increased cytokine production that leads to oncogenic changes in the cerebral microenvironment, including aberrant microvasculature development and infiltration of tumor cells into the perivasculature matrix;
5. the dysregulation of different cellular signalling pathways (e.g., Wnt/β-catenin, Sonic Hedgehog (SHH), Notch homolog 1, translocation-associated (Drosophila) (Notch)) leading the Glioma Initiating Cells (GICs) to a self-renewal capacity that can support tumor development, increase resistance to drugs and to all current therapies and promote recurrences;
6. robust DNA repair mechanisms and self-renewing capabilities because of the O (6)-MethylGuanine-DNA MethylTransferase (MGMT) that promote methylation in malignant gliomas, MisMatch Repair systems (MMR) and Checkpoint Kinases (Chks) of glioblastoma cells (Wu et al., 2021);
7. poorly oxygenated tissues, which creates perfect GIC niches, which induces autophagy to maintain cellular homeostasis;
8. a series of mutations, including overexpression of receptors, dysregulation of Vascular Endothelial Growth Factor (VEGF), Epidermal Growth Factor Receptor (EGFR), alteration of the Poly-ADP Ribose Polymerase (PARP) (Ayub and Wettig, 2022).

This Review offers new perspectives on nanotechnology-based systems for Gliobastoma Multiforme (GBM) treatments. Particular emphasis is given to nanoparticles systems that, among all the new investigated strategies for the GBM treatment, are one of the most promising approaches. A specific focus is dedicated to the Dual-Targeting Systems (DTSs). In particular, the Dual-Targeting Polymeric-based NanoParticles (DT-PNPs) and the Dual-Targeting Lipid-based NanoParticles (DT-LNPs) are evaluated as a promising targeted-delivery strategy. DT-PNPs and DT-LNPs are designed to use one or more target ligands promoting at the same time BBB crossing and GBM cells targeting. In this review, we also give practical examples of different dual-targeted nanosystems developed in the recent literature for treating GBM, such as Liposomes, SLNs, NLCs, Polymeric Nanoparticles, Polymeric Micelles, Dendrimers, and Hybrid Systems (HSs). Their potential use in the treatment of GBM is also reviewed here. We describe the complex disease of GBM that, so far, has limited treatment options and offered poor patient survival. We also provide summaries of the challenging aspects that need to be addressed in multitargeted systems to support their translation to clinical testing.

2. The Blood-Brain Barrier (BBB) and the Blood-Brain Tumor Barrier (BBTB)

Delivering drugs to the brain has been a major challenge for researchers for decades. Different technologies and delivery systems have been studied but, therapeutic success still seems to be far away. One of the main reasons for the failure of current therapies for the treatment of GBM is closely related to the difficulty in reaching the site of action due to brain barriers that act to prevent the passage of drugs to the brain, namely the BBB and also the BBTB (Arvanitis et al., 2020; Marcucci et al., 2021; Zhang et al., 2023).

The BBB is defined as a highly intricate interface between the blood capillary network and the brain parenchyma. Physiologically, the main BBB functions are to act as a:

- **Physical barrier**, which prevents the entry of potentially harmful substances.
- **Pump to remove and eliminate all toxic substances and metabolites** that could damage the integrity of the brain, *via* different active efflux pumps (Jena et al., 2020).
- **Enzymatically detoxify neuroactive molecules**, which can bypass the barrier, to protect the brain.
- **Immunologically stabilize** the brain by means of immune cells including perivascular macrophages, microglia, T-cells, and mast cells.

The integrity of BBB is very crucial to perform its function and to adequately protect and maintain in a healthy status the brain tissue.

The BBB comprises a continuous layer of endothelial cells that function as a good capillary network. Endothelial cells are linked together by tight junctions and adherent junctions and are enclosed by astrocytes-end-feet and basal membrane.

These structures have been classified into three barriers (Fig. 1, A):

- **Glycocalyx**, which is rich in carbohydrates and composed of negatively charged glycoproteins, proteoglycans, glycosaminoglycans;
- **Endothelium**, which is characterized by tight junctions, adherent junctions and transport mechanisms, including efflux pumps that

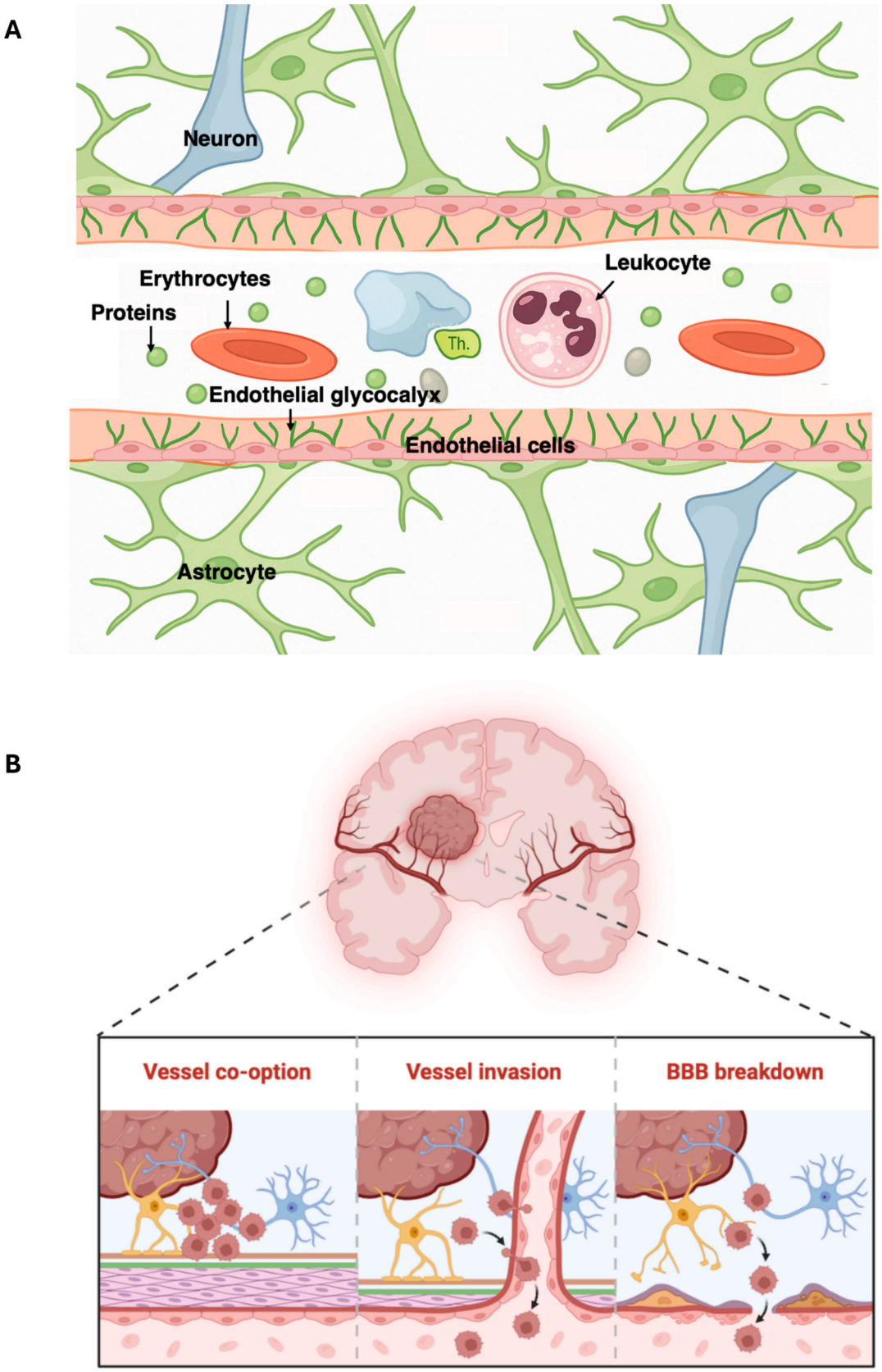

Fig. 1. Schematic representation of: A) the main structural features of the BBB. Readapted from Wiley (Zhao et al., 2021); B) a possible tumor progression through vascular co-option, vessel invasion and disruption of the BBB.

allow the exchange of nutrients and other molecules through the BBB;

- **NeoVascular Unit (NVU)**, which is made up of arteries, veins, arterioles that are the main blood supply to the brain; as well as pericytes and astrocytes that help to maintain homeostasis in the brain and microglia that have a role in the immune responses of the brain (Mehrdadi, 2023; Jena et al., 2020; Zhao et al., 2021)

The BBTB is a specialized barrier that forms within brain tumors, specifically gliomas, to protect the tumor from the surrounding brain tissue.

As the tumor progresses, the infiltration of cancer cells through the BBB leads to a loss of its integrity and a consequent alteration of the neural structure leading to the formation of BBTB which is more permeable than BBB but has a more heterogeneous and unpredictable permeability (Tincu (Iurciuc) et al., 2023).

During the formation of BBTB several modifications occur leading the cerebral microvascular endothelial cells to lose its original composition; indeed, pericytes weaken their tight and adherent joints and high concentrations of pro-inflammatory cytokines lead to the alteration of NVU and, therefore, to an altered permeability of BBB. In particular, cancer cells can also use vascular co-option using existing blood vessels to obtain nutrients without inducing the formation of new vessels. In vessel invasion, cancer cells can penetrate the walls of the blood vessel, enter the circulation and spread. All these changes can lead to a break and alteration of the BBB (Ribatti and Pezzella, 2022) (Fig. 1, B).

These abnormalities contribute to the degradation of the vascular structure as the tumor progresses.

The intensity of these changes strongly depends on the type of tumor, volume, phase and location. The damage is more relevant in high-grade gliomas, which are the most aggressive types. In these, the tumor grows very aggressively, producing a condition of hypoxia that regulates the Hypoxia Inducible Factor 1 (HIF-1). HIF-1 then stimulates VEGF production, angiogenesis, and abnormal vessel formation to compensate the growing demand for nutrients and oxygen that cause further disruption of BBB (Reddy et al., 2021).

3. Current challenges in the treatment of GBM

The current standard treatment (in use) of GBM includes maximal surgical resection, followed by a combination of radiotherapy and/or chemotherapy (Fig. 2).

Surgery, however, it is not always possible (it depends on the location of the glioblastoma, for example those located in the basal ganglia or in the brain stem are more difficult to remove). Above all, surgery does not allow to completely remove all the cells associated with the tumor because of its invasive nature.

Surgery is usually followed by fractional radiation therapy at 60 Gray in 30 fractions with concurrent and adjuvant chemotherapy (Narsinh et al., 2024). Radiotherapy is used in patients with residual tumors to eliminate the remaining cancer cells, by breaking apart double stranded DNA and causing apoptosis. However, about 50 % of GBMs express Epidermal Growth Factor Receptor gene amplification (EGFR), in particular the truncated variant III, EGFRvIII, which induces radiation resistance and can repair double stranded DNA (Hsu et al., 2021).

The chemotherapy standard treatment recognized by the Food and Drug Administration (FDA) is oral Temozolomide (TMZ). TMZ is a small lipophilic alkylating agent that acts on the DNA of GBM cells. Unfortunately, more than 50 % of patients do not respond to TMZ due to the presence of the numerous DNA repair systems that restore DNA damage.

Specifically, TMZ is an alkylating agent that damages tumor cells by methylating the purine base of DNA leading to the formation of O6-methylguanine that result in autophagy and apoptosis. The DNA repair enzyme O6-Methyl-Guanine DNA MethylTransferase (MGMT) can reverse this alkylation by removing O6-methylguanine adducts. In the same way, the presence of MisMatch Repair (MMR), another DNA repair system, is responsible for maintaining proper base pairing hampers and reduces the activity of TMZ as well as other drugs. DNA damages can be also removed by Poly ADP Ribose Polymerase system (PARP) and the checkpoint system, which uses checkpoint kinases (Chk1 and Chk2) that prevents cells from dividing in an uncontrolled manner (Wu et al., 2021).

Among the treatments currently used for GBM, there is also the use of bevacizumab that is a monoclonal antibody that binds specifically to Vascular Endothelial cell Growth Factor (VEGF). Despite the FDA's accelerated approval of bevacizumab for brain tumors, based on its effectiveness against recurrent glioblastoma, this anti-angiogenic therapy failed to improve the overall survival of the patient, even if it has effectively reduced or stopped the growth of the tumor.

In fact, bevacizumab and antiangiogenic therapy, according to 11 studies included in the paper, did not show an improvement in overall survival. However, pooled analysis of 10 studies (3595 participants) showed improved progression-free survival with the addition of anti-angiogenic therapy.

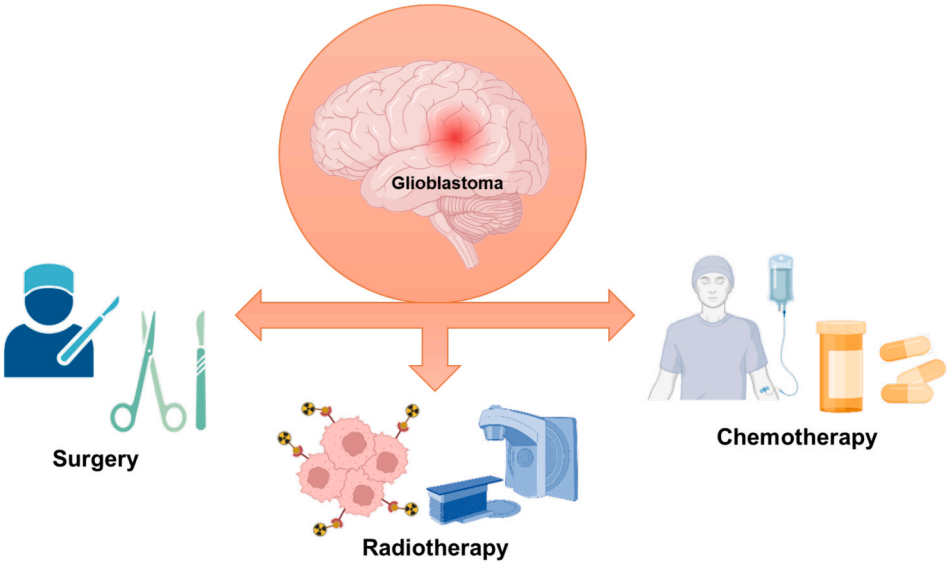

Fig. 2. Current proposed therapies for the treatment of GBM.

While there is strong evidence that bevacizumab prolongs progression-free survival in newly diagnosed and recurrent glioblastoma, the impact of this on quality of life and clinical benefit for patients remains unclear (Ameratunga et al., 2018).

In 1996, the FDA approved the Gliadel® wafer, a biodegradable polyanhydride- based intracranial implant loaded with the alkylating agent Carmustine (BCNU). Each Gliadel wafer contains 7.7 mg of BCNU and it can be intracranially implanted for a maximum dose of 61.6 mg (corresponding to 8 wafers). This implant is indicated for newly-diagnosed high-grade glioma as an adjunct to surgery and radiation and for recurrent glioblastoma as an adjunct to surgery.

Patients with recurrent tumors benefited from an increased survival of 8 weeks when wafers were implanted after the second surgery and in patients where the wafers were implanted after the first surgery the survival was increased by 2\3 months (Tincu (Iurciuc) et al., 2023). The main problems of such implants, however, is the high rate of complications such as risk of seizures, cerebral edema, asthenia, nausea, vomiting, constipation, wound healing abnormalities and depression and the limited therapeutic efficacy (Narsinh et al., 2024), as well as the absence of data on the safety and effectiveness of repeated administration.

Therefore, the main failures of the above reported therapies are due to the presence of numerous DNA repair systems that restore the DNA damages and to the fact that by administering the drug alone and not having specific targets, that drugs can reach the GBM cells only if administered in large quantities, which in most cases result in high likelihoods of toxic side effects.

Moreover, the overexpression by the Glioma Initiating Cells (GICs) of ATP binding cassette (ABC transporter), which are a family of protein pumps that allow passage through the membrane, is a problem for the drugs administration. These transporters prevent drug entry and promote the efflux of compounds out of the cells, representing a further obstacle to the success of chemotherapy against GBM (Jena et al., 2020).

The above reported issues, associated with the difficulty in crossing the BBB and other specific characteristics of GBM such as faster growth, the presence of GICs, high infiltration, high intratumor heterogeneity, the hypoxic condition and the epigenetic signalling alterations, contribute to making GBM very difficult to treat (Fig. 3).

4. Nanoparticles as a promising approach for GBM treatment

GBM is a very difficult tumor to treat using standard technologies, which warrants consideration of innovative treatment options. Researchers have considered new therapies and innovative Drug Delivery Systems (DDS) that can overcome these barriers and selectively target the tumor site to reduce some of the problems associated with ineffective current therapies.

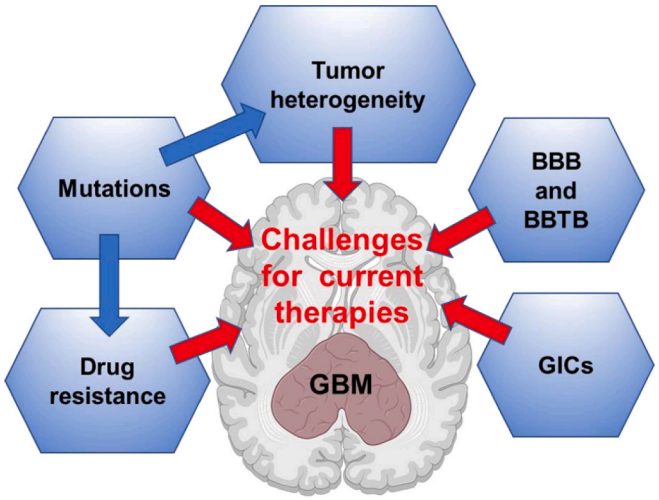

Fig. 3. Main reasons of the failure of GBM current therapies.

This Review focuses on dual-targeted polymeric and lipidic nanosystems, as well as their hybrids, for the treatment of GBM. Other treatment options, such as therapy with inhibitors, immunotherapy, gene therapy, phototherapy, and metallic nanoparticles, are beyond the scope of this review. Readers seeking information on these alternatives are referred to other review articles (Nozhat et al., 2023; Pinel et al., 2019; Shabani et al., 2022).

Nanoparticles (NPs) are small particulate systems usually sized from 10 to 100 nm able to load and release in a controlled way the loaded drugs.

Over the years, nanoparticle features enable them to be extremely versatile systems useful in different fields of application, from pharmaceutical to cosmetic or nutraceutical. As such, NPs are attractive vectors for consideration to treat GBM because of their:

- ability to encapsulate different drugs at higher concentrations, improving their solubility and protecting the cargo from attacks of the immune system and degradation of the surrounding environment (Qamar et al., 2023)
- ability to be functionalized with specific ligands useful for overcoming not only the BBB but also for reaching GBM cells directly. In fact, the NPs can bind specific ligands that allow the system to reach the tumor target more easily and precisely, thus reducing systemic side effects and increasing the antitumor activity to have a more controlled administration of the drug compared to conventional treatments (Ayub and Wettig, 2022)
- small size, allowing their easy pass-through cellular membranes, as well as the surface charge can facilitate the interaction with cells and the cellular uptake, being generally higher for positively charged NPs than for negatively charged NPs.

Generally, the NPs cross the BBB via either passive or active transport mechanisms. The most common passive transport is due to the Enhanced Permeability and Retention (EPR) effect, which consists in the fact that some nanoparticles smaller than 100 nm can spontaneously accumulate in the tumor tissue more than in healthy tissues. This effect exploits specific features of the tumor tissues such as high vascular density, a well-developed vascular network, ineffective or lower lymphatic drainage, as well as the unique properties of the nanoparticles themselves like their size and shape (Lahooti et al., 2023; Zheng et al., 2024).

However, the EPR effect is not always possible or effective. In particular, it is often weaker in brain tumors due to the dense matrix of the brain, which impairs the spread of drugs. In addition, increased interstitial fluid pressure in the tumor due to the ineffective lymphatic drainage leads to the accumulation of larger particles in the tumor and the spread of the smaller ones. As a result, once injected intravenously, most nanoparticles accumulate in other organs and do not reach the brain tumor, upstream of any potential BBB breakthrough (Shlapakova et al., 2021).

Nanoparticles can cross the BBB via two main types of the transcytosis mechanisms (active transport), the Adsorption-Mediated Transcytosis (AMT) and the Receptor-Mediated Transcytosis (RMT). The AMT is a transport activated by electrostatic interactions between cationic molecules and negatively charged membrane surface domains on the BBB. It therefore allows the transport of cationic molecules through the BBB.

The RMT is based on the presence of selective receptors towards specific ligands that allow large molecules to pass through the BBB. As such, NPs functionalized with a proper ligand agent can selectively bind a specific transmembrane receptor (e.g., Glucose Transporter 1, GLUT-1; Transferrin receptor, TfR; Lactoferrin receptor, LfR; Sodium Dependent Multivitamin Transporter, SMVT), thus facilitating cellular internalization (Wiwatchaitawee et al., 2021).

Regardless of the advancement of clinical research in this area, to the

best of our knowledge, no nanoparticles reached the market, though few liposomal systems are currently undergoing clinical testing.

For example, a liposome system called SGT-53, first developed by Senzer and colleagues and still on Phase II of clinical trial, is a cationic liposome loaded with a p53 tumor suppressor plasmid (Senzer et al., 2013). The tumor suppressor gene p53 is involved in the control of DNA damage and is able to induce apoptosis. SGT-53 is also decorated with a TfR ligand. This study showed minimal side effects in patients with advanced solid tumors and an accumulation of the system in the tumors, demonstrating an effective targeting (Wu et al., 2021).

Another system currently on clinical trials was developed first by Gaillard (Gaillard et al., 2014). This system involves a glutathione-pegylated liposomal Doxorubicin (Dox) (2B3-101), which is based on the already marketed pegylated liposomal Dox (Doxil®/Caelyx®) used for treating breast and ovarian cancers. The key feature of this new system is the glutathione coating, which aims to enhance drug delivery across the blood–brain barrier.

Dox is an anthracycline able to inhibit the growth of many cancerous cells, including glioblastoma. Glutathione is an endogenous tripeptide that possesses antioxidant properties and can easily cross the BBB. Because of this promising aspects, 2B3-101 is on a phase I/IIa of clinical study to explore the antitumor activity in brain metastases or recurrent malignant glioma (Miao et al., 2023).

The preceding examples focused on technologies with innovative mechanisms for entry to the brain. In the next section, we focus on the dual-targeting strategy, analysing the different potential systems that can capably surpass the BBB and are designed to directly target and treat GBM.

5. Dual-Targeting Systems (DTSs)

As above-mentioned, the main difficulties of the proposed therapies for GBM are related to the crossing of the BBB and reaching the target site. An ideal system, for a better chance of success should first permeate the BBB and selectively reach the GBM cells by eluding the repair mechanisms and by acting on the altered signal pathways, restoring them and, to date, this represents one of the main challenges for the researchers in the field.

So, hundreds of nanoparticle systems have been formulated with the dual-targeting technique. The structure of nanoparticles indeed is flexible enough to allow surface decoration with specific ligands to improve the tumor targeting (Reddy et al., 2021).

The dual-targeting strategy, schematically represented in Fig. 4, refers to the possibility to use one or more target ligands able to transport the system beyond the BBB and, at the same time, to direct it to the GBM cells and it seems to be a very promising technique for delivering drugs to the GBM.

Starting from this concept, the possible operative strategies for obtaining dual targeting NPs concern the functionalization of the system with the:

- combination of two ligands, one that allows the crossing of the BBB and the other allowing the achievement of the GBM cells.
- use of a single ligand that allows both, based on the expression of common receptors on endothelial and cancer cells (Rodà et al., 2023).

Dual-Targeting Systems present different potential benefits such as the reduction of drug resistance, which is one of the major problem in the treatment of glioblastoma, due to the protective effect of the nano-system towards loaded drugs from the attack by the immune systems and from the efflux pumps; a higher chance of the therapy success by directing the system towards the site of action and, in addition, a significant cost reduction due to the use of only one system to convey drugs through the BBB and directly to the GBM cell. Among the most used systems for dual-targeting strategy application there are lipid- and polymeric-based nanoparticle systems that will be described below, and some significant examples are summarised in Table 1.

5.1. Dual-Targeting Lipid-based NanoParticles (DT-LNPs)

Among all nanocarriers, Lipid-based NanoParticles (LNPs) present

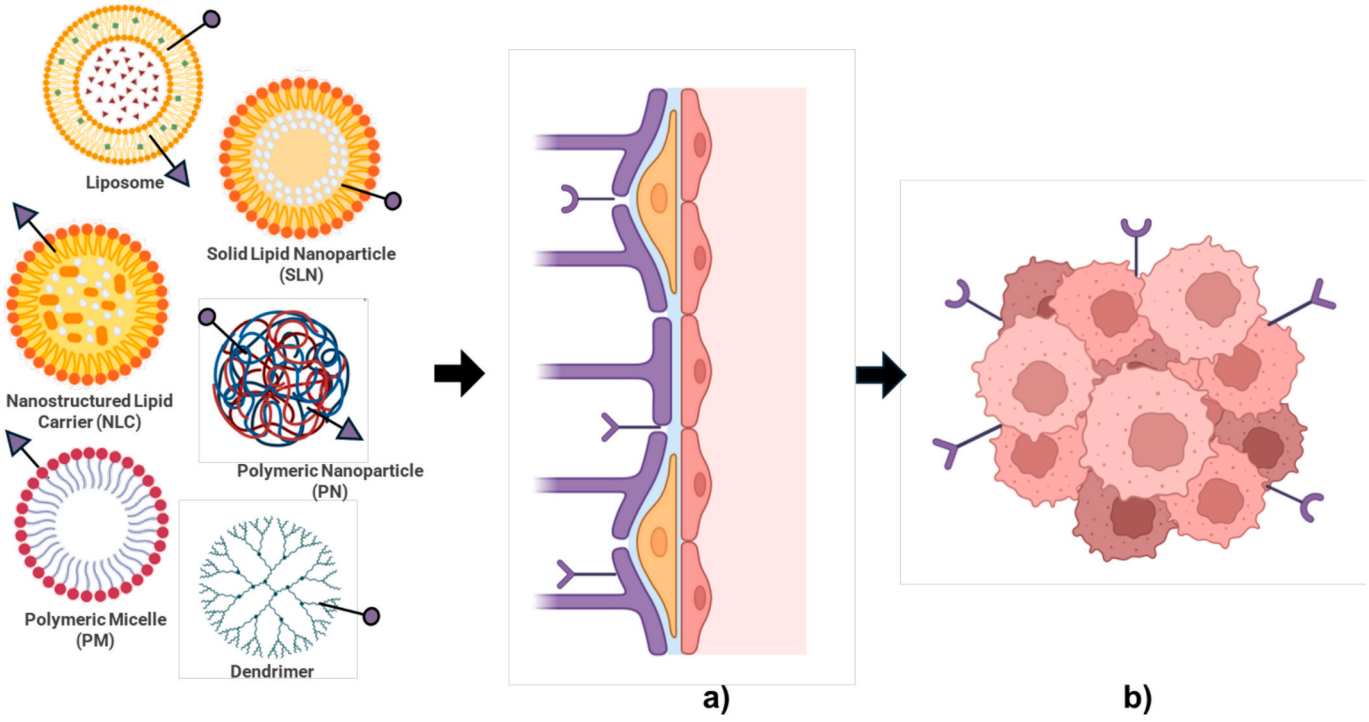

Fig. 4. Schematic representation of generic functionalized Dual-Targeted polymeric or lipidic nanosystems containing one or two different ligand molecules that make it able to: a) cross the BBB via RMT; b) selectively reach the GBM cells by interacting with receptors over-expressed on cancer cell surfaces.

Table 1
Main ligands used to functionalize Dual-Targeting Nanosystems for GBM treatment.

Nanosystems	Main components of the nanosystems	Ligands to cross the BBB	Ligands to reach the GBM cells	References
Liposomes	DSPE-PEG(2000)-NHS	Transferrin (Tf) TAT and QLPVM peptides	Transferrin (Tf)	(Lakkadwala et al., 2020)
	SPC/cholesterol/DSPE-mPEG2000	Rabies Virus Glycoprotein (RVG)	Rabies Virus Glycoprotein (RVG)	(Xin et al., 2021)
	Artesunate- phosphatidylcholine (ARTPC), mPEG2k-DSPE, chloroform, methanol mixture	Apolipoprotein E (Apo E)	Apolipoprotein E (Apo E)	(Ismail et al., 2022)
	Phospholipids (Egg Yolk Phospholipid, EPC), cholesterol or ginsenoside Rg3	Ginsenoside Rg3	Ginsenoside Rg3	(Zhu et al., 2021)
Solid Lipid Nanoparticles (SLNs)	Glyceryl monostearate (GMS), Stearic acid (SA), chloroform and Tween 80	Angiopep-2 (ANG)	ANG	(Kadari et al., 2018)
	Beeswax (BW), SA and Palmitic Acid (PA), ethanol, Tween80	Transferrin (Tf)	Folic Acid (FA)	(Kuo et al., 2022)
Nanostructured Lipid Carriers (NLCs)	Compritol®, Tween 80, Oleic acid, Dichloromethane	Transferrin (Tf)	Transferrin (Tf)	(Emami et al., 2017)
	Soybean phosphatidylcholine (SPC) COMPRITOL® 888 ATO (888 ATO), Cremophor ELP, PEG-DSPE	Lactoferrin (Lf)	Arginine–Glycine–Aspartic acid (RGD)	(Zhang et al., 2018)
Polymeric Nanoparticles (PNs)	Poly Lactic-co-Glycolic Acid (PLGA)	Transferrin (Tf)	Transferrin (Tf)	(Ramalho et al., 2023)
	Poly-ε-Caprolactone (PCL)	Transferrin (Tf)	Transferrin (Tf)	(Heggannavar et al., 2019)
		Wheat Germ Agglutinin (WGA)	Folic Acid (FA)	(Kuo et al., 2019)
	Albumin	Albumin	Albumin	(Shariatnasery et al., 2020)
Polymeric Micelles (PMs)	Dithiolane Trimethylene Carbonate (DTC) copolymers	Angiopep-2 TAT peptide	Angiopep-2	(Zhu et al., 2018)
	PEG-b-poly(PCL-co-dithiolane trimethylene carbonate)-mefenamate (PEG-P(CL-DTC)-MA) copolymer	Apolipoprotein E peptide	Apolipoprotein E peptide	(Wei et al., 2021)
Dendrimers	Polyamide amine (PAMAM)	Glucose, Mannose, Galactose	Glucose, Mannose, Galactose	(Sharma et al., 2021)
		Angiopep- 2	Angiopep- 2	(Sahoo et al., 2023)

several advantages such as reduced immunogenicity, toxicity, and enhanced biodegradability and biocompatibility.

Such systems consist essentially of lipids such as phospholipids that, when properly functionalized, can load and transport drugs to the site of action. Moreover, one of the major advantages of these systems is that often their nature makes them a carrier system capable of loading both hydrophobic and hydrophilic substances, improving their solubility and stability (Zhao et al., 2024).

The most studied lipid-based systems include Liposomes, Solid Lipid Nanoparticles (SLNs) and Nanostructured Lipid Carriers (NLCs); these have been explored so far based on their capacity to incorporate several drugs simultaneously and due to the presence of sufficient space on the surface to allow the functionalization (Jnaidi et al., 2020).

In the following part of this Review, we will analyze the main applications in the Dual-Targeting of these systems that have been proposed in the literature of recent years for treating GBM.

5.1.1. Liposomes

Liposomes are spherical structures composed of a double layer of amphiphilic phospholipids enclosing in an aqueous core (Piwowarczyk et al., 2022). They are the first nanomedicine approved for clinical use and are still considered a useful and versatile approach that can be tailored to specific needs by tuning their size, morphology, composition, and surface modification (Zylberberg and Matosevic, 2016; Allen and Cullis, 2013). They present an amphiphilic structure that makes possible encapsulation of both hydrophilic and hydrophobic drugs, they are versatile and can be functionalized on surface with specific targeting agents and possess great biocompatibility and biodegradability characteristics.

However, they also have some critical aspects such as low drug loading capacity, poor shelf life and the tendency for burst release of the encapsulated drug (De Leo et al., 2022).

The preparation of liposomes involves physical techniques in which lipids and other components (e.g., cholesterol, modified lipids, drugs) are placed in organic solvents in a homogeneous solution at defined temperatures and concentrations. Drugs can usually be inserted both before and after liposome formations.

Several studies were aimed to upgrade and amplify the transport of anti-tumor drug by liposomes. During the past few years, various liposomal preparations have been explored for treating gliomas and for facilitating their diffusion across the BBB.

Lakkadwala *et al.* developed two dual functionalized liposomes to treat GBM, containing cell-penetrating peptides (CPPs) (TAT or QLPVM peptides) and Transferrin (Tf) as ligand agents for co-delivery of Doxorubicin (Dox) and Erlotinib (Erlo) (Lakkadwala et al., 2020). This approach was aimed considering that a combined therapy, by using drugs that act by different mechanisms, in the treatment of complex tumors, such as GBM, can significantly contributes to reduce resistance phenomena. In fact, Dox is an anthracycline that can disrupt DNA and RNA by interpolating base pairs of DNA strands and inhibiting topoisomerase II, while Erlo is an antibody directed against Epidermal Growth Factor Receptor (EGFR) whose activation increases cell proliferation, migration and invasiveness, and decreases apoptosis.

The liposomes were prepared using thin film hydration method of a mixture between phospholipids and cholesterol while dual functionalized liposomes were formulated via post-insertion method of an active ligand into the preformed liposomes.

The use of the serum glycoprotein Transferrin to functionalize nanosystems, improved their transport across the BBB and increased drug uptake by GBM cells. However, this approach had limited effectiveness due to receptor saturation. As a result, other ligands such as CPPs were tested to improve the delivery of the nanocarriers to the target site.

Moreover, the systems are functionalized with Polyethylene Glycol (PEG) to improve the stability of liposome due to its ability to act as a steric stabilizer that reduces the protein absorption as well as interactions with macrophages, preventing its removal from the circulation and increasing its time in the brain.

In particular, PEG is an approved polymer for human use that can improve the aqueous solubility of the system, increase the blood circulation time of liposomes by providing steric stability, thereby enhancing their accumulation in tumors. However, PEG can reduce the transport

rate of liposome-loaded biomolecules.

To address this issue, it is possible to combine the PEGylated liposomes with specific ligands for ligand-mediated targeting, which can help retain the drug at the site of action (Shi et al., 2022; Zalba et al., 2022; Pavón et al., 2024).

Lakkadwala *et al.* found that liposomes functionalized by two CPPs (TAT and QLPVM), Transferrin and PEG, after i.v. administration, showed higher brain distribution with no significant difference between the use of TAT and QLPVM as a penetrating agent and a 10- to 2.7-fold increase in Dox and Erlo accumulation. Thus, dual-targeted liposomes can effectively improve the delivery of drugs across the BBB into tumor cells via receptor and adsorptive mediated transcytosis pathways (Lakkadwala et al., 2020).

Reported in 2021, Xin *et al.* developed a liposome system loaded with Paclitaxel (PTX), a natural taxan with antitumor activity and low water solubility, poor BBB penetration ability and several side effects (Xin et al., 2021). The systems were prepared using thin film hydration method and functionalized with Rabies Virus Glycoprotein (RVG), a derivative peptide able to specifically bind the nicotinic Acetylcholine Receptor (nAChR) overexpressed on BBB and glioma cells. The presence

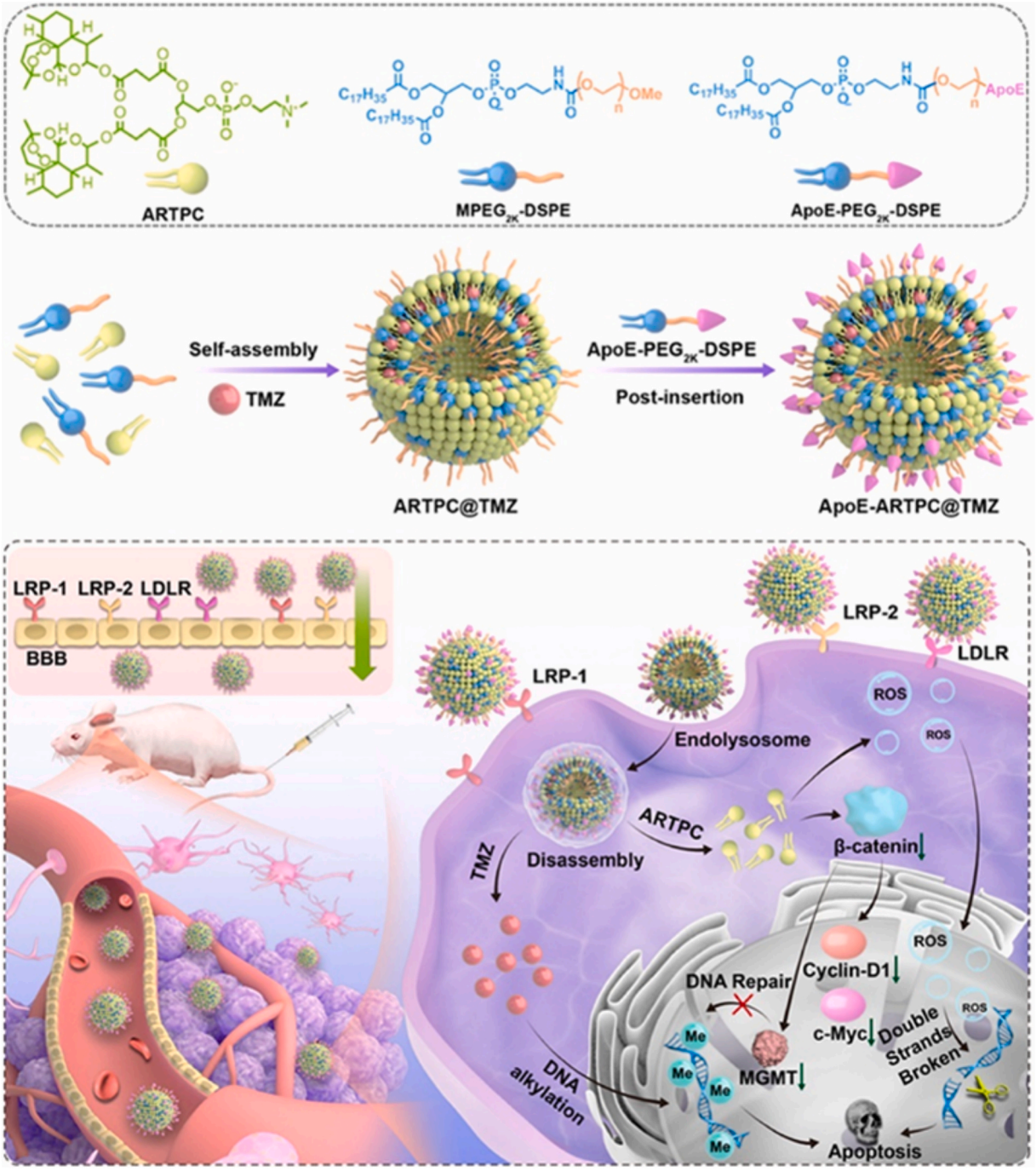

Fig. 5. Schematic illustration displaying the preparation of ApoE-peptide decorated combination liposomes for GBM targeting and therapeutics. The chemical structures of starting materials, the subsequent self-assembly with encapsulation of TMZ and further functionalization with ApoE-PEG2k-DSPE are shown. The targeted combination liposomes (ApoE-ARTPC@TMZ) efficiently traverse the BBB through LDLRs-mediated transcytosis and target the glioma cells, where the liposomes undergo disassembly releasing TMZ, intensifying the DNA damage and apoptosis by the synergistic effect of both ART and TMZ. Adapted with kind permission from Elsevier (Ismail et al., 2022).

of RVG increased drug permeability through BBB and drug selectivity and penetration for GBM cells.

Moreover, using *in vitro* and *in vivo* studies, authors observed that, compared with free PTX, functionalized liposomes exhibit excellent efficiency and safety of use in Human Brain Microvascular Endothelial Cells (HBMEC) and C6 cells and improved transport efficiency (by *in vitro* BBB model studies). In addition, based on *in vivo* imaging tests, the prepared system favored the accumulation of PTX in the brain, by promoting the penetration of chemotherapy drug in C6 glioma cells. *In vivo* studies showed that liposome significantly inhibit glioma growth, improving the survival rate of tumor-carrying mice.

Ismail *et al.* developed an Apolipoprotein E (ApoE)-functionalized liposomal nanoplatform based on Artesunate Phosphatidylcholine (ARTPC) and loaded with TZM (ApoE-ARTPC@TMZ) to avoid the GBM drug resistance (Ismail et al., 2022). The systems were produced by the thin-film hydration method. As reported in the previous paragraphs of this review, one of the main problems of GBM resistance to TMZ is due to the presence of DNA repair systems such as the Methylguanine Methyltransferase (MGMT). In particular, it has been demonstrated that Wnt/β-catenin signaling pathway could enhance the MGMT expression and induce pharmaceutical resistance in glioma cells. For this reason, the system by Ismail *et al.* was loaded with TMZ and Artesunate (ART). This latter design strategy possesses strong antitumor activity due to its ability to inhibit the Wnt/β-catenin pathway and, additionally, it is able to induce cytotoxic activity in cancer cells by creating several DNA damages (e.g., inducer of Reactive Oxygen Species (ROS)) that inhibit cellular proliferation. Then, with the aim of directing the system and allowing it to cross the BBB and reach the GBM cells, it has been functionalized with Apolipoprotein E (Apo E). Apo E is a ligand capable of binding to Low Density Lipoprotein Receptors (LDLR) present in large quantities on the BBB and on the GBM cells promoting the effective brain tumor accumulation of both ART and TMZ *via* LDLRs-mediated transcytosis. This study showed that such constituted and functionalized systems manage to cross the BBB and reach the GBM cells with an anti-glioma activity improving the chemosensitivity of TMZ *in vivo,* evidenced by a significant reduction in tumor mass in mice in an intracranial U251-TR mouse model; consequently, this nanosystem is also able to reduce the dose of TMZ, decreasing the risk of TMZ-associated toxicity (Fig. 5).

Ginsenoside Rg3-based liposomal systems (Rg3-LPs) also offer a dual-targeting approach for glioblastoma treatment by using GLUT-1 transporters, which are overexpressed in both the BBB and GBM cells. By replacing cholesterol, Rg3 enhances membrane stability and facilitates BBB penetration, improving drug delivery to the brain. Co-loaded with paclitaxel (PTX), Rg3-LPs increase tumor accumulation, reprogram tumor-associated macrophages (TAMs) from M2 to M1, and enhance immune response, prolonging survival in GBM models. Given its anticancer and immunomodulatory properties, Rg3 in this case is both a structural component and a bioactive molecule, making Rg3-LPs a promising strategy for targeted GBM therapy (Zhu et al., 2021).

5.1.2. Solid Lipid Nanoparticles (SLNs)

These nanoparticles, having diameters ranging from 50 nm to 100 nm, are usually composed of 3 main components such as solid lipids, surfactants, and water. The SLNs consist of biodegradable and biocompatible lipids that are safe and biocompatible, they can be functionalized and loaded with both hydrophilic and lipophilic drugs (by dispersing them in the lipid matrix or in the outer shell), allowing a controlled release. They possess a long-term stability and are able to protect what they contain from the clearance of the Reticulo Endothelial System (RES).

SLNs also present a low efficiency of encapsulation because of the crystallization process that leaves the internal structure of the lipid nucleus without sufficient space for the load of the therapeutic substance, and they can cause allergic reactions and immune responses within the human body (Qamar et al., 2023; Mehrdadi, 2023).

The choice of the type of lipids and surfactants used and, therefore, also the final composition of the SNLs systems influences their release profile, drug encapsulation, stability over time, surface charge, polydispersity, size and physical–chemical characteristics.

As an example of a Dual-Targeting SLNs addressed to GBM treatment, in 2018 Kadari *et al.* developed a docetaxel-loaded SLNs functionalized with Angiopep-2 (ANG), a 19 amino acids peptide which has great affinity for the LRP1 (LDLR Related Protein 1) receptor, expressed both on the endothelial cells of the BBB and at the level of the GBM cells (Kadari et al., 2018). Docetaxel is a taxan with cytotoxic effect on the GBM as it interrupts the microtubular network of the cells. The SLNs systems were prepared by the emulsion-solvent evaporation technique. Thus, the study was aimed at obtaining an Agiopep-2-functionalized nanoparticles (A-SLN) to be used for binding to the LRP1 receptor and, therefore, to be able both to overcome the BBB and to selectively reach the tumor site with the dual-targeting strategy. The obtained A-SLN showed increased cytotoxicity, improved cellular internalization and improved apoptosis against human U87MG glioblastoma and mouse glioma GL261 cells much more than unconjugated nanoparticles. Moreover, pharmacokinetic and tissue distribution studies showed selective targeting of A-SLN with an increased accumulation in the brain compared to other free docetaxel formulations. After treatment with A-SLN, the average survival time of the mice was significantly increased to 39 days compared to 24 days with normal docetaxel administration. Therefore, such ANG-conjugated nanosystems seem to be a very promising system for the treatment of GBM, at least in mice models.

Another example of promising dual-targeting SLN was developed in 2022 by Kuo *et al.* (Kuo et al., 2022). The author prepared a Transferrin (Tf) and Folic Acid (FA) double-functionalized SLNs able to cross the BBB and promote targeting toward Inhibitors of Apoptosis Proteins (IAP) in GBM cells. Tf is a serum glycoprotein that has been widely used to functionalize nanosystems to improve their transport across BBB and promote increased drug uptake by GBM cells through specific receptors overexpressed in cellular membranes. FA is known to have a high affinity for folate receptor which is usually overexpressed on the surface of human cancer cells such as lung, colon and glioblastoma multiforme.

The systems were loaded with BV6 and GDC0152 directly against the inhibitors of apoptosis proteins (IAPs). These two potent drugs can inhibit the expression of IAPs, X-linked Inhibitor of Apoptosis Protein (XIAP), and cellular Inhibitor of Apoptosis Protein 1/2 (cIAP-1/2), which are each involved in an important pathway for chemotherapy resistance of the drugs directly to GBM.

Results from SLN conjugated with Tf and FA on the surface demonstrated their ability to cross the BBB. In addition, BV6-GDC0152-Tf-FA-SLNs showed the ability to act at the level of U87MG cells and HBCSCs of GBM by providing the down regulation of XIAP and cIAP-1/2.

5.1.3. Nanostructured Lipid Carriers (NLCs)

NLCs are configured as the new generation of SLNs to address and ideally overcome the limitations presented with earlier prototype designs. NLCs are irregularly shaped, lipid-based nanovectors composed of liquid and solid lipids in different parts to incorporate a greater dosage of both hydrophilic and lipophilic drugs.

The possibility of lipid fusion with various physical–chemical properties improves the stability of drug-loaded NLCs and the effectiveness of encapsulation (Qamar et al., 2023).

Optimized Paclitaxel-loaded NLCs functionalized with Transferrin (Tf) was developed by Emami et al 2017. The NLCs were prepared through the emulsification-solvent evaporation method and were conjugated with Tf that, as already reported, is widely used to functionalize nanosystems to improve their transport across BBB and promote increased drug uptake by GBM cells. *In vitro* cytotoxicity assays indicated the higher cytotoxic effect against U-87 brain cancer cell line of functionalized NLCs when compared to the unmodified NLCs and free paclitaxel, suggesting its potential for GBM therapy (Emami et al., 2017).

Zhang *et al.*, designed a nanostructured lipid carrier to carry TZM and vincristine for GBM combination therapy. The NLC system was functionalized with Lactoferrin (Lf) and arginine–glycine–aspartic acid (RGD) and was prepared by solvent-diffusion method (Zhang et al., 2018).

Lf is a glycoprotein that has affinity for iron, and it can reversibly chelate iron and transport it. Being part of the family of transferrins, Lf can cross the BBB by transcytosis; in addition, the Lf receptors have also been found on GBM cells. So, the functionalization of the system with this ligand allows to cross not only the BBB but also to reach the GBM cells. Finally, Lf is also able to inhibit the multiplication of malignant cells U87MG of GBM via the downregulation of cyclin D1 and D4.

RGD is a peptide that strongly bind αvβ3 and αvβ5 receptors, which are typically overexpressed on endothelial cells of angiogenic tumor vessels and then on GBM cells (e.g., U87MG cell line). These systems are thus composed by sustained-release behavior, high cellular uptake, high cytotoxicity and synergistic effects, increased drug accumulation in the tumor tissue, and obvious tumor inhibition efficiency with minimal reported systemic toxicity.

5.2. Dual-Targeting Polymeric-based NanoParticles (DT-PNPs)

Polymer-based nanosystems can be obtained using natural or synthetic polymers, usually with biodegradable characteristics such as Poly Lactic-co-Glycolic Acid (PLGA), Poly (ε- Caprolactone) (PCL) or Albumin.

Additionally, polymer-based nanocarriers can be modified on their surface with the addition of specific ligands useful for conveying the drug-loaded system at the tumor level (Di Filippo et al., 2021). The main advantages of polymeric NPs include high temperature and pH stability, sustained and controllable release of encapsulated drugs, easy surface modification, non-immunogenicity, and ability to avoid the immune system clearance.

5.2.1. Polymeric Nanoparticles (PNs)

Polymeric nanoparticles are colloidal systems, characterized by small particle sizes, usually ranging from 10 nm to 500 nm. Commonly referred to as nanospheres or nanocapsules, in which the drug is homogeneously dispersed in the polymer matrix or encapsulated in the inner core of a system to form a core–shell structure, polymeric nanoparticles have been the focus of an extensive biomedical research field for several decades.

These systems are often proposed for the treatment of Glioblastoma Multiforme as they can load drugs increasing their stability, solubility and bioavailability, and can be designed to allow a controlled release of drugs by reducing their systemic toxicity, be easily functionalized with specific ligands, directing the drug to the target site.

In addition, polymeric systems offer advantages in terms of size and safety. Indeed, their small size can promote bioaccumulation in the tumor through the Enhanced Permeability and Retention (EPR) effect and their constitution, usually made of biodegradable polymers, can facilitate the release of loaded drugs and, at the same time, the *in vivo* degradation of the system to non-toxic metabolites promote high safety profiles.

However, the main disadvantage of polymer-based DDS is the difficulty of crossing BBB by passive diffusion due to: i) their low propensity to breach the BBB and; ii) the brain matrix that is quite dense and reduces the spreading and amount of medication available at the site of action.

Ramalho *et al.* developed PLGA-based nanoparticles loaded with Bortezomib (BTZ) and TMZ. The systems were prepared with the emulsion-solvent evaporation method (Ramalho et al., 2023).

Results showed that BTZ can increase TMZ's therapeutic efficacy in GBM patients by downregulating MGMT expression. The systems were further conjugated with Tf to ensure the passage through the blood – brain barrier (BBB) and to direct them to the GBM cells.

The NPs exhibited suitable features (sizes lower than 200 nm, low polydispersity, and negative surface charge) and a controlled and sustained release for 20 days. *In vitro* studies have been conducted on human GBM cells to assess the antiproliferative potential and thus the absorption of systems and it has been shown that PLGA-based systems are rapidly absorbed by GBM cells, promote synergistic effects of the loading drugs leading to the inhibition of survival and proliferation of cancer cells.

In addition, the biocompatibility of discharged NPs has been evaluated in healthy brain cells, demonstrating the safety of the nanovectors. These results show that co-delivery of BTZ and TMZ in Tf-derivatized PLGA nanoparticles is a promising approach to treat GBM, overcoming the limitations of current therapeutic strategies, such as drug resistance and increased side effects.

Heggannavar *et al.* developed a Paclitaxel-loaded PCL nanoparticles, functionalized with different concentration of Tf on the surface (Heggannavar et al., 2019). By binding to its overexpressed receptors, Tf allows to easily reach both BBB and cancer cells. The PCL is synthesized from ε-caprolactone via a ring-opening polymerization technique and the nanocarriers were prepared by following the emulsion-solvent evaporation method. *In vitro* release studies have shown that paclitaxel in these nanosystems presents prolonged release up to 72 h, suggesting that developed nanoparticles have the potential to improve long-term anticancer efficiency. MTT assay performed against U87 cells revealed that the Tf- conjugated nanoparticles exhibited extremely low cytotoxicity with over 90 % cell viability. The nanosystems tested have different concentrations of Tf on the surface, the greater the amount, the greater the cellular absorption of nanoparticles (up to 160 μg) thus emphasizing the importance of the functionalized system with specific target. This was further confirmed by the *in vitro* BBB cell absorption study.

Another example of dual-targeting PCL-based nanoparticles was developed by Kuo *et al.* In their study, a Methoxy Poly (Ethylene Glycol) (MPEG)-PCL nanoparticles functionalized with Wheat Germ Agglutinin (WGA) and Folic Acid (FA) were prepared in order to transport anticancer drugs across the BBB and direct it to the GBM cells (Kuo et al., 2019).

In particular, PCL was copolymerized with MPEG, and MPEG-PCL NPs were produced using a microemulsion-solvent evaporation technique and stabilized with Pluronic F127. The systems were loaded with Etoposide (ETO), Carmustine (BCNU) and Dox and functionalized with WGA, that is a lectin agglutinin protein able to bind to *N*-acetylglucosamine receptors overexpressed in human brain endothelial cells and with FA, able to bind folate receptor overexpressed in malignant U87MG cells.

The authors' design and ligand surface decoration were intended to create a system possible to cross the BBB and reach the GBM cell.

The results showed that the length of the PCL chain played a role in the encapsulation and delivery profile of the drug. The shorter the chain, the more the systems had a smaller size with less entrapment, but they showed a faster drug release rate.

The permeability of the BBB of the three loaded drugs appeared in the order of WFNPs > WNPs > MPEG-PCL NPs > free drug. Also, the dual-targeting ability of drug-charged WFNPs increased penetration through the BBB and inhibited the survival of U87MG cells. In this context, WFPNs can be proposed as an effective colloidal vector in the transfer of anticancer drugs to the brain and targeting cancer cells for the treatment of GBM.

Albumin is a natural biopolymer widely used to produce NPs because it is non-toxic, non-immunogenic and biodegradable. In addition, albumin NPs can interact with Secreted Protein Acidic and Rich in Cysteine (SPARC) and GlycoProtein (gp60), two proteins that are overexpressed on glioma cells (Madani et al., 2022).

For this reason, albumin Cationic Bovine Serum Albumin (CBSA) is used as a ligand that can promote the transport of drug-loaded systems through BBB. CBSA shows a better accumulation profile and a high

degree of selectivity for brain tissue than others (e.g., the heart). CBSA seems to easily cross the BBB through an absorption mediated transcytosis (Agarwal et al., 2011). Moreover, it was observed that albumin NPs are able to breach the BBB and be uptaken by glioma cells. In this context, Shariatnasery *et al.* investigated the effectiveness of Paclitaxel-loaded albumin NPs on the U251 GBM cell line (Shariatnasery et al., 2020). The system was also loaded with MicroRNAs miR-34a (a small endogenous single strand non-coding RNA molecules), a tumor-suppressor miR with different functions: inhibiting cell proliferation, inducing apoptosis, cell cycle arresting, reducing cell migration and reversing the chemoresistance to some drugs by targeting important oncogenes in different cancer types. This study showed that the viability of U251 cells decreased significantly with the use of this system compared with the use of free drug (paclitaxel).

5.2.2. *Polymeric Micelles (PMs)*

Polymeric micelles derived by the spontaneous association in water of amphiphilic copolymers and consist of a hydrophobic nucleus and hydrophilic shell. PMs have a very small size, usually ranging from 10 to 50 nm, typically smaller than all other polymer nanosystems and for this reason micelles can easily cross the BBB by passive diffusion for an effective delivery of the loaded drug.

Moreover, micelles can also be functionalized with target ligands specific for some overexpressed BBB receptors or at the GBM cell level.

The main advantages of micelles are that they: i) improve the water solubility of drugs, ii) modulate the kinetic release, iii) protect the loaded drug from degradation and, iv) decrease their toxicity.

However, major limitations of these systems are that they can mainly encapsulate hydrophobic drugs and without any surface modification they easily underwent opsonization in the bloodstream and trapped in the spleen or liver, thus becoming ineffective for the treatment of GBM and dangerous for the possibility of causing side effects in other organs (Di Filippo et al., 2021).

Zhu *et al.* in 2018 developed tandem nanomicelles based on co-polymers of Dithiolane Trimethylene Carbonate (DTC) by dialysis method co- functionalized with two brain tumor-targeting agents, the ANG, and the TAT peptides (a CPP), to obtain an efficient system for anti-glioma chemotherapy (Fig. 6, left). ANG peptide is able to target LRP1 receptor overexpressed not only on the BBB but also on the GBM cells, for these reasons it was used to functionalize these micelles together with TAT peptide that has been shown to improve both the penetration of BBB and cancer cells.

To avoid non-specific penetration during blood circulation, TAT peptide was associated with short PEG2000, protected by long PEG6000. Once the micelles reach the target site, the binding of ANG with LRP-1 will bring the TAT close to the cell membrane, facilitating its penetration into the cells.

Studies have shown a long blood circulation time of micelles, increased BBB permeation, improved accumulation and absorption of glioma cells. Studies using U87MG orthotopic glioma-bearing human mice also show that Docetaxel (DTX) tandem-loaded nanomicelles cause superior tumor inhibition compared with ANG single peptide functionalized (Zhu et al., 2018).

In 2021 Wei *et al.* developed small micelles loaded with Sorafenib (SF), a kinases inhibitor capable of restoring altered GBM signaling pathways. The micelles were obtained by the dialysis method, the dispersions were then dialyzed against PBS for 5 h (Wei et al., 2021).

To improve SF therapy of GBM by enhancing BBB penetration, GBM accumulation, and cell uptake, the micelles were functionalized with ApoE peptide. The overexpression of receptors such as LDLRs, (including LRP-1, LRP-2, and LDLR), the transferrin receptor, and integrins on BBB and on GBM cells, prompted the research team to functionalize the micelles with apolipoprotein E peptide, which is the tandem-repeat dimer peptide of the apolipoprotein E protein able to bind to LDLRs with high affinity.

In vitro and *in vivo* studies have shown that such micelles have greatly improved BBB permeability and uptake by U-87MG cells and have shown greater accumulation in GBM cells than free SF and non-functionalized micelles. Treatment at a safe dosage of 15 mg SF/kg significantly delayed tumor progression and improved tumor suppression by inducing apoptosis of cancer cells and inhibiting tumor angiogenesis. These micelles present themselves as a potential solution to

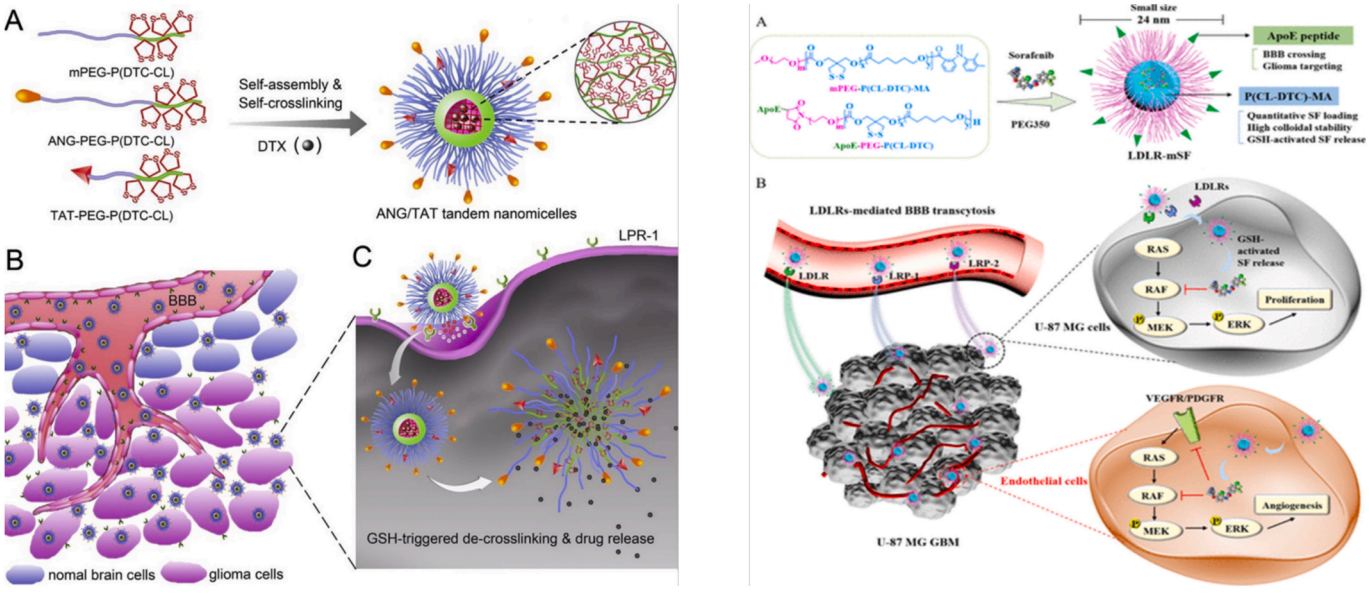

Fig. 6. Schematic illustration of: LEFT) tandem nanomicelles co-functionalized with brain tumor-targeting and cell- penetrating peptides, Angiopep-2 and TAT, for highly effective and specific anti-glioma chemotherapy. (A) Formation of ANG/TAT-Ms micelles through self-assembly of three PEG-P(DTC-CL) co-polymers with different PEG lengths. ANG peptide is fully exposed at the outer surface while TAT peptide is hidden by longer PEG shells, (B) Tandem nanomicelles while maintaining a high glioma cell selectivity show significantly improved BBB and U87MG glioma cell penetration, (C) DTX is quickly released into the cytoplasm as a result of GSH-triggered de-crosslinking of ANG/TAT-Ms; RIGHT) (A) Small, smart, and LDLR-specific micelles loading SF (LDLR-mSF), (B) Its enhanced BBB penetration and tumor targeting resulting in better inhibition of U-87 MG tumor cells and tumor angiogenesis. Adapted with permission: Fig. 6 left with kind permission from Elsevier (Zhu et al., 2018); Fig. 6 right with kind permission from ACS publications (Wei et al., 2021).

improve targeted GBM therapy (Wei et al., 2021) (Fig. 6, right).

5.2.3. Dendrimers

Dendrimers are highly branched synthetic polymer macromolecules with sizes ranging from a few nanometers to 100 nm. They provide a large area available for functionalization with specific target ligands and high loading capacity (Akhter et al., 2021). There are many different types of dendrimers, including polyamidoamine (PAMAM), polypropylene (PPI), phosphorus (PHH) dendrimers, etc.

PAMAM dendrimers have shown positive results in effective drug delivery to the brain by crossing the BBB. In fact, surface changes with specific ligands and the size of dendrimers can ensure optimized and targeted drug delivery to the tumor site (Sahoo et al., 2023).

Dendrimers, compared to traditional linear polymers, allow better functionalization as they guarantee an attachment of a greater number of functional groups. In addition, recent studies showed that increasing the number of generations of dendrimers would increase the circulating residence time and the accumulation in the brain (Miao et al., 2023).

Sharma *et al.* in 2021 developed three hydroxyl PAMAM dendrimers modified with glucose, mannose, or galactose sugar moieties as promising ligands to target upregulated sugar transporters on the BBB and on the GBM cells (Sharma et al., 2021).

The BBB presents an overexpression of glucose receptors (GLUT) that mediates the transport of substances with glucose-like structures. If the system binds to these receptors, it has been shown to have a much-improved BBB crossing capacity. Crossing and toxicity studies have been carried out on BBB, by using murine microglia BV2. Regarding cytotoxicity, the vitality of microglia cells *in vitro* after 24 h of exposure was assessed and the systems showed no toxicity other than D-GAL systems which gave mild toxicity at high doses. In general, it has been observed that so functionalized systems significantly increase the internalization of these dendrimers through the BBB.

Macrophages associated with cancer (TAMs) are an important target because they regulate the immune response of the tumor. Tumors secrete signals that recruit macrophages and then turn them into TAMs, which suppress immune activation that kills cancer and promotes tumor growth and invasion but also drug resistance. Therefore, by targeting these TAMs it is possible to reprogram the immune response of the tumor.

TAMs express high levels of surface receptors that can interact with sugars such as glucose, mannose, and galactose.

Sharma *et al.* demonstrate that glucose modification enhanced targeting TAMs and microglia by increasing brain penetration and cellular internalization, while galactose modification shifts targeting away from TAMs towards galectins on glioblastoma tumor cells. Mannose modification did not alter TAMs and microglia targeting of these dendrimers, but they can alter the kinetics of accumulation in GBM. So, studies on these systems have indeed shown better achievement and accumulation of the system at the target site level as well as a much greater crossing of the BBB.

A few years later, Sahoo *et al.* developed a PEGylated and non-PEGylated PAMAM dendrimers conjugated to ANG for the delivery of encapsulated TMZ (Sahoo et al., 2023).

The cytotoxicity of pegylated conjugates (Den-PEG2-ANG) and not (Den-ANG) has been evaluated on U87MG cells, demonstrating that Den-PEG2-ANG loaded TMZ has significant anticancer activity with minimal IC50 values. From *in vivo* studies, it has been seen that the pharmacokinetic properties of TMZ have been improved by the pegylated nanocarrier in terms of half-life, bioavailability, and circulation time, as well as the delivery potential of the Den-brainPEG2-ANG that was significantly higher than Den-ANG; in fact, PEGylation also improved drug permeation and contributed to biocompatibility. So, functionalization with ANG, which possesses affinity for the LRP-receptor1 present both on the endothelial cells of the BBB and at the level of the GBM cells, has brought good results both *in vitro* and *in vivo* leading to increased brain penetration of TMZ thus also increasing its therapeutic efficacy proving a good therapeutic target in GBM therapy.

6. Hybrid Systems (HSs)

In addition to Dual-Targeting, another possible strategy under study for the treatment of GBM is the formulation of Hybrid Systems (HSs), in which nanoparticles of different nature are combined in a single system with the aim of synergistically optimizing the advantages of both vectors and making them as functional as possible to achieve the set objectives. In particular, this review focuses on HSs composed of a single delivery system that combines polymeric and lipidic nanoparticles.

Fig. 7 depicts a schematic representation of a generic double-functionalized polymeric/lipidic Hybrid NanoSystem and its mechanism of interaction with both endothelial cells of BBB and with GBM cell receptors. HSs usually are constituted by two main layers, a polymeric nucleus coated with a lipid layer, and both can add to their surface different ligands that aim at specific targeting. In particular, the central material (polymeric core) may be encapsulated in single and/or multiple lipid layers and it can be functionalized on its surface with different targeting moieties and ligands. When more than one lipid layer is present, the additional layers are considered as the third layer of the HS.

Thus, in a HS we can identify the main following layers:

1. The innermost first layer consists of several polymers, organic and inorganic materials that can be coated with other agents and\or can form a structure that can be functionalized with different target ligands to direct the system towards a specific site. Hydrophilic or hydrophobic drugs may be encapsulated in this inner layer
2. The second layer consists of natural or derived lipid material which confers the desired pharmacokinetic properties to the DDS. This layer encapsulates the polymer core, regulates the release of the encapsulated drug and improves biocompatibility
3. The third layer, if present, is composed by other lipid layers that can load other polymeric systems and should be useful to increase the functionalization of the system surface and to increase the loading capacity of the system

However, hybrid lipid-polymeric (HSs) systems are not limited to this specific structure as other hybrid configurations are possible, incorporating both polymeric and lipid components. The specific design of these systems depends on the intended application and the desired properties.

Considering their structure, it is clear that HSs have strength in combining the advantages of both the polymeric and lipid systems, while reducing the disadvantages they present as isolated systems. In particular, these systems combine the advantages of the lipid vesicles as a better drug carrying capacity, amphiphilic characteristics, minimal loss of drug at time of production or storage, excellent biocompatibility and low immunogenicity with the benefits of polymeric systems such as biocompatibility and biodegradability, high structural integrity and storage stability and good candidates for the passive and active targeting drug delivery (Gajbhiye et al., 2023).

Compared to polymeric or lipid nanosystems, the HSs offer greater protection from the attack of the immune system, leading to an increased stability in the blood circulation, allow higher loading capacity of either hydrophilic and lipophilic drugs which could improve the antitumor effect thanks to the synergistic effect of different drugs, a better control of drug release, and a high potential of surface functionalization, which reduce the need of high drug doses and toxicity due to active targeting to cells (Liu et al., 2021). However, the structural complexity of the HS leads to some critical aspects like the difficulty of scaling-up and higher costs of manufacturing, compared to the single polymeric or lipid systems (Fig. 8) (Date et al., 2018).

In 2021 Yang *et al.* developed a Hybrid NanoSystem for the treatment of GBM to deliver the Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR/Cas9), a gene-editing technique that has been

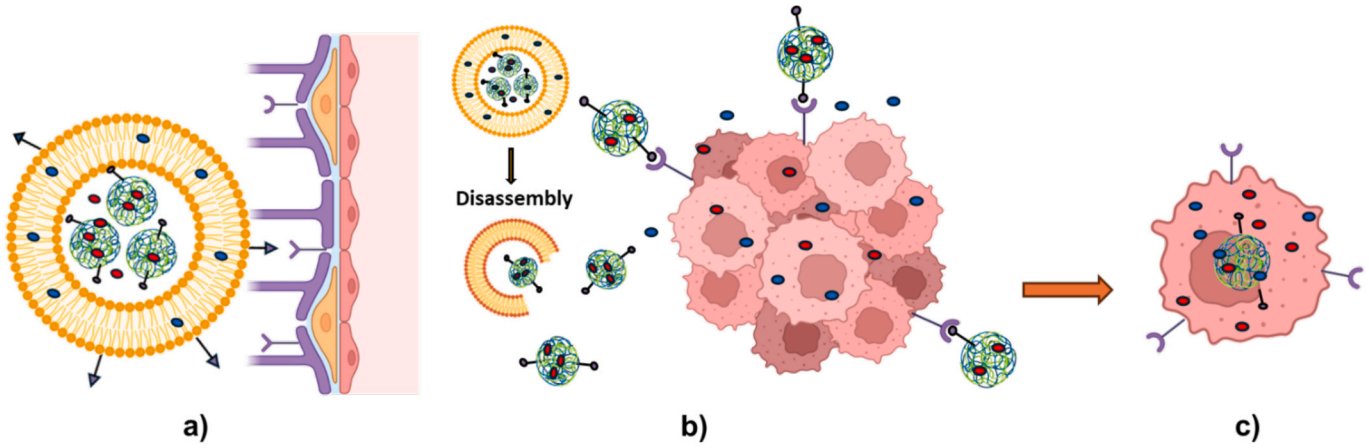

Fig. 7. Schematic representation of a double-functionalized Hybrid NanoSystem with the polymeric nanoparticles core encapsulated on a single lipid layer able to: a) cross the BBB via RMT; b) Liposome disassemble realizing drug-loaded nanoparticles which selectively reach the GBM cells by interacting with overexpressed surface cell receptors; c) be internalized by cancer cells and exert its therapeutic effect.

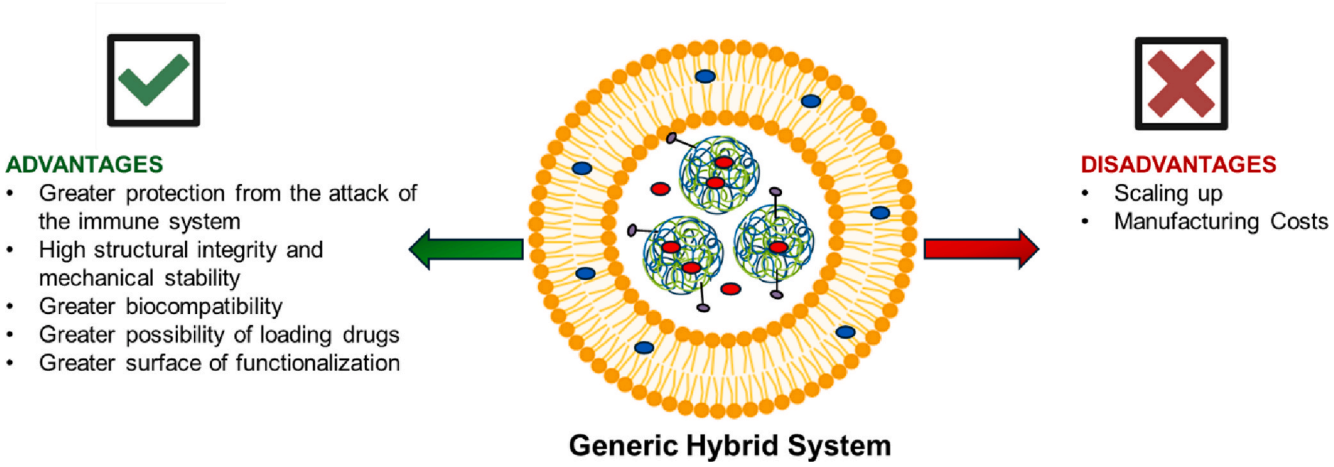

Fig. 8. Advantages and Disadvantages of the Hybrid Systems (HSs).

revolutionized in many tumors (Yang et al., 2021). In particular, a lipid-polymer hybrid nanoparticles (LPHNs-cRGD) were used to deliver CRISPR/Cas9 plasmids to TZM that target O6-MethylGuanine-DNA MethylTransferase (MGMT), a drug resistance gene. Focused ultrasound (FUS) microbubbles (MBs) were utilised to open the BBB and the systems were functionalized with RGD, a peptide that strongly bind $\alpha v\beta 3$ and $\alpha v\beta 5$ receptors, which are usually overexpressed on GBM cells. LPHNs-cRGD were prepared via nanoprecipitation method using PLGA, lecithin, DSPE-PEG-cRGD, and DSPE-PEG-biotin. *In vitro* and *in vivo* studies have shown that LPHNs-cRGD are able to convey CRISPR/Cas9 plasmids directly to GBM cells and downregulate MGMT expression by increasing GBM cell sensitivity to TMZ. This treatment has been shown to be effective and to inhibit tumor growth and prolong survival in mice. A biotoxicity study has also been conducted and data reported that the hybrid system is biocompatible and safe in mice models and able to reach the GBM supported by its functionalization.

Ahmed T. *et al* in 2021 developed a polymeric-lipid hybrid nanoparticles (TPLNs) to enhance the delivery of doxorubicin (DOX) to GBM. The polymeric part of the TPLNs consists of poly(methacrylic acid) (PMAA)-PS80-grafted-starch, where Pluronic F-68 (PF68) is also used as a stabilizer and to facilitates LDL receptor-mediated transcytosis across the blood–brain barrier (BBB).The lipidic part is composed of ethyl arachidate (EA), a long-chain fatty acid ester selected for its optimal hydrophobic interactions with the polymer, leading to high drug loading and encapsulation efficiency. The lipid component forms a solid matrix core, enabling controlled drug release and improved penetration into tumor spheroids and brain tissues. TPLNs exploit LDL receptor-mediated transcytosis via PF68 but also thanks to Apolipoprotein E for efficient BBB penetration. *In vitro* studies showed a higher efficacy than free DOX and deep tumor penetration. *In vivo*, TPLNs accumulated effectively in GBM tumors within a mouse model. This study highlights TPLNs as a promising strategy for targeted GBM treatment (Ahmed et al., 2021).

Polymeric-Lipid Hybrid NanoParticles (PLHNPs) as an advanced Drug Delivery System for GBM treatment were developed by Bhattacharya in 2021. The structure consists of a PLGA polymeric core loaded with methotrexate (MTX), surrounded by a lipid shell of glyceryl tripalmitate and phosphatidylcholine, ensuring controlled drug release and enhanced biocompatibility (Fig. 9, A). The formulation includes Tween 80 to improve brain penetration and polyvinyl alcohol (PVA) for stability. Effectiveness studies show high entrapment efficiency (70.56–86.34 %), increased MTX brain concentration, sustained drug release (49.37 % over 72 h), and strong anticancer activity against U-87MG glioma cells (Bhattacharya, 2021).

Pucci *et al.* in 2022 developed a piezoelectric Hybrid lipid-polymeric Nanoparticles, loaded with Nutlin-3a, a non-genotoxic drug antagonist of the Murine Double Minute-2 (MDM2) protein, which is a negative regulator of the tumor suppressor protein p53 (Pucci et al., 2022). Piezoelectric nanosystems, in particular, are a stimuli-responsive systems capable of converting external mechanical stimuli into bioactive signals. These systems belong to an innovative class of nanotransducers

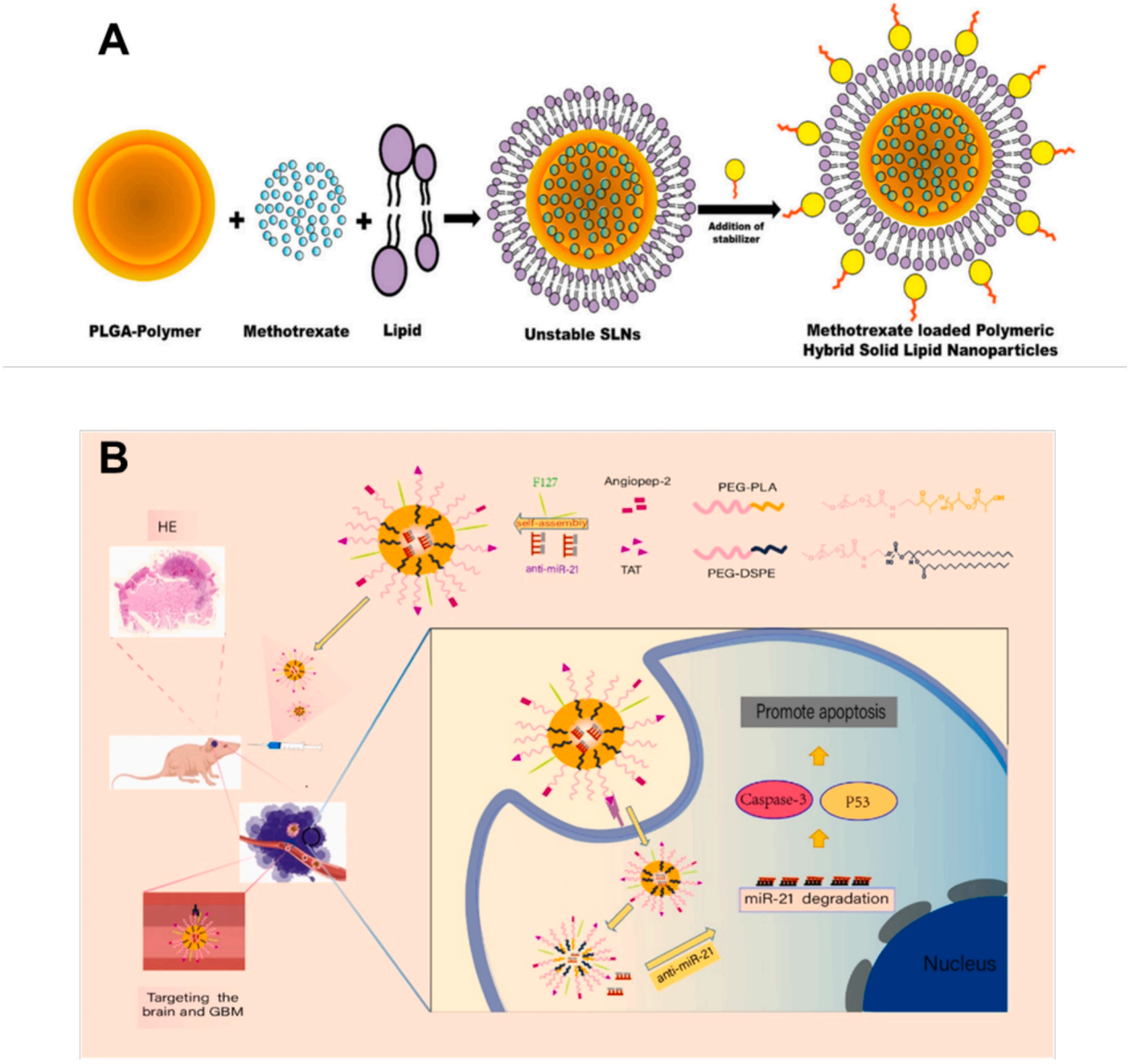

Fig. 9. Example of Hybrid Systems: A) Schematic presentation of methotrexate-loaded poly(lactic-co-glycolic acid) lipid hybrid nanoparticles preparation and drug loading (Bhattacharya, 2021); B) Schematic illustration of the preparation and therapeutic mechanism of BTMLPMS. Adapted with kind permission from Elsevier (Zhang et al., 2024).

that respond to external cues, such as ultrasound, and transform them into therapeutic effects. In this study, piezoelectric lipid-polymer hybrid nanoparticles were developed, consisting of a Polyvinylidene fluoride-trifluoroethylene (P(VDF-TrFE)) polymeric core and a biocompatible lipid shell. Functionalized with ApoE that binds the LDLR, which is overexpressed on the BBB and on GBM cells, these nanoparticles were loaded with Nutlin-3a and remotely activated by ultrasound. This activation induced controlled drug release and localized anticancer electric stimulation, inhibiting glioblastoma cell proliferation and reducing drug resistance. These hybrid nanoparticles integrate the advantages of polymeric nanoparticles, such as structural integrity, superior stability, and controlled drug release, with those of liposomes, including enhanced biocompatibility and biomimetic properties. Additionally, they incorporate a stimuli-responsive mechanism, allowing a precise drug release and localized therapeutic effects.

The study demonstrated that Nut-PNPs were able to reduce cell migration, actin polymerization, and invasion ability of T98G cells, while fostering apoptotic and necrotic events. Through a BBB model obtained by co-culture of human endothelial cells (hCMEC/D3) and astrocytes derived from the human brain, it has been shown that functionalization with ApoE allowed the system to easily cross the BBB.

Zhang *et al.* in 2024 developed an orally administered, Brain-Targeted anti-miRNA-21 Lipid Polymer Micelle System (BTMLPMS) based on PLA-PEG2000 and DSPE-PEG2000, employing the thin film hydration technique (Zhang et al., 2024) (Fig. 9, B). The HS systems were functionalized to obtain a dual targeting system, with ANG and TAT peptides. ANG is a specific peptide able to bind the LRP1 overexpressed on both BBB and glioma cells. This interaction facilitates receptor-mediated cellular uptake, ensuring targeted delivery. At the same time, TAT, a cell-penetrating peptide, is used to improve micelle internalization. The nanosystems were loaded with miRNA-21 (non-coding RNAs) antisense oligonucleotides (anti-miR-21). mir-21 has been shown to play a key role in the progression of glioma, using a mir-21 inhibitor is possible to reduce GBM progression and induce apoptosis. A model of orthotopic glioma xenograft model using nude mice showed that oral administration of these micelles activated pro-apoptotic proteins p53 and Caspase-3, effectively inducing apoptosis in tumor tissues reducing tumor progression. *In vitro* studies confirmed that these

micelles were efficiently uptaken by glioma cells, resulting in induced apoptosis.

Through the analysis of physical and chemical properties, safety in mice, and the study of the absorption mechanism, it has been shown that the micelle had a good shape, stable properties and good safety and which were able to enter the cells via endocytosis mediated by the clathrin and caveolin protein mediated transport.

The cited examples of HSs are summarized in Table 2.

7. Other multifunctional nanosystems for GBM treatment

In recent years extensive research has been conducted on multifunctional nanoparticles for the treatment of glioblastoma multiforme (GBM). This section reports additional examples of multifunctional systems developed for GBM, further confirming the need for a complex, multilevel strategy to find more effective treatments.

The investigations reported here have shown that nanoparticles can enhance drug delivery, increase the effectiveness of therapy, and overcome the challenges associated with the treatment of glioblastoma.

Rizvi *et al.* in 2021 developed novel dual-imaging and dual-targeting self-assembled cyclic peptide nanoparticles with improved diagnostic and therapeutic (theranostic) capabilities, as well as enhanced specificity and efficiency for GBM.

First, they prepared a dual-targeting peptide sequence. This sequence consisted of the cyclic arginine-glycine-aspartic acid ligand (cRGD), which targets the αvβ3-integrin receptor present on the surface of many tumors, including breast and lung carcinomas, melanomas, osteosarcomas, and glioblastoma. The αvβ3-integrin serves as a receptor for extracellular matrix proteins with an exposed RGD sequence, making it a molecular target of interest for early cancer diagnosis and selective attachment and internalization of RGD-containing peptides and peptidomimetics for cancer therapy.

Additionally, the KLAK ligand was used as a pro-apoptotic motif for targeting the mitochondria, resulting in the induction of cancer cell apoptosis through the activation of the caspase-3 enzyme.

Then, this dual-targeting peptide probe was further modified with DTPA. The cyclic peptide–DTPA complex was self-assembled into peptide nanoparticles via co-assembly with NIRF-dye Cy5.5 to form uniform, spherical-shaped nanoparticles (Cy5.5@SAPD). These nanoparticles were then radiolabeled with ^{99m}Tc (Cy5.5@SAPD-^{99m}Tc), creating a novel dual-imaging probe for molecular imaging studies.

The results demonstrated that the Cy5.5@SAPD nanoparticles specifically and efficiently internalized into U87MG glioma tumor cells, compared to HEK-293 kidney tumor cells, suggesting their potential as agents for the early diagnosis of glioblastoma multiforme. Furthermore, the Cy5.5@SAPD-^{99m}Tc nanoparticles, after SPECT/NIRF diagnostic studies in tumor-bearing mouse models, showed promising characteristics for the diagnosis of brain tumors and can be proposed as theranostic agents (Rizvi et al., 2021).

Beola L. *et al.* in 2023 developed a multifunctional lipid nanovector functionalized with the peptide angiopep-2 which is able to promote glioma targeting by interacting with the low-density lipoprotein receptor protein 1, LRP1, expressed in both brain capillaries endothelial cells and glioma cells. Additionally, the nanovector was loaded with Temozolomide (TMZ) (Ang-TMZ_LMNVs) and superparamagnetic iron oxide nanoparticles (SPIONs) to confer magnetic properties suitable for hyperthermia. These multifunctional nanovectors exhibited a synergistic intratumoral chemo-hyperthermia effect, leading to an antiglioma effect in a human GBM mouse model. The results showed good tumor retention of the nanovectors without presence in other organs or in outer region of the tumor, thereby overcoming the common issue of drug leakage often associated with this approach. The synergistic effect of chemotherapy and hyperthermia within a single nanosystem appears to be a safe and effective strategy with durable benefits after a single administration (Fig. 10, A) (Beola et al., 2023).

Zhang Y. *et al.* in 2021 designed a biocompatible cRGD/Pt + DOX@GFNPs (RPDGs) nanoformulation to disrupt redox homeostasis in GBM cells and promote the simultaneous occurrence of efficient apoptosis and ferroptosis (Fig. 10, B). First, they prepared gallic acid (GA)/Fe2 + nanocomplexes (GFNPs) as a substrate for sustained Fenton reaction to induce GBM cell ferroptosis. In addition, the GA/Fe2 + nanoparticles (GFNPs) demonstrated an excellent photothermal ability by a significant increase of the Fe2 + released from nanoparticles after near-infrared (NIR) light irradiation (808 nm) leading to a local

Table 2
Examples of HSs reported in the literature for the treatment of Glioblastoma Multiforme (GBM).

Hybrid systems and their main components	Targeting receptors	Targeting ligands	Loaded molecules (Drug\gene)	Key findings	References
Core: PLGA Shell: Ethyl arachidate	LDL receptors	Apolipoprotein E (ApoE)	Doxorubicin (DOX)	• higher efficacy than free DOX, • enhanced BBB penetration, • tumor accumulation.	(Ahmed et al., 2021)
Core: PLGA Shell: Glyceryl tripalmitate, Phosphatidylcholine	–	Tween 80 (for BBB penetration)	Methotrexate (MTX)	• Enhanced brain penetration, • sustained release (49.37 % over 72 h), • increased anticancer efficacy.	(Bhattacharya, 2021)
Core: PLGA, DC-Chol (3beta-[N-(N',N'-dimethylaminoethane)-carbamoyl] cholesterol) Shell: DPPC (1,2-Dipalmitoyl-*sn*-glycero-3-phosphatidylcholine), DSPE-PEG2000	Integrin αvβ3 receptors	cRGD peptide	CRISPR/Cas9 plasmids targeting MGMT	• Increased GBM sensitivity to TMZ, • effective tumor growth inhibition, • prolonged survival in mice.	(Yang et al., 2021)
Core: P(VDF-TrFE) (Poly(vinylidene fluoride-trifluoroethylene)) Shell: DSPE-PEG	LDL receptors	Apolipoprotein E (ApoE)	Nutlin-3a	• Reduced GBM cell migration and invasion, • apoptosis induction, • successful BBB crossing.	(Pucci et al., 2022)
Core: PLA-PEG2000 (Poly(lactic acid)-polyethylene glycol) Shell: DSPE-PEG2000	LRP1 receptors	Angiopep-2 (Ang-2), TAT peptide	Anti-miR-21 oligonucleotides	• Induced apoptosis in glioma cells, • reduced tumor progression, • activated p53 and Caspase-3.	(Zhang et al., 2024)

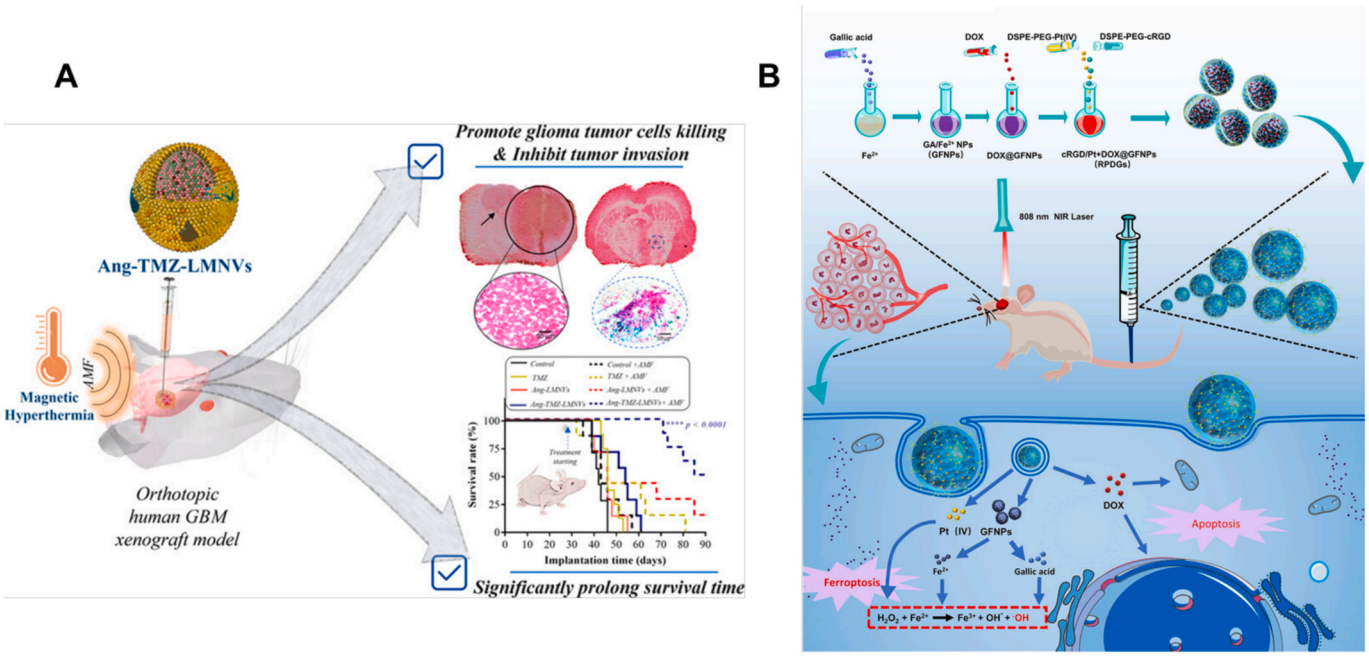

Fig. 10. Example of multifunctional drug-loaded lipid-based nanovectors: A) multifunctional lipid-based magnetic nanovectors functionalized with the peptide angiopep-2 and loaded with TMZ (Ang-TMZ-LMNVs). *In vivo* studies have demonstrated synergistic effects of magnetic hyperthermia after alternating magnetic fields (AMFs) stimulation combined with the chemotherapeutic agent. This allows the Ang-TMZ-LMNVs to accumulate and remain within the tumor following local administration, suppressing tumor invasion and proliferation while prolonging the median survival time (Beola et al., 2023); B) the design and synthesis of DOX-loaded liposome-based nanoformulations functionalized with cyclic arginine-glycine-aspartic acid (cRGD) ligand (RPDGs) and the underlying mechanism that induces GBM cell ferroptosis and apoptosis. Adapted with kind permission from Elsevier (Zhang et al., 2021).

temperature increase causing tumor cell death. Doxorubicin (DOX) was then loaded onto GFNPs via electrostatic interaction and the nanocomplexes were incorporated into a liposome system to improve their tumor accumulation. In particular, the liposomes were prepared by mixing of DSPE-PEG (2000)-Pt (IV), where Pt (IV) acted as a prodrug that can be reduced to cytotoxic Pt (II) specifically within tumor cells and DSPE-PEG-cRGD, to facilitate receptor-mediated endocytosis via the cyclic arginine-glycine-aspartic acid (cRGD) ligand. The results from *in vivo* studies on mice suggested that the nanoformulations RPDGs could be a promising multifunctional system that combines apoptosis, ferroptosis and photothermal therapy for treating GBM (Zhang et al., 2021).

8. Conclusions and future perspectives

The complex molecular heterogeneity, aggressive infiltrative growth, presence of the BBB, the difficulty of reaching the target site and the problem of the resistance to drugs that characterize the GBM lead to the failure of current therapies. Given these challenges, nanoparticles have emerged as a promising strategy for drug delivery, offering the potential to improve treatment effectiveness. Their success largely depends on the design of the formulation and the ability to precisely target therapeutic agents at the tumor site. In the case of GBM, this requires overcoming BBB and selective accumulation within cancer cells to maximize the impact of anti-cancer drugs.

In this context, the Dual-Targeting seems to be a promising strategy because, through the functionalization of a nanoparticle system with one or more ligands, it is possible to create a system (containing one or more drugs) able to bind the overexpressed receptors on BBB and/or on GBM cells, thus ensuring targeted and accurate drug delivery in the site of action by reducing the off-target effects.

Another useful approach seems to be the use of Hybrid Systems, composed of nanoparticles able to combine in one single system both the advantages of the different components that constitute them and, at the same time, reduce the disadvantages of the isolated systems. HSs are also potentially useful to obtain greater protection of the system from the attack by the immune system, greater biocompatibility, increasing drug loading and a higher surface of functionalization.

Although the use of complex systems, such as hybrid ones, and highly functionalized systems, such as Dual-Targeting carriers, seems to be very promising for GBM treatment, it is still necessary a continue study of developing of new, perhaps more complex, and combined therapies.

The treatment of Glioblastoma Multiforme continues to evolve, with promising advancements in hybrid and Dual-Targeting systems. Recent studies highlight their potential, yet ongoing research is essential to refine and expand therapeutic strategies. Given the multifaceted nature of GBM, characterized by numerous dysregulated signaling pathways, integrating innovative and synergistic approaches can help address tumor heterogeneity, enhance efficacy, minimize cytotoxicity, and ultimately improve patient outcomes by extending survival and preserving quality of life.

To overcome the challenges of GBM treatment, a deeper investigation into the molecular mechanisms underlying resistance and therapeutic failure is crucial. This will enable the development of more effective multitargeted systems, such as those discussed in this review.

We endeavor that this research movement can give valid viable options of care to patients affected by this lethal disease.

CRediT authorship contribution statement

Paola Riccobelli: Writing – original draft, Investigation. **Benjamin Franklin Pierce:** Supervision, Writing – review & editing. **Emanuela Fabiola Craparo:** Writing – review & editing. **Sara Anna Bonini:** Writing – review & editing. **Gianfranco Pasut:** Writing – review & editing. **Alessandro Fanzani:** Writing – review & editing. **Delia Mandracchia:** Supervision, Writing – review & editing, Conceptualization, Formal analysis.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Gianfranco Pasut reports a relationship with NOF Corporation Kawasaki Operation Center that includes: board membership and funding grants. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

All the data used to write this review article were found on peer reviews scientific papers reported in references section of the manuscript. Each reference includes the DOI code.

References

Agarwal, A., Agrawal, H., Tiwari, S., Jain, S., Agrawal, G.P., 2011. Cationic ligand appended nanoconstructs: a prospective strategy for brain targeting. Int. J. Pharm. 421 (1), 189–201. https://doi.org/10.1016/j.ijpharm.2011.09.039.

Ahmed, T., Liu, F.-C.-F., He, C., Abbasi, A.Z., Cai, P., Rauth, A.M., Henderson, J.T., Wu, X.Y., 2021. Optimizing the design of blood–brain barrier-penetrating polymer-lipid-hybrid nanoparticles for delivering anticancer drugs to glioblastoma. Pharm. Res. 38 (11), 1897–1914. https://doi.org/10.1007/s11095-021-03122-9.

Akhter, M.H., Rizwanullah, Md., Ahmad, J., Amin, S., Ahmad, M.Z., Minhaj, M.A., Mujtaba, M.A., Ali, J., 2021. Molecular targets and nanoparticulate systems designed for the improved therapeutic intervention in glioblastoma multiforme. Drug Res. 71 (03), 122–137. https://doi.org/10.1055/a-1296-7870.

Allen, T.M., Cullis, P.R., 2013. Liposomal drug delivery systems: from concept to clinical applications. Adv. Drug Deliv. Rev. 65 (1), 36–48. https://doi.org/10.1016/j.addr.2012.09.037.

Ameratunga, M., Pavlakis, N., Wheeler, H., Grant, R., Simes, J., Khasraw, M., 2018. Anti-angiogenic therapy for high-grade glioma. Cochrane Database Syst. Rev. 2018 (11). https://doi.org/10.1002/14651858.CD008218.pub4.

Arvanitis, C.D., Ferraro, G.B., Jain, R.K., 2020. The blood–brain barrier and blood–tumour barrier in brain tumours and metastases. Nat. Rev. Cancer 20 (1), 26–41. https://doi.org/10.1038/s41568-019-0205-x.

Ayub, A., Wettig, S., 2022. An overview of nanotechnologies for drug delivery to the brain. Pharmaceutics 14 (2), 224. https://doi.org/10.3390/pharmaceutics14020224.

Beola, L., Iturrioz-Rodríguez, N., Pucci, C., Bertorelli, R., Ciofani, G., 2023. Drug-loaded lipid magnetic nanoparticles for combined local hyperthermia and chemotherapy against glioblastoma multiforme. ACS Nano 17 (18), 18441–18455. https://doi.org/10.1021/acsnano.3c06085.

Bhattacharya, S., 2021. Methotrexate-loaded polymeric lipid hybrid nanoparticles (PLHNPs): a reliable drug delivery system for the treatment of glioblastoma. J. Exp. Nanosci. 16 (1), 344–367. https://doi.org/10.1080/17458080.2021.1983172.

Date, T., Nimbalkar, V., Kamat, J., Mittal, A., Mahato, R.I., Chitkara, D., 2018. Lipid-polymer hybrid nanocarriers for delivering cancer therapeutics. J. Control. Release 271, 60–73. https://doi.org/10.1016/j.jconrel.2017.12.016.

De Leo, V., Maurelli, A.M., Giotta, L., Catucci, L., 2022. Liposomes containing nanoparticles: preparation and applications. Colloids Surf. B Biointerfaces 218, 112737. https://doi.org/10.1016/j.colsurfb.2022.112737.

Di Filippo, L.D., Duarte, J.L., Luiz, M.T., de Araújo, J.T.C., Chorilli, M., 2021. Drug delivery nanosystems in glioblastoma multiforme treatment: current state of the art. Curr. Neuropharmacol. 19 (6), 787–812. https://doi.org/10.2174/1570159X18666200831160627.

Emami, J., Rezazadeh, M., Sadeghi, H., Khadivar, K., 2017. Development and optimization of transferrin-conjugated nanostructured lipid carriers for brain delivery of paclitaxel using Box–Behnken design. Pharm. Dev. Technol. 22 (3), 370–382. https://doi.org/10.1080/10837450.2016.1189933.

Gaillard, P.J., Appeldoorn, C.C.M., Dorland, R., van Kregten, J., Manca, F., Vugts, D.J., Windhorst, B., van Dongen, G.A.M.S., de Vries, H.E., Maussang, D., van Tellingen, O., 2014. Pharmacokinetics, brain delivery, and efficacy in brain tumor-bearing mice of glutathione pegylated liposomal doxorubicin (2B3-101). PLoS One 9 (1), e82331. https://doi.org/10.1371/journal.pone.0082331.

Gajbhiye, K.R., Salve, R., Narwade, M., Sheikh, A., Kesharwani, P., Gajbhiye, V., 2023. Lipid polymer hybrid nanoparticles: a custom-tailored next-generation approach for cancer therapeutics. Mol. Cancer 22 (1), 160. https://doi.org/10.1186/s12943-023-01849-0.

Grochans, S., Cybulska, A.M., Simińska, D., Korbecki, J., Kojder, K., Chlubek, D., Baranowska-Bosiacka, I., 2022. Epidemiology of glioblastoma multiforme–literature review. Cancers 14 (10), 2412. https://doi.org/10.3390/cancers14102412.

Heggannavar, G.B., Vijeth, S., Kariduraganavar, M.Y., 2019. Preparation of transferrin-conjugated poly-ε-caprolactone nanoparticles and delivery of paclitaxel to treat glioblastoma across blood–brain barrier. Emerg. Mater. 2 (4), 463–474. https://doi.org/10.1007/s42247-019-00033-9.

Hsu, J.-F., Chu, S.-M., Liao, C.-C., Wang, C.-J., Wang, Y.-S., Lai, M.-Y., Wang, H.-C., Huang, H.-R., Tsai, M.-H., 2021. Nanotechnology and nanocarrier-based drug delivery as the potential therapeutic strategy for glioblastoma multiforme: an update. Cancers 13 (2), 195. https://doi.org/10.3390/cancers13020195.

Ismail, M., Yang, W., Li, Y., Chai, T., Zhang, D., Du, Q., Muhammad, P., Hanif, S., Zheng, M., Shi, B., 2022. Targeted liposomes for combined delivery of artesunate and temozolomide to resistant glioblastoma. Biomaterials 287, 121608. https://doi.org/10.1016/j.biomaterials.2022.121608.

Iturrioz-Rodríguez, N., Bertorelli, R., Ciofani, G., 2021. Lipid-based nanocarriers for the treatment of glioblastoma. Adv. NanoBiomed Res. 1 (2). https://doi.org/10.1002/anbr.202000054.

Jena, L., McErlean, E., McCarthy, H., 2020. Delivery across the blood-brain barrier: nanomedicine for glioblastoma multiforme. Drug Deliv. Transl. Res. 10 (2), 304–318. https://doi.org/10.1007/s13346-019-00679-2.

Jnaidi, R., Almeida, A.J., Gonçalves, L.M., 2020. Solid lipid nanoparticles and nanostructured lipid carriers as smart drug delivery systems in the treatment of glioblastoma multiforme. Pharmaceutics 12 (9), 860. https://doi.org/10.3390/pharmaceutics12090860.

Kadari, A., Pooja, D., Gora, R.H., Gudem, S., Kolapalli, V.R.M., Kulhari, H., Sistla, R., 2018. Design of multifunctional peptide collaborated and docetaxel loaded lipid nanoparticles for antiglioma therapy. Eur. J. Pharm. Biopharm. 132, 168–179. https://doi.org/10.1016/j.ejpb.2018.09.012.

Kuo, Y.-C., Chang, Y.-H., Rajesh, R., 2019. Targeted delivery of etoposide, carmustine and doxorubicin to human glioblastoma cells using methoxy poly(ethylene glycol)–poly(ε–caprolactone) nanoparticles conjugated with wheat germ agglutinin and folic acid. Mater. Sci. Eng. C 96, 114–128. https://doi.org/10.1016/j.msec.2018.10.094.

Kuo, Y.-C., Yang, I.-S., Rajesh, R., 2022. Suppressed XIAP and cIAP expressions in human brain cancer stem cells using BV6- and GDC0152-encapsulated nanoparticles. J. Taiwan Inst. Chem. Eng. 135, 104394. https://doi.org/10.1016/j.jtice.2022.104394.

Lahooti, B., Akwii, R.G., Zahra, F.T., Sajib, M.S., Lamprou, M., Alobaida, A., Lionakis, M. S., Mattheolabakis, G., Mikelis, C.M., 2023. Targeting endothelial permeability in the EPR effect. J. Control. Release 361, 212–235. https://doi.org/10.1016/j.jconrel.2023.07.039.

Lakkadwala, S., dos Santos Rodrigues, B., Sun, C., Singh, J., 2020. Biodistribution of TAT or QLPVM coupled to receptor targeted liposomes for delivery of anticancer therapeutics to brain in vitro and in vivo. Nanomed. Nanotechnol. Biol. Med. 23, 102112. https://doi.org/10.1016/j.nano.2019.102112.

Liu, Z., Chu, W., Sun, Q., Zhao, L., Tan, X., Zhang, Y., Yin, T., He, H., Gou, J., Tang, X., 2021. Micelle-contained and PEGylated hybrid liposomes of combined gemcitabine and cisplatin delivery for enhancing antitumor activity. Int. J. Pharm. 602, 120619. https://doi.org/10.1016/j.ijpharm.2021.120619.

Louis, DN., Ohgaki, H., Wiestler, OD., Cavenee, WK., Burger, PC., Jouvet, A., Scheithauer, BW., Kleihues, P., 2007. The 2007 WHO classification of tumours of the central nervous system. Acta Neuropathol. 114, 97–109. https://doi.org/10.1007/s00401-007-0243-4.

Madani, F., Esnaashari, S.S., Webster, T.J., Khosravani, M., Adabi, M., 2022. Polymeric nanoparticles for drug delivery in glioblastoma: state of the art and future perspectives. J. Control. Release 349, 649–661. https://doi.org/10.1016/j.jconrel.2022.07.023.

Marcucci, F., Corti, A., Ferreri, A.J.M., 2021. Breaching the blood–brain tumor barrier for tumor therapy. Cancers 13 (10), 2391. https://doi.org/10.3390/cancers13102391.

Mehrdadi, S., 2023. Drug delivery of Solid lipid nanoparticles (SLNs) and nanostructured lipid carriers (NLCs) to target brain tumors. Adv. Pharm. Bull. 13 (3), 512–520. https://doi.org/10.34172/apb.2023.062.

Miao, Y.-B., Zhao, W., Renchi, G., Gong, Y., Shi, Y., 2023. Customizing delivery nano-vehicles for precise brain tumor therapy. J. Nanobiotechnol. 21 (1), 32. https://doi.org/10.1186/s12951-023-01775-9.

Mittal, S., Ali, J., Baboota, S., 2021. Overcoming the challenges in the treatment of glioblastoma via nanocarrier- based drug delivery approach. Curr. Pharm. Des. 27 (45), 4539–4556. https://doi.org/10.2174/1381612827666210603152056.

Narsinh, K.H., Perez, E., Haddad, A.F., Young, J.S., Savastano, L., Villanueva-Meyer, J.E., Winkler, E., de Groot, J., 2024. Strategies to improve drug delivery across the blood–brain barrier for glioblastoma. Curr. Neurol. Neurosci. Rep. 24 (5), 123–139. https://doi.org/10.1007/s11910-024-01338-x.

Nozhat, Z., Heydarzadeh, S., Shahriari-Khalaji, M., Wang, S., Iqbal, M.Z., Kong, X., 2023. Advanced biomaterials for human glioblastoma multiforme (GBM) drug delivery. Biomater. Sci. 11 (12), 4094–4131. https://doi.org/10.1039/D2BM01996E.

Pavón, C., Benetti, E.M., Lorandi, F., 2024. Polymer brushes on nanoparticles for controlling the interaction with protein-rich physiological media. Langmuir 40 (23), 11843–11857. https://doi.org/10.1021/acs.langmuir.4c00956.

Pinel, S., Thomas, N., Boura, C., Barberi-Heyob, M., 2019. Approaches to physical stimulation of metallic nanoparticles for glioblastoma treatment. Adv. Drug Deliv. Rev. 138, 344–357. https://doi.org/10.1016/j.addr.2018.10.013.

Piwowarczyk, L., Mlynarczyk, D.T., Krajka-Kuźniak, V., Majchrzak-Celińska, A., Budzianowska, A., Tomczak, S., Budzianowski, J., Woźniak-Braszak, A., Pietrzyk, R., Baranowski, M., Goslinski, T., Jelinska, A., 2022. Natural compounds in liposomal nanoformulations of potential clinical application in glioblastoma. Cancers 14 (24), 6222. https://doi.org/10.3390/cancers14246222.

Pucci, C., Marino, A., Şen, Ö., De Pasquale, D., Bartolucci, M., Iturrioz-Rodríguez, N., di Leo, N., de Vito, G., Debellis, D., Petretto, A., Ciofani, G., 2022. Ultrasound-responsive nutlin-loaded nanoparticles for combined chemotherapy and

piezoelectric treatment of glioblastoma cells. Acta Biomater. 139, 218–236. https://doi.org/10.1016/j.actbio.2021.04.005.
Qamar, Z., Sartaj, A., Iqubal, M.K., Qizilbash, F.F., Sabir, S., Ali, J., Ali, A., Baboota, S., 2023. Combination drug loaded lipid-based nanocarriers as treatment entity for battling glioblastoma multiforme. J. Drug Delivery Sci. Technol. 87, 104800. https://doi.org/10.1016/j.jddst.2023.104800.
Ramalho, M.J., Torres, I.D., Loureiro, J.A., Lima, J., Pereira, M.C., 2023. Transferrin-conjugated PLGA nanoparticles for co-delivery of temozolomide and bortezomib to glioblastoma cells. ACS Appl. Nano Mater. 6 (15), 14191–14203. https://doi.org/10.1021/acsanm.3c02122.
Reddy, S., Tatiparti, K., Sau, S., Iyer, A.K., 2021. Recent advances in nano delivery systems for blood-brain barrier (BBB) penetration and targeting of brain tumors. Drug Discov. Today 26 (8), 1944–1952. https://doi.org/10.1016/j.drudis.2021.04.008.
Ribatti, D., Pezzella, F., 2022. Vascular co-option and other alternative modalities of growth of tumor vasculature in glioblastoma. Front. Oncol. 12. https://doi.org/10.3389/fonc.2022.874554.
Rizvi, S.F.A., Ali, A., Ahmad, M., Mu, S., Zhang, H., 2021. Multifunctional self-assembled peptide nanoparticles for multimodal imaging-guided enhanced theranostic applications against glioblastoma multiforme. Nanoscale Adv. 3 (20), 5959–5967. https://doi.org/10.1039/D1NA00597A.
Rodà, F., Caraffi, R., Picciolini, S., Tosi, G., Vandelli, M.A., Ruozi, B., Bedoni, M., Ottonelli, I., Duskey, J.T., 2023. Recent advances on surface-modified GBM targeted nanoparticles: targeting strategies and surface characterization. Int. J. Mol. Sci. 24 (3), 2496. https://doi.org/10.3390/ijms24032496.
Sahoo, R.K., Kumar, H., Jain, V., Sinha, S., Ajazuddin, Gupta, U., 2023. Angiopep-2 grafted PAMAM dendrimers for the targeted delivery of temozolomide: *in vitro* and *in vivo* effects of PEGylation in the management of glioblastoma multiforme. ACS Biomater Sci. Eng. 9 (7), 4288–4301. https://doi.org/10.1021/acsbiomaterials.3c00263.
Senzer, N., Nemunaitis, J., Nemunaitis, D., Bedell, C., Edelman, G., Barve, M., Nunan, R., Pirollo, K.F., Rait, A., Chang, E.H., 2013. Phase I study of a systemically delivered p53 nanoparticle in advanced solid tumors. Mol. Ther. 21 (5), 1096–1103. https://doi.org/10.1038/mt.2013.32.
Shabani, L., Abbasi, M., Amini, M., Amani, A.M., Vaez, A., 2022. The brilliance of nanoscience over cancer therapy: novel promising nanotechnology-based methods for eradicating glioblastoma. J. Neurol. Sci. 440, 120316. https://doi.org/10.1016/j.jns.2022.120316.
Shariatnasery, M., Irani, S., Soleimani, M., Goodarzi, N., Dinarvand, R., 2020. Synergistic effect of microRNA and albumin-bound nanoparticles for inhibition of glioblastoma cancer cell proliferation. Braz. J. Pharm. Sci. 56. https://doi.org/10.1590/s2175-97902019000318306.
Sharma, R., Liaw, K., Sharma, A., Jimenez, A., Chang, M., Salazar, S., Amlani, I., Kannan, S., Kannan, R.M., 2021. Glycosylation of PAMAM dendrimers significantly improves tumor macrophage targeting and specificity in glioblastoma. J. Control. Release 337, 179–192. https://doi.org/10.1016/j.jconrel.2021.07.018.
Shi, D., Beasock, D., Fessler, A., Szebeni, J., Ljubimova, J.Y., Afonin, K.A., Dobrovolskaia, M.A., 2022. To PEGylate or not to PEGylate: Immunological properties of nanomedicine's most popular component, polyethylene glycol and its alternatives. Adv. Drug Deliv. Rev. 180, 114079. https://doi.org/10.1016/j.addr.2021.114079.
Shlapakova, T.I., Tyagunova, E.E., Kostin, R.K., Danilova, D.A., 2021. Targeted antitumor drug delivery to glioblastoma multiforme cells. Russ. J. Bioorg. Chem. 47 (2), 376–379. https://doi.org/10.1134/S1068162021020254.
Tincu (Iurciuc), C.-E., Andrițoiu, C.V., Popa, M., Ochiuz, L., 2023. Recent advancements and strategies for overcoming the blood–brain barrier using albumin-based drug delivery systems to treat brain cancer, with a focus on glioblastoma. Polymers 15 (19), 3969. https://doi.org/10.3390/polym15193969.
Wei, J., Xia, Y., Meng, F., Ni, D., Qiu, X., Zhong, Z., 2021. Small, smart, and LDLR-specific micelles augment sorafenib therapy of glioblastoma. Biomacromolecules 22 (11), 4814–4822. https://doi.org/10.1021/acs.biomac.1c01103.
Wiwatchaitawee, K., Quarterman, J.C., Geary, S.M., Salem, A.K., 2021. Enhancement of therapies for glioblastoma (GBM) using nanoparticle-based delivery systems. AAPS PharmSciTech 22 (2), 71. https://doi.org/10.1208/s12249-021-01928-9.
Wu, W., Klockow, J.L., Zhang, M., Lafortune, F., Chang, E., Jin, L., Wu, Y., Daldrup-Link, H.E., 2021. Glioblastoma multiforme (GBM): an overview of current therapies and mechanisms of resistance. Pharmacol. Res. 171, 105780. https://doi.org/10.1016/j.phrs.2021.105780.
Xin, X., Liu, W., Zhang, Z.-A., Han, Y., Qi, L.-L., Zhang, Y.-Y., Zhang, X.-T., Duan, H.-X., Chen, L.-Q., Jin, M.-J., Wang, Q.-M., Gao, Z.-G., Huang, W., 2021. Efficient anti-glioma therapy through the brain-targeted RVG15-modified liposomes loading paclitaxel-cholesterol complex. Int. J. Nanomed. 16, 5755–5776. https://doi.org/10.2147/IJN.S318266.
Yang, Q., Zhou, Y., Chen, J., Huang, N., Wang, Z., Cheng, Y., 2021. Gene therapy for drug-resistant glioblastoma via lipid-polymer hybrid nanoparticles combined with focused ultrasound. Int. J. Nanomed. 16, 185–199. https://doi.org/10.2147/IJN.S286221.
Zalba, S., ten Hagen, T.L.M., Burgui, C., Garrido, M.J., 2022. Stealth nanoparticles in oncology: facing the PEG dilemma. J. Control. Release 351, 22–36. https://doi.org/10.1016/j.jconrel.2022.09.002.
Zhang, C., Zhao, X., Zhang, J., Zhang, Q., Jin, C., Li, D., Chen, X., Hai, X., Chen, X., Tang, X., Zhao, Y., 2024. Dual-modified brain-targeting lipid polymer micelle delivery of anti-miRNA-21 for achieving oral treatment of glioma. J. Drug Delivery Sci. Technol. 96, 105721. https://doi.org/10.1016/j.jddst.2024.105721.
Zhang, J., Xiao, X., Zhu, J., Gao, Z., Lai, X., Zhu, X., Mao, G., 2018. Lactoferrin- and RGD-comodified, temozolomide and vincristine-coloaded nanostructured lipid carriers for gliomatosis cerebri combination therapy. Int. J. Nanomed. 13, 3039–3051. https://doi.org/10.2147/IJN.S161163.
Zhang, L., Dimberg, A., Rasouli, J., 2023. Editorial: the blood-brain barrier in brain tumors: molecular mechanisms and therapeutic strategies. Front. Neurol. 14. https://doi.org/10.3389/fneur.2023.1225594.
Zhang, Y., Xi, K., Fu, X., Sun, H., Wang, H., Yu, D., Li, Z., Ma, Y., Liu, X., Huang, B., Wang, J., Li, G., Cui, J., Li, X., Ni, S., 2021. Versatile metal-phenolic network nanoparticles for multitargeted combination therapy and magnetic resonance tracing in glioblastoma. Biomaterials 278, 121163. https://doi.org/10.1016/j.biomaterials.2021.121163.
Zhao, C., Zhu, X., Tan, J., Mei, C., Cai, X., Kong, F., 2024. Lipid-based nanoparticles to address the limitations of GBM therapy by overcoming the blood-brain barrier, targeting glioblastoma stem cells, and counteracting the immunosuppressive tumor microenvironment. Biomed. Pharmacother. 171, 116113. https://doi.org/10.1016/j.biopha.2023.116113.
Zhao, F., Zhong, L., Luo, Y., 2021. Endothelial glycocalyx as an important factor in composition of blood-brain barrier. CNS Neurosci. Ther. 27 (1), 26–35. https://doi.org/10.1111/cns.13560.
Zheng, Y., Oz, Y., Gu, Y., Ahamad, N., Shariati, K., Chevalier, J., Kapur, D., Annabi, N., 2024. Rational design of polymeric micelles for targeted therapeutic delivery. Nano Today 55, 102147. https://doi.org/10.1016/j.nantod.2024.102147.
Zhu, Y., Jiang, Y., Meng, F., Deng, C., Cheng, R., Zhang, J., Feijen, J., Zhong, Z., 2018. Highly efficacious and specific anti-glioma chemotherapy by tandem nanomicelles co-functionalized with brain tumor-targeting and cell-penetrating peptides. J. Control. Release 278, 1–8. https://doi.org/10.1016/j.jconrel.2018.03.025.
Zhu, Y., Liang, J., Gao, C., Wang, A., Xia, J., Hong, C., Zhong, Z., Zuo, Z., Kim, J., Ren, H., Li, S., Wang, Q., Zhang, F., Wang, J., 2021. Multifunctional ginsenoside Rg3-based liposomes for glioma targeting therapy. J. Control. Release 330, 641–657. https://doi.org/10.1016/j.jconrel.2020.12.036.
Zylberberg, C., Matosevic, S., 2016. Pharmaceutical liposomal drug delivery: a review of new delivery systems and a look at the regulatory landscape. Drug Deliv. 23 (9), 3319–3329. https://doi.org/10.1080/10717544.2016.1177136.