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REVIEW

Engineered Extracellular Vesicles in Glioma Therapy: Recent Advances and Applications

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Abstract: Gliomas are a highly heterogeneous group of primary tumors of the central nervous system. The blood-brain barrier and the complex tumor microenvironment restrict drug penetration and reduce the effectiveness of standard chemotherapy. Extracellular vesicles (EVs) have gained attention as potential delivery vehicles because they can move across biological barriers, are generally well tolerated, and naturally shuttle signals between cells. However, unmodified EVs face practical hurdles for clinical use, including limited tissue targeting, modest drug payload capacity, low manufacturing yield, and imperfect control over what they carry. To overcome these constraints, growing efforts have focused on engineering EVs to improve delivery performance and therapeutic precision. This review outlines key EV characteristics and commonly used isolation methods, with an emphasis on engineering approaches for glioma therapy. We also summarize recent progress in engineered EV-based treatments for glioma and discuss the main barriers to clinical translation.

Keywords: glioma, extracellular vesicles, engineering, glioma therapy, multimodal therapy

Introduction

Gliomas are the most common primary tumors of the central nervous system, arising from glial cells or their precursors. They account for about 24% of all central nervous system tumors and 80.9% of malignant cases.[1] Under the World Health Organization classification, gliomas range from low-grade (grades 1–2), which generally grow more slowly, to high-grade (grades 3–4), which are more aggressive. Patients with low-grade gliomas often have relatively favorable five-year survival (70% to 97%), yet complete surgical removal is frequently not feasible. Recurrence is common, and 17% to 32% of recurrent tumors progress to higher-grade disease through malignant transformation.[2–4] Glioblastoma (GBM) is the most aggressive subtype, with a median overall survival (OS) of 12–15 months and a five-year relative survival below 7%.[1,3,5–7] Standard treatment includes maximal safe resection followed by radiation and chemotherapy, typically with temozolomide (TMZ), but the benefit is limited for most patients. Tumor relapse remains the rule, and effective options after recurrence are scarce.[5,8] Together, these realities highlight the need for more effective therapies.

The blood-brain barrier (BBB) is formed by brain microvascular endothelial cells, pericytes, and astrocyte end-feet. It acts as a tight barrier that prevents most chemotherapeutic agents from reaching brain-tumor tissue, which remains a major obstacle to effective drug delivery in the brain.[9] Synthetic nanocarriers, including liposomes, polymeric micelles, and inorganic

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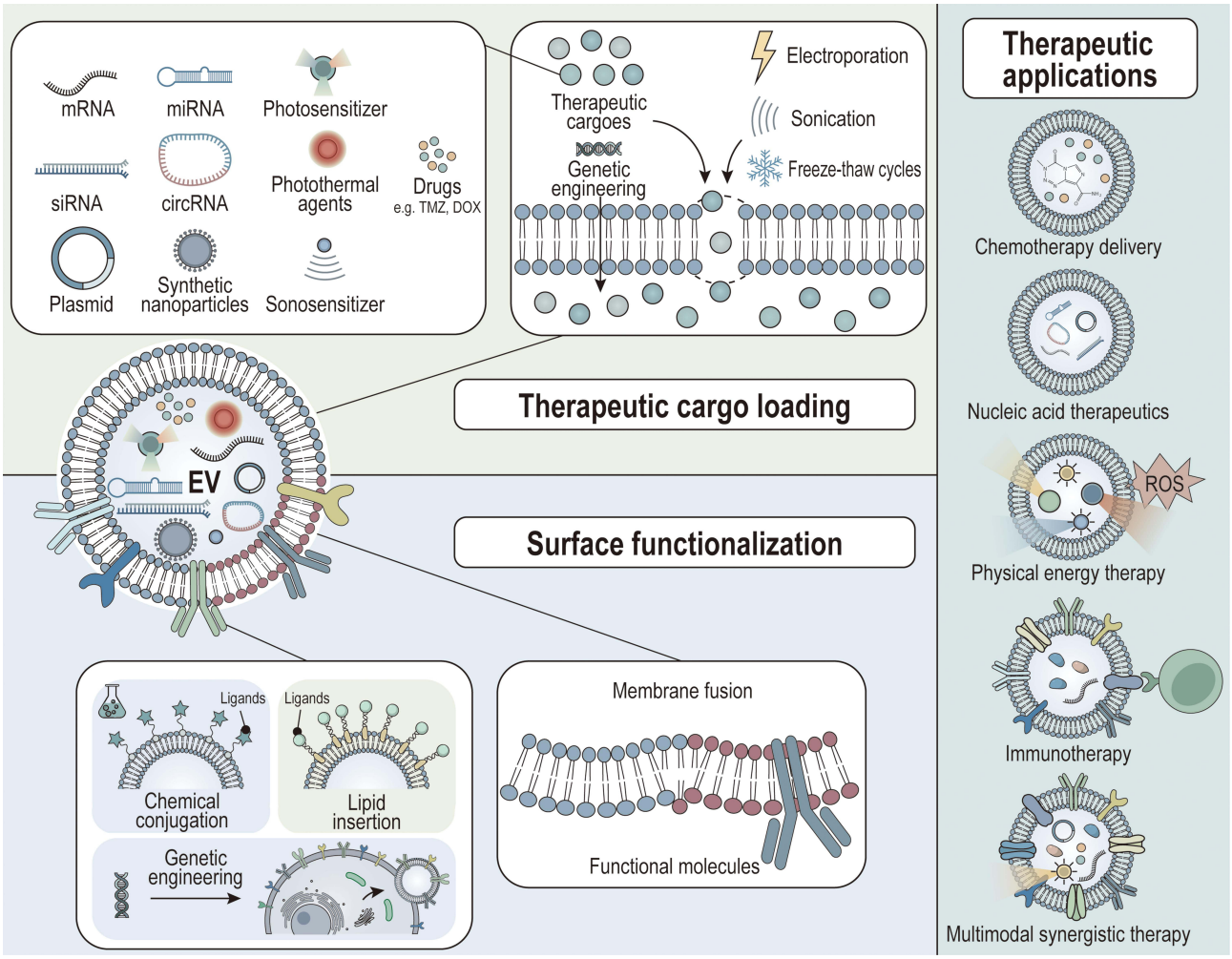
Graphical Abstract



nanoparticles, can improve BBB transport and increase tumor accumulation to some extent. However, overall delivery efficiency is still modest, and these materials are often recognized and cleared by the immune system.[10] Because gliomas are highly heterogeneous and the tumor microenvironment (TME) is complex, improved delivery platforms are needed. An effective system should cross the BBB efficiently and localize to tumor tissue with high precision in this challenging setting.

Extracellular vesicles (EVs) were once viewed mainly as cellular waste products. They are now recognized as important messengers that transfer bioactive cargo, including proteins, lipids, and nucleic acids, between cells and thereby influence both normal physiology and disease processes.[11] In GBM, EVs can shape the immune and invasive programs that support tumor progression. Zhao et al reported that ITGA5 carried by GBM-derived exosomes is taken up by macrophages and activates FAK and RUNX1 signaling, which drives an immunosuppressive macrophage state and promotes SPP1 release, contributing to T-cell dysfunction.[12] EVs have also been implicated in tumor invasion by altering Connexin-43-associated cell communication, increasing calcium signaling and PYK2 phosphorylation.[13] These properties have prompted interest in EVs as drug-delivery vehicles. Compared with synthetic carriers such as liposomes, EVs may offer advantages related to their natural origin, including lower immune activation, good biocompatibility, and reduced clearance by phagocytic cells.[14,15] Surface proteins such as integrins and tetraspanins may also support transport across the BBB, making EVs attractive candidates for glioma therapy.[16]

Native EVs have therapeutic promise, but their natural cargo is often insufficient for clinical needs. As a result, growing efforts have focused on engineering EVs to improve targeting, payload capacity, and therapeutic performance. Given the rapid pace of progress, a clear summary of current EV engineering approaches is needed to support clinical translation. This review first outlines key EV characteristics, major cargo types, and commonly used isolation methods. We then summarize recent advances in EV engineering strategies for glioma therapy and review their applications in glioma treatment. Finally, we discuss the main barriers to clinical use and highlight priorities for developing EV-based platforms for glioma diagnosis and therapy.

Characteristics, Cargo, and Isolation of EVs

Characteristics and Cargo of EVs

EVs are lipid bilayer particles released by cells that do not replicate. They are widespread in biological systems and span a broad size range of roughly 30 nm to 10 μm.[16] In their native state, EVs are typically round with a bilayer membrane, although conventional transmission electron microscopy can show an artificial cup-shaped appearance because of dehydration during sample preparation.[14] EVs are often grouped into three main classes based on how they are produced: exosomes, microvesicles, and apoptotic bodies (Figure 1A). These categories are not always defined consistently across studies. Exosomes are generally described as forming within the endosomal system through a stepwise process in which intraluminal vesicles (ILVs) accumulate inside multivesicular bodies (MVBs) before release. This process is commonly linked to Endosomal Sorting Complex Required for Transport (ESCRT) machinery, although ESCRT-independent routes have also been reported.[14,17,18] Exosomes are usually reported to be 40 nm to 150 nm in diameter, with some studies citing 50 nm to 150 nm.[14,19] Microvesicles form by outward budding from the plasma membrane and are typically 50 nm to 1000 nm. Apoptotic bodies are released during programmed cell death and are less often used therapeutically, in part because they are larger and carry more complex cargo.[17] Several surface proteins are frequently used to characterize EV preparations, including tetraspanins such as CD9, CD63, and CD81.[14,16,20] However, size ranges overlap substantially between exosomes and microvesicles, and these markers are not exclusive to a single subtype. Even with guidance from

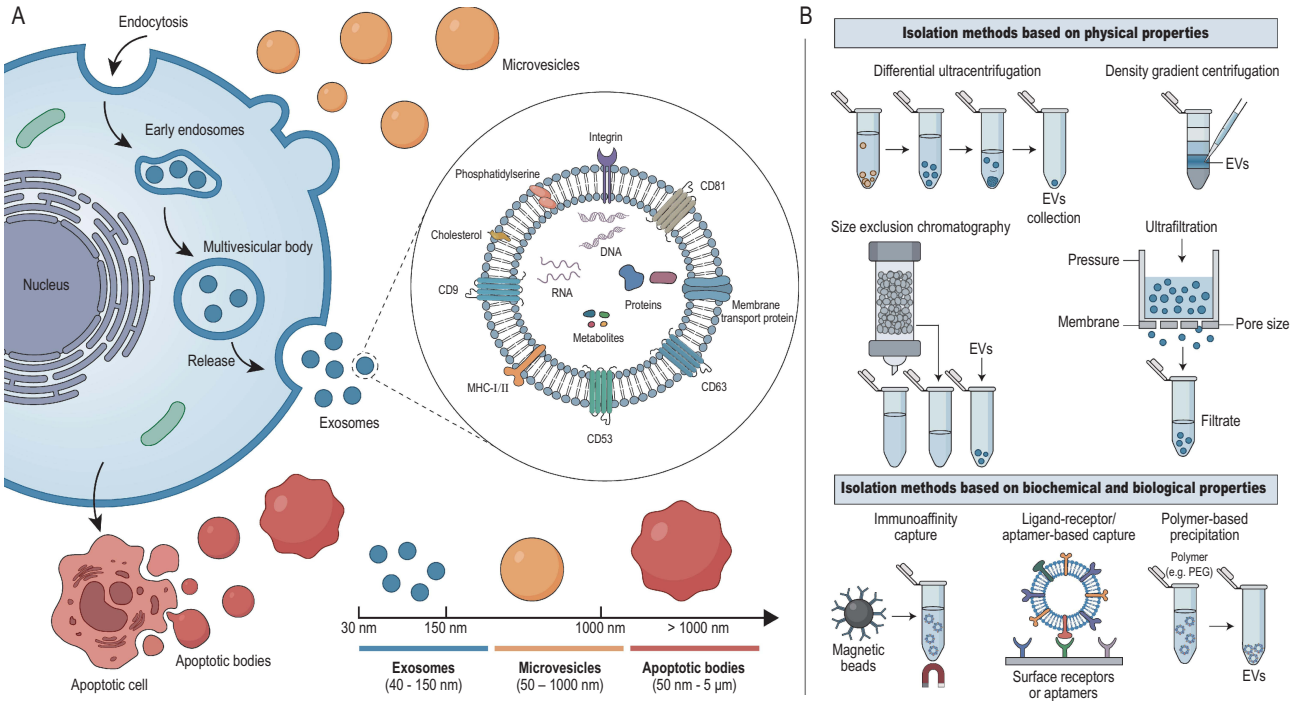


Figure 1 Biogenesis, classification, structure, and isolation strategies of EVs. (**A**) EVs originate from distinct cellular pathways. Exosomes are generated through endocytosis and inward budding of early endosomes, forming MVBs that fuse with the plasma membrane to release ILVs. Microvesicles are shed directly from the plasma membrane via outward budding, whereas apoptotic bodies are released during programmed cell death. (**B**) Common EV isolation strategies are categorized by their underlying principles, including methods based on physical properties and biochemical or biological properties.

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MISEV, vesicle origin cannot be assigned reliably using size and tetraspanin expression alone. For clarity, this review uses an inclusive definition of EVs and considers vesicles released through plasma-membrane-related pathways, including exosomes and microvesicles.

EVs carry a wide range of cargo, including proteins, lipids, and nucleic acids. Increasing evidence suggests that cargo loading is selective rather than a passive capture of cytoplasmic material. For proteins, sorting into EVs has been linked to ubiquitination and ESCRT-related pathways, as well as co-transport with chaperones such as HSP70 and HSC70.[14,21,22] EV membranes have a distinct lipid profile and are often enriched in cholesterol, sphingomyelin, and ceramide. These lipids help maintain membrane stability and can influence how molecules are recruited into vesicles through ordered membrane microdomains.[14,23–25] EVs also contain nucleic acids, with miRNAs being particularly prominent. In plasma-derived EVs, miRNAs can represent a substantial fraction of small RNA sequencing reads, reported at approximately 40% in some datasets.[26] Proposed sorting mechanisms include recognition of sequence features, 3' end modification, and association with the miRNA-induced silencing complex (miRISC).[27–29] Because EV cargo mirrors the physiological or pathological state of the source cell and can change over time, EVs have translational value in two ways. They may contribute to therapy through their endogenous bioactivity, and they can also serve as biomarker reservoirs for tracking disease progression.

Isolation of EVs

High-quality EV preparations with adequate yield and minimal heterogeneity are essential for translation (Figure 1B). Current isolation approaches rely on either physical properties or biochemical binding, as summarized in Table 1. Ultracentrifugation (UC) remains the most widely used reference method.[30] Differential ultracentrifugation (DUC) applies increasing centrifugal force to remove debris and larger particles and then pellets EVs.[31] It is simple and inexpensive, but purity can be compromised by co-pelleting of protein aggregates. Density gradient centrifugation (DGC) improves purity by separating particles in sucrose or iodixanol gradients, which helps remove non vesicular contaminants, although the workflow is more complex and time consuming.[32] Several size-based methods offer gentler processing. Size-exclusion chromatography (SEC) separates EVs on porous bead columns, preserves vesicle integrity, and removes soluble proteins, but it cannot reliably separate EVs from similarly sized lipoproteins.[33,34] Ultrafiltration (UF) uses membranes with defined pore sizes for rapid concentration, but membrane fouling can reduce performance. Tangential flow filtration (TFF) reduces clogging by directing flow parallel to the membrane surface and can increase yield while limiting shear related damage.[35,36] Polymer-based precipitation, most commonly using polyethylene glycol (PEG), is widely used because it produces high yield with simple handling. However, it often co-isolates non-EV components and therefore reduces purity.[37] In contrast, immunoaffinity capture uses antibodies against EV-surface markers to isolate high-purity EVs or specific subpopulations, but it is limited by low yield, higher cost, and practical challenges in releasing intact EVs from the capture surface.[38] Newer platforms, including microfluidic chips, aim to combine isolation and detection with high sensitivity and low sample-volume requirements, which is attractive for liquid-biopsy applications.[39,40] Overall, no single method achieves high purity and high yield at low cost with simple operation. For many diagnostic and therapeutic workflows, combining methods is often necessary to obtain EV preparations that are fit for purpose.

Native EVs for Glioma Therapy

Native EVs have shown early therapeutic activity in glioma models, which has been linked to their biocompatibility, reported ability to cross the BBB, and cell-specific cargo.[41–43] EVs from different sources, including tumor cells, immune cells, stromal cells, and plants, may act by reshaping the TME, promoting tumor cell death, or delivering bioactive molecules.

Tumor cell-derived EVs (TDEVs) contain tumor-associated antigens and other cancer-related components, which has prompted interest in their use as therapeutic agents. Mantile et al isolated EVs from human teratocarcinoma cells and identified a CRIPTO-positive EV subpopulation that reduced GBM cell migration without affecting proliferation or TMZ sensitivity.[44] Wang et al reported that TDEVs enriched in immune-activating factors such as HSP70 promoted dendritic cell maturation, improved antigen presentation, and induced antigen-specific cytotoxic T-cell responses.[45] However, safety remains a concern because tumor-derived EVs can also support glioma progression by promoting drug resistance, immune evasion, and tumor growth.

Table 1 Categorization of EVs Isolation Techniques Based on Physicochemical and Biochemical Mechanisms

Category	Strategy	EVs Integrity	Advantages	Limitations	References
Density	DUC	Shear stress damage; Aggregation risk	Simple operation; Low cost; Large sample capacity	Low reproducibility; Time-consuming; Protein co-sedimentation; Low purity	[30,31]
	DGC	Less mechanical damage than DUC	High purity; Non-vesicular contaminant removal	Complex operation; Time-consuming; Low throughput	[32]
Size	SEC	Biological activity preserved	High reproducibility; Soluble protein removal; Simple operation; No chemical contamination	Lipoprotein co-elution; Sample dilution; Sample volume limitation	[33,34]
	UF	Shear stress damage	Fast speed; Sample concentration; No extra reagents needed	Low purity; Membrane clogging; Non-EV protein trapping	[35]
	TFF	High; Reduced shear stress	High yield; Scalable; High purity; Minimal clogging	Specialized equipment cost	[36]
Solubility	Precipitation	Polymer encapsulation	High yield; Simple; Large volume compatible	Low purity; Polymer residue toxicity; Protein co-precipitation; Hard to remove polymer	[37]
Affinity	Immunoaffinity	pH-dependent elution damage	Highest purity; Specific subpopulation isolation; High specificity	Low yield; High cost; Marker bias; Non-scalable; Low throughput	[38]
Integrated	Microfluidics	High; Minimal loss	Low sample volume; High sensitivity; Rapid analysis; Integration with detection	Complex fabrication; Low throughput; High cost	[39]

Abbreviations: DUC, Differential Ultracentrifugation; DGC, Density Gradient Centrifugation; SEC, Size-Exclusion Chromatography; UF, Ultrafiltration; TFF, Tangential Flow Filtration.

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Immune cell-derived EVs have also been studied for their ability to remodel the TME and suppress glioma. Yan et al showed that EVs from M1-polarized macrophages are enriched in miR-150, which downregulates Matrix Metalloproteinase 16 (MMP16) and reduces glioma cell proliferation and migration.[46] Zhang et al found that neutrophil-derived EVs carry cytotoxic proteins that can trigger apoptosis through caspase activation in tumor cells.[47] Seo et al reported that CD8-positive T-cell-derived EVs deliver miR-298-5p, activate caspase-3 signaling, and induce apoptosis in mesenchymal stem cells (MSCs) and cancer-associated fibroblasts (CAFs), thereby weakening tumor-supportive stroma.[48]

Resident glial cells and MSCs can also release EVs with anti-tumor effects. Astrocyte-derived EVs enriched in miR-124 were shown to transfer this miRNA into GBM cells and reduce migration and invasion by downregulating LRRC8C, a subunit of the volume-regulated anion channel (VRAC).[49] EVs from adipose-derived MSCs have also been reported to inhibit GBM proliferation and invasion, potentially through reduced integrin expression and downregulation of the VEGF receptor KDR.[50]

Plant-derived EVs have attracted attention because they can be produced at scale and may carry bioactive cargo that remains functional across species. Kim et al reported that ginseng-derived EVs cross the BBB, accumulate in glioma, and deliver cargo including ptc-miR396f, leading to tumor cell apoptosis and a shift toward a more inflammatory macrophage state, with improved survival in tumor-bearing mice.[51] EVs from ginger were reported to enhance immunogenic tumor cell death and promote dendritic cell maturation and cytotoxic T-cell responses.[52] EVs from mangosteen pericarp were taken up by microglia and glioma cells, suppressed tumor growth by inhibiting PI3K/Akt signaling, and reduced M2-like polarization in microglia.[53] Despite these findings, further work is needed to clarify biodistribution, delivery mechanisms, and long-term safety for plant-derived EVs.

Engineering Strategies for EVs

While Native EVs offer inherent advantages such as biocompatibility and the ability to cross biological barriers, their native form often falls short of meeting the complex demands of glioma therapy. Key limitations include low drug loading efficiency, insufficient targeting capability, and limited therapeutic functionality.[54] To overcome these constraints, researchers have developed engineering approaches that tailor EVs for more precise and effective treatment (Table 2). These strategies generally fall into two connected directions (Figure 2): loading EVs with therapeutic cargo and modifying the EV surface to improve targeting or add new functions.[55]

Cargo Loading into EVs

Efficient and stable loading of therapeutic agents into EVs, either within the vesicle lumen or on the surface, is essential for their use as delivery vehicles. Loading approaches are commonly classified as passive or active, depending on when the cargo is introduced and how it is incorporated.

Active Loading

Active loading is a cell-based approach in which producer cells are modified so that they continuously release EVs containing a therapeutic payload. The most direct strategy is genetic engineering. By introducing sequences that encode therapeutic RNAs or proteins, the producer cells can express these molecules and package them into EVs. However, loading levels are often variable and difficult to control, so optimization is usually required. For example, human MSCs were transduced to overexpress the tumor-suppressive miR-1208, and the resulting EVs contained higher miR-1208 levels and showed enhanced anti-glioma activity through effects on the METTL3/NUP214 axis.[58]

Beyond genetic modification, physical or chemical stimulation of producer cells can increase EV output and shift cargo composition, thereby altering biological activity.[81] In one study, calcium phosphate particles stimulated macrophages to secrete more EVs and promoted a pro-inflammatory phenotype, yielding EVs enriched in inflammatory factors. After intranasal administration, these EVs crossed the BBB, reshaped the tumor immune microenvironment, and improved antitumor effects in animal models.[82] In another report, irradiated bone-marrow MSCs released EVs enriched in phosphorylated STING, which enhanced immune activation at tumor sites.[61] These approaches are comparatively simple but remain constrained by the intrinsic response capacity of the producer cells.

Table 2 Engineering Strategies for EVs

Drug Loading Category	EVs Source	EVs Type	EVs Isolation Method	Modifiers	Modification Strategy	Cargo	Cargo Loading Method	Therapeutic Outcome	References
Active	HEK293T cells	Engineered	DGC	VSVg or Chimeric RB19g; HIV-Gag	Genetic engineering	Polycistronic plasmid (miR-124-2, miR-135a-2, let-7i)	Genetic engineering	Inhibited tumor growth and significantly prolonged survival in mouse intracranial glioma models	[56]
	HEK293T cells	Engineered	Immunocapture; SEC	E-NoMi	Genetic engineering	Cre recombinase; SaCas9; sgRNA; EGFP	Genetic engineering	Achieved functional gene editing and recombination in brain tumor models	[57]
	BMSCs	Engineered	DGC	-	-	miR-1208	Genetic engineering	Combined with focused ultrasound, inhibited malignant progression and prolonged survival in nude mice	[58]
	MSCs	Engineered	SEC	-	-	HSVTK mRNA	Genetic engineering	Effectively inhibited glioma cell lines and primary GBM cells	[59]
	Umbilical cord MSCs	Engineered	Polymer-based precipitation	PD-1	Genetic engineering	miR-124	Genetic engineering	Achieved tumor inhibition and prolonged survival in GBM models	[60]
	BMSCs	Engineered	DUC	Anti-CD47 nanobody	Genetic engineering	STING protein; BafA1	Genetic engineering; Incubation	Significantly prolonged survival and induced complete tumor remission in glioma models	[61]
	iNSCs	Engineered	DUC	TRAIL	Genetic engineering	TRAIL	Genetic engineering; Incubation	Reduced brain tumor xenografts and significantly prolonged animal survival	[62]
	NSCs	Engineered	DUC; Polymer-based precipitation	-	-	CpG-STAT3ASO conjugates	Incubation	Inhibited tumor growth and activated glioma-associated microglia in mice	[63]
Active/ Passive	M1 macrophages	Engineered	DUC	CPPO; Ce6	Incubation	AQ4N	Incubation; Passive Diffusion	Markedly prolonged survival times in CDX and PDX glioma models	[64]
Passive	DCs and GL261 hybrid cells	Biomimetic	DUC	-	-	cGAMP	Electroporation	Achieved almost complete obliteration of intracranial primary lesions	[65]
	C6 cells	Biomimetic	Extrusion	T7 peptide	Membrane fusion	pHSVtk plasmid DNA	Extrusion	Enhanced tumor targeting and inhibited GBM growth in vivo	[66]
	THP-1 cells	Biomimetic	Extrusion	Anti-EGFRvIII CAR	Genetic engineering	DOX	Extrusion; Passive Diffusion	Increased tumor inhibition efficacy with reduced cardiotoxicity in GBM models	[67]
	C6 cells	Biomimetic	Extrusion	T7 peptide	Incubation	AMO21c	Passive Diffusion	Enhanced delivery to brain tumor across the BBB and significant reduction in tumor size	[68]
	Serum	Biomimetic	DUC	CpG oligonucleotides	Hydrophobic insertion	Tanshinone IIA-Glycyrrhizic acid nanomicelles	Sonication; Passive Diffusion	Improved BBB traversal, sustained drug release, significant reduction in tumor volume	[69]
	HEK293T cells	Engineered	Ultrafiltration	T7 peptide	Genetic engineering	AMO21c	Electroporation	Achieved significant tumor reduction and prolonged survival in intracranial GBM rat models	[70]

(*Continued*)

Table 2 (Continued).

Drug Loading Category	EVs Source	EVs Type	EVs Isolation Method	Modifiers	Modification Strategy	Cargo	Cargo Loading Method	Therapeutic Outcome	References
	Blood	Engineered	DUC	-	-	RASGRP1 siRNA; VPS28 siRNA; TMZ; EPIC-0412	Electroporation	Quadruple combination therapy significantly reduced tumor burden and prolonged survival in vivo	[71]
	DCs	Engineered	SEC; DUC	-	-	VEGF-A siRNA; DOX	Electroporation; Passive Diffusion	Reduced tumor angiogenesis, induced apoptosis in tumoral cells, and normalized vessel morphology	[72]
	U87MG cells	Engineered	DUC	-	-	Irinotecan	Extrusion	Enhanced encapsulation efficiency (45%) and improved cytotoxicity at lower concentrations	[73]
	GBM cells	Engineered	DUC	-	-	TMZ or EPZ015666	Passive Diffusion	Significant reduction in cancer cell proliferation compared to free drugs	[74]
	BV2 cells	Engineered	DUC	Redox-responsive oligopeptides	Hydrophobic insertion and chemical crosslinking	DOX	Passive Diffusion	Significant anti-cancer activity in orthotopic glioma, high targeting efficiency, and no cardiotoxicity	[75]
	Canine plasma	Engineered	DUC	-	-	Gd-DOTA or ICG	Passive Diffusion	Selective accumulation in intracranial tumors for enhanced MRI contrast	[76]
	U87 cells	Engineered	DUC	cRGDyC	Hydrophobic insertion and thiol-maleimide conjugation	DOX	Passive Diffusion	2.4-fold higher cellular targetability and 22.9-fold higher potency compared to free DOX	[77]
	BMSCs	Engineered	Polymer-based precipitation	-	-	Celastrol	Sonication	Inhibited GBM growth while decreasing systemic toxicity compared to free drug	[78]
	BMDCs	Engineered	Ultrafiltration	MHC-I; CCR7	Genetic engineering	IL-12	Sonication	Delayed murine GBM growth and prolonged survival without toxicity	[79]
	Citrus limon L.	Engineered	DGC	-	-	TMZ	Sonication	Enhanced drug stability and achieved strong cytotoxicity on GBM cells	[80]

Abbreviations: AMO21c, Anti-microRNA-21 oligonucleotides; BMDCs, Bone marrow-derived dendritic cells; BMSCs, Bone marrow-derived mesenchymal stem cells; cGAMP, 2'3'-cyclic guanosine monophosphate–adenosine monophosphate; cRGDyC, cyclic-RGDyC; DCs Dendritic cells; DGC, Density gradient centrifugation; DOX, Doxorubicin; DUC, Differential ultracentrifugation; ICG, Indocyanine green; IL-12 Interleukin-12; iNSCs, Induced Neural stem cells; MSCs, Mesenchymal stem cells; NSCs, Neural stem cells; RB19g, Rabies B19 Glycoprotein; SEC, Size-exclusion chromatography; TMZ, Temozolomide.

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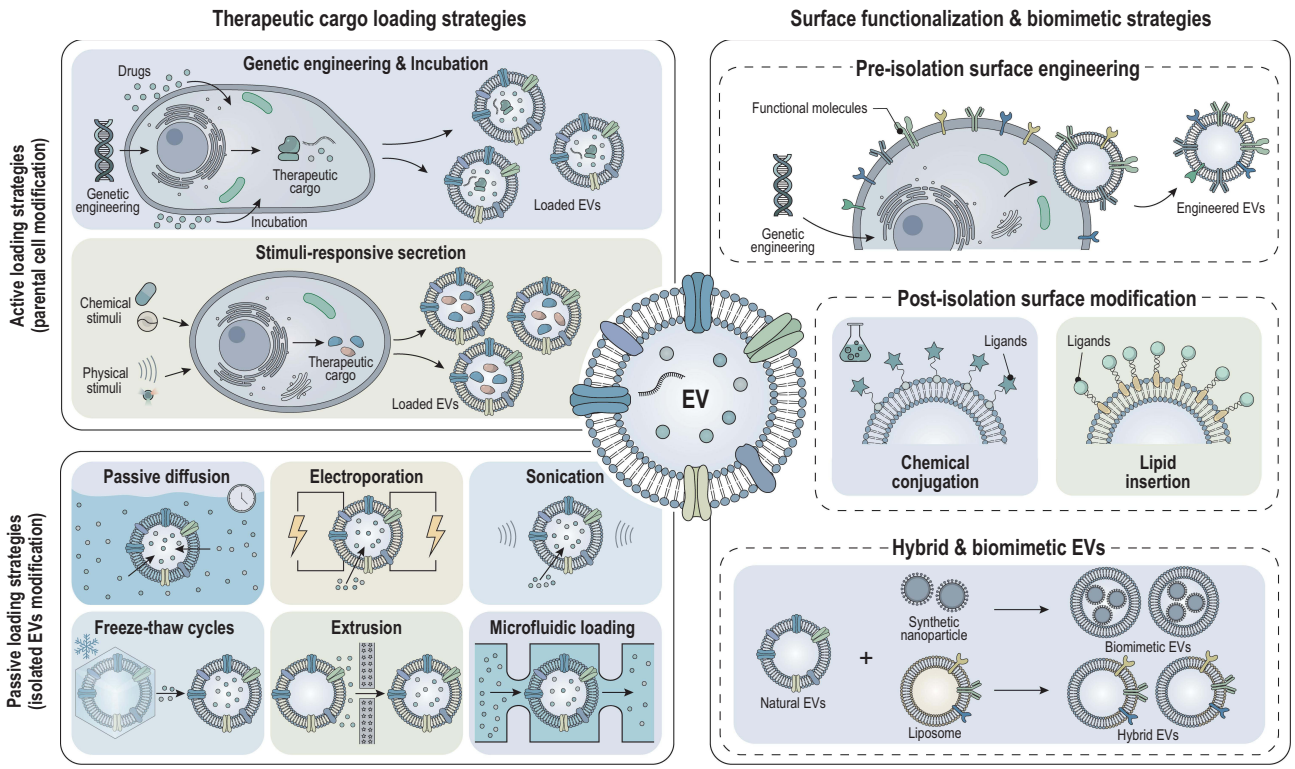

Figure 2 Engineering strategies for EV functionalization. Engineering approaches for EVs primarily involve optimized cargo loading and membrane functionalization. Cargo loading can be achieved through active strategies, including genetic modification, drug co-incubation with donor cells and donor cell stimulation. Alternatively, post-isolation passive methods such as electroporation, sonication, and microfluidic loading can be employed. Membrane engineering enhances targeting and functionality via genetic engineering, chemical conjugation, or biomimetic membrane fusion. These strategies collectively transform native EVs into versatile and programmable therapeutic platforms.

A third option is to incubate producer cells with a therapeutic agent so that the cells take it up and subsequently load it into secreted EVs through endogenous sorting. For instance, a STAT3 antisense oligonucleotide conjugated to CpG was taken up by neural stem cells and then incorporated into exosomes.[63] However, loading efficiency depends on cargo properties and incubation conditions, and limited understanding of intracellular sorting restricts precise control over how much cargo is packaged.

Passive Loading

Passive loading is a vesicle-based approach in which EVs are first isolated and then loaded with therapeutic cargo using post-isolation methods. Although these procedures can alter membrane properties, they are widely used because they are practical and can accommodate many drug classes.

The simplest method is passive diffusion, which relies on diffusion of drug molecules across the membrane and is most suitable for hydrophobic small-molecule drugs. For example, rapamycin was incubated with exosomes to generate Exo-Rapa, which crossed the BBB and the blood-brain tumor barrier. Exo-Rapa inhibited GBM cell proliferation and induced G1 arrest in vitro. After intravenous administration, it accumulated in tumors, reduced angiogenesis and tumor growth through effects on VEGF and its receptors, and prolonged survival. Drug release was also faster under acidic tumor-like conditions.[83]

Electroporation is another widely used technique. Brief electrical pulses create transient pores that allow drug molecules to enter EVs. Lee et al loaded selumetinib into exosomes derived from U87MG cells and observed enhanced uptake by the parental tumor cells compared with exosomes from non-glioma sources, which improved delivery and increased antitumor activity in GBM models.[84] However, electroporation can increase vesicle size and size heterogeneity relative to sonication-based loading, which may raise safety and quality-control concerns.[85]

Mechanical methods, including sonication, freeze-thaw cycles, and extrusion, can also promote cargo entry by temporarily disrupting the membrane. Sonication uses acoustic energy to increase membrane permeability and can

support loading while maintaining some functional features of the parent cell-derived vesicles. For instance, doxorubicin (DOX) was loaded into EVs by sonication, and the resulting vesicles retained chemotactic responsiveness to inflammatory cues and crossed both an in vitro BBB model and the zebrafish BBB in vivo.[86] Freeze-thaw loading has been used to incorporate atorvastatin through repeated freezing and thawing cycles,[87] and extrusion-based methods can force vesicles or lipid suspensions through defined pores to promote loading and generate more uniform particles.[88]

Microfluidic platforms have emerged as an alternative that can increase throughput and improve control over loading conditions. By combining chemical permeabilization with shear forces inside microchannels, one study achieved efficient loading of DOX into glioma-derived EVs.[89] Another approach used a disruption-reassembly process to generate uniformly sized vesicles while co-loading antimiRNA-21 and miRNA-100 into neural stem cell EVs, achieving markedly higher loading than conventional transfection-based methods.[90]

Modification and Transformation of EVs

To confer new functional capabilities, such as enhanced tumor targeting, improved BBB penetration, or immunomodulatory effects, EVs can be engineered through various modification strategies, particularly those targeting the membrane surface.

Surface Engineering of EVs

EV surface modification is commonly achieved in two ways. One approach engineers the producer cells before EV release, and the other chemically modifies EVs after isolation. Producer cell engineering enables surface display of functional molecules by expressing fusion proteins that combine a targeting or effector domain with an EV membrane protein such as Lamp2b, CD9, or CD64. After secretion, these fusion proteins are incorporated into the EV membrane and presented on the vesicle surface. This strategy has been used to add several types of functions. First, targeting peptides such as Angiopep-2, which binds LRP-1 on the BBB and glioma cells, and the cell-penetrating peptide TAT have been displayed on EVs. Combining these peptides can improve brain delivery and tumor penetration.[91–93] Second, immunoregulatory proteins have been presented to modulate antitumor immunity. Examples include PD-1 displayed on EV surfaces, which can bind PD-L1 on glioma cells, and anti-CD47 nanobodies, which block the CD47 signal and promote macrophage uptake of tumor cells.[61,92] Third, EVs have been engineered to display antibody-binding adapters such as CD64, which binds the Fc region of antibodies. This creates a modular platform that can be paired with different antibodies to retarget EVs as needed.[94]

Post-isolation chemical modification provides a direct way to engineer EV surfaces after purification. For example, click chemistry has been used to attach the c(RGDyK) peptide, which binds integrin αvβ3, to EV membranes and improve targeting to GBM.[95] Because native EVs are coated with surface proteins that can limit access for chemical coupling, one study used a mild enzymatic pretreatment to improve labeling. EVs were briefly treated with trypsin to reduce surface proteins and then modified with folate using cholesterol-anchored DNA linkers. This approach increased coupling efficiency and improved targeting performance.[96] In a related strategy, cholesterol has been used as a membrane anchor to insert cholesterol-modified T7 targeting peptides into EV-based hybrid membranes to enhance tumor targeting.[66] Surface engineering can also be used to present therapeutic cargo on the EV exterior, which can increase effective loading and strengthen targeting. In one example, heparin-based nanoparticles loaded with DOX and decorated with cRGD were attached to grapefruit-derived EVs using a patch-like assembly method. The resulting constructs accumulated efficiently in intracranial glioma tissue and showed improved tumor penetration.[97]

Hybrid and Biomimetic EVs

Despite advances in EV engineering, using native EVs as drug carriers still faces two practical constraints. First, common loading methods such as electroporation and sonication often achieve low encapsulation efficiency, which can limit the delivered dose and reduce the likelihood of reaching therapeutic levels.[73] Second, EV supply is restricted by the time and effort required to isolate and purify vesicles from cell culture supernatants, which complicates scale up for clinical use.[98] To address these barriers, biomimetic EV platforms and hybrid nanovesicles have been developed. These systems aim to combine the biological benefits of natural membranes, such as biocompatibility and targeting, with the strengths of synthetic nanomaterials, including higher loading capacity and more scalable manufacturing. In practice, this is achieved by fusing cell derived membranes with engineered nanoparticles or coating functional cores with biological membranes to create delivery vehicles that leverage both components.

Membrane-fusion approaches can generate hybrid vesicles by combining native EVs with synthetic liposomes. These hybrids are designed to merge the strengths of both components, including the loading capacity and stability of liposomes and the biocompatibility and inherent targeting features of EVs. For example, liposomes carrying the ferroptosis inducer RSL3 were fused with natural killer cell-derived EVs (NK-EVs) using PEG-mediated fusion.[99] In another study, camptothecin-loaded, tLyp-1-modified liposomes were fused with blood-derived exosomes containing SAB via sonication-induced fusion.[100]

Extrusion is another practical route to generate biomimetic EVs. In this method, intact cells, such as CAR-expressing monocytes, are repeatedly forced through nano-sized pores. The shear forces fragment the cells, and membrane components reassemble into uniformly sized vesicles. These vesicles can retain key membrane proteins and tumor-homing features of the donor cells, while allowing drug encapsulation during processing.[67] Liu et al used this strategy to produce drug-loaded biomimetic EVs with improved yield and antitumor activity. HEK293T cells were engineered to display an ANG-TRP-PK1 fusion peptide on the membrane, in which Angiopep-2 served as the targeting ligand and TRP-PK1 provided membrane anchorage. DOX was introduced into the cells by electroporation, and the cells were then repeatedly extruded under high pressure. During extrusion, the membrane fragments reassembled around the intracellular drug payload to form uniformly sized vesicles. This workflow increased EV yield by approximately 40-fold compared with natural secretion and produced vesicles that retained surface targeting peptides and showed stronger antitumor efficacy than TMZ in animal models.[98]

One hybrid strategy uses a core-shell design in which drug-loaded EVs are encapsulated within synthetic lipid nanoparticles to form NLP-EXOSOME complexes. This approach can improve siRNA stability and delivery efficiency and may also change in vivo pharmacokinetics.[101] Other designs combine EVs with engineered nanoparticle cores. In one study, magnetic mesoporous silica nanoparticles (MNPs) were loaded with the DHODH inhibitor BQR. In parallel, producer cells were engineered to secrete exosomes displaying Angiopep-2 on the surface, and siGPX4 was loaded into these exosomes by electroporation. The nanoparticle surface was functionalized with anti-CD63 antibodies to capture CD63-positive exosomes, thereby decorating the nanoparticles with the engineered exosomes.[102] Overall, these hybrid and biomimetic platforms provide practical routes to improve yield and enable more controlled manufacturing of engineered EV formulations.

Engineered EVs for Glioma Therapy

EVs are being developed as multifunctional delivery vehicles for glioma therapy because of their favorable biological properties. With appropriate engineering, EVs can deliver chemotherapeutics and nucleic acid drugs and can also support physical and immune-based interventions. Together, these approaches enable both single-modality and combination strategies for GBM treatment (Figure 3). Representative studies of engineered EVs in glioma therapy are summarized in Table 3.

EV-Based Delivery Systems for Chemotherapeutic Drugs

Engineered EVs for Chemotherapeutic Drug Delivery

Conventional chemotherapeutics used in glioma, including DOX, are limited by poor penetration across the BBB and off-target toxicity due to insufficient tumor selectivity. Encapsulating these agents in EV-based carriers can improve pharmacokinetics, reduce systemic exposure, and enhance antitumor activity. For example, EVs isolated from glioma cell lines were co-loaded with TMZ and EPZ015666, which reduced the required dose and showed therapeutic activity across multiple cancer models.[74] A major barrier to effective delivery is rapid EV clearance in vivo. To prolong circulation, Zhao et al introduced a pH-responsive PEG coating in which PEG was reversibly attached to EV surfaces through acid-labile cis-aconitic anhydride linkages for DOX delivery.[103] At physiological pH, the PEG layer reduced macrophage uptake and extended circulation time. In the acidic TME, the linkage hydrolyzed and the PEG detached, restoring the native EV surface and improving cellular uptake. In vitro and in vivo results showed increased tumor accumulation and improved treatment efficacy.[103] In a related strategy, redox-responsive oligopeptides were incorporated into exosome membranes to retain DOX during circulation and reduce premature leakage.[75]

To overcome chemoresistance in GBM, Liang et al developed engineered exosomes with dual targeting and dual drug loading. Macrophage-derived exosomes were co-loaded with TMZ and the resistance-modulating agent O6-

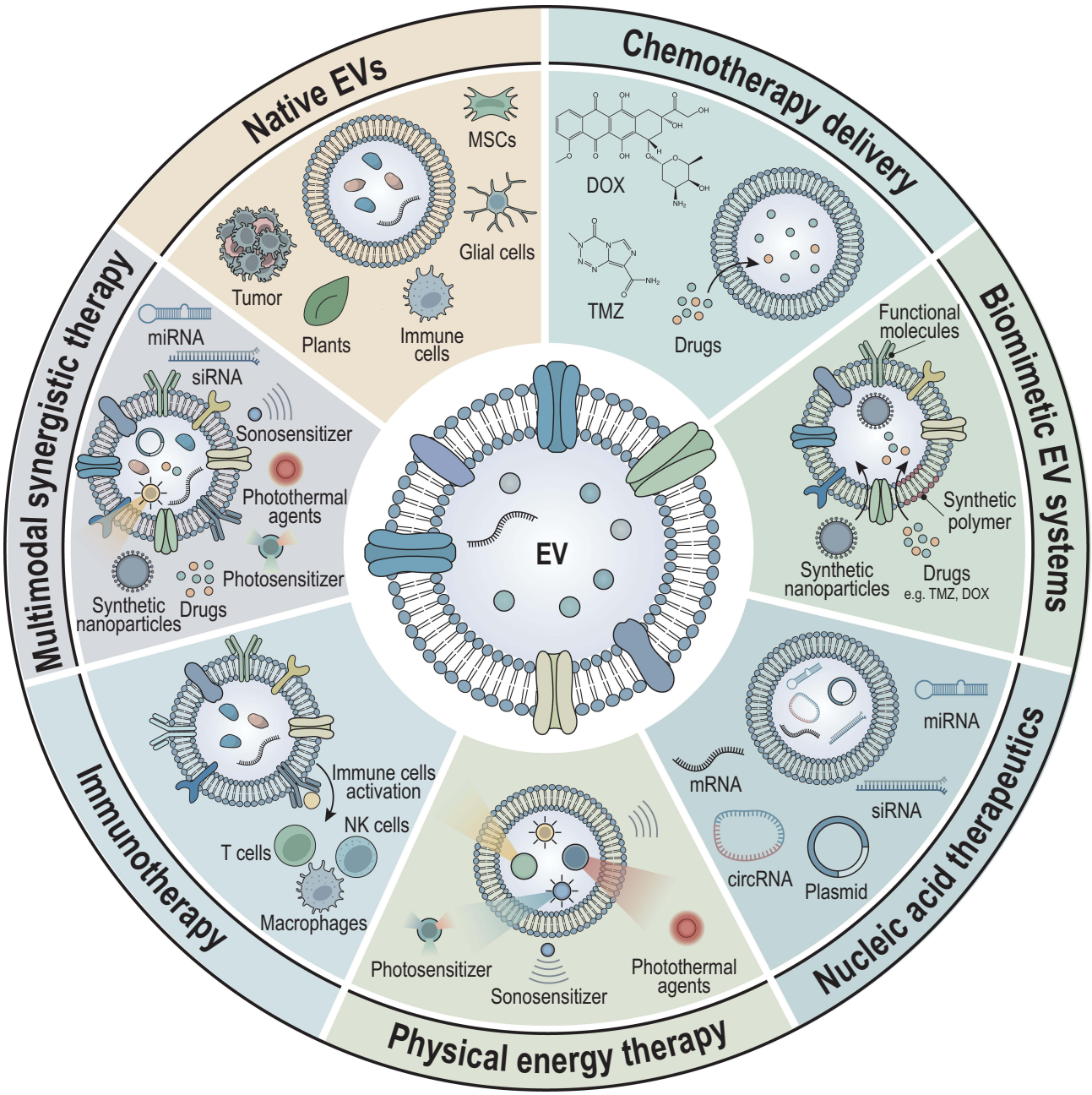

Figure 3 EVs as an Emerging Therapeutic Platform for Glioma. Native EVs provide a biologically compatible delivery scaffold, but their therapeutic potential is constrained by inherent functional limitations. Engineering interventions, including cargo loading, surface modification, and biomimetic integration, redefine EVs as versatile and tunable nanotherapeutic platforms, expanding their application landscape in glioma therapy.

benzylguanine. The exosome surface was then functionalized with Angiopep-2 and a CD133-binding ligand through chemical conjugation.[105] This dual-targeting design improved transport across the BBB, increased tumor accumulation, and enhanced uptake by both GBM cells and CD133-positive cancer stem-like cells. In resistant GBM models, the platform suppressed tumor growth and prolonged survival in tumor-bearing mice.[105]

Biomimetic EVs for Chemotherapeutic Drug Delivery

Cell-derived biomimetic EVs can resemble native EVs in morphology, size, and membrane composition, including key proteins and lipids. Their main practical advantage is manufacturability. Compared with naturally secreted EVs, they can be produced more readily with higher yield, more uniform particle size, and purification that is better suited for scale-up.

Table 3 Engineered EVs for Glioma Therapy

Treatment Type	EVs Source	EVs Type	Modifiers	Cargo	Therapeutic Mechanism	Therapeutic Outcome	References
Chemotherapy	Blood	Biomimetic	tLyp-1 peptide	Salvianolic acid B; Cryptotanshinone	STAT3 pathway inhibition and anti-angiogenesis	Significantly prolonged median survival time in orthotopic GBM xenograft mice	[100]
	Grapefruit	Biomimetic	DNs	DOX	Bypassing BBB via receptor-mediated transcytosis and membrane fusion	Resulted in enhanced therapeutic efficacy and prolonged survival in glioma-bearing mice	[97]
	HEK293T cells	Biomimetic	ANG2	DOX	LRP1 receptor-mediated BBB penetration and targeted chemotherapy	Effective suppression of orthotopic GBM and improved survival rate (57.1%)	[98]
	NK cells	Biomimetic	-	RSL3	Synergistic ferroptosis and immune activation via GPX4 inhibition	Enhanced tumor ablation and extended survival in orthotopic C6 glioma mice	[99]
	U87MG cells	Biomimetic	ANG2	DTX	LRP1 receptor-mediated transcytosis and targeted chemotherapy (G2/M arrest)	Significant suppression of orthotopic GBM growth with reduced side effects	[88]
	GL261 cells or iPSCs	Engineered	mPEG-CDM	DOX	pH-responsive PEG detachment for passive enrichment at acidic disease sites	Enhanced anti-tumor efficacy in glioma and improved angiogenesis in stroke models	[103]
	HEK293T cells	Engineered	ANG2; TAT peptide	DOX	LRP1-mediated targeting and cell penetration for BBB crossing	Improved survival of glioma-bearing mice by more than 2-fold with reduced cardiotoxicity	[104]
	MSCs	Engineered	-	Rapamycin	Inhibition of angiogenesis via VEGF/VEGFRs axis	Suppressed tumor growth and prolonged survival in GBM models	[83]
	NEs	Engineered	-	DOX	Inflammatory chemotaxis to target glioma	Efficiently suppressed tumor growth and prolonged survival in a glioma mouse model	[86]
	SF7761 cells; U251 cells	Engineered	-	DOX; PTX	Inhibition of cell proliferation	Inhibited parent glioma cells' proliferation more effectively than heterologous cells	[89]
	THP-1 cells	Engineered	ANG2; CD133 RNA aptamer	TMZ and O6-benzylguanine	Targeting LRP-1 and CD133 to overcome TMZ resistance	Extended median survival time of U87MG-bearing mice without adverse effects	[105]
	U87 cells; U251 cells; GL261 cells	Engineered	-	DOX	Induction of tumor cell apoptosis and interference with EV-mediated inter-cellular communication	Significant tumor suppression in subcutaneous and orthotopic GBM models with improved survival	[106]

(*Continued*)

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Table 3 (Continued).

Treatment Type	EVs Source	EVs Type	Modifiers	Cargo	Therapeutic Mechanism	Therapeutic Outcome	References
Gene therapy	U87MG cells	Biomimetic	Ionizable lipid nanoparticle coating	STAT3-siRNA	STAT3 downregulation and gene silencing	Reduced tumor proliferation and extended survival in GBM mice	[101]
	DCs	Engineered	ANG2	MIR-21 inhibitor	Disruption of miR-21/SP1/DNMT1 feedback loop to reduce CD73-mediated immunosuppression	Synergized with anti-PD-1 therapy to decrease MDSC infiltration and significantly prolong survival	[107]
	HEK293 cells	Engineered	FA	Mir-138 mimics	Targeting FA receptor and reprogramming TAMs	Inhibited tumor growth and enhanced antitumor immunity by depolarizing TAMs	[96]
	HEK293T cells	Engineered	T7 peptide	Galectin-9 siRNA	Polarized macrophages to M1 phenotype	Restricted GBM growth and reversed immunosuppression in tumor microenvironment	[108]
	HEK293T cells	Engineered	ANG2	Multi-siRNA (targeting circCABIN1 and OLFML3)	Suppressing circCABIN1/OLFML3 axis	Sensitized GBM cells to TMZ and improved antitumor activity	[109]
	M1 macrophages	Engineered	ANG2	STAT3 siRNA	STAT3 gene silencing and BBB crossing	Inhibited orthotopic GBM growth and significantly prolonged median survival	[110]
	MSCs	Engineered	-	iF3T3 siRNA	Silencing FGFR3-TACC3 fusion gene	Functional depletion of F3-T3 RNA and protein without affecting wild-type genes	[111]
	NSCs	Engineered	CXCR4 receptor	Anti-miRNA-21; miRNA-100	MIRNA-mediated sensitization of GBM to TMZ	Achieved prominent tumor regression and improved overall survival in mice	[90]
Immunotherapy	MEFs/ HEK293T cells	Engineered	CD64; anti-CD71 and anti-PD-L1 antibody	IFN-γ mRNA	Restoration of MHC-I expression and T-cell infiltration	Inhibited tumor growth and extended survival in preclinical GBM models	[94]
	RAW264.7 cells	Engineered	-	Anti-PD-L1 antibody; anti-CTLA-4 antibody	PD-L1/CTLA-4 blockade and macrophage repolarization	Combinatory treatment reduced tumor volume and prolonged survival in glioma-bearing mice	[82]

Physical energy therapy	GSCs	Biomimetic	-	Sodium borocaptate	Boron neutron capture therapy	Achieved cell-specific targeting and successful boron loading for GBM therapy	[85]
	RAW264.7 cells	Biomimetic	cRGD and PEG	NIR-C12 dye	NIR-II fluorescence imaging-guided targeted PTT	Complete inhibition of tumor growth and 100% survival rate on day 60	[112]
	U87MG cells	Biomimetic	-	Prussian Blue Nanoparticles	NIR laser-induced photothermal ablation and ROS scavenging	Successful photoacoustic imaging of brain tumor and effective tumor mass reduction in vivo	[113]
	J774.A.1 cells	Engineered	AS1411 aptamer	Catalase-encapsulated SiO2 nanoparticles and ICG	GSH depletion and O2 self-supply for enhanced SDT	Significant inhibition of orthotopic GBM growth and prolonged survival time	[114]
Multimodal synergistic therapy	MSCs	Biomimetic	ANG2; Magnetic nanoparticles	GPX4 siRNA; Brequinar; Fe3O4 nanoparticles	Disrupting DHODH and GPX4 axes to induce ferroptosis	Demonstrated effective tumor growth suppression and enhanced ferroptosis in GBM models	[102]
	Raw264.7 cells	Biomimetic	ANG2	DOX, Lauric acid, and Ru/Pt-TiOx nanoparticles	Synergistic multimodal therapy (PTT/PDT/Chemo/Immuno); H2O2 consumption to produce O2 and ROS; M2 to M1 polarization	Efficient BBB penetration, targeted accumulation in glioma, >80% tumor inhibition, and prolonged survival	[115]
	Raw264.7 cells	Biomimetic	cRGD peptide	Panobinostat and PPM1D-siRNA	Targeted delivery of HDAC inhibitor and gene silencing to overcome mutation-driven proliferation	Significant tumor growth inhibition and prolonged survival in orthotopic DIPG mice	[116]
	GL261 cells	Engineered	ACT	DOX and siTGF-β	Reprogramming immunosuppressive microenvironment via TGF-β downregulation; Chemo-immunotherapy synergism	Enhanced BBB transcytosis, targeted GBM accumulation, significant survival prolongation, and M2-to-M1 polarization	[117]
	HEK293T cells	Engineered	ANG2; TAT peptide	Cas9 protein; GSS sgRNA	GSS gene editing to trigger ferroptosis	Sensitized GBM to radiotherapy and achieved high gene editing efficiency in vivo	[91]
	HEK293T cells	Engineered	PD-1; ANG2	Cas9 protein; sgPLK1; sgVEGF	CRISPR gene editing and PD-L1 blockade	Suppressed tumor growth and remodeled the immunosuppressive microenvironment	[92]
	HMC3 cells	Engineered	HFn	siMCT4 and ultrasmall Au/MnO2 nanoparticles	Synergistic LA metabolic therapy, starvation therapy, and chemodynamic therapy	Significant inhibition of GBM growth and 75% long-term survival in combination with anti-PD-1	[118]

Abbreviations: ACT, Acid-cleavable Transferrin; ANG2, Angiopep-2; DCs Dendritic Cells; DNs, DOX-loaded heparin-based nanoparticles; DOX, Doxorubicin; DTX Docetaxel; FA, Folate; GBM Glioblastoma; GSCs, Glioblastoma stem-like cells; HFn, H-ferritin; ICG, Indocyanine green; MEFs, Mouse embryonic fibroblasts; NEs, Neutrophils; NK, Natural killer; NSCs, Neural stem cells; PTX, Paclitaxel; PTT, Photothermal therapy; SDT, Sonodynamic Therapy; TMZ, Temozolomide.

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Zhang et al prepared DOX-loaded PEG-PLA nanoparticles and coated them with exosome membranes from mouse brain endothelial cells using extrusion. After in vivo administration, these biomimetic vesicles crossed the BBB, accumulated in tumor tissue, and triggered systemic antitumor immune responses, leading to reduced tumor growth and prolonged survival in mice.[119] Similarly, Cheng et al developed a high-yield biomimetic EV platform with dual targeting, based on chemotaxis and CAR-mediated recognition, and reduced toxicity. After intranasal administration, the system bypassed the BBB, increased DOX accumulation in GBM tumors, and mitigated cardiotoxicity.[67]

Hybrid EVs are typically created by combining cell-derived membranes or membrane proteins with functional nanomaterials. This design aims to retain EV-like biocompatibility and biological interactions while adding the physicochemical advantages of the synthetic component. In one study, membrane proteins from U87MG cells were incorporated into DOX-loaded liposomes, which conferred exosome-like features, including CD47-associated immune evasion. The vesicles were further functionalized with Angiopep-2 to target LRP1 on the BBB.[88] This biomimetic design reduced nonspecific protein adsorption and helped preserve ligand activity, improving BBB transport and GBM targeting while limiting systemic toxicity of docetaxel.[88] In another study, hybrid biomimetic nanovesicles were generated by fusing RSL3-loaded liposomes with NK-EVs. The resulting vesicles showed improved penetration across the BBB and enhanced accumulation in brain tumor tissue.[99] Mechanistically, RSL3 promoted ferroptosis by inhibiting GPX4, while NK-EV-derived IFN-γ supported antitumor immunity and further reduced GPX4, increasing tumor sensitivity to ferroptosis. In an orthotopic glioma model, this combination suppressed tumor growth and extended survival by more than threefold without detectable systemic toxicity.[99]

In addition to vesicle-based designs, cell-based biomimetic delivery systems are being explored for glioma therapy. Microglia, the resident macrophages of the central nervous system, can cross the BBB and are naturally recruited to glioma lesions, making them potential cellular carriers. Du et al designed paclitaxel-loaded liposomes that incorporated dipalmitoyl phosphatidylserine (DPPS), an "eat-me" signal that promoted microglial uptake while limiting toxicity to the carrier cells. After internalization, microglia transferred paclitaxel to glioma cells through tunneling nanotubes and EV-mediated transport. In vivo, intravenous administration increased brain accumulation and suppressed glioma growth through direct chemotherapy and remodeling of the immune microenvironment.[120]

EV-Based Delivery Systems for Nucleic Acid Therapeutics

Nucleic-acid therapies for glioma are limited by instability in vivo, inefficient cellular uptake, and the risk of immune activation, underscoring the need for safe and effective delivery systems. One approach used exosomes derived from M1 macrophages to deliver STAT3-targeting siRNA, leveraging the tumor-homing behavior of these cells. The exosomes accumulated in the brain and were internalized by U87MG cells via macropinocytosis, leading to STAT3 knockdown and increased apoptosis.[110] EV-based delivery has also been applied to overcome TMZ resistance. Liu et al reported that EIF4A3 promotes the production of circCABIN1, which sequesters miR-637 and thereby increases OLFML3 expression. This axis activated ErbB signaling, enhanced stem-like features, and contributed to chemoresistance.[109] Based on these findings, the authors designed cholesterol-modified siRNAs targeting circCABIN1 and OLFML3 and loaded them into EVs. This platform reversed TMZ resistance and prolonged survival in orthotopic glioma models.[109]

For miRNA delivery, Nguyen et al used folate-modified EVs to deliver miR-138. This approach produced two complementary effects, including reduced GBM cell proliferation and reprogramming of tumor-associated macrophages from an M2-like state toward an M1-like phenotype, which was associated with enhanced CD8-positive T-cell responses.[96] In another study, Wang et al engineered neural stem cell-derived EVs to display CXCR4 and co-loaded them with antimiRNA-21 and miRNA-100. After intranasal administration, these EVs entered the brain through olfactory and trigeminal nerve pathways and targeted glioma via CXCR4 binding to tumor-derived SDF-1, supporting a route for brain-directed nucleic-acid delivery.[90]

EVs have also been explored for circular-RNA delivery. One report showed that loss of circPRKD3 sustains STAT3 activation and supports glioma stem-cell programs. Exosomes engineered to overexpress circPRKD3 were taken up by tumor-associated macrophages, where circPRKD3 suppressed STAT3 signaling and shifted macrophages from an M2-like to an M1-like phenotype. This remodeling increased CXCL10 production and promoted recruitment of CD8-positive T cells into the tumor core.[121]

For mRNA and plasmid-DNA delivery, one study introduced the herpes simplex virus thymidine kinase (HSVTK) gene into MSCs. The engineered MSCs continuously released exosomes carrying HSVTK mRNA, which were internalized by glioma cells and delivered functional transcripts.[59] In a related approach, Lee et al used a histidine and arginine linked polyamidoamine (PHR) polymer to condense pHSVTK and then encapsulated the complex within C6 glioma cell-derived exosome membranes, followed by surface modification with T7 peptides to enhance targeting.[66] After intravenous administration, pHSVTK/PHR-EM-T7 showed increased accumulation in brain tumors with limited distribution to liver and kidney and no obvious hemolysis or organ toxicity, supporting EV-based platforms for brain-targeted gene delivery.[66]

Engineered EV-Based Physical Therapies

Photothermal therapy (PTT) and photodynamic therapy (PDT) are light-activated treatment modalities that have been explored for glioma. In one study, Hill et al encapsulated Prussian blue nanoparticles (PBNPs), which also support photoacoustic imaging, within U87 glioma cell-derived exosomes. The resulting Exo:PB formulation was prepared by extrusion, measured approximately 120 nm, and retained key exosome membrane proteins.[113] After intravenous administration in an orthotopic GBM model, Exo:PB accumulated in brain tumors and, upon 808 nm laser irradiation, induced tumor-cell death and tumor ablation, supporting a non-invasive theranostic strategy for GBM.[113] A related approach used an exosome-liposome hybrid nanocarrier loaded with lipophilic NIR-II dyes to enable NIR-II imaging-guided PTT for GBM.[112] Conventional PDT photosensitizers often show limited tumor selectivity, which can lead to off-target tissue damage. To improve specificity, one study conjugated chlorin e6 (Ce6) to the mitochondria-targeting ligand triphenylphosphonium (TPP) to generate TPP-Ce6 and then loaded it into bEnd.3 cell-derived EVs.[122] This system crossed the BBB and, under 660 nm light irradiation, generated reactive oxygen species (ROS) that impaired mitochondrial function and triggered apoptosis in tumor cells.[122]

Sonodynamic therapy (SDT) is conceptually related to PTT and PDT but uses ultrasound as the trigger, which can reach deeper tissue. Wu et al designed silica-coated catalase nanoparticles loaded with the sonosensitizer indocyanine green (ICG). Disulfide bonds were incorporated into the silica network to enable glutathione-responsive degradation.[114] The particles were cloaked with macrophage-derived exosomes and functionalized with AS1411 aptamers to support BBB transport and GBM targeting. After uptake by GBM cells, elevated intracellular glutathione promoted silica-shell breakdown, releasing catalase. Catalase converted endogenous hydrogen peroxide to oxygen, which reduced hypoxia and lowered HIF-1α signaling. Together with glutathione depletion, this increased ultrasound-induced ROS production. In mouse models, the platform suppressed tumor growth and metastasis and extended survival.[114]

EV-based platforms have also been explored to deliver agents for radiotherapy-related approaches. Balboni et al developed a biomimetic delivery system using membranes from patient-derived GBM cells. Sodium borocaptate was loaded into these vesicles by electroporation, and the vesicles retained membrane proteins from the source cells.[85] This membrane-matched design promoted preferential uptake by homologous GBM cells compared with non-tumor cells. Intracellular boron levels reached approximately 6×10^{11} atoms per cell, exceeding the reported threshold for effective boron-neutron-capture therapy. Together, these results support EV-based delivery as a potential route for more precise GBM treatment.[85]

Engineered EV-Based Immunotherapy

EVs are being explored as delivery vehicles for cancer immunotherapy. Interleukin-12 (IL-12) is a potent antitumor cytokine, but clinical use is limited by a short half-life and dose-limiting systemic toxicity. To improve delivery, Barnwal et al loaded IL-12 into dendritic cell-derived EVs using sonication and achieved an encapsulation efficiency of approximately 20%.[79] In vivo, the IL-12 loaded EVs reduced immunosuppression and promoted a Th1-type immune response. The treatment increased infiltration of CD8-positive T cells and NK cells, shifted macrophages toward an M1-like state, reduced regulatory T cells and myeloid-derived suppressor cells, lowered PD-L1 expression, and inhibited angiogenesis. Compared with free IL-12, the EV formulation provided sustained release and higher tumor accumulation, reaching concentrations 3.5- to 4.5-fold greater and leading to stronger tumor growth inhibition and improved survival.[79] In a related strategy, Dong et al used microfluidic nanoelectroporation to generate EVs at scale for delivery of IFN-γ mRNA. This approach increased MHC-I expression on tumor cells and, when combined with PD-L1 blockade, enhanced antitumor immune activity.[94]

Engineered EVs Nanosystems for Multimodal Synergistic Therapy

Combining agents with different mechanisms can target GBM through multiple pathways, improve efficacy, and help delay or overcome resistance. Recent work has highlighted the value of pairing chemotherapy with gene-based interventions for glioma. Shan et al developed nanoparticles loaded with panobinostat and PPM1D-siRNA and coated them with functionalized macrophage exosome membranes. The macrophage exosome coating supported BBB transport, with cRGD surface modification resulting in improved tumor targeting and increased accumulation in brain tumors, thereby enhancing therapeutic activity.[116] Similarly, Yang et al used GBM cell-derived exosomes and attached an acid-labile transferrin (Tf) ligand to the surface using metabolic glycoengineering and click chemistry. DOX and siTGF-β were co-loaded by electroporation. The Tf ligand promoted Tf receptor-mediated transcytosis across the BBB and was then cleaved in endothelial lysosomes, releasing Tf-free exosomes that relied on homotypic targeting for more selective GBM cell uptake.[117]

Combining chemotherapy with energy-based modalities can provide synergistic effects in glioma treatment. Wang et al developed RGE peptide-modified EVs co-loaded with ICG and paclitaxel. Under near-infrared irradiation, the platform produced a combined PTT and chemotherapeutic response, increasing median survival from 25 days in the PBS group to 48 days with a favorable safety profile.[123] Han et al designed a multi-component system built on titanium-oxide nanoparticles with oxygen vacancies that carried ruthenium and platinum bimetallic nanoparticles (Ru/Pt-TiOx). The core was encapsulated in a lauric-acid phase-change material containing DOX and then coated with Angiopep-2 modified macrophage membranes to improve delivery and uptake.[115] Upon near-infrared irradiation, the core generated heat that triggered controlled DOX release and improved oxygenation, which reduced HIF-1α and P-glycoprotein expression and helped reverse multidrug resistance. The same treatment also increased ROS generation to support photodynamic activity.[115] Beyond light-based approaches, Dong et al used ultrasound microbubbles to transiently open the BBB and enable stimulus-responsive release within the glutathione-rich glioma TME. This strategy achieved approximately 40% tumor regression and induced long-term immune memory.[124]

Combining gene-based interventions with immunotherapy is technically demanding but offers complementary mechanisms for GBM treatment. One strategy integrates tumor targeting, immune checkpoint blockade, and gene editing within a single EV platform. Liu et al engineered HEK293T cells to express HA-Angiopep-2-Lamp2b and MYC-PD-1-CD9 fusion proteins, yielding EVs that displayed Angiopep-2 and PD-1 on the surface after UC-based isolation. The EVs were then loaded by electroporation with Cas9 ribonucleoprotein complexes carrying sgPLK1 and sgVEGF. This design supported dual gene knockout to directly impair GBM cells while also inhibiting angiogenesis.[92] In a related approach, umbilical cord MSC-derived exosomes were used for dual delivery of miR-124 and PD-1. The combination produced synergistic effects by suppressing GBM growth through miR-124 targets, including CDK6, while PD-1 blockade reduced immunosuppression and enhanced both systemic and local antitumor immune responses.[60] Together, these studies illustrate the potential of combining gene therapy with immunotherapy to improve GBM outcomes.

In addition, Lu et al developed a multifunctional platform that links metabolic intervention with immune modulation. Using click chemistry, they conjugated Au/MnO2-loaded H-ferritin (HFn) to the surface of microglia-derived EVs loaded with siMCT4. This design combined EV-based delivery with HFn targeting through Tf receptor 1 (TFR1) and increased brain-tumor accumulation by 3.1-fold compared with unmodified EVs.[118] Mechanistically, siMCT4 limited lactate export from tumor cells, while the ultrasmall Au and MnO2 components supported starvation-related and chemodynamic effects, respectively. Together, the platform enhanced GBM killing and reshaped the immune microenvironment, illustrating the potential of metabolism-focused, multimodal combination therapy.[118]

Conclusions and Perspectives

Gliomas, particularly GBM, remain among the most difficult tumors to treat in neuro-oncology. Their infiltrative growth, marked intratumoral heterogeneity, and the BBB together limit the effectiveness of many therapies. EVs have gained attention as therapeutic carriers because of their biocompatibility and innate tissue-tropism, which can support transport across the BBB and penetration into tumor tissue. This review summarizes key EV characteristics, major cargo types, and commonly used isolation methods, with an emphasis on engineering strategies and therapeutic applications. Current evidence indicates that engineered EVs can be adapted to different treatment goals and can improve efficacy in GBM

models. Multimodal designs that combine more than one therapeutic mechanism within a single EV platform are especially promising. For instance, combining chemotherapeutic agents with gene-silencing or gene-editing cargo allows simultaneous targeting of multiple tumor pathways, enhancing efficacy and potentially overcoming resistance. Similarly, platforms integrating gene-based interventions with immunomodulatory molecules offer complementary mechanisms to directly impair tumor cells while activating antitumor immune responses. Such strategies illustrate the clinical potential of multimodal EVs for glioma therapy.

Despite encouraging progress, several barriers must be addressed before engineered EVs can be widely used for glioma therapy. First, standardized manufacturing and quality-control workflows are still limited. Common isolation methods such as UC and SEC often cannot deliver both high purity and the scale needed for clinical production, and EV heterogeneity complicates batch-to-batch consistency. Second, drug-loading efficiency remains a key bottleneck because it directly influences the achievable dose and therapeutic effect. Loading methods should be optimized to maximize cargo incorporation while minimizing the amount of EV material required. Third, safety requires careful evaluation. EVs from different sources and subtypes can behave differently, and they may trigger immune responses or carry bioactive cargo with unintended effects, including potential tumor-supporting signals. These risks should be assessed through rigorous toxicology studies and early-phase clinical trials. Addressing these challenges will be essential for EV-based precision therapies to realize their potential in glioma treatment.

Abbreviations

BBB, Blood-Brain Barrier; CAFs, Cancer-Associated Fibroblasts; Ce6, Chlorin e6; DGC, Density Gradient Centrifugation; DOX, Doxorubicin; DPPS, Dipalmitoyl Phosphatidylserine; DUC, Differential Ultracentrifugation; ESCRT, Endosomal Sorting Complex Required for Transport; EVs, Extracellular Vesicles; GBM, Glioblastoma; HSVTK, Herpes Simplex Virus Thymidine Kinase; ICG, Indocyanine Green; IL-12, Interleukin-12; ILVs, Intraluminal Vesicles; miRISC, miRNA-Induced Silencing Complex; MMP16, Matrix Metalloproteinase 16; MNPs, Magnetic Mesoporous Silica Nanoparticles; MSCs, Mesenchymal Stem Cells; MVBs, Multivesicular Bodies; NK-EVs, Natural Killer Cell-Derived Extracellular Vesicles; OS, Overall Survival; PBNPs, Prussian Blue Nanoparticles; PDT, Photodynamic Therapy; PEG, Polyethylene Glycol; PTT, Photothermal Therapy; ROS, Reactive Oxygen Species; SDT, Sonodynamic Therapy; SEC, Size-Exclusion Chromatography; TDEVs, Tumor Cell-Derived Extracellular Vesicles; Tf, Transferrin; TFF, Tangential Flow Filtration; TME, Tumor Microenvironment; TMZ, Temozolomide; TPP, Triphenylphosphonium; UC, Ultracentrifugation; UF, Ultrafiltration; VRAC, Volume-Regulated Anion Channel.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare that there are no conflicts of interest.

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