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Review

Extracellular vesicles: Navigating new frontiers in glioblastoma therapy

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ABSTRACT

The profound challenge in treating glioblastoma (GBM) stems from a confluence of obstacles. The formidable blood-brain barrier (BBB) limits drug access, while the tumor's inherent inter- and intra-tumoral heterogeneity, profound immunosuppression, invasive growth, and frequent recurrence all contribute to dismal prognoses and severely hamper therapeutic efficacy. Extracellular vesicles (EVs), naturally occurring nano-sized messengers between cells, offer a novel therapeutic avenue by addressing these key obstacles. Their inherent ability to cross the BBB, deliver diverse cargo, and modulate the immune system positions them as promising vehicles for targeted drug delivery, immunotherapy, and even cancer vaccination. This review explores the therapeutic potential of various EV subtypes, including those derived from dendritic cells, T cells, brain endothelial cells, and mesenchymal stem cells, emphasizing their unique properties and preclinical successes in GBM models. We discuss current engineering strategies to enhance EV targeting, delivery, and therapeutic efficacy, alongside the emerging potential of EV-based cancer vaccines for GBM. Finally, we address the challenges and future directions of EV-based therapies for GBM, including standardized isolation and characterization protocols, scalable production, and rigorous safety assessments. Despite these challenges, the burgeoning field of EV research holds immense promise for transforming GBM treatment paradigms and improving patient outcomes.

Introduction

Glioblastoma (GBM) remains a formidable clinical challenge, characterized by its aggressive growth, diffuse infiltration, and dismal prognosis [1]. Despite decades of research, therapeutic options remain limited, underscoring the urgent need for innovative treatment strategies. Extracellular vesicles (EVs) have recently emerged as promising therapeutic candidates for a wide range of diseases [2,3], including cancer [4]. These naturally occurring nano-sized vesicles mediate intercellular communication [5] by transferring bioactive cargo, including proteins, lipids, and nucleic acids, between cells. This inherent ability to act as natural delivery vehicles, coupled with their capacity to traverse biological barriers like the blood-brain barrier (BBB) [3], makes EVs particularly attractive for GBM therapy.

EVs are a heterogeneous group (Fig. 1), broadly categorized by size and biogenesis into exosomes (EXs; 30–150 nm, endosomal origin), microvesicles (MVs; 50–10000 nm or larger, plasma membrane budding), oncosomes (100–400 nm, cancer-cell derived), with a notable subpopulation, and large oncosomes (LOs; 1–10 μm), released by tumor cells like GBM in advanced stages [6–9]. Although EV subtypes are often classified according to size, they are more fundamentally distinguished by mechanistically distinct biogenesis pathways. Exosomes originate within the endosomal system as intraluminal vesicles of multivesicular bodies and are released following fusion of these bodies with the plasma membrane through both ESCRT-dependent and ESCRT-independent, ceramide- and tetraspanin-driven mechanisms [3,10]. By contrast, microvesicles bud directly from the plasma membrane under the control of ARF6 and RhoA signaling and calcium-dependent calpain activation [11,12]. Large oncosomes arise from non-apoptotic membrane blebs of migratory, amoeboid tumor cells, with their biogenesis promoted by loss of the cytoskeletal regulator DIAPH3 and by oncogenic AKT1, MYC, and EGFR signaling [13–15]. Although large oncosomes share elements of the microvesicle biogenesis program, they remain distinct from similarly sized apoptotic bodies, which arise from dying rather than viable cells. These distinct origins are also reflected in EV cargo, as large oncosomes carry a proteomic payload substantially different from that of nano-sized EVs and efficiently transfer functional miRNA, proteins and other oncogenic cargo to recipient cells, thereby promoting tumor progression

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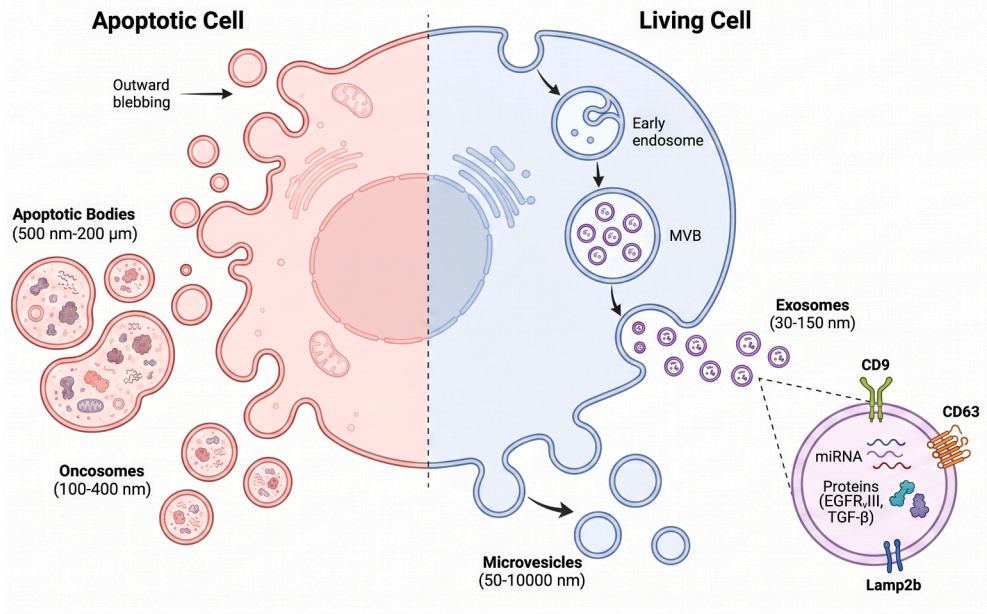

Fig. 1. Overview of EV Biogenesis and Molecular Composition. The schematic depicts the primary pathways for EV formation. ***Left:*** Apoptotic cells undergo outward blebbing of the plasma membrane to release large apoptotic bodies (500 nm-200 μm). Tumor-derived oncosomes are also shown as cancer-associated EVs; however, large oncosomes are generally generated from viable tumor cells through non-apoptotic membrane blebbing and should be distinguished from apoptotic bodies. ***Right:*** In living cells, exosomes (30–150 nm) originate from the endosomal system; endocytosis forms early endosomes, which mature into late endosomes. Invagination of the late endosomal membrane creates intraluminal vesicles (ILVs) within multivesicular bodies (MVBs). Fusion of MVBs with the plasma membrane releases ILVs as exosomes. Microvesicles (50–10000 nm) are formed by direct outward budding and fission from the plasma membrane. The magnified view highlights the molecular complexity of exosomes, showing characteristic transmembrane surface markers and the encapsulation of bioactive cargo, including miRNA and specific functional proteins that facilitate targeted intercellular communication. Created in BioRender (https://BioRender.com/gtyqbyv).

and remodeling the TME [8,16–19]. This functional divergence is particularly consequential for the pro-tumorigenic activities of GBM-derived EVs discussed below. Despite these mechanistic distinctions, the current isolation methods cannot reliably separate these biogenesis-defined EV populations. Accordingly, the International Society for Extracellular Vesicles (MISEV2018 and MISEV2023), recommends that isolated EV preparations be described using operational criteria such as size ("small" versus "large" EVs) or biochemical composition rather than by presumed origin [3,20].

Irrespective of their subtype, EVs interact with recipient cells via receptor-ligand binding, membrane fusion, or endocytosis [21], delivering their cargo to modulate recipient cell pathways and functions [22–26]. Given that therapeutic design often focuses on the collective intercellular communication roles of EVs involving the brain and tumor microenvironment (TME), this review will refer to EVs as a general population, without differentiation into specific subtypes unless explicitly stated.

While EVs show broad therapeutic potential across various diseases, including cardioprotection, liver regeneration, kidney repair, wound healing, and as adjuncts in cancer immunotherapy [27–40], their application to GBM is of particular interest. This review will delve specifically into the current advances, inherent complexities (such as the dual role of GBM-derived EVs), engineering strategies, challenges, and future prospects of employing EVs as therapeutics for glioblastoma.

GBM-Associated Extracellular Vesicles: A Double-Edged Sword

While EVs hold significant therapeutic potential, GBM-derived EVs (GBM-EVs) themselves play a complex and often detrimental role in tumor development and progression [25]. GBM-EVs have been shown to facilitate tumor growth, promote angiogenesis, and enable immune evasion. These pro-tumorigenic effects are attributed to their cargo, comprising a plethora of tumor-promoting molecules. For instance, GBM-EVs enriched with miR-451 and miR-21 have been implicated in promoting tumor growth and suppressing anti-tumor immune responses [41]. Transforming Growth Factor-β (TGF-β), a potent immunosuppressive cytokine found in GBM-EVs [42], further contributes to immune evasion and facilitates tumor progression. In addition, GBM-EVs carrying the oncogenic epidermal growth factor receptor variant III (EGFRvIII) promote angiogenesis, a hallmark of cancer, by activating pro-angiogenic signaling pathways in endothelial cells [43]. Similarly, GBM-EVs can reprogram monocytic cells, inducing their differentiation into tumor-associated macrophages (TAMs) with an M2-like phenotype, which fosters tumor growth and progression [44]. These GBM-EVs are also enriched with pro-tumorigenic factors, including VEGF and IL-6. Furthermore, GBM-derived EVs have been shown to upregulate proteins involved in invasion and extracellular matrix (ECM) interactions, facilitating tumor initiation and spread [45].

Despite their tumor-promoting roles, GBM-EVs hold diagnostic potential due to their ability to mirror the molecular landscape of the TME. Several studies have demonstrated a strong correlation between elevated plasma EV concentration and GBM presence, with levels decreasing post-tumor resection and increasing upon recurrence [46–48]. These findings suggest that EV concentration could serve as a non-invasive biomarker for GBM diagnosis and monitoring. Additionally, the identification of specific molecular markers within GBM-EVs, such as EGFRvIII and $IDH1^{R123H}$ mutations [49], offers promise for the development of more precise and personalized diagnostic tools. In line with Shao et al. [49], Skog and colleagues demonstrated that serum microvesicles from GBM patients carry GBM-specific mRNA variants and miRNAs, including EGFRvIII, suggesting their utility as diagnostic markers [50]. Furthermore, specific miRNAs (miR-194-5p and miR-205-5p) in transferrin receptor (TfR)-positive EVs could serve as potential biomarkers for brain metastatic breast cancers (BMBC) [51]. While discrepancies exist regarding the correlation between plasma EV concentration and temozolomide (TMZ) treatment response [46,49], the

growing body of evidence underscores the diagnostic and prognostic potential of GBM-EVs, warranting further investigation to delineate their clinical utility.

Therapeutic Potential of EVs: Harnessing Their Intrinsic Properties

The inherent ability of EVs to traverse biological barriers, including the BBB, makes them attractive vehicles for delivering therapeutic cargo to the brain. For instance, a recent study showed that brain metastatic cancer cells release EVs containing microRNA-181c, which disrupts the BBB, facilitating access to brain parenchyma [52]. This ability to bypass or modulate the BBB addresses the challenge of ineffective drug delivery associated with current brain tumor therapies, making EVs an exceptionally promising vehicle to deliver therapeutic agents for GBM treatment. Several subtypes of EVs have demonstrated therapeutic potential in preclinical models of GBM, including dendritic cell-derived EVs (DEVs), T cell-derived EVs, brain endothelial cell-derived EVs (BEC EVs), and mesenchymal stem cell-derived EVs (MEVs).

DEVs are particularly appealing for their role in antigen presentation and T cell activation. They possess a unique surface marker profile (MHC-I/II, CD86, HSP70-90) enabling them to present antigens, activate T cells, and induce DC maturation [9]. DEVs can also enhance anti-tumor immunity by secreting IL-2 and exosomal CD80, facilitating the transfer of MHC-I-peptide complexes to $CD8^+$ T cells and boosting their proliferation effects *in vivo* [53]. While most DEV research has not focused on brain tumors, a recent study demonstrated their potential as a GBM vaccine. DEVs loaded with chaperone-rich GBM cell lysates significantly prolonged survival in mice with intracranial tumors [27]. This, along with the emerging use of tumor-derived EVs as vaccine platforms, necessitates comparative studies to determine the optimal approach. Notably, although DEVs typically lack naturally expressed tumor antigens unless specifically loaded, they offer a potentially safer alternative due to their general lack of inherent immunosuppressive and tumor-promoting effects [9]. Further translational research is needed to fully elucidate their therapeutic potential in GBM.

T cell-derived EVs, particularly those derived from cytotoxic $CD8^+$ T cells, hold promise for boosting anti-tumor immunity. Previous studies show that these EVs can reduce tumor metastasis and invasion [54], activate cytotoxic T lymphocytes (CTLs) to release IFNγ and granzyme B through IL-12 delivery [55], and enhance antigen presentation via specialized miRNAs [56]. Similarly, $CD4^+$ T cell EVs, rich in immunologically relevant markers such as CD4, TCR, LFA-1, CD25, and FasL, enhance communication between $CD4^+$ T cells and antigen-presenting cells, boosting overall immunogenicity [57]. Collectively, these findings highlight the potential of T cell-derived EVs in amplifying anti-tumor immune responses.

Moreover, EVs derived from chimeric antigen receptor (CAR) T cells have emerged as a promising "off-the-shelf" therapeutic option. While CAR T-cell therapy has shown remarkable efficacy in hematological malignancies, its success in solid tumors like GBM has been limited by challenges, including toxicity and immunosuppression [58]. In contrast, CAR T-cell-derived EVs retain the anti-tumor efficacy of their parent cells while exhibiting lower toxicity and resistance to immunosuppressive mechanisms. Notably, exosomes derived from CAR-T cells, expressing CAR molecules and cytotoxic proteins on their surface, have demonstrated anti-tumor activity by inhibiting tumor growth [12]. Importantly, CAR T-cell exosomes, unlike their parent cells, lack PD-1 expression and are not susceptible to PD-L1-mediated immunosuppression. This highlights their potential to overcome this common resistance mechanism in the TME [59]. Given their inherent anti-tumor properties, particularly their nanoscale size facilitating tumor penetration, and resistance against immunosuppressive mechanisms, CAR T-cell exosomes represent a promising avenue for future GBM immunotherapy research.

While tumor-associated brain endothelial cells (BECs) can contribute to tumor progression [60], BEC-derived EVs offer a unique advantage for drug delivery due to their inherent ability to traverse BBB. These EVs exhibit enriched expression of CD63 and other surface receptors involved in *trans*-BBB transport, enhancing their brain-targeting ability and internalization compared to EVs from other brain cell-types [61]. This enhanced brain-targeting ability translated to improved therapeutic outcomes, for instance, BEC EVs loaded with doxorubicin or paclitaxel demonstrated significantly increased cytotoxicity against GBM cells in zebrafish models [61]. Additionally, BEC EVs engineered to overexpress ECRG4 inhibited GBM cell proliferation and invasion both *in vitro* and *in vivo*, reducing both inflammation and angiogenesis [62]. While promising, further research is needed to fully elucidate the intrinsic properties of BEC EVs and optimize their therapeutic application in GBM.

MEVs are another promising EV subtype for GBM therapy due to their inherent homing ability to tumor sites, high biocompatibility, and scalability for production. Mesenchymal stem cells (MSCs) are widely known for their immunosuppressive properties and have been widely explored for tissue regeneration and cancer therapy [63]. MSCs are of particular interest for EV-based therapies due to their inherent tumor-homing properties [64], ability to integrate into the tumor microenvironment [64], and capacity for large-scale EV production [65], which helps to overcome a key challenge in clinical translation. MEVs inherit these beneficial properties and can be readily engineered to deliver a diverse range of therapeutic cargo [63], including anti-tumor molecules, chemotherapeutic agents, and miRNAs. MEVs exhibit natural tumor-homing abilities, paracrine signaling functions, and can penetrate the disrupted BBB, often associated with brain tumors. Notably, Do et al. demonstrated superior brain penetration and parenchyma integration of MEVs compared to MSCs after intranasal delivery in mice [66]. Despite limited research specifically on GBM, the inherent properties of MEVs, particularly their brain penetration and biocompatibility, highlight their promising potential as engineered vehicles for delivering anti-tumor therapeutics to brain tumors.

While the inherent advantages of these various EV subtypes, including the natural tumor-homing of MEVs and the immune-stimulating properties of DEVs, highlight their therapeutic promise, fully appreciating their clinical utility must be assessed against other established delivery systems, such as lipid nanoparticles (LNPs), polymeric nanoparticles, and viral vectors (Table 1). Viral vectors, including adeno-associated viruses (AAVs) and adenoviruses, have been at the forefront of gene therapy because of their high gene-transfer efficiency and sustained transgene expression capacity [67,68]. However, their clinical application in neuro-oncology is constrained by vector-specific limitations, including restricted cargo capacity, particularly for AAV, pre-existing or treatment-induced anti-vector immunity, inflammatory toxicity, and challenges with repeated dosing [69–71]. Insertional mutagenesis is primarily a concern for integrating viral vectors, whereas AAV vectors generally persist mainly as episomes but may undergo low-frequency genomic integration, and adenoviral vectors are largely non-integrating [72–74]. On the other hand, synthetic non-viral platforms like LNPs and polymeric nanoparticles offer important advantages, including scalable manufacturing, tunable physicochemical properties, relatively defined composition, and flexible loading of nucleic acids or chemotherapeutic agents [75–77]. LNPs have transformed systemic RNA delivery, but efficient delivery across the intact BBB remains challenging and often requires chemical optimization or ligand-mediated targeting strategies [78]. In addition, PEGylated (polyethylene glycol-modified) lipid components, although useful for colloidal stability and circulation time, may in some settings contribute to anti-PEG antibody responses, hypersensitivity reactions, complement activation-related pseudoallergy, or accelerated blood clearance upon repeated administration [79,80]. In contrast, EVs uniquely bridge the gap between viral and synthetic vectors [81–83]. They may retain

Table 1
Comparison of EVs with established delivery platforms for GBM therapy.

Platform	Strengths	Limitations	GBM relevance	References
EVs	1. Biocompatible 2. Low immunogenicity 3. Potential BBB crossing	1. Heterogeneous 2. Variable yield 3. Non-specific biodistribution 4. Immature GMP production	Promising for targeted delivery and immunotherapy	[20,81,82,84,86,87]
LNPs	1. Scalable 2. Defined composition 3. Efficient nucleic acid delivery	1. Limited intrinsic BBB penetration 2. PEG-related immune reactions may occur	Brain delivery usually requires further targeting	[75,76,78–80]
Polymeric NPs	1. Tunable 2. Scalable 3. Flexible cargo loading	1. Potential toxicity 2. Variable degradation and release 3. Limited BBB penetration	Useful for controlled drug delivery but requires brain targeting	[77,144]
AAV vectors	1. Efficient gene transfer 2. Sustained expression	1. Small cargo capacity 2. Anti-vector immunity 3. Limited repeat dosing 4. Rare integration	Effective for CNS gene delivery but limited for repeated or large-cargo therapy	[67–72]
Adenoviral vectors	1. High transduction efficiency 2. Larger cargo capacity 3. Largely non-integrating	1. Immunogenicity 2. Inflammatory toxicity 3. Limited repeat dosing	Potential for gene delivery and oncolytic therapy, but safety remains a concern	[74,145–147]

Abbreviations: AAV, adeno-associated virus; BBB, blood–brain barrier; EV, extracellular vesicle; GBM, glioblastoma; GMP, good manufacturing practice; LNP, lipid nanoparticle; PEG, polyethylene glycol.

parent-cell-associated properties such as biocompatibility, relatively low immunogenicity, and context-dependent BBB-interacting or BBB-crossing potential, making them promising platforms for brain-directed therapeutic delivery [82,84,85]. Nevertheless, EVs still face their unique limitations, including biological heterogeneity, variable yield, batch-to-batch inconsistency, incomplete control over cargo loading, non-specific biodistribution, systemic clearance, route-dependent CNS exposure, and less mature GMP manufacturing and regulatory frameworks [20,81,86,87]. Therefore, targeted engineering and standardized manufacturing will be essential to determine whether EV-based platforms can achieve clear clinical advantages over existing viral and synthetic delivery systems in GBM.

Overcoming Limitations: Engineering EVs for Enhanced Therapeutic Efficacy

Despite their inherent therapeutic potential, several limitations hinder the clinical translation of native EVs. One major obstacle is their tendency for non-specific biodistribution, often leading to accumulation in organs like the liver and spleen [88]. This results in rapid clearance from circulation [89] and consequently, reduced therapeutic efficacy at the target site. This off-target effect underscores the need for strategies to enhance EV targeting specificity, several of which are being developed through the engineering approaches detailed below. Furthermore, current methods for EV isolation and purification often yield low quantities [90] and are challenging to scale up for clinical use [91], posing a significant hurdle for large-scale production. Additionally, EV populations are inherently heterogeneous in terms of their size, cargo, and function [91], making it challenging to standardize their production and therapeutic application.

To address these limitations, researchers are actively developing engineering strategies to enhance the therapeutic efficacy of EVs. Specifically, EVs can be precisely tailored to encapsulate and release therapeutic cargo such as RNA, DNA, proteins, and small drugs. Engineering can also aim to amplify EV production, enhance targeting capabilities, and ultimately improve their deployment as a treatment modality for GBM. These strategies primarily focus on two key approaches: cargo loading and membrane modification.

Cargo loading involves equipping EVs with diverse therapeutic payloads, including nucleotides, proteins, drugs, and nanoparticles. This can be achieved by two primary approaches: endogenous loading, where parent cells are genetically or chemically modified to produce the desired cargo, which is subsequently packaged into EVs, or direct loading of pre-formed EVs via various physical or chemical methods, such as electroporation, sonication, extrusion, or click chemistry [92]. Complementing these strategies, membrane modification aims to enhance EV targeting specificity, stability, and delivery efficiency by modifying surface proteins or lipids or by conjugating targeting ligands to the EV surface. Commonly expressed transmembrane proteins like CD9, CD63, and Lamp2b, which are also used as EV biomarkers [93], serve as attractive targets for surface engineering. For example, fusing cargo to the extracellular domain of CD63, a technique known as EV display technology, allows for targeted delivery and tracking, as demonstrated by studies using GFP-fused EVs [94]. Similarly, *Lamp2b* has been exploited for RNA display, while the C1C2 domain of Lactadherin has been used to present a broad array of proteins on EV surfaces [95], including GM-CSF, IL-2, and HER2, highlighting the versatility of this approach.

These engineering approaches have already demonstrated significant promise in preclinical models of brain cancer. For instance, MEVs engineered to overexpress the chemokine receptor CXCR4 exhibited enhanced homing to tumor sites in a breast cancer brain metastasis model [65]. Similarly, MEVs loaded with the death receptor ligand TRAIL effectively induced apoptosis in neuroblastoma cells [96]. Beyond MEVs, melanoma-derived EVs engineered to express OX40 ligand and 4-1BB immunostimulatory molecules successfully promoted T-cell activation and overcame regulatory T-cell-mediated immunosuppression [97]. While promising in melanoma, this strategy holds potential for brain tumors, given the conserved roles of OX40 and 4-1BB in T-cell function, warranting further investigation in GBM models.

Furthermore, the unique structure of EVs accommodates both hydrophobic and hydrophilic drugs [98]. Yang et al. elegantly demonstrated the superior BBB-crossing ability of EVs using a zebrafish model of GBM(61). Doxorubicin and paclitaxel, ineffective when administered alone, effectively reached tumor cells and reduced tumor growth when delivered within BEVs.

EVs also show promise for delivering therapeutic miRNA to brain tumors. GBM-derived EVs naturally carry miRNA [99], highlighting their potential as therapeutic payloads. Preclinical studies have demonstrated the efficacy of MEVs loaded with miR-146b-5p in reducing glioblastoma growth [100] and anti-mir-9 in overcoming TMZ resistance [101]. Similarly, engineered tumor-derived EVs (TEVs) delivering miR-7a [102] and miR-1 [103] have shown anti-tumor effects in other models, suggesting potential applications in GBM. These findings underscore the potential of EVs as a platform for miRNA-based brain tumor therapies.

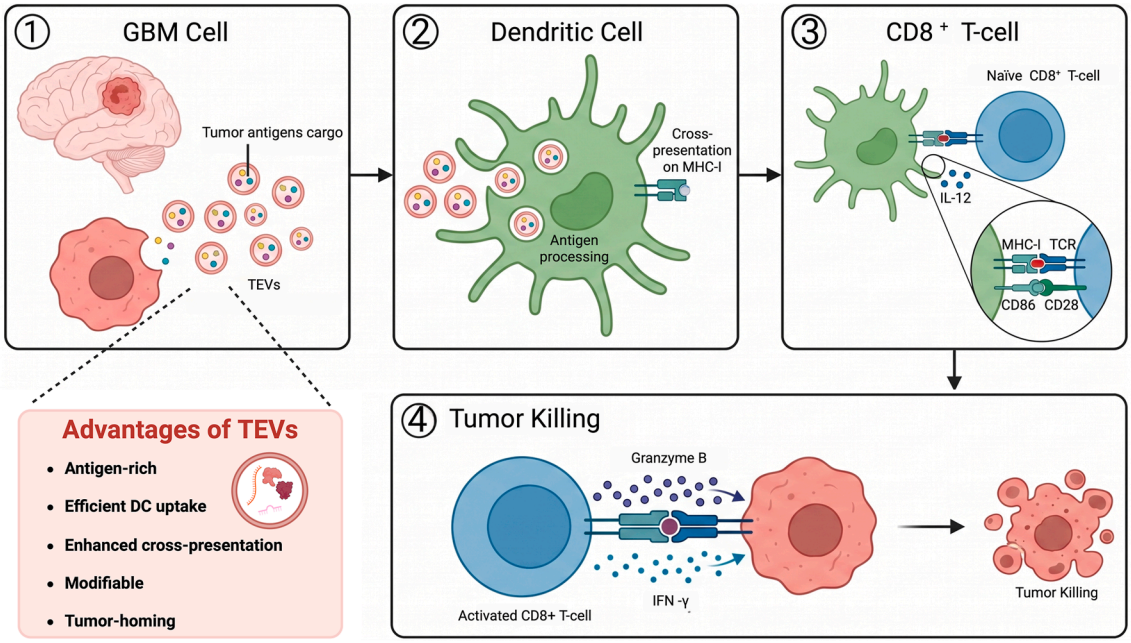

Fig. 2. Mechanistic overview of the immunotherapeutic potential of GBM-TEVs. The schematic illustrates the multi-step process by which tumor-derived EVs (TEVs) induce targeted anti-tumor immunity. **(1)** Glioblastoma (GBM) cells release TEVs carrying a rich cargo of tumor antigens. These TEVs offer significant advantages, including concentrated neoantigens, efficient cellular uptake, tunability via engineering, and inherent tumor-homing properties. **(2)** Dendritic cells (DCs) internalize these TEVs, process the encapsulated antigens, and cross-present them on major histocompatibility complex class I (MHC-I) molecules. **(3)** The DCs prime and activate naïve $CD8^+$ T-cells via a detailed immunological synapse: MHC-I interacts with the T-cell receptor (TCR), accompanied by essential co-stimulation between CD86 (on DCs) and CD28 (on T-cells), and the targeted release of the stimulatory cytokine IL-12. **(4)** Finally, the activated $CD8^+$ T-cells traffic back to the tumor site, where they recognize and eliminate GBM cells through the release of cytotoxic effector molecules, including interferon-gamma (IFN-γ) and Granzyme B, resulting in tumor destruction. Created in BioRender (https://BioRender.com/gtyqbyv).

GBM-EVs as Cancer Vaccines: A Novel Frontier

Beyond their role as therapeutic delivery vehicles, GBM-EVs are gaining traction as potential cancer vaccines (Fig. 2). TEVs naturally harbor an array of tumor-associated antigens (TAAs) and tumor-specific antigens (TSAs) [104], reflecting the antigenic fingerprint of the tumor cells from which they originate. This inherent characteristic makes them attractive candidates for stimulating targeted anti-tumor immune responses.

Preclinical studies have provided compelling evidence supporting this concept. For instance, previous *in vitro* studies have demonstrated that TEVs isolated from murine GBM models can effectively activate dendritic cells (DCs) [105], leading to the upregulation of maturation markers and enhanced antigen presentation. Furthermore, these activated DCs, when co-cultured with T cells, can prime tumor-specific CTLs that are capable of recognizing and killing GBM cells [105]. These findings have been replicated in various other cancer models [106–108], highlighting the broad applicability of this approach. Similar approaches are being explored in other cancers. Lung cancer-derived EVs have been shown to activate DCs and induce anti-tumor immunity in mouse tumor models [109,110], while melanoma-derived EVs loaded with the MART-1 antigen have elicited potent anti-tumor responses and inhibited tumor growth in mice [108].

Building on these promising preclinical results, significant efforts are now dedicated to developing EV-based cancer vaccines. This strategy typically involves isolating TEVs from patients, either directly from resected tumor tissue [111] or from bodily fluids like blood or ascites [107] and engineering them to enhance their immunostimulatory potential. This can involve loading them with additional TAAs or TSAs, incorporating immunostimulatory molecules like cytokines or Toll-like receptor agonists, or modifying their surface with targeting ligands to enhance their uptake by DCs or other antigen-presenting cells (APCs) [112]. One notable example is a Phase I trial investigating the use of autologous tumor-derived EVs loaded with the immune adjuvant granulocyte-macrophage colony-stimulating factor (GM-CSF) in patients with colorectal cancer [113]. Preliminary results from this trial have shown that the vaccine is safe and can induce tumor-specific immune responses in some patients. Another clinical trial using a single intrapleural infusion of TEV loaded with methotrexate demonstrated safety and showed clinical activity in patients with malignant pleural effusion from advanced lung cancer [114]. Although these initial clinical findings are promising, further rigorous exploration of tumor-derived EVs as a therapeutic platform for treating malignancies is essential.

While EV-based cancer vaccines are still in their early stages, their potential for inducing potent and personalized anti-tumor immunity is undeniable. The growing interest and progress in this field, as evidenced by the numerous preclinical studies underway (Table 2), underscore the transformative potential of this approach for treating GBM and other challenging malignancies.

Challenges and Future Directions

Despite significant progress in EV research, several challenges must be overcome before these promising therapeutic agents can be effectively translated from bench to bedside and transform GBM treatment paradigms. A major obstacle remains the lack of universally standardized protocols for EV isolation and characterization [20,115]. Currently, there is no consensus on the optimal method for isolating EVs from complex biological fluids [116–118]. Blood plasma, while easily accessible for liquid biopsies, contains vast quantities of lipoproteins and plasma proteins that overlap with EVs in size and density, confounding downstream analysis [119]. In contrast, cerebrospinal fluid (CSF) offers a more direct representation of the brain tumor microenvironment but requires invasive sampling and typically yields relatively low EV concentrations [84,120,121].

Table 2
Pre-clinical EV-based therapeutic strategies for GBM.

EV source	Agent(s) loaded	Therapeutic target	References
GBM	TMZ, EPZ015666	Enhance Drug Delivery	[148]
Engineered GSCs	miR-302-367	Cell Growth and Invasion	[149]
GBM	miR-124, miR-128, miR-137	Cell Survival, Chemotherapy Response	[150]
GBM	miR-151a	Chemoresistance	[151]
VB-treated GBM	miR-7-5p	Tumor Growth & Metastasis	[152]
Saponin-treated GBM	Doxorubicin	Tumor Growth	[153]
GSC	Paclitaxel, Doxorubicin	Targeted Drug Delivery, Homing	[154]
U87	Selumetinib	Targeted Cytotoxicity	[155]
U87 Spheroids	Hydroxychloroquine	Targeted Drug Delivery	[156]
GL261	Arsenic trioxide, Chlorin e6	Tumor Growth, Immune Modulation	[157]
GL261	TGF-β siRNA, DOX	Immune Modulation, Chemotherapy	[158]
GBM	TMZ, DOX	Tumor Growth, Survival	[159]
GBM	N/A	Immune Activation	[160]
MSC	miR-124, miR-145	Cell Invasion	[161]
BMSC	miR-512-5p	Cell Growth, Survival	[162]
MSC	miR-146b	Tumor Growth	[100]
MSC	miR-199a	Cell Growth, Chemosensitivity	[163]
GBM	AMO-21	Tumor Growth	[164]
EL-4	Exo-cur, Exo-JSI124	Tumor Growth	[165]
b.END3	Doxorubicin	Tumor Growth	[61]
MSC	Anti-miR-9	Chemoresistance	[101]
GBM	Anti-miR-21	Tumor Growth	[166]
Serum	Doxorubicin	Tumor Growth	[167]
DC	N/A	Immune Activation, Tumor Elimination	[168]
DC	N/A	Apoptosis	[169]
Raw264.7	SPIONs, Cur, RGERPPR	Tumor Growth, Theranostics	[170]
Blood	SAB, CPT	Tumor Growth, Angiogenesis	[171]
MSC	GPX4 siRNA, DHODH inhibitor	Ferroptosis, BBB Crossing	[172]
ESC	Paclitaxel (PTX)	Targeted Drug Delivery	[173]
BMSC	TMZ, STAT3 siRNA	TMZ Resistance, Targeted Therapy	[174]

Abbreviations: GBM (Glioblastoma); TMZ (Temozolomide); GSCs (Glioma Stem Cells); miR (Micro RNA sequences); VB (Verbascoside); ZnS (Zinc Sulfide); GL261 (GL261, murine glioblastoma cell line); TAM (Tumor-Associated Macrophage); TGF-β (Transforming Growth Factor Beta); siRNA (small interfering RNA); DOX (Doxorubicin, chemotherapy drug); MSC (Mesenchymal Stem Cells); BMSC (Bone Marrow Stem Cells); EL-4 (EL-4, murine lymphoma cell line); U87 (U87, human glioblastoma cell line); Exo-cur, Exo-JSI124 (Exosome-curcumin, Exosome-JSI124, therapeutic exosome formulations); b.END3 (bEnd.3 cells, Murine Brain Endothelial Cell Line); DC (Dendritic Cells); Raw264.7 (Raw264.7, murine macrophage cell line); SPIONs (Superparamagnetic Iron Oxide Nanoparticles); Cur (Curcumin); SAB (Sorafenib, Anticancer Drug); CPT (Camptothecin, chemotherapy agent); GPX4 siRNA (small interfering RNA targeting Glutathione Peroxidase 4); DHODH (Dihydroorotate Dehydrogenase); ESC (Embryonic Stem Cells); PTX (Paclitaxel); cRGD (Cyclic Arg-Gly-Asp peptide); STAT3 siRNA (small interfering RNA targeting Signal Transducer and Activator of Transcription 3); AMO-21 (Antisense MicroRNA Oligonucleotides targeting miR-21); PDCD4 (Programmed Cell Death 4); PTEN (Phosphatase and Tensin Homolog).

Conventional isolation techniques often fail to meet the purity, scalability, and reproducibility requirements needed for clinical implementation. Ultracentrifugation (UC), historically considered one of the most widely used benchmark methods for EV enrichment, often results in the co-isolation of non-EV contaminants such as protein aggregates, and high shear forces can induce EV structural damage and aggregation [116,119]. These contaminants can confound characterization efforts and lead to inconsistent results [122]. To address these challenges, considerable effort has been directed toward developing more refined isolation techniques tailored for GBM-specific EVs. Size-exclusion chromatography (SEC) has emerged as a useful alternative that can preserve EV integrity and reduce soluble protein contamination, although it often requires secondary concentration steps [116,123,124]. Similarly, microfluidic platforms and immunoaffinity-based capture technologies that exploit GBM-associated surface markers are being developed to enrich tumor-derived EV populations and improve disease specificity [125–127]. However, many of these approaches currently suffer from low throughput, limited recovery, and high cost, limiting their therapeutic scalability [84,120,128]. A summary of the primary EV isolation techniques, alongside their advantages and limitations for clinical translation, is provided in Table 3.

Looking ahead, successful clinical translation of EV-based platforms will require not only improved isolation and characterization technologies, but also the establishment of regulatory and manufacturing frameworks. Advanced characterization tools, such as high-resolution flow cytometry, nanoparticle tracking analysis, and proteomic profiling, are essential for elucidating GBM-EV heterogeneity and for identifying EV subpopulations with enhanced therapeutic potential.

Another significant challenge in advancing EV-based therapeutics for GBM is the scalability and cost-effectiveness of EV production for clinical applications. Traditional cell culture methods, while suitable for preclinical studies, are often insufficient to produce the high EV doses [129] required for therapeutic efficacy, particularly given GBM's infiltrative nature and high recurrence rates. To address these issues, several promising avenues are being explored. One approach involves identifying or engineering high-yielding cell sources [130], such as patient-derived GBM stem cells or GBM cell lines modified for enhanced EV secretion. Another strategy focuses on transitioning from traditional 2D cell culture to 3D culture systems like spheroids or organoids [131, 132]. These systems more accurately recapitulate the TME and show potential for enhancing EV production while preserving functional properties. Additionally, scalable bioreactor systems specifically designed for EV production, potentially incorporating microfluidic technologies or biocompatible scaffolds, are being developed [129,133], holding the potential to enable large-scale, cost-effective EV production without compromising EV quality and therapeutic efficacy.

Finally, although EVs are generally considered safe due to their natural origin, the long-term safety and potential toxicity of engineered EVs destined for GBM treatment require rigorous evaluation. The off-target biodistribution of EVs noted earlier warrants closer consideration in the context of brain-directed therapies. Systematic analyses across diverse cell sources, vesicle sizes, and administration routes have consistently shown that systemically administered EVs accumulate preferentially in the liver, spleen, lungs, and kidneys, where they are rapidly cleared by the mononuclear phagocyte system, leaving only a small fraction that reaches the CNS(86, 87). Furthermore, the route of administration is a major determinant of brain exposure, with intranasal and local intracranial delivery outperforming intravenous injection. Interpretation of biodistribution studies also requires caution, as the apparent tissue biodistribution can vary considerably depending on the labeling strategy used [134]. The GBM microenvironment introduces a further layer of complexity. Notably, the BBB in GBM is heterogeneously disrupted rather than uniformly compromised. Although the contrast-enhancing tumor core is often permeable, the infiltrative margin that harbors the invasive cells responsible for recurrence retains a largely intact barrier, and enhanced permeability therefore cannot be assumed to provide uniform EV access [135]. Moreover, EVs within the tumor are preferentially internalized by tumor-associated macrophages and microglia, an interaction that can be harnessed for immunomodulation, but that also represents a source of off-target distribution requiring careful characterization [136]. These biodistribution patterns have implications for long-term safety. The concern over neurotoxicity is mechanistically plausible, as endogenous EVs released by activated

Table 3
EV isolation methods and key translation challenges in GBM.

Isolation method	Strengths	Limitations	GBM translation challenges	References
UC	1. Widely used 2. Handles larger volumes 3. Low reagent cost	1. Time-consuming 2. Co-isolates proteins or lipoproteins 3. May damage or aggregate EVs	Low purity and variability may reduce biomarker specificity and therapeutic potency	[20,115, 119]
SEC	1. Gentle 2. Preserves EV integrity 3. Reduces soluble protein contamination	1. Diluted fractions 2. Incomplete separation from some lipoproteins	Requires concentration and standardized column or fraction selection for clinical use	[124,175, 176]
DGUC	1. Higher purity than simple UC 2. Separates by buoyant density	1. Slow, labor-intensive, and low-throughput	Useful for research-grade EVs but difficult for routine diagnostics or manufacturing	[20,124, 177]
Polymer Precipitation	1. Rapid, simple, high yield 2. No specialized equipment	1. Low purity 2. Co-precipitates proteins or lipoproteins and polymer residues	Contamination limits omics reliability and therapeutic-grade application	[119,178]
Immunoaffinity and Microfluidics	1. Enriches specific EV subpopulations using surface markers	1. Costly 2. Marker-biased 3. Limited yield/throughput	Promising for GBM liquid biopsy, but heterogeneity and scalability remain barriers	[84,179, 180]

Abbreviations: UC (Ultracentrifugation); SEC (Size-Exclusion Chromatography); DGUC (Density Gradient Ultracentrifugation); EV (Extracellular vesicles); GBM (Glioblastoma).

microglia can propagate neuroinflammation and glial activation and can shuttle pathogenic proteins between cells, illustrating that EV cargo may itself perturb CNS homeostasis [137,138]. However, whether repeated dosing of engineered therapeutic EVs provokes chronic microglial or astrocytic activation, elicits immune responses against engineered surface modifications, or causes off-target gene silencing in normal neural cells remains largely unknown and represents a critical knowledge gap for clinical translation [139]. Encouragingly, early-phase clinical experience with EV-based therapeutics, including dendritic cell- and mesenchymal stromal cell-derived exosomes administered systemically or intranasally, has generally been well tolerated without dose-limiting toxicity [140–142]. To date, however, no EV therapeutic has been evaluated in a registered clinical trial involving direct CNS delivery for GBM, and regulatory authorities have cautioned against the use of unapproved exosome products outside rigorously controlled studies [143].

Addressing the safety and toxicity concerns surrounding engineered EVs, particularly for brain tumor applications, requires a multifaceted approach. Rigorous preclinical safety testing in relevant GBM models, particularly in immunocompetent models that closely mimic human immune responses, is paramount. Such testing should comprehensively assess the biodistribution, pharmacokinetics, pharmacodynamics, and toxicity of engineered EVs within the complex brain TME. Furthermore, clinical trials evaluating EV-based therapies for GBM must prioritize patient safety by implementing robust monitoring protocols. These protocols should include thorough neurological assessments and long-term follow-up to detect and manage potential adverse events meticulously.

Conclusion

Extracellular vesicles represent a promising frontier in GBM therapy, offering a versatile platform to address this challenging disease. Their inherent ability to cross biological barriers, including the blood-brain barrier, positions them as potentially ideal candidates for delivering therapeutic cargo directly to the tumor site, overcoming a major obstacle encountered by conventional therapies. Moreover, their natural capacity to transport a wide range of bioactive agents, including therapeutic molecules, genetic material, and even tumor antigens, opens a vast array of therapeutic possibilities, ranging from targeted drug delivery and gene editing to immune modulation and vaccination.

As highlighted throughout this review, various subtypes of EVs, including those derived from dendritic cells, T cells, brain endothelial cells, and mesenchymal stem cells, have demonstrated promising therapeutic potential in preclinical GBM models (Table 2), paving the way for potential clinical translation. Furthermore, the emerging field of EV engineering, which encompasses strategies for cargo loading, membrane modification, and production optimization, holds immense promise for enhancing the therapeutic efficacy, targeting specificity, and scalability of EV-based therapies.

Nevertheless, several challenges must be addressed before EVs can be fully realized as clinical therapeutics for GBM patients. Standardizing EV isolation and characterization protocols, particularly in the context of the heterogeneous GBM tumor microenvironment, is crucial for ensuring the reproducibility of preclinical research findings and facilitating the development of standardized therapeutic products. Similarly, establishing scalable and cost-effective EV production platforms, potentially leveraging 3D culture systems or bioreactor technology, will be essential for meeting the demands of clinical trials and future therapeutic applications. Moreover, the long-term safety and potential toxicity of engineered EVs must be rigorously assesses through comprehensive preclinical studies and robust safety monitoring protocols in clinical trials.

Despite these challenges, the future of EV-based therapies for GBM remains bright. The rapid pace of research in this field, coupled with the increasing recognition of EVs' therapeutic versatility, suggests that these naturally occurring nanocarriers are well positioned to revolutionize the GBM treatment landscape. Continued exploration into EV biology, engineering, and clinical application is warranted to unlock their full therapeutic potential and pave the way for more effective, personalized, and hopefully curative therapies for GBM patients.

Author contributions

N.C.M., K.C., L.L.: literature review and interpretation, manuscript writing, final approval of manuscript; K.C., K.S.: conception and design, literature review and interpretation, manuscript writing, final approval of manuscript.

Search Strategy and Selection Criteria

References for this Review were identified by searches of PubMed up to July 2026 and by screening references from relevant articles. There were no language restrictions. The final reference list was generated based on relevance to the topics covered in this Review.

Declaration of generative AI and AI-assisted technologies in the manuscript preparation process

During the preparation of this work, the author(s) used Gemini 3.1 Pro for writing clarity. The author(s) reviewed and edited the output as needed and take full responsibility for the content of the published article.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Khalid Shah reports financial support was provided by National Institutes of Health. 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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