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Review Article

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Engineering Dendritic Cells and Their Exosomes for Gene Therapy in Glioblastoma: Emerging Strategies and Translational Perspectives

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ABSTRACT

Glioblastoma (GBM) is the most aggressive and malignant primary cancer of the nervous system and is highly heterogeneous in terms of tumor phenotype. Dendritic cells (DCs) are professional antigen-presenting cells (APCs) that are components of the innate immune system and coordinate the adaptive immune response; however, their function is impaired in the GBM tumor microenvironment (TME). DC-derived exosomes (DEXs) contain APCs and co-stimulatory molecules; they have emerged as cell-free immunotherapeutic vesicles offering potential advantages in terms of stability, safety, and manufacturability. Engineering strategies are being developed through ongoing research. The ability of DEXs to carry tumor antigens, siRNA, miRNA, mRNA, CRISPR/Cas components, cytokines, and targeting ligands engages immune activation through genetic transfer methods. This review article summarizes the biological rationale for DEX-based gene therapy in GBM, targeting blood-brain barrier permeability, the development of engineering strategies, preclinical findings, and GMP principles.

Keywords: Glioblastoma, Dex, Tme, Apc, Therapy

Introduction

The World Health Organization (WHO) classifies glioblastoma as a grade 4 diffuse glioma in adults. Because of features including extensive invasion, significant heterogeneity, intensive angiogenesis, and treatment resistance, glioblastoma continues to be a clinically difficult illness to treat. The largest and safest surgical resection is followed by radiation and temozolomide as part of the current standard treatment protocol. Even while these therapies increase survival, long-term illness control is rarely attained. The majority of immune-based treatments for GBM have demonstrated little efficacy, despite immunotherapy's notable success in treating numerous cancer types. Low immune cell infiltration, immunosuppressive myeloid cell populations, lymphocyte malfunction, and the challenge of medicines passing through the blood-brain barrier are the main causes of this. The mechanisms of antigen collection, processing, presentation, and T-cell activation are all heavily dependent on dendritic cells (DCs). Dendritic cell vaccines have been used to study their potential therapeutic uses in the treatment of glioblastoma. Furthermore, dendritic cell-derived exosomes (DEXs) may be employed as a cell-free therapeutic strategy, according to developments in extracellular vesicle research. DEXs can be designed to act as transporters for immunomodulatory compounds and nucleic acids, as well as retain MHC-peptide complexes and costimulatory proteins [1-6].

Glioblastoma Biology and Tumor Microenvironment

Molecular Definition and Histopathological Characteristics

Glioblastoma (GBM) is a primary malignant tumor of the central nervous system that is extremely infiltrative and physiologically diverse. The term "glioblastoma" is limited to adult-type diffuse astrocytic tumors that are isocitrate dehydrogenase wild-type (IDH-wildtype) and assigned CNS WHO grade 4, according to the fifth edition of the World Health Organization Classification of Tumors of the Central Nervous System (WHO CNS5). Histologically, the presence of necrosis and/or microvascular growth may establish a diagnosis. However, if an IDH-wildtype diffuse astrocytic tumor in an adult patient has at least one of three distinctive molecular changes telomerase reverse transcriptase promoter mutation, epidermal growth factor receptor amplification, or combined whole-chromosome 7 gain and whole-chromosome 10 loss (+7/−10) it may still be categorized as glioblastoma even if it lacks

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these traditional histological characteristics. Significant cellular pleomorphism, rapid mitotic activity, diffuse infiltration of the surrounding brain tissue, vascular anomalies, and geographically dispersed necrotic areas are the histological characteristics of GBM. Pseudopalisading tumor cells that encircle necrotic foci are thought to be a morphological sign of tumor-cell migration away from locations that are extremely hypoxic. Malignant cells often spread beyond radiographically visible boundaries by migrating along white-matter tracts, perivascular gaps, and pre-existing brain structures, even if contrast-enhanced magnetic resonance imaging often shows a core tumor mass. The high incidence of local recurrence has a biological basis since even comprehensive surgical resection is unable to eradicate all infiltrating tumor cells.[7] Glioblastoma, IDH-wildtype, is distinguished from astrocytoma, IDH-mutant, CNS WHO grade 4, which is a physiologically and clinically unique disease entity, using this comprehensive method.

Genomic Architecture and Oncogenic Signaling

The p53 route, the retinoblastoma cell-cycle regulatory pathway, and receptor tyrosine kinase–RAS–phosphoinositide 3-kinase signaling are the three main regulatory networks that dominate the complex genomic architecture of GBM. EGFR amplification or mutation, PDGFRA amplification, NF1 loss, PTEN deletion or mutation, PIK3CA and PIK3R1 alterations, TP53 mutation, MDM2 or MDM4 amplification, CDKN2A/CDKN2B homozygous deletion, and RB1 disruption are examples of recurrent abnormalities [8,9].

Additionally common are TERT promoter mutations, which facilitate telomerase reactivation and replicative immortality. Nevertheless, these anomalies are not always present in all malignant cells within a single tumor, nor are they consistently distributed among individuals. This genetic mosaicism restricts the longevity of therapies aimed at a single biological target and permits concurrent oncogenic programs to coexist [8].

GBM was first divided into proneural, classical, mesenchymal, and neural expression subtypes by bulk transcriptome investigations. The mesenchymal subtype was linked to inflammatory signaling and NF1 loss, the proneural subtype to PDGFRA-associated programs, and the classical subtype to EGFR abnormalities [10]. These classifications do not reflect mutually exclusive or permanently fixed tumor identities, as later single-cell investigations showed. Rather, a tumor may have more than one transcriptional program, and the relative abundance of each program depends on anatomical location, treatment exposure, genetic changes, and interactions with non-malignant cells. Rather than being a permanent tumor-intrinsic subtype, the previously suggested neural subtype is now widely understood to be heavily impacted by the admixture of non-neoplastic brain tissue [11,12].

Intratumoral Heterogeneity and Cellular-State Plasticity

GBM's biological interpretation has been radically altered by single-cell RNA sequencing. Even among cells isolated from the same surgical tissue, early single-cell investigations showed significant cell-to-cell diversity in the expression of immune-related transcripts, proliferation-associated genes, hypoxia-response programs, and carcinogenic pathways [11]. Four primary malignant cellular states that resemble neural progenitor cells, oligodendrocyte progenitor cells, astrocytes, and mesenchymal cells were found by a later integrative model. The terms neural progenitor cell-like (NPC-like), oligodendrocyte progenitor cell-like (OPC-like), astrocyte-like (AC-like), and mesenchymal-like (MES-like) are frequently used to characterize these states [12]. Tumor genetics has an impact on how these states are distributed. NPC-like programs are linked to CDK4 amplification, OPC-like programs to PDGFRA amplification, AC-like programs to EGFR amplification, and MES-like programs to NF1 loss. However, GBM cells can switch between these phenotypes in response to environmental stressors, according to lineage-tracing and experimental perturbation investigations. As a result, the four-state paradigm should not be seen as a strict categorization where every tumor falls into a single group. Instead, a single tumor is a dynamic blend of biological states that can be converted. This flexibility contributes to treatment resistance by allowing GBM cells to adjust to nutrition restriction, hypoxia, inflammation, radiation, and pharmaceutical stress. Within this dynamic framework, the idea of glioma stem cells must also be taken into account. Although they are abundant in particular cellular populations, stem-like characteristics including self-renewal, tumor initiation, multilineage differentiation, and resistance to cytotoxic stress are not always limited to a permanently defined cell subpopulation. According to single-cell investigations, GBM can partially replicate the developmental hierarchies present in the normal brain system, and under the right microenvironmental circumstances, malignant cells that appear differentiated may regain stem-like characteristics. Therefore, rather than being an invariant phenotype determined by a single surface marker, cellular stemness in GBM is better characterized as a reversible and context-dependent functional state [12,13].

Spatial Organization, Hypoxia and the Perinecrotic Niche

Instead of being dispersed at random, GBM heterogeneity is spatially ordered. Localized tumor patches are often enriched in specific malignant cell states, and recurrent state combinations produce repeatable tissue architectures, according to spatial transcriptomic and proteomic investigations. Cellular-state distributions were coordinated over both short and long distances in a multilayered organization found in a new integrative spatial investigation. Necrotic areas, mesenchymal-like tumor programs, vascular remodeling, and immune-cell localization are all connected by hypoxia, which has been identified as a key long-range organizer of this architecture [14]. The tumor core and infiltration front are biologically separate compartments, as demonstrated by the discovery of radial glial-like stem-cell programs enriched within neuron-rich invasive margins using spatial profiling [15].

Significant oxygen gradients are produced within GBM by rapid tumor growth, vascular failure, and insufficient tissue perfusion. Transcriptional systems involved in glycolysis, angiogenesis, cell survival, invasion, and maintenance of stem-like phenotypes are activated by hypoxia-inducible factors, specifically HIF-1α and HIF-2α. Although the degree and transcriptional makeup of this response differ among tumors generated from patients, hypoxic stress may also encourage a transition toward mesenchymal-like states. Because hypoxia actively reorganizes malignant and non-malignant compartments and creates spatial niches that favor treatment-resistant phenotypes, it should not be seen as solely a passive effect of tumor growth [14-21].

Vascular Microenvironment and Blood–Tumor Barrier

Although GBM is one of the most vascularized cancers in humans, its vasculature is both physically and functionally aberrant. Tumor-associated arteries often have damaged basement membranes, uneven endothelial junctions, variable pericyte covering, and are tortuous, dilated, and poorly structured. Alongside places where the blood–brain barrier or blood–tumor barrier is largely intact; these anomalies result in regions of enhanced permeability. As a result, systemic drug distribution is extremely variable across the infiltrated peritumoral brain as well as within the augmenting tumor core. The vascular compartment does more than just carry oxygen and medicinal substances. Tumor-cell survival and stemness are supported by cytokines, growth factors, and extracellular matrix molecules released by endothelial cells and perivascular stromal cells. Through processes dependent on CSF-1 and IL-6, tumor-associated endothelial cells can stimulate macrophage polarization. Mesenchymal-like endothelial populations that create an immunosuppressive vascular niche, encourage macrophage accumulation, and lessen response to immunotherapy have been found by more recent single-cell and geographic investigations. These findings suggest that vascular anomalies in GBM concurrently affect medication penetration, immune cell function, stem cell maintenance, nutrition delivery, and invasion [13,16].

Immune Composition of the Glioblastoma Microenvironment

Effective lymphocyte-mediated antitumor responses are somewhat lacking in the GBM immunological milieu, which is mostly myeloid. A significant amount of the tumor mass is made up of tumor-associated macrophages, resident microglia, infiltrating monocyte-derived macrophages, dendritic-cell-like populations, neutrophils, and myeloid-derived suppressor cells. Myeloid cells may make up as much as half of all cells in some instances [18,19,21]. The immunological makeup of primary brain tumors is significantly different from that of brain metastases and other extracranial malignancies, as shown by high-dimensional single-cell investigations. This difference reflects both tumor-driven reprogramming and tissue-specific immune limitations [18,19].

The diversity of myeloid populations linked to GBM is not well represented by the conventional binary classification of macrophages as pro-inflammatory M1 or anti-inflammatory M2 cells. Inflammatory, phagocytic, lipid-metabolic, angiogenic, and immunoregulatory processes can all be expressed concurrently by a single myeloid cell. Several recurring immunomodulatory programs, such as microglial inflammatory, scavenger-immunosuppressive, systemic inflammatory, and complement-associated immunosuppressive states, were found by a thorough cross-species examination. Crucially, the developmental origin of the myeloid cell or the tumor's mutational state had less of an impact on these programs than local signals such hypoxia, IL-1β, transforming growth factor-β, and corticosteroid exposure. Because dexamethasone, which is often used to treat cerebral edema, may further alter antitumor immunity, this discovery has significant clinical ramifications [21].

In GBM, lymphocyte infiltration is typically restricted and varies in function. Reduced proliferative ability, decreased cytokine production, and expression of inhibitory receptors linked to long-term antigen exposure and cellular dysfunction can all be seen in tumor-infiltrating T cells. However, the degree of T-cell dysfunction varies among tumors and geographic niches, therefore not all infiltrating lymphocytes should be referred to as "exhausted." HMOX1-positive myeloid cells that release IL-10, colocalize with MES-like tumor areas, and aid in the inhibition of effector T-cell responses have been found by mechanistic and geographic investigations. Therefore, intrinsic T-cell deficiencies cannot be the exclusive cause of poor antitumor immunity in GBM; interactions between the malignant, myeloid, vascular, and lymphoid compartments actively sustain it [20].

The overall quantity of immune cells may not be as significant as the spatial architecture of the immune system. Clinical outcome has been linked to particular cellular neighborhoods and cell-to-cell connections, as shown by imaging mass cytometry of large patient cohorts. Notably, myeloid infiltration is not always harmful because some macrophage populations may be associated with better survival rather than tumor growth. These findings encourage therapies that reprogram specific immunosuppressive states while maintaining or improving potentially tumor-restrictive capabilities rather than tactics intended to randomly eliminate all macrophages or microglia [22].

Extracellular Matrix and Stromal Components

The normal brain's extracellular matrix contains more hyaluronan, proteoglycans, and specific glycoproteins than fibrillar collagen. By producing and redistributing tenascin-C, chondroitin sulfate proteoglycans, laminins, fibronectin, and matrix-degrading enzymes, GBM significantly alters this environment. These modifications affect cell adhesion, migration, integrin signaling, and tissue stiffness. Additionally, matrix components control immune-cell trafficking and may play a role in the development of immune-excluded areas. Pericyte-like and other stromal populations significantly contribute to the expression of extracellular matrix-associated transcriptional programs, which can become more prominent during recurrence [30].

Single-cell RNA sequencing and spatial transcriptomics have revealed cancer-associated fibroblast-like cells in human GBM, despite the fact that traditional fibroblasts are rare in normal brain parenchyma. These populations may encourage tumor-cell invasion, proliferation, and vascular remodeling because they are concentrated near blood arteries. Since the exact developmental origins and frequency of these cells are still being studied, it is not appropriate to presume that their biological function in extracranial carcinomas is the same as that of fibroblasts. However, their discovery shows that the non-malignant compartment goes beyond immune and vascular cells and broadens the acknowledged stromal complexity of GBM [23].

Neuron–Glioma Interactions

The GBM microenvironment is made up of activated neurons and neural circuits. High-grade glioma growth is stimulated by neural activity through both direct electrochemical communication and paracrine signaling, according to experimental research. A portion of malignant glioma cells establish functional synaptic connections with neurons that are dependent on AMPA receptors. Intracellular calcium signaling and depolarizing currents are induced by synaptic input, and these signals may spread

over greater distances through electrically linked tumor-cell networks. As a result, the tumor can functionally incorporate the surrounding neural tissue into the malignant ecosystem rather than just displacing or destroying it [24,25].

It is a reciprocal relationship. GBM cells have the ability to change functional neural networks, restructure cortical connections, and increase neuronal excitability. Increased functional connection between tumor-infiltrated areas and the surrounding brain has been linked to worse cognitive function and a lower chance of survival in human studies. These results offer a biological foundation for thinking of GBM not just as a proliferative mass but as an electrically and synaptically integrated illness. However, depending on the location of the tumor, the malignant cell state, and the makeup of the neural circuit, the frequency and clinical significance of specific neuron–glioma communication routes may differ [21].

Metabolic Heterogeneity

To maintain development in the face of varying oxygen and food availability, GBM cells go through a significant metabolic reprogramming. GBM metabolism is not always glycolytic, despite the fact that aerobic glycolysis is common in many tumors. Metabolically different cancers, such as mitochondrial phenotypes that heavily rely on oxidative phosphorylation and glycolytic or plurimetabolic phenotypes that employ glucose, amino acid, and lipid metabolic pathways, have been discovered using integrated molecular analysis. This variation can be found within the same tumor as well as between people. In addition to malignant cells, immunological and stromal compartments are also impacted by lactate generation, extracellular acidification, lipid accumulation, and altered amino acid consumption. Tumor-associated myeloid cells can adapt to low-oxygen and lipid-rich conditions, while hypoxia and nutritional competition may limit T-cell function. Thus, metabolic flexibility is a collective characteristic of the tumor ecology. Metabolic therapies necessitate biomarker-based patient categorization and assessment of both malignant and non-malignant cells, as therapeutic suppression of a specific metabolic pathway may favor alternate nutrition sources or mitochondrial adaptations [25-30].

Dendritic Cell Biology in Glioblastoma

Biological Functions of Dendritic Cells in Antitumor Immunity

Specialized antigen-presenting cells called dendritic cells (DCs) create the functional link between adaptive antitumor immunity and innate immunological sensing. Tumor-derived material is captured, tumor antigens are processed intracellularly, antigenic peptides are presented via major histocompatibility complex class I and class II molecules, costimulatory signals are delivered, and cytokines that control the differentiation and effector capacity of responding lymphocytes are produced. Because naïve tumor-specific T lymphocytes typically require priming by professional antigen-presenting cells rather than direct stimulation by malignant cells, these roles are especially crucial in cancer. In addition to antigen availability, the presenting DC's maturation state, lineage identification, metabolic fitness, and anatomical location all influence the immune response to antigen presentation. Three synchronized signals are needed for T-cell priming to be effective. The T-cell receptor's identification of peptide–MHC complexes produces Signal 1. Costimulatory molecules, especially CD80 and CD86, engage with CD28 on T cells to provide Signal 2. Cytokines like interleukin-12, type I interferons, and other inflammatory mediators that influence T-cell development, persistence, and cytotoxic activity make up Signal 3. Instead of producing effective anticancer immunity, antigen presentation in the lack of adequate costimulation or inflammatory signaling may cause T-cell anergy, deletion, or regulatory differentiation. Therefore, the existence of DC-like cells in glioblastoma does not always mean that antigen presentation is functionally effective [31-33].

Dendritic Cell Subsets Relevant to Glioblastoma

Multiple ontogenetically and functionally different populations make up human DCs. CLEC9A, XCR1, CD141, BATF3, and IRF8 are expressed by conventional type 1 dendritic cells (cDC1s). They are skilled at absorbing material from dying cells and presenting foreign antigens to CD8-positive T lymphocytes via MHC class I molecules. Additionally, cDC1s stimulate the recruitment of cytotoxic T cells and type 1 immunological responses by producing interleukin-12 and chemokines including CXCL9 and CXCL10. Because of these characteristics, cDC1s are especially important for immune-checkpoint blockage effectiveness and anticancer immunity. CD1c, CLEC10A, FCER1A, and IRF4 expression are frequently used to identify conventional type 2 dendritic cells (cDC2s). They are effective in activating CD4-positive T lymphocytes and presenting MHC class II-dependent antigens, yet under some circumstances they may also cross-present antigens. Depending on the tissue context and environmental cues, cDC2s can promote either inflammatory or regulatory immune responses. They are phenotypically and functionally diverse. The differentiation between genuine DC lineages and monocyte-derived antigen-presenting cells in malignancies has been made more difficult by recent single-cell investigations that have shown DC3-like and transitional populations that share characteristics with both conventional DCs and monocytes. Plasmacytoid dendritic cells are characterized by expression of GZMB, GZMB-associated transcriptional programs, IL3RA/CD123, GZMB, TCF4, CLEC4C and GZMB under resting conditions, although their phenotype changes substantially following activation. Their defining functional property is the capacity to produce large amounts of type I interferons in response to nucleic-acid sensing through endosomal Toll-like receptors. In cancer, however, plasmacytoid DCs frequently display impaired interferon production and may promote regulatory T-cell responses. Their biological contribution to human GBM remains incompletely resolved and may differ according to activation state, treatment exposure and tumor-associated cytokine signaling. Another important population, especially in the production of therapeutic vaccines, is monocyte-derived dendritic cells. These cells are produced experimentally from circulating CD14-positive monocytes, usually by exposing them to interleukin-4 and granulocyte–macrophage colony-stimulating factor. Monocyte-derived DCs are ontogenetically different from circulating cDC1 and cDC2 populations, despite the fact that they can have strong antigen-presenting abilities ex vivo. Therefore, it is not always appropriate to extend findings from monocyte-derived DC vaccines to the biology of endogenous conventional DCs in GBM [31-36].

Anatomical Distribution and Immune Surveillance of the Central Nervous System

Because of the blood-brain barrier, the apparent lack of traditional lymphatic vessels in the brain parenchyma, and the comparatively small number of professional antigen-presenting cells under physiological conditions, the central nervous system was once thought to be an immune-privileged organ. This idea has undergone significant revision. Anatomically specialized compartments such as the meninges, choroid plexus, perivascular spaces, cerebrospinal fluid, and bone marrow associated with the skull are used for immune surveillance. Although dendritic cells are rare in the normal brain parenchyma, they can be found in the meninges and other vascularized border tissues, where they can take part in the surveillance of CNS antigens. The discovery of functioning meningeal lymphatic arteries has made it possible for antigen-presenting cells and CNS-derived antigens to enter both superficial and deep cervical lymph nodes. Tumor-derived antigens in DCs found in the tumor, dura, and cervical lymph nodes can be found in experimental brain tumors. This suggests that peripheral adaptive immunity and brain malignancies are not entirely separate. However, compared to similar processes in many extracranial tissues, antigen drainage and DC migration from the brain seem to be less effective and more physically limited. According to preclinical data, effective anticancer immune priming requires intact meningeal lymphatic drainage. Meningeal lymphatic artery disruption hinders CD8-positive T-cell activation and reduces tumor-associated DC migration to cervical lymph nodes. On the other hand, in experimental brain cancers, meningeal lymphatic vessel enlargement via VEGF-C-dependent processes can augment the immunological effects of radiation therapy and CCL21-mediated DC trafficking. These results lend credence to the idea that DC function in GBM is influenced not only by antigen presentation within the tumor but also by the effectiveness of antigen transport and cellular migration between the tumor, meningeal interfaces, and lymph nodes that drain the tumor [32-37]

Dendritic Cell Infiltration in the Glioblastoma Microenvironment

Numerous DC populations have been seen in both newly diagnosed and recurring high-grade gliomas, according to single-cell transcriptomic, cytometric, and geographic studies. These include migratory, plasmacytoid, cDC1-like, cDC2-like, and DC-like states produced from monocytes. In contrast to tumor-associated macrophages, resident microglia, and infiltrating monocytes, DCs often make up a comparatively minor percentage of the GBM immune infiltrate. Furthermore, because these populations may share HLA-DRA, CD1C, FCER1A, and inflammatory gene-expression patterns, it can be difficult to distinguish between cDC2s, DC3-like cells, activated monocytes, and monocyte-derived macrophages [33,35,38]. DCs linked to gliomas are not always removed from the tumor. They can interact with lymphoid and myeloid populations, enter tumor tissue, and obtain material derived from malignant cells, according to experimental and human studies. However, they often have insufficient antigen-presenting function. DCs derived from tumor tissue showed detectable MHC class I-restricted cross-presentation in mouse glioma models, but their ability to elicit strong antigen-specific T-cell responses was constrained. In human high-grade gliomas, intratumoral DC populations were associated with immature or defective transcriptional states, suggesting that physical presence and antigen uptake may not necessarily translate into successful T-cell priming [33].

The Specialized Role of cDC1 Cells

The most compelling experimental evidence for a non-redundant function in brain-tumor immunity among DC populations is seen in cDC1s. In preclinical GBM models, cDC1s were found in the dura and cervical lymph nodes that drain the central nervous system in addition to capturing detectable tumor antigens within the tumor. The priming of neoantigen-specific CD8-positive T cells was hampered by a genetic or functional loss of cDC1s, which also decreased the survival advantage of immune-checkpoint blocking [34]. Chemokines produced by activated cytotoxic lymphocytes play a role in regulating cDC1 recruitment. XCL1 and XCL2, which interact with XCR1 on cDC1s, can be produced by natural killer cells and activated T cells. Activated cDC1s then generate CXCL9, CXCL10, and CXCL11, which attract effector T cells that express CXCR3. A positive immune-feedback circuit including tumor-cell death, antigen absorption, cDC1 activation, and further T-cell recruitment can be established via this reciprocal interaction. However, due to restricted lymphocyte infiltration, inadequate DC maturation, and the predominance of suppressive myeloid populations, this circuit is weak or incomplete in the majority of untreated GBMs. Analysis of recurrent GBM after neoadjuvant PD-1 inhibition showed enhanced T-cell infiltration along with activation-associated alterations in cDC1 populations. T-cell-attracting chemokines were expressed by interferon-responsive myeloid and DC populations, while activated T cells expressed XCL1, XCL2, and CCL5. Tumor-associated macrophages, however, maintained to be the predominant myeloid population and to express ligands that might bind to T cell inhibitory receptors. These findings imply that although cDC1 activation can be produced in GBM, it might not be enough as a stand-alone therapeutic approach to overcome the more extensive suppressive microenvironment [39].

Antigen Capture, Processing and Cross-Presentation

Mutated proteins, aberrantly produced developmental antigens, cancer-testis antigens, viral-associated antigens described in specific investigations, and proteins released after spontaneous or treatment-induced tumor-cell death are some of the probable sources of antigen found in GBM. DCs can get tumor material by extracellular vesicles, phagocytosis, macropinocytosis, uptake of apoptotic bodies, or transfer of peptide–MHC complexes. After internalization, antigens can either go into the cytosol for proteasomal processing and MHC class I cross-presentation or enter endosomal–lysosomal pathways for MHC class II presentation [31,34]. Because malignant glial cells typically lack the whole set of signals needed to activate naïve CD8-positive T cells, cross-presentation is particularly crucial in GBM. Antigen transfer into MHC class I-processing pathways is made possible by cDC1s' adaptation to protect imported proteins from quick lysosomal breakdown. Antigen abundance, however, is not enough on its own. Pattern-recognition receptor activation, costimulatory molecule overexpression, cytokine production, and migration to an anatomical region with responding T cells must all occur simultaneously for cross-priming to be productive [31]. When opposed to highly immunogenic cancers like melanoma or smoking-associated lung cancer, GBM often has a comparatively low tumor mutational burden. As a result, the neoantigen repertoire

that is accessible may be both geographically and numerically diverse. Only a subset of cancerous cells may express subclonal antigens, which raises the possibility that antigen-specific immunity will eradicate antigen-positive cells while allowing antigen-negative populations to grow. Although they may also contain a large amount of non-immunogenic self-proteins and possibly tolerogenic material, whole-tumor lysate and entire tumor-RNA techniques aim to address this heterogeneity by offering a broad antigenic repertoire [36-40].

Glioblastoma-Induced Dendritic Cell Dysfunction

Numerous elements in the GBM microenvironment have the ability to inhibit DC maturation, differentiation, and antigen presentation. Interleukin-10, transforming growth factor-β, prostaglandin E2, vascular endothelial growth factor, colony-stimulating factors, adenosine, lactate, and other metabolites linked to hypoxia and necrosis are among them. These signals can favor the accumulation of immature or regulatory antigen-presenting states, decrease the expression of MHC molecules, CD80, CD86, and CCR7, and hinder the synthesis of interleukin-12 [32,33,36]. Because it can promote tolerogenic antigen presentation while inhibiting DC maturation and interleukin-12 production, interleukin-10 is especially important. Tumor-associated myeloid populations, rather than only malignant cells, are the primary producers of IL-10 in GBM. In an immunoregulatory milieu where DC-mediated priming is unlikely to provide long-lasting cytotoxic immunity, spatially structured IL-10-producing myeloid cells can restrict effector T-cell function [41]. DC function is further compromised by hypoxia via transcriptional and metabolic pathways. Lactate buildup, extracellular acidification, and glucose restriction can all lower the oxidative and biosynthetic processes needed for DC activation. While long-term exposure to damage-associated chemicals may result in dysfunctional rather than productive maturation, lipid buildup may impede antigen processing and cross-presentation. Therefore, metabolic circumstances that limit efficient antigen presentation may coexist with the necrotic and hypoxic areas that provide an abundance of tumor antigen. DC activity may also be indirectly suppressed by monocytes and macrophages linked to GBM. These cells significantly outnumber traditional DCs, produce immunoregulatory cytokines, and compete for tumor-derived material. Certain monocytes develop DC-like surface markers without attaining the functional characteristics of fully developed conventional DCs. As a result, lymphocytes that may exhibit MHC molecules but are ineffective at priming naïve tumor-specific T cells numerically dominate the resulting antigen-presenting compartment. Another clinically significant suppressive factor is dexamethasone, which is frequently used to lessen cerebral edema. Glucocorticoids inhibit the maturation of antigen-presenting cells, lymphocyte activation, and the generation of inflammatory cytokines. Corticosteroid exposure should be taken into account when evaluating immune-monitoring data and planning DC-based immunotherapy trials, however the extent of this effect depends on dose, duration, and timing. Although certain lymphodepleting regimens may theoretically produce homeostatic conditions that encourage the formation of antigen-specific clones, temozolomide-induced lymphopenia may also restrict the pool of T cells accessible for vaccine-induced expansion. Thus, the overall effect depends on the context [33,38].

Dendritic Cells and Treatment-Induced Immunity

By promoting tumor-cell death, antigen release, and inflammatory signaling, radiation therapy can affect DC biology. DNA damage may increase DC maturation and cross-presentation by triggering type I interferon production and cyclic GMP–AMP synthase–stimulator of interferon genes signaling. Radiation therapy, however, can potentially harm lymphocytes, change the integrity of blood vessels, and encourage the recruitment of suppressive myeloid populations. The integrity of meningeal lymphatic drainage, radiation dose, fractionation, and therapy timing all affect the immunological outcome [37]. Temozolomide and DC-mediated immunity have a similarly complicated connection. Tumor antigens may be released more often as a result of cytotoxic treatment, but it can also impede immune cell proliferation and result in systemic lymphopenia. Because DC-induced T-cell priming necessitates a sufficiently wide and functioning lymphocyte repertoire, severe or protracted lymphopenia is unlikely to favor vaccination effectiveness. Thus, a crucial part of DC-vaccine protocols is the scheduling of leukapheresis, vaccine manufacture, chemoradiotherapy, and vaccine administration. Inadequate antigen presentation is not directly replaced by immune-checkpoint blockage. Rather, it mostly eliminates inhibitory signals from T cells that have already been activated. As a result, when tumor antigens are not effectively collected and delivered, checkpoint inhibitors have a lower chance of success. This interpretation is supported by preclinical evidence that cDC1 deficiency decreases the therapeutic effect of checkpoint blockage. Therefore, rational combinations might need to simultaneously increase DC recruitment or activation and block T-cell checkpoints [34].

Biological Basis of Dendritic Cell Vaccination

The majority of clinical DC vaccinations for GBM are produced using leukapheresis-derived autologous peripheral-blood monocytes. GM-CSF and IL-4 are used to differentiate monocytes ex vivo before they are loaded with tumor-associated antigen. Autologous whole-tumor lysate, acid-eluted tumor peptides, specified synthetic peptides, entire tumor RNA, messenger RNA encoding specific antigens, tumor-derived extracellular vesicles, and preparations enriched for glioma stem-like-cell antigens are examples of antigen-loading techniques [36,42]. The generated immune response's specificity and range are determined using the antigen-loading technique. Defined peptide vaccines are limited by HLA type and susceptible to antigen loss, yet they allow for standardized production and immunological monitoring. A wider range of common and patient-specific antigens are present in whole-tumor lysate, which may lessen the possibility of single-antigen escape. Nevertheless, different patients have different lysate compositions, and not all of the proteins are immunogenic. Endogenous antigen processing and presentation across all MHC classes can be supported by whole tumor RNA, but this needs adequate high-quality tumor material and consistent manufacturing processes. DC maturation is just as significant. Although they can promote tolerance and offer insufficient costimulation, immature DCs are effective in absorbing antigens. Mature DCs are more able to migrate toward CCL19 and CCL21 in lymphoid tissues and express larger quantities of MHC molecules, CD80, CD86, and CCR7. TNF-α, IL-1β, IL-6, and prostaglandin E2 are examples of traditional maturation cocktails.

Prostaglandin E2 increases the ability to migrate, although it may also decrease the production of IL-12. To create more immunogenic DC products, alternative maturation techniques employing Toll-like receptor agonists, type I interferons, interferon-γ, CD40 activation, or combinations of these agents are being researched. Biological activity is also influenced by the administration site and route. Although subcutaneous and intradermal injection are frequently employed, only a small percentage of injected DCs are able to move to draining lymph nodes. In a small GBM research, preconditioning the vaccination site with tetanus–diphtheria toxoid enhanced DC migration to lymph nodes and was linked to better immunological and clinical outcomes, partially via a CCL3-dependent mechanism [43]. This finding shows that the capacity of given cells to reach lymphoid tissues is just as important to vaccine efficacy as antigen loading.

Clinical Evidence for Dendritic Cell Vaccination

Autologous DC vaccination is often viable and not linked to the systemic toxicities frequently seen with rigorous cytotoxic treatment or CAR-T-cell therapy, according to early-phase research. Injection-site responses, fever, exhaustion, and brief inflammatory signs have typically been reported side effects. However, in terms of illness stage, antigen source, DC production technique, concurrent treatment, and survival calculations, early studies were often small, single-arm, and varied. Therefore, it is important to interpret comparisons with historical controls with caution [36,42]. Six tumor-associated peptide epitopes from AIM2, HER2, IL-13Rα2, MAGE1, TRP2, and gp100 were included in the autologous DC product ICT-107. ICT-107 increased median progression-free survival by around 2.2 months in a randomized, double-blind, placebo-controlled phase II trial of individuals with recently diagnosed GBM, but it did not result in a statistically significant improvement in the primary overall-survival goal. Although these subgroup data were insufficient to create conclusive predictive biomarkers, exploratory analysis indicated that benefit might vary depending on HLA type and antigen expression.[44] Cytomegalovirus pp65 RNA-loaded DC vaccines have also been evaluated on the basis of reports that CMV-associated proteins are detectable in a subset of GBMs. Sequential early-phase studies reported reproducible immune responses and prolonged survival in selected patient cohorts, particularly when vaccination was combined with tetanus–diphtheria preconditioning. However, these trials were relatively small, and the prevalence, biological relevance and consistency of CMV antigen expression in GBM remain debated. Larger randomized trials are required before CMV-directed DC vaccination can be considered an established treatment strategy [43,45]. DCVax-L was assessed in patients with newly diagnosed or recurrent GBM in the largest documented study of an autologous tumor-lysate-loaded DC vaccination. According to the study, newly diagnosed patients receiving DCVax-L had a median overall survival of 19.3 months from randomization, while a contemporaneous matched external-control sample had a median overall survival of 16.5 months. The median survival time for recurrent GBM was 7.8 months for external controls and 13.2 months for vaccinated patients [46]. Although these results show promise for therapeutic activity, cautious methodological interpretation is necessary. Since the majority of control patients eventually received the vaccine, the final overall-survival analysis relied on external control cohorts rather than an internally randomized contemporaneous comparison. The original study featured randomization and considerable crossover. Although they cannot totally eliminate variations in eligibility, supportive care, imaging evaluation, post-progression treatment, and unmeasured prognostic variables, external controls can be instructive, especially when crossover jeopardizes the original control group. Additionally, the trial's first progression-free-survival endpoint did not show a discernible advantage, in part because radiographic evaluation was hampered by immune-associated inflammatory alterations and pseudoprogression. As a result, while the DCVax-L results are clinically favorable, they are not comparable to confirmation from a traditional randomized phase III overall-survival comparison.[47] DC immunization in conjunction with standard therapy was linked to better overall survival, according to an updated 2024 meta-analysis that included randomized evidence and the externally controlled phase III dataset. The evidence gathered was statistically consistent with a real survival benefit, according to trial-sequential analysis. There was still significant variation in vaccination composition, antigen source, maturation technique, treatment timing, and patient selection, notwithstanding the small number of randomized studies that were available. There was less consistent evidence of progression-free survival [48].

Determinants of Response and Resistance

Both patient-specific and vaccine-intrinsic factors are anticipated to influence the clinical activity of DC immunization. DC lineage, maturation phenotype, viability, antigen-loading efficiency, IL-12 production, CCR7-dependent migration, and the ability to excite polyfunctional T cells are all pertinent vaccination attributes. Age, performance status, tumor burden, MGMT promoter methylation, degree of resection, corticosteroid exposure, lymphocyte numbers, HLA genotype, and the initial makeup of the tumor immune microenvironment are all patient-related factors. Because the ratio of effector lymphocytes to malignant cells is higher and systemic immunosuppression may be less severe, minimal residual illness may be a more favorable environment for vaccination than bulky recurring tumor. On the other hand, recurrent GBM is often enriched in cellular states resistant to treatment and may be linked to increased exposure to corticosteroids, lymphopenia, and myeloid-mediated suppression. The capacity of vaccine-induced T lymphocytes to infiltrate and function inside the tumor may be restricted by these parameters. One of the key mechanisms of resistance is still antigen heterogeneity. Target antigens may be absent from some malignant cells or may be downregulated under immunological pressure, even in cases when vaccination produces detectable peripheral T-cell responses. Tumor recognition may be further hampered by defects in antigen-processing machinery, decreased expression of MHC class I, and local inhibitory ligands. Peripheral immune activation is therefore not always indicative of successful intratumoral cytotoxicity [40-48].

Emerging Strategies to Improve Dendritic Cell-Based Immunotherapy

The creation of medicinal compounds that are cDC1-enriched or cDC1-like is one new tactic. Because monocyte-derived DCs can be produced in comparatively large amounts, they are used in the majority of current GBM vaccines. Nonetheless, cDC1s have a more distinct non-redundant role in CD8-positive T-cell priming and exhibit higher cross-presenting capability. Biologically different alternatives to traditional monocyte-derived vaccines may be offered by strategies based on FLT3

ligand-mediated expansion, isolation of naturally circulating CD141-positive cDC1s, differentiation of DC progenitors, or genetic programming of DC-like cells [31,34]. Improving DC migration and maturation is another major priority. Toll-like receptor 3 or 7/8 agonists, STING agonists, CD40 agonism, type I interferons, IL-12, FLT3 ligand, and modification of the CCL19–CCL21–CCR7 axis are examples of possible therapies. Although this approach is still primarily preclinical in GBM, manipulation of meningeal lymphatic function may also enhance the transport of tumor antigen and migratory DCs to cervical lymph nodes. Since DC vaccination only tackles one aspect of the cancer-immunity cycle, combination therapy will likely be necessary. Radiation therapy, checkpoint inhibitors, oncolytic viruses, tumor-treating fields, myeloid-cell reprogramming agents, and therapies that lessen metabolic suppression are examples of possible partners. The timing of injection is crucial: checkpoint suppression should occur concurrently with the growth and tumor entrance of vaccine-induced T cells, while tumor-cell death should produce antigen when DCs are able to mature. Standardized DC-product characterisation, prospective molecular stratification, and integrated immune monitoring should all be included in future trials. Vaccine-cell phenotype, IL-12 production, migratory capacity, T-cell receptor clonality, antigen-specific T-cell responses, circulating myeloid populations, and geographic examination of tumor tissue after therapy are all pertinent metrics. Improvements in supportive care and separating treatment effects from patient-selection bias still require randomized designs with well-defined control groups [37].

Dendritic Cell-Derived Exosomes: Biogenesis, Composition and Therapeutic Potential Terminological and Biological Framework Dendritic cell-derived exosomes, also known as DC-Exo or DEX, are membrane-bound extracellular vesicles that are released by dendritic cells via the endosomal route. Peptide–major histocompatibility complex complexes, adhesion molecules, immune-regulatory proteins, lipids, and nucleic acids are among the molecular traits of their parent cells that they maintain. Due to these characteristics, DEX has attracted a lot of attention as physiologically produced nanocarriers for cancer immunotherapy and as cell-free antigen-presenting platforms. However, methodological caution should be exercised while using the term "exosome." Extracellular vesicles should not be categorized based merely on particle size, density, or the presence of frequently used markers as CD9, CD63, CD81, TSG101, or ALIX, according to the Minimal Information for Studies of Extracellular Vesicles 2023 guidelines. Technically speaking, the term "exosome" refers to vesicles produced as intraluminal vesicles inside multivesicular endosomes and expelled once these endosomes fuse with the plasma membrane. The more cautious words "small extracellular vesicles" or "dendritic cell-derived extracellular vesicles" are better when endosomal origin has not been proven. While recognizing that many preparations referred to as exosomes are operationally isolated small-EV-enriched fractions rather than homogeneous vesicle populations, DEX is used in this section to maintain consistency with the previous literature [49-51].

Biogenesis of Dendritic Cell-Derived Exosomes

The uptake of extracellular material and components of the plasma membrane through endocytosis is the first step in DEX biogenesis. As early endosomes develop into late endosomes or multivesicular endosomes, certain proteins, lipids, and nucleic acids are integrated into membrane domains that invade the endosomal lumen. Intraluminal vesicles are produced by this inward budding mechanism. Multivesicular endosomes can then merge with the plasma membrane to release intraluminal vesicles into the extracellular space as exosomes, or they can fuse with lysosomes to degrade their contents [50-52]. Cargo selection and intraluminal vesicle production are regulated by a number of partially overlapping molecular mechanisms. ESCRT-0, ESCRT-I, ESCRT-II, and ESCRT-III, the endosomal sorting complexes necessary for transport, identify specific membrane-associated cargo, encourage membrane deformation, and facilitate vesicle-neck constriction and scission. Cargo organization and vesicle formation are facilitated by accessory proteins such as TSG101, ALIX, VPS4, and elements of the syndecan–syntenin axis. The number and molecular makeup of secreted extracellular vesicles are changed by experimental interference with certain ESCRT components, indicating that vesicle synthesis and cargo selection are mechanistically related rather than separate processes.[50] Additionally, DEX production may occur via ESCRT-independent or largely ESCRT-independent processes. Neutral sphingomyelinase activity produces ceramide, which encourages the development of membrane microdomains with biophysical characteristics that favor inward budding.

Tetraspanins, including as CD9, CD63, and CD81, may help selectively incorporate proteins and lipids by organizing membrane proteins into specific domains. The relative contribution of each process appears to rely on the dendritic-cell subtype, maturation status, external stimulation, and cargo being integrated; these mechanisms should not be seen as mutually incompatible [49,52]. Multivesicular endosomes are moved in the direction of the plasma membrane after intraluminal-vesicle production. Different stages of multivesicular endosome placement, docking, and secretion are regulated by Rab-family small GTPases, specifically RAB27A and RAB27B. While RAB27A aids in docking at the plasma membrane, RAB27B has been linked to the redistribution of multivesicular compartments toward the cell periphery. The ultimate membrane-fusion process involves additional Rab proteins, cytoskeletal elements, tethering factors, and soluble N-ethylmaleimide-sensitive factor attachment protein receptor proteins. However, as these molecules may also be involved in other secretory processes, it is not possible to assume that blocking RAB27-dependent pathways will exclusively repress exosomes [53].

Influence of Dendritic-Cell Maturation on Exosome Production

The condition of the parental dendritic cell has a significant impact on the molecular and functional characteristics of DEX. Extracellular antigens are regularly sampled by immature dendritic cells, which are highly endocytic. Their vesicles often exhibit lesser quantities of certain costimulatory and adhesion molecules, although they may contain MHC molecules and antigenic content. Immature or tolerogenic DC-derived vesicles may support regulatory immunological responses, encourage antigen-specific unresponsiveness, or cause mild immune activation, depending on the culture circumstances [50,54]. Dendritic cells experience extensive transcriptional, metabolic,

and membrane-trafficking alterations after being exposed to microbial products, inflammatory cytokines, Toll-like receptor agonists, CD40 activation, or other maturation signals. As a result, DEX produced from activated DCs may have higher concentrations of ICAM-1, MHC–peptide complexes, and certain immune-stimulatory molecules. Compared to vesicles generated by immature DCs, vesicles formed from mature DCs have demonstrated significantly higher antigen-specific T-cell-stimulatory activity in experimental systems. Because it promotes sustained contact with recipient cells that express LFA-1 and enables the effective transfer of vesicle-associated antigens to antigen-presenting cells, ICAM-1 expression is especially significant [55]. However, not all favorable characteristics of DEX are consistently improved with maturation. Vesicle yield, particle heterogeneity, cytokine-associated cargo, and biodistribution can all be affected by strong activation. Certain maturation techniques alter the quantity of particular MHC-peptide complexes or decrease antigen absorption while increasing costimulatory capacity. Therefore, rather than assuming that maximal phenotypic maturation always results in the best therapeutic vesicle preparation, DEX manufacture necessitates choosing a parental-DC maturation technique based on the intended biological mechanism.

Molecular Composition

Membrane and Antigen-Presentation Molecules

DEX possess a lipid bilayer whose transmembrane proteins generally maintain the same orientation as those on the plasma membrane or limiting membrane of the parental endosomal compartment. Characteristic DEX-associated proteins include MHC class I and MHC class II molecules, CD9, CD63, CD81, TSG101, ALIX, flotillins, heat-shock proteins and cytoskeletal or membrane-trafficking proteins. Nevertheless, no single protein constitutes a universally specific exosome marker, and the abundance of these proteins varies among preparations [49,51,56]. The main characteristic that sets DEX apart from the majority of other extracellular-vesicle populations in the context of immunotherapy is the presence of peptide-loaded MHC molecules. While MHC class II-peptide complexes can support CD4-positive T-cell responses, MHC class I-peptide complexes may aid in the activation of antigen-specific CD8-positive T cells. Both MHC classes were found on vesicles released by dendritic cells and within multivesicular endosomes, according to early ultrastructural and biochemical investigations [56-58]. Costimulatory and adhesion molecules, including as CD80, CD86, and ICAM-1, may also be seen in DEX, albeit their quantity varies depending on DC origin and maturity. Integrins, MFGE8, CD40, CD83, and complement-regulatory molecules are possible additional surface components. Vesicle binding, cellular absorption, lymphoid-tissue distribution, and the quality of the ensuing immune response can all be influenced by these proteins. Therefore, rather than being a fixed set of universal indicators, DEX composition should be viewed as an integrated molecular phenotype reflecting both dendritic-cell lineage and manufacturing conditions.

Lipid Composition

Lipids linked to organized membrane microdomains, such as ceramide, cholesterol, sphingomyelin, and glycosphingolipids, are abundant in the DEX membrane. These lipids support connections with recipient cells, vesicle formation, and membrane integrity.

Phosphatidylserine can mediate binding to bridging molecules, scavenger receptors, or phosphatidylserine-recognizing receptors when it is exposed on the outer surface of vesicles. Nevertheless, phosphatidylserine is not exclusive to DEX; it is also present on apoptotic particles and a number of extracellular vesicle types. DEX's lipid makeup may have an impact on immunological response, cellular absorption, and circulation. Lipids are more than just structural elements; they can alter recipient immune cells' metabolism or act as signaling mediators. However, vesicle characterisation may be complicated by lipid contamination from blood, lipoproteins, or culture additives [49].

Nucleic-Acid and Soluble Cargo

Messenger RNAs, microRNAs, various non-coding RNAs, and, in certain experimental settings, DNA-associated material can all be found in DEX. Cargo integration may be selective since the nucleic-acid profile may not always replicate the entire transcriptome of the parental DC. When transported at biologically adequate amounts, vesicle-associated RNAs may modify gene expression in recipient cells; however, the functional significance of individual RNA molecules must be demonstrated experimentally rather than deduced exclusively from sequencing data. DEX may contain immune-regulatory proteins, chaperones, and cytosolic enzymes. While certain DC-derived vesicle preparations have been found to contain molecules including TNF, Fas ligand, and TRAIL, heat-shock proteins can aid in antigen processing and innate immune activation. These TNF-superfamily ligands activated caspase in vulnerable tumor cells and aided in the activation of natural killer cells in experimental animals. It is not appropriate to generalize the existence and functional importance of such molecules to all DEX products because they are extremely reliant on the parental-cell activation technique [59].

Mechanisms of Antigen Presentation

DEX may activate the adaptive immune system in a number of non-exclusive ways. Vesicle-associated peptide–MHC complexes engage with antigen-specific T-cell receptors in the direct pathway. Because isolated DEX lack cellular motility, persistent cytokine secretion, and the dynamic construction of an immunological synapse, they are typically less effective than complete mature dendritic cells at stimulating naïve T cells, despite the physiologic possibility of this technique. In the indirect pathway, recipient dendritic cells or other antigen-presenting cells internalize DEX, process their protein cargo and present DEX-derived peptides on newly synthesized host MHC molecules. This mechanism enables antigen presentation by endogenous dendritic-cell subsets that possess efficient migratory and costimulatory functions. It may also permit the processing of multiple proteins rather than limiting immunity to the preformed peptide–MHC complexes carried on the vesicle surface [57,60]. Cross-dressing, often known as semi-direct presentation, is a third mechanism. This procedure transfers intact peptide–MHC complexes from DEX to recipient antigen-presenting cells' surfaces without fully degrading and reprocessing the antigen. The adhesion molecules, costimulation, and cytokines needed for T-cell activation are then supplied by the recipient cell. Antigen-

bearing DC-derived vesicles have been shown in experiments to primarily activate naïve CD4-positive and CD8-positive T lymphocytes through contacts with recipient dendritic cells [55,57,60]. The maturation status of the donor DC, recipient-cell population, antigen abundance, MHC compatibility, and vesicle administration method all affect how important direct, indirect, and semi-direct antigen presentation is. Because DEX can serve as both transferable antigen storage and preassembled antigen-presenting structures, this mechanistic diversity has therapeutic benefits. On the other hand, it makes potency testing more difficult because in vivo immunological activity may not be predicted by particle quantity or total MHC abundance alone.

Activation of Innate Immunity

DEX can affect both antigen-specific T lymphocytes and natural killer cells. Certain DEX preparations include ligands for IL-15 receptor α, NKG2D, and other molecules involved with the survival, proliferation, and cytotoxicity of NK cells. Antitumor responses in mouse systems were aided by DEX-dependent NK-cell activation, especially in cases when adaptive T-cell responses were insufficient. The NKp30 ligand BAG6 was present in IFN-γ-matured DEX utilized in phase II non-small-cell lung cancer research. BAG6 abundance and NKp30-dependent NK-cell activities were associated with higher MHC class II expression in the finished product. These findings imply that features of DEX products may affect innate immune response without regard to the tumor-antigen load of those products. However, a vesicle marker's validity as a predictive potency assay is not shown by a link between the marker, immune activation, and clinical result [61,62].

DEX as Cell-Free Cancer Vaccines

Exosomes generated by tumor-peptide-pulsed dendritic cells stimulated antigen-specific cytotoxic T lymphocytes and reduced or eliminated existing tumors in animal experiments, which provided the first evidence for DEX-based cancer immunotherapy. These studies demonstrated that the immunological information needed for tumor-specific immunity might be transferred via a subcellular product. Theoretically, DEX is superior to living DC vaccines in a number of ways. They cannot proliferate after administration or differentiate into unwanted cell states since they are non-replicating vesicular products. Their function is less reliant on the migration and survival of given dendritic cells in vivo. DEX may be cryopreserved, kept, standardized according on particle and molecular properties, and altered following isolation. These benefits, however, are still dependent on the creation of proven manufacturing, storage, and potency protocols. By pumping parental DCs with synthetic peptides, recombinant proteins, tumor lysates, tumor-derived extracellular vesicles, or apoptotic tumor material, tumor antigens can be added to DEX. Moreover, mRNA or DNA expressing tumor-associated antigens can be transfected into parental DCs. Although it is limited by HLA type and predetermined antigen selection, peptide loading provides comparatively accurate control. While whole-tumor preparations increase antigen coverage, they also bring patient-to-patient variability and may transfer immunosuppressive or non-immunogenic material. Exogenous peptides may attach directly to accessible MHC molecules without going through natural intracellular processing, which is a significant drawback of surface peptide loading. This may produce vesicle products that target a limited selection of epitopes, even if it may enhance the density of defined peptide–MHC complexes. Reliance on one or a small number of antigens raises the risk of antigen-negative escape in heterogeneous malignancies like GBM [60].

DEX as Therapeutic Delivery Systems

DEX are being studied as carriers for proteins, nucleic acids, and small-molecule medications in addition to immunization. After vesicle isolation, cargo can be added directly or indirectly by altering the primary dendritic cell. Targeting ligand expression, transfection with antigen-encoding RNA, and genetic fusion of therapeutic proteins with vesicle-associated membrane proteins are examples of parental-cell strategies. Electroporation, sonication, membrane permeabilization, incubation with hydrophobic medications, and chemical conjugation to surface molecules are examples of post-isolation techniques. Every loading technique has its limitations. In general, passive incubation is more effective for hydrophobic chemicals than hydrophilic ones; electroporation might result in RNA aggregation and membrane degradation; sonication may change vesicle integrity; and genetic editing of the parental DC may change its physiology and vesicle-production profile. Therefore, it is necessary to quantify both the maintenance of biological activity and the efficiency of cargo loading.

Improved tissue targeting or immune-cell interaction can be achieved through surface modification. Antibodies, antibody fragments, integrin-binding motifs, receptor-binding peptides, and ligands for tumor-associated receptors are examples of potential targeting ligands. Nevertheless, the selective accumulation of intravenously delivered DEX in the targeted tumor is not guaranteed by the addition of a targeting ligand. Circulating vesicles are frequently eliminated by the liver, spleen, and other reticuloendothelial tissues. Particle size, surface chemistry, dosage, administration method, and host immunological status all affect biodistribution [65].

Clinical Experience

Autologous DEX was assessed in patients with advanced non-small-cell lung cancer and metastatic melanoma in the initial phase I clinical trials. Autologous monocyte-derived DCs were loaded with MAGE-associated peptides and utilized to generate DEX in the melanoma study. DEX containing MAGE peptides were similarly given as an autologous product in the NSCLC experiment. Both investigations showed that repeated dosing and clinical-grade output were possible with generally acceptable safety profiles. The purpose of these phase I trials was not to offer conclusive proof of clinical efficacy. Objective tumor regressions were rare, and tumor-specific T-cell responses were uneven. Small cohort sizes, advanced disease, and the lack of randomized control groups made it impossible to reliably attribute clinical benefits to DEX therapy, while some patients showed signs of innate immune activation or disease stabilization [64,65]. A later phase II study examined IFN-γ-matured DEX as maintenance immunotherapy for patients with advanced inoperable non-small cell lung cancer (NSCLC) following platinum-based chemotherapy. A progression-free survival rate of at least 50% four months following the conclusion of treatment was the predetermined primary outcome. The trial did not demonstrate a strong enough clinical benefit to classify DEX as a viable

conventional maintenance medication, despite the fact that the treatment was well tolerated and evidence of NK-cell activation was seen. Longer progression-free survival was associated with NKp30-dependent NK activity, although these associations were hypothesis-generating rather than confirmatory. Therefore, while the clinical research shows a typically controllable safety profile and practicality, it does not yet offer high-level proof of efficacy. No DEX-based cancer treatment has established itself as a standard of care [62].

Relevance to Glioblastoma

Because GBM combines significant antigenic heterogeneity with impaired dendritic-cell maturation, restricted T-cell invasion, and a highly immunosuppressive myeloid milieu, DEX are conceptually relevant to this disease. Preformed peptide–MHC complexes and more extensive tumor-derived antigenic material might conceivably be delivered by a DEX-based vaccine without just relying on the migration and survival of ex vivo-generated DCs.

However, there are still a number of GBM-specific obstacles. Systemic injection does not guarantee therapeutically meaningful vesicle accumulation across invaded brain tissue, as the blood–brain and blood–tumor barriers are spatially diverse. Therefore, it is important to exercise caution when interpreting claims that exosomes naturally pass the blood-brain barrier. Under some experimental circumstances, some EV populations can enter the central nervous system; however, transport is dependent on the vesicle source, surface molecules, delivery route, breakdown of disease-associated barriers, and experimental detection technique. Tumor-reactive T cells must travel to the brain, enter the tumor, and continue to operate in the context of TGF-β, IL-10, hypoxia, adenosine, corticosteroid exposure, and suppressive myeloid populations, even if peripheral immunization is successful in expanding these cells. Therefore, when employed as a monotherapy, DEX immunization is unlikely to defeat the entire GBM immune-evasion network. Additionally, endogenous and therapeutically given dendritic-cell responses may be compromised by extracellular vesicles produced from GBM. According to a 2024 preclinical study, glioblastoma-derived vesicles stimulated lipid accumulation, transported lipid-associated material to dendritic cells, and caused ferroptosis-associated dysfunction via the NRF2–SLC7A11–GPX4 axis. In a rat model, lowering RAB27A-dependent tumor-vesicle secretion was linked to higher dendritic cell abundance, more CD8-positive T-cell infiltration, and slower tumor progression. Direct evaluation of DEX-based approaches in glioblastoma has started in recent preclinical research. Dendritic cells exposed to glioma cells treated with photodynamic treatment generated a DEX preparation high in immune-regulatory microRNAs in a 2026 murine investigation. In immunocompetent, but not immunodeficient, glioma-bearing mice, the delivery of these vesicles was linked to reduced tumor growth and longer survival. They also increased DC maturation and T-cell activation. It was suggested that miR-152-3p contributes via controlling the DNMT1–MyD88 axis. These results are preclinical and do not prove efficacy in human GBM, although supporting an immune-dependent mechanism [66,67].

Isolation, Purification and Characterization

Differential ultracentrifugation, density-gradient centrifugation, size-exclusion chromatography, ultrafiltration, tangential-flow filtration, precipitation, immunoaffinity capture, or combinations of these techniques can be used to remove DEX from DC-conditioned media. According to vesicle biogenesis, no single technique offers total separation. While precipitation-based techniques often produce preparations with significant non-vesicular contamination, high-speed centrifugation may co-isolate protein aggregates and lipoprotein-like particles. Although it may necessitate extra concentration steps, size-exclusion chromatography typically enhances separation from soluble proteins. A scalable combination, such as conditioned-medium clarification, filtration, ultrafiltration, or tangential-flow concentration, followed by purification based on density or chromatography, is frequently needed in clinical production. Yield, purity, biological activity, scalability, affordability, and regulatory compatibility must all be balanced in the procedure that is chosen. Because process modifications may affect the vesicle subpopulations retained in the finished product, they cannot be considered merely technical. Complementary characterisation techniques are advised by MISEV2023. Nanoparticle tracking analysis, adjustable resistive pulse sensing, and other verified single-particle techniques can be used to evaluate particle concentration and size distribution. Atomic-force microscopy or electron microscopy can be used to study vesicle morphology. Membrane-associated EV proteins, cytosolic EV-associated proteins, and an evaluation of potential contaminants or non-vesicular components should all be included in protein analysis. MHC class I and II, antigen-specific peptide–MHC complexes when detectable, CD80, CD86, ICAM-1, and pertinent engineered molecules should all be evaluated further for DEX products. Dosage or efficacy should not be determined exclusively by absolute particle number. Antigen density, costimulatory proteins, RNA cargo, contaminating proteins, and biological activity can vary significantly across two preparations with comparable particle densities. Particle-to-protein ratios cannot determine identity or function, but they can offer evidence of purity [49].

Engineering Strategies for DEX-Based Gene Therapy Conceptual Framework and Terminological Considerations

Dendritic cell-derived exosomes (DEX) are a physiologically active subclass of extracellular vesicles that may be used as platforms for gene transfer and cell-free immunotherapy. Their ability to simultaneously transport therapeutic nucleic acids or proteins and retain immunologically significant components derived from the parental dendritic cell, such as adhesion molecules, peptide–major histocompatibility complex complexes, and specific costimulatory proteins, sets them apart from many synthetic nanocarriers. As a result, designed DEX may serve as both delivery systems and immune-modulating structures that can affect tissue-specific immunological responses, cell activation, and antigen presentation. Nevertheless, care should be used when using the term "DEX." Vesicles should not be given an exosomal origin based just on size, density, or the presence of CD9, CD63, CD81, TSG101, or ALIX, according to the Minimal Information for Studies of Extracellular Vesicles 2023 guidelines. The more general term "dendritic cell-derived extracellular vesicles," often known as DC-EVs or DEVs, is more methodologically correct unless endosomal biogenesis has been proven. Although many experimentally isolated preparations might be better classified as small-EV-enriched

fractions, DEX is kept in this section due to its widespread use in the medicinal literature. Additionally, there are many biological and regulatory interpretations of the phrase "DEX-based gene therapy." Messenger RNA, microRNA, small interfering RNA, antisense oligonucleotides, splice-modifying oligonucleotides, plasmid DNA, gene-editing components, and intracellular regulatory proteins are all included in this broad experimental environment. However, not all regulatory jurisdictions classify oligonucleotide-based therapies as gene-therapy pharmaceutical goods. Therefore, therapies that alter gene expression, RNA processing, protein synthesis, or genomic sequence via a DEX-mediated delivery mechanism are collectively referred to here as DEX-based gene therapy [68-70].

Engineering Objectives

An effective DEX-based gene-delivery system must satisfy several interconnected requirements. The therapeutic cargo must be incorporated at a reproducible and biologically meaningful level; the vesicle must remain structurally intact during production and storage; the engineered surface must direct the product toward the intended tissue or cell population; and the internalized cargo must reach the appropriate intracellular compartment. For RNA-interference agents and messenger RNA, delivery to the cytosol is required. Plasmid DNA generally requires subsequent nuclear entry, whereas CRISPR-associated systems must deliver an active ribonucleoprotein complex, editor-encoding RNA or nucleic-acid template to the relevant subcellular compartment. These requirements demonstrate that vesicle uptake is not equivalent to functional delivery. A DEX preparation may bind to target cells and enter endosomal compartments without releasing a sufficient proportion of its cargo into the cytoplasm. Similarly, high loading efficiency does not guarantee biological activity if the loaded nucleic acid is aggregated, chemically damaged, incorrectly oriented or inaccessible after internalization. The engineering process should therefore be evaluated as a complete sequence comprising parental-cell modification, cargo loading, vesicle isolation, tissue targeting, cellular uptake, endosomal escape and molecular activity. A second design consideration is whether DEX are intended primarily as gene-delivery vehicles, immune vaccines or multifunctional products. In a conventional gene-silencing platform, the principal objective may be cytosolic delivery of a defined siRNA to malignant cells. In contrast, an immunotherapeutic DEX product may be expected to transfer antigenic material to antigen-presenting cells while simultaneously delivering an immune-regulatory RNA or cytokine. The optimal dendritic-cell activation state, targeting ligand and route of administration may differ substantially between these applications [69].

Engineering the Parental Dendritic Cell

By genetically or pharmacologically modifying their paternal dendritic cells, DEX can be indirectly altered. By utilizing normal vesicle biogenesis, this endogenous approach may allow the incorporation of therapeutic proteins, RNAs, and membrane ligands prior to vesicle release. Viral vectors or messenger RNA, plasmid DNA, or non-integrating genetic constructs can be used to transfect dendritic cells. Transgene time, cell viability, innate immune activation, vesicle yield, and the molecular profile of the resultant DEX are all impacted by the engineering technique selected. Transient protein expression is achieved through messenger RNA transfection without the need for nuclear entrance or genomic integration. Thus, it can be used to introduce RNA-binding proteins, cytokines, targeting ligands, or tumor antigens into dendritic cells generated from monocytes. The inherent immunological sensitivity of dendritic cells to foreign RNA and its comparatively brief expression time are its main drawbacks. Although excessive activation may be lessened by modified nucleosides, optimized untranslated sections, and regulated transfection circumstances, the ensuing DC maturation state must be observed rather than presumed. Because nuclear transport is necessary, plasmid DNA often exhibits poorer transfection effectiveness in primary dendritic cells, while it can produce longer transgenic expression than messenger RNA. Nucleofection and electroporation can boost intracellular delivery, but they may also change the amount or makeup of released vesicles and reduce cell survival. In the purified product, residual plasmid DNA must also be assessed, especially if the encoded protein has strong biological activity.

Engineered surface and cargo-loading proteins may be expressed more effectively or sustainably using viral-vector transduction. While adenoviral and adeno-associated viral vectors can produce mostly non-integrating expression depending on the system, lentiviral vectors can integrate steadily. However, clonal selection and insertional mutagenesis are dangers associated with sustained integration in the producer-cell population. Through verified purification and release testing, viral proteins, vector particles, and leftover nucleic acids must be eliminated from the final DEX formulation. An similarly significant engineering variable is the paternal DC's immunological condition. There is no functional exchange between DEX generated by immature, mature, inflammatory, or tolerogenic dendritic cells. While tolerogenic DCs may produce vesicles with larger concentrations of regulatory molecules, mature DCs often produce vesicles with enhanced antigen-presenting and T-cell-stimulatory properties. Therefore, a defined maturation method and a mechanism-based evaluation of MHC expression, CD80, CD86, ICAM-1, cytokine production, and metabolic fitness should be integrated with parental-cell engineering for cancer gene therapy [70].

Endogenous Loading of Therapeutic RNA

Overexpressing the therapeutic RNA in the parental dendritic cell and depending on endogenous integration into secretory vesicles is one method of RNA loading. Technically simple, this approach is frequently ineffective since the majority of intracellular RNA is not immediately sorted into DEX. RNA length, quantity, subcellular location, sequence motifs, secondary structure, and interactions with RNA-binding proteins all affect cargo loading. The selective sorting of RNA into extracellular vesicles has been linked to a number of endogenous RNA-binding proteins. Certain microRNAs, such as miR-223, are incorporated into tiny EVs by Y-box-binding protein 1. While SYNCRIP detects an extra sequence-dependent RNA-sorting signal, sumoylated heterogeneous nuclear ribonucleoprotein A2B1 identifies certain motifs in a subset of vesicle-associated microRNAs. These routes offer a theoretical foundation for recruiting endogenous RNA-binding proteins or creating therapeutic RNAs with vesicle-enrichment patterns. Nevertheless, it is not possible to presume that the RNA-sorting mechanisms found in other producer-cell types function as well in dendritic cells. RNA-binding proteins, endosomal trafficking, cytokine synthesis, and RNA metabolism are all altered by dendritic cell activation. Therefore, it is necessary to validate each created sorting motif in the desired DC source and

maturation state. An RNA-binding domain and an EV-associated membrane protein can be coupled to construct active RNA-loading systems. Cognate MS2 stem-loop sequences were introduced into the cargo RNA and an MS2 bacteriophage coat protein was fused to EV-associated proteins in the targeted and modular EV-loading platform. Although shorter RNA molecules were loaded more effectively than messenger RNA-sized payloads, this design boosted RNA enrichment inside EV preparations. Several synthetic biology modules were coupled in the EXOsomal transfer into cells system, also known as the EXOtic platform, to boost EV synthesis, actively recruit messenger RNA into vesicles, and target specific recipient cells with the final product. The technique added C/D-box sequences to the therapeutic mRNA by fusing a L7Ae RNA-binding protein to an EV-associated protein. To boost vesicle production and facilitate cytosolic distribution, extra ingredients were added. Even though the initial platform was not created in dendritic cells, its modular design might be modified to produce DEX if it has been established that the added proteins do not interfere with antigen presentation or cause an unwanted DC phenotype. In order to enhance the selective loading and purification of EVs carrying desired protein, RNA, or lipid payloads, modified CD63 systems have been developed more recently. An altered CD63-based approach was employed in a 2025 study to improve transport to recipient cells and enrich certain cargo-containing vesicle populations. Because bulk preparations are diverse and only a portion of released vesicles may contain the therapeutic payload, this strategy is pertinent to DEX production. However, comparison with unmodified immunogenic DEX and direct validation in primary human dendritic cells are still required [71-76].

Exogenous Loading After Vesicle Isolation

Another option is to load therapeutic compounds into DEX following vesicle isolation. This method allows the use of standardized DEX batches with various medicinal compounds while separating vesicle formation from cargo inclusion. Passive incubation, electroporation, sonication, extrusion, repeated freeze-thaw cycles, transitory membrane permeabilization, and chemical conjugation are examples of common exogenous loading techniques. Technically, passive incubation is straightforward and results in comparatively little physical disturbance. While some cholesterol-conjugated oligonucleotides may bind to the lipid bilayer, hydrophobic small molecules can partition into the vesicle membrane. On the other hand, unaltered hydrophilic RNAs typically exhibit restricted spontaneous encapsulation. Nuclease-protection tests and the separation of free cargo are crucial because passive association may also produce surface-bound rather than intraluminal cargo. Electroporation has been used extensively to transfer siRNA, microRNA, and certain medications into isolated vesicles while temporarily increasing membrane permeability. In the seminal DEX investigation by Alvarez-Erviti et al., extracted vesicles were electroporated with siRNA after murine dendritic cells were modified to express a Lamp2b–rabies viral glycoprotein peptide fusion. Targeted DEX was able to deliver functionally active siRNA to the mouse brain through intravenous delivery, which resulted in gene silencing in neurons, microglia, and oligodendrocytes.

However, inadequate removal of free RNA, oligonucleotide aggregation, and possible alterations in vesicle integrity have been linked to electroporation. As a result, unless external cargo is eliminated and aggregation restrictions are implemented, apparent loading efficiency may be overstated. Total associated RNA, protected intravesicular RNA, and RNA that is functionally transported to recipient-cell cytosol should all be distinguished by measurements. By momentarily distorting or rupturing the vesicle membrane, sonication and extrusion can enhance cargo integration. Larger molecules or compounds with subpar passive loading properties might benefit from these techniques. On the other hand, they might produce vesicle–vesicle fusion products, eliminate surface-associated MHC complexes, or change the orientation of membrane proteins. Because immune surface protein preservation may be a component of the desired mechanism of action, these implications are especially pertinent to DEX. Although freeze-thaw loading is easy to apply, it can lead to aggregation and uneven particle growth. Although saponin and other membrane-permeabilizing chemicals may boost intravesicular loading, they must be effectively removed since leftover permeabilizer can harm recipient cells. The planned cargo and the significance of maintaining natural DEX membrane organization must thus be taken into consideration while choosing the loading technique [77].

Post-Isolation Surface Functionalization

Targeting ligands can be applied without genetically altering the parental dendritic cell thanks to post-isolation functionalization. This could make producer-cell engineering easier and allow for the adaptation of a single DEX batch for several indications. Covalent conjugation, affinity binding, lipid insertion, electrostatic adsorption, and membrane fusion are other methods. Through interactions between complementary chemical groups on the vesicle and the targeted molecule, click chemistry can introduce ligands. It has been shown that EVs can be surface functionalized utilizing azide–alkyne chemistry without totally losing their vesicle integrity. However, as excessive surface modification can interact with native DEX proteins and speed up clearance, reaction conditions, solvent exposure, and the density of added molecules must be adjusted. Through hydrophobic interactions, lipid-conjugated peptides, polyethylene glycol compounds, or antibodies can spontaneously enter the DEX membrane. This method may result in varying ligand orientation and slow dissociation in biological fluids, but it does not alter vesicle-associated proteins. Although excessive PEG density may decrease target-cell absorption and cause immunological reactions after repeated exposure, polyethylene glycol can extend circulation and decrease non-specific protein adsorption. The CP05 peptide was found to be an affinity ligand for CD63's second extracellular loop. It can be coupled to targeting peptides or therapeutic oligonucleotides to enable cargo attachment and tissue-specific functionalization of EVs from various sources. CP05-mediated loading of a splice-modifying phosphorodiamidate morpholino oligomer enhanced dystrophin restoration in a mouse model lacking dystrophin, especially when a muscle-targeting peptide was added [78-81].

Engineering DEX With Cytokines and Immune-Checkpoint Modulators

In addition to carrying nucleic acid payloads, DEX can be designed to carry immune-regulatory proteins on their surface. This approach is pertinent to gene therapy because, while

surface cytokines or antibodies concurrently control immune-cell activation, a therapeutic RNA may alter a tumor-intrinsic pathway. In 2025, surface-associated interleukin-12 and an anti-CTLA-4 antibody were added to extracellular vesicles generated from dendritic cells.

Tumor neoantigens, lipopolysaccharide, and interferon-γ were used to develop the parental DCs. In animal models, the resultant DEV@IL-12-aCTLA-4 formulation suppressed tumor development, boosted CD8-positive T-cell activity, and improved type 1 immune responses. Additionally, the vesicles facilitated the transport of related immune-regulatory chemicals to lymphatic regions. This study shows that DEX may be transformed from passive delivery vehicles to multifunctional immunotherapeutic platforms. Nevertheless, surface-conjugated IL-12 and anti-CTLA-4 do not function as a gene-delivery system on their own. Their potential to combine surface immune modulation with inside RNA or gene-editing cargo makes them relevant to DEX-based gene therapy. While systemic exposure to IL-12 or checkpoint blockage may result in significant damage, these combinations necessitate careful dose optimization because local immunity augmentation may be desired [82].

Small Interfering RNA and MicroRNA Delivery

One of the most sophisticated gene-regulatory cargoes for engineered DEX is siRNA, which is easier to load than full-length messenger RNA or plasmid DNA. Once released into the cytosol, siRNA can be incorporated into the RNA-induced silencing complex and direct sequence-specific degradation of target messenger RNA. The RVG–Lamp2b DEX study showed that systemic administration of targeted siRNA-loaded vesicles reduced BACE1 messenger RNA and protein in the mouse brain. Despite the disease model was neurological rather than oncological, this experiment is still the most direct evidence of DEX-mediated functional gene silencing across the blood–brain interface. In cancer applications, potential siRNA targets encompass oncogenic receptors, transcription factors, DNA repair proteins, anti-apoptotic agents, and immune-suppressive mediators. In glioblastoma multiforme, pertinent targets include EGFR or EGFRvIII, STAT3, SOX2, OLIG2, MGMT, BCL2 family members, elements of the PI3K–AKT–mTOR pathway, and certain immune checkpoint molecules. These targets, however, should not be regarded as interchangeable. Their expression is spatially variable, and the inhibition of a specific pathway may favor resistant cellular states. MicroRNAs can concurrently regulate numerous transcripts, therefore providing a more extensive mechanism than siRNA. Therapeutic delivery may entail the substitution of tumor-suppressive microRNAs or the suppression of oncogenic microRNAs through the use of antisense compounds. Their pleiotropic activity may be beneficial in heterogeneous tumors, although it also heightens the danger of unexpected consequences in non-malignant recipient cells. Consequently, sequence selection must be integrated with cell-specific targeting and transcriptome-wide toxicity assessment [77].

Antisense and Splice-Modifying Oligonucleotides

Antisense oligonucleotides can inhibit translation, facilitate RNase H-mediated transcript degradation, or alter pre-mRNA splicing. In comparison to siRNA, several antisense chemistries have enhanced extracellular stability and do not necessitate incorporation into the RNA-induced silencing complex. Nevertheless, they must still access the nucleus for splice modification or the relevant cytoplasmic compartment for transcript inhibition. The CP05 study indicated that EV-associated phosphorodiamidate morpholino oligomers could enhance splicing correction in skeletal muscle. While the EVs were not explicitly sourced from dendritic cells, the methodology offers a transportable way for affixing splice-modifying cargo to DEX. In the context of GBM, antisense cargo could potentially target oncogenic splice variants, fusion transcripts, or regulatory long non-coding RNAs; however, direct data based on DEX is still scarce [81].

Messenger RNA Delivery

The transport of messenger RNA facilitates the temporary synthesis of therapeutic proteins without integrating into the genome. Potential applications encompass the expression of tumor suppressors, cytokines, bispecific engagers, prodrug-converting enzymes, genome editors, or proteins that elicit immunogenic tumor-cell death. Nonetheless, full-length mRNA is far bigger and structurally more delicate than siRNA. Basic overexpression in the parental cell frequently results in modest and inconsistent mRNA loading. Active RNA-binding technologies, such as MS2-based loading and EXOtic devices, enhance enrichment but may still preferentially select for shorter transcripts. Extensive mRNA molecules may be fragmented or inadequately packaged, necessitating the evaluation of transcript integrity instead of quantifying total RNA abundance. Cellular nanoporation was devised to enhance the yield of functional mRNA-containing extracellular vesicles. Producer cells were activated via localized electrical nanoporation while receiving plasmid templates that encode therapeutic messenger RNA. The approach produced significantly greater EV and mRNA yields compared to traditional transfection methods. In an orthotopic PTEN-deficient glioma model, targeted mRNA-laden extracellular vesicles reinstated PTEN expression and suppressed tumor proliferation. The producer cells in this investigation were not dendritic cells; thus, adaptation to DEX necessitates an assessment of DC survival, activation, and antigen-presenting capabilities. The glioma findings are significant as they illustrate that EV-mediated mRNA transport can generate a functional tumor-suppressor protein in an intracranial model. However, there is currently no proof that the identical loading output, tissue distribution, and therapeutic impact can be replicated using primary human dendritic cells [74,75,83].

DNA and Plasmid Delivery

Loading DNA into DEX is typically more challenging than loading tiny RNA due to the size and high charge of plasmids, which necessitate protection from nucleases and subsequent transport into the recipient cell's nucleus. Certain EV preparations include endogenous genomic or mitochondrial DNA; nevertheless, the presence of naturally related DNA does not confirm that full therapeutic plasmids may be consistently packaged and therapeutically delivered. Producer-cell transfection can lead to the association of plasmid fragments or DNA protein complexes with secretory vesicles, as well as the contamination of the preparation with free plasmid DNA, apoptotic bodies, or virus particles. DNase treatment, density-based separation, and tests

that can differentiate intravesicular DNA from externally linked DNA are necessary. In non-dividing brain cells, nuclear entrance constitutes an extra obstacle since the nuclear envelope remains intact during mitosis. As a result, DEX-mediated plasmid transfer is less developed than messenger RNA delivery. Messenger RNA is typically favored for transitory protein production, while DNA-based methods are appropriate for prolonged expression or when a DNA repair template is necessary [80].

Delivery of CRISPR and Other Gene-Editing Systems

DEX could potentially provide CRISPR-associated elements in the form of plasmid DNA, Cas messenger RNA accompanied by guide RNA, or preformed ribonucleoprotein complexes. Each format possesses a unique equilibrium of editing duration, cargo size, off-target danger, and intracellular prerequisites. Plasmid administration results in sustained nuclease expression but necessitates nuclear entrance and poses an increased danger of prolonged off-target editing. Messenger RNA and guide RNA facilitate temporary expression, whereas ribonucleoprotein delivery provides swift action and reduced intracellular longevity. The primary technological issue is the synchronized loading of all necessary components at a biologically effective stoichiometry. Cas9 messenger RNA is substantial, guide RNAs are prone to degradation, and ribonucleoprotein complexes may diminish in activity during vesicle loading or endosomal transport. Base editors and prime editors are larger than traditional Cas nucleases, hence imposing further packing limitations. Multiple non-dendritic extracellular vesicle platforms have demonstrated proof-of-concept genome editing in recipient cells. Nonetheless, concrete evidence for effective, targeted, and therapeutically significant CRISPR editing with primary human DEX remains limited. Consequently, it would be premature to characterize DEX-mediated genome editing as a validated platform.

The primary advantage of DEX may reside in providing smaller regulatory RNAs or compact editing systems while advancements in cargo-loading and endosomal escape technologies are pursued [79].

Protein and Ribonucleoprotein Loading

Certain gene-therapy applications necessitate the direct administration of an intracellular protein instead of a nucleic acid. Protein delivery circumvents the necessity for transcription and translation, enabling swift, temporary action. The passive integration of soluble proteins into extracellular vesicles is typically ineffective. The EXPLOR platform employs optically reversible protein–protein interactions to incorporate therapeutic proteins into extracellular vesicles during biogenesis. A cargo protein is conjugated to the light-sensitive CRY2 domain, whereas CIBN is conjugated to the EV-associated protein CD9. Blue-light stimulation briefly directs the cargo to locations of vesicle production. The elimination of illumination liberates the protein into the vesicle lumen, facilitating its subsequent transport to target cells. The effective delivery of proteins, such as Cre recombinase and a dominant NF-κB inhibitor, was established. While EXPLOR was created utilizing modified cell lines instead of dendritic cells, it exemplifies a potential approach for incorporating transcription factors, recombinases, nucleases, or gene-editing proteins into DEX. Adapting to primary DCs necessitates consistent and reproducible lighting conditions, verification of protein function post-purification, and assessment of whether optogenetic components influence vesicle immunogenicity.

Cellular Uptake and Endosomal Escape

Engineered DEX may infiltrate recipient cells by clathrin-dependent endocytosis, caveolin-associated routes, macropinocytosis, phagocytosis, or direct membrane contacts. The primary pathway differs based on the recipient cell type, vesicle dimensions, surface proteins, ligand concentration, and local microenvironment. The majority of internalized vesicles transit through endosomal compartments. Cargo retained in late endosomes may undergo degradation in lysosomes or be recycled to the extracellular environment. For siRNA, mRNA, antisense oligonucleotides, CRISPR components, and the majority of therapeutic proteins, effective delivery necessitates translocation into the cytosol. The enhancement of endosomal escape can be achieved through the integration of pH-responsive fusogenic peptides, viral fusion proteins, ionizable lipids, or membrane-disruptive polymers. The glycoprotein of vesicular stomatitis virus has been utilized experimentally to enhance membrane fusion and cargo transport, whereas pH-sensitive peptides exhibit membrane activity under acidic endosomal conditions. Nonetheless, viral fusogens may enhance immunogenicity, activate complement, and induce non-specific membrane fusion. The TAMEL investigation revealed that enhanced RNA loading does not necessarily result in proportional protein production in recipient cells, suggesting that absorption and intracellular release may become rate-limiting despite improved cargo enrichment. Consequently, effective potency assays must assess functional target modulation instead of solely depending on vesicle uptake or cargo copy quantity. Complete cytosolic escape may not always be requisite for immunotherapeutic DEX. Vesicles transporting antigens to dendritic cells may undergo processing within endosomal pathways for MHC class II presentation. Nonetheless, cross-presentation via MHC class I might be enhanced by the translocation of antigens into the cytosol. The preferred intracellular pathway should be determined based on whether the primary target is a tumor cell, dendritic cell, macrophage, or lymphocyte [74].

Extending Circulation and Modifying Biodistribution

After systemic dosing, numerous EV formulations concentrate in the liver, spleen, and lungs. Uptake by macrophages and other elements of the mononuclear phagocyte system may diminish delivery to the targeted tumor. DEX may interact with lymphoid tissues due to its dendritic-cell-derived surface makeup, which can be beneficial for vaccination but detrimental when direct tumor-cell transfection is necessary. Modification of polyethylene glycol, CD47-related "do not eat me" signals, and changes in surface glycosylation have been explored in extensive extracellular vesicle engineering to extend circulation time. These methods may diminish quick phagocytic clearance while potentially decreasing absorption by antigen-presenting cells. The ideal circulation profile consequently varies between a DEX vaccine designed to target lymph nodes and a DEX gene-delivery product aimed at infiltrating an intracranial tumor. Ligand density must be regulated. Minimal expression may be inadequate to modify biodistribution, while excessive ligand presentation may enhance non-specific binding, vesicle

aggregation, or receptor-mediated sequestration in non-target organs.

Quantitative assessment of surface-ligand copy number and receptor occupancy is superior to qualitative validation using Western blotting alone [74].

Engineering DEX for Glioblastoma Gene Therapy

GBM poses multiple challenges pertinent to DEX engineering: variable permeability of the blood–tumor barrier, extensive infiltration into healthy brain tissue, significant antigenic heterogeneity, hypoxic and necrotic areas, numerous tumor-associated myeloid cells, and fast adaptive resistance. A successful DEX platform must be engineered for both anatomical delivery and biological variability. RVG-based surface engineering is significant as it has facilitated DEX-mediated siRNA transport to brain cells following systemic injection [10].

However, effective distribution to normal neurons, microglia, and oligodendrocytes may not guarantee selective delivery to GBM cells. For oncological applications, RVG may necessitate combination with tumor-associated ligands or localized injection to minimize exposure to healthy brain tissue. Integrin-targeting peptides, such as iRGD, may enhance infiltration into tumors that express pertinent αv integrins and neuropilin-1 [11]. Due to the variability of integrin expression among GBM cell states, endothelial cells, and stromal compartments, iRGD targeting is improbable to be entirely tumor-cell selective. Additional potential targeting agents comprise ligands or antibodies specific to EGFRvIII, IL13Rα2, B7-H3, ephrin type-A receptor 2, transferrin receptor, or certain vascular indicators. Their application necessitates verification that the target is reachable from the vascular or extracellular compartment and well preserved across malignant cells. A rational GBM platform may integrate a comprehensive tissue-targeting ligand with a molecular payload aimed at a tumor-specific pathway. A brain-targeting ligand may be combined with siRNA directed against a pathway prevalent in malignant cells. Alternatively, a tumor-associated antibody fragment may be presented on DEX that carries mRNA encoding a tumor suppressor. The initial technique emphasizes anatomical delivery, whereas the subsequent strategy focuses on cellular selectivity. Tumor-intrinsic gene targets may encompass EGFR-related signaling, components of the PI3K–AKT–mTOR pathway, STAT3, transcription factors linked to stemness, anti-apoptotic pathways, and DNA repair processes. Targeting a singular driver is improbable to eradicate all malignant populations, as GBM comprises numerous genetic clones and interconvertible cellular states. Combination of multiplex siRNA, microRNA, or mRNA expressing broadly active apoptotic and immune-stimulatory proteins may be necessary. DEX may also be utilized to modify the GBM microenvironment instead of directly eliminating tumor cells. Possible payloads are siRNA targeting immunosuppressive myeloid pathways, mRNA encoding IL-12 or proteins related with type I interferon, STING-activating components, or compounds that augment antigen presentation. This method may leverage the inherent interaction of DC-derived vesicles with immune cells; however, it is essential to prevent excessive inflammatory activation within the CNS. The amalgamation of antigenic and genetic components is very appealing. DEX may provide GBM-associated peptide–MHC complexes while transporting RNA that mitigates local immune suppression. This substance may excite or reactivate tumor-specific T lymphocytes while modifying the tumor microenvironment. Nonetheless, this multifunctionality complicates dose determination, as the optimal amounts for antigen-presentation activity and gene-delivery activity may differ [77,78].

Route of Administration

Intravenous injection is minimally intrusive and suitable for repeated treatment; however, it subjects DEX to systemic clearance and affects non-target organs. It necessitates the vesicles to traverse or circumvent diverse areas of the blood–tumor barrier. The identification of a fluorescent or radioactive signal in the brain does not adequately prove the effective and functioning transport of cargo to cancer cells. Intratumoral delivery enhances local exposure; nevertheless, it is constrained by the infiltrative nature of GBM and may not effectively target malignant cells beyond the injection site. Local injection may generate elevated levels of immune-stimulatory DEX in a confined brain area, heightening the risk of edema or inflammation. Administering medication into the surgical resection cavity may facilitate the eradication of remaining cells at the tumor margin. DEX may be integrated into hydrogels or alternative local depots to enhance retention and facilitate controlled release. Local platforms may diminish systemic clearance while introducing increased manufacturing and regulatory complexities, as the final product comprises a blend of a biological vesicle and biomaterial.

Convection-enhanced delivery employs a pressure gradient to disseminate medicinal medicines into brain interstitial regions. This approach has the potential to enhance regional DEX distribution, but necessitates meticulous tuning of particle size, infusion rate, catheter placement, and tissue binding. Intranasal injection has been explored for many EV types; however, consistent delivery to human GBM has not been confirmed [76].

Hybrid Vesicle and Biomaterial Strategies

DEX can be integrated with liposomes or synthetic nanoparticles to merge biological targeting with enhanced loading capacity and membrane modification. Hybrid systems may integrate ionizable lipids that facilitate nucleic acid encapsulation and endosomal egress while preserving specific DEX surface proteins. Nonetheless, hybridization alters the identity of the product. A DEX-liposome fusion cannot be presumed to exhibit identical biodistribution, immunological activity, or safety profile as unaltered DEX. The degree of fusion, protein orientation, and ratio of synthetic lipid must be quantified. Hybrid preparations may additionally include unfused liposomes, native DEX, and partially fused particles unless rigorous selective purification is implemented. Hydrogel-based depots may prolong local retention of DEX and diminish the necessity for recurrent administration. Metabolically or chemically modified extracellular vesicles have been crosslinked into injectable hydrogels that gradually release vesicle-associated antigens and attract immune cells in preclinical animals. While these systems were not explicitly founded on DEX gene therapy, they exemplify a methodology for prolonged administration following GBM resection [84,85].

Manufacturing and Scale-Up

Autologous DEX synthesis typically initiates with the differentiation of peripheral-blood monocytes into dendritic cells utilizing GM-CSF and IL-4. Autologous synthesis may diminish alloreactivity but results in patient-specific variability in monocyte quantity, cellular viability, maturation, transfection efficacy, and vesicle yield. Patients with advanced GBM may have undergone corticosteroid treatment, temozolomide administration, or radiotherapy, all of which can affect the quality of immune cells. Allogeneic standardized producer cells may offer enhanced production consistency and facilitate the availability of an off-the-shelf product. Nevertheless, vesicles harboring allogeneic HLA molecules may elicit undesirable immunological responses, especially upon repeated administration. Gene editing could potentially eliminate specific HLA molecules; nevertheless, further modifications pose genomic and manufacturing hazards. The culture media must be devoid of contaminating extracellular vesicles or utilize a meticulously specified EV-depleted supplement. Standard fetal bovine serum can introduce bovine vesicles, RNA, and proteins that may be co-isolated with DEX. Serum-free or chemically specified conditions are advantageous for clinical manufacturing but may affect dendritic cell maturation and product yield. Scalable purification may employ clarifying, tangential-flow filtering, size-exclusion chromatography, ion-exchange chromatography, or affinity techniques. Ultracentrifugation is extensively employed in laboratory research; nonetheless, it may provide challenges in achieving reproducible scaling and can induce aggregation. Immunoaffinity purification can enhance a specific DEX subset but may skew the final product towards vesicles that express a chosen marker.

Characterization of Engineered DEX

Engineered DEX must be evaluated in terms of identity, purity, amount, structure, cargo, targeting ligand, and function. Particle concentration and size distribution can be evaluated using nanoparticle-tracking analysis, resistive-pulse sensing, or verified single-particle techniques. Morphology can be assessed using electron microscopy or atomic force microscopy. Protein characterization must encompass several EV-associated membrane and cytosolic proteins, pertinent dendritic-cell markers, tailored targeting ligands, and indicators of potential contaminants. The absence or depletion of endoplasmic reticulum, nuclear, serum, and lipoprotein-associated components must be assessed in accordance with the production procedure. Cargo analysis must ascertain the quantity of therapeutic compounds per particle or per unit of relevant DEX protein. RNA products should be evaluated for transcript integrity, sequence identity, and resistance to nuclease degradation. For surface ligands, orientation and binding affinity provide more insight than overall protein abundance. The heterogeneity of cargo distribution renders an average measurement potentially deceptive. A formulation with a substantial quantity of vacant particles and a minimal quantity of densely filled particles may exhibit an equivalent average cargo concentration to that of a more homogeneous formulation. Analysis targeted to single vesicles or subpopulations may thus become crucial for enhanced DEX products [68].

Potency Assays

Potency evaluation must align with the intended mechanism of action. For siRNA-loaded DEX, an appropriate assay must exhibit sequence-specific diminution of the target transcript and protein in the designated recipient cell. The assay for mRNA-loaded DEX must confirm the synthesis of the properly folded and physiologically active encoded protein. For genome-editing tools, potency evaluation must measure editing frequency, allelic distribution, functional outcomes, and pertinent off-target occurrences. Assessing Cas protein or guide RNA within DEX is inadequate to determine editing capability.

The multifunctional immunotherapeutic DEX may necessitate many potency assays. A product intended to transport siRNA to GBM cells and activate tumor-specific lymphocytes must be evaluated for gene silencing and antigen-dependent T-cell activation. An individual assay focused on particle quantity, total protein, or cytokine release would insufficiently encapsulate the entire mechanism. Establishing reference standards and acceptance ranges will be challenging due to the biological variety exhibited by basic DC products. Clinically effective DEX platforms necessitate established release criteria instead than retrospective comparisons of experimental batches [69].

Blood–Brain Barrier Targeting Strategies Biological and Therapeutic Context

The blood–brain barrier (BBB) is a specialized vascular interface that preserves central nervous system homeostasis by controlling the passage of ions, nutrients, macromolecules, xenobiotics, and immune cells between systemic circulation and neural tissue. The restrictive features are principally produced by brain microvascular endothelial cells linked by tight and adherens junctions, with support from pericytes, astrocytic endfeet, basement membrane components, microglia, and neural signals within the neurovascular unit. Brain endothelial cells have low rates of non-selective vesicular transport, polarized transporter expression, and elevated amounts of ATP-binding cassette efflux systems, such as P-glycoprotein and breast cancer resistance protein. Collectively, these attributes significantly limit the infiltration of the majority of biologics, nucleic acids, and traditional anticancer medicines. The blood–brain barrier (BBB) is a specialized vascular interface that preserves central nervous system homeostasis by controlling the passage of ions, nutrients, macromolecules, xenobiotics, and immune cells between systemic circulation and neural tissue. The restrictive features are principally produced by brain microvascular endothelial cells linked by tight and adherens junctions, with support from pericytes, astrocytic endfeet, basement membrane components, microglia, and neural signals within the neurovascular unit. Brain endothelial cells have low rates of non-selective vesicular transport, polarized transporter expression, and elevated amounts of ATP-binding cassette efflux systems, such as P-glycoprotein and breast cancer resistance protein. Collectively, these attributes significantly limit the infiltration of the majority of biologics, nucleic acids, and traditional anticancer medicines. In glioblastoma (GBM), the vascular barrier is typically referred to as the blood–brain tumor barrier (BBTB). This language denotes tumor-related alterations, including aberrant angiogenesis, uneven pericyte covering, modified basement membranes, damaged endothelial junctions, and heightened vascular permeability. Nonetheless, the BBTB exhibits geographic heterogeneity. The angiogenic and contrast-enhancing tumor core may include highly permeable arteries,

while infiltrating malignant cells at the tumor margin often exist behind vasculature that maintain significant blood-brain barrier features. Consequently, drug concentrations assessed within the augmenting tumor core cannot be presumed to reflect exposure across the invasive compartment accountable for recurrence. The discovery that contrasts agents infiltrate specific GBM regions does not confirm that bigger therapeutic compounds, nanoparticles, or extracellular vesicles may access all malignant cells at effective quantities. Gadolinium-based contrast enhancement indicates permeability to a relatively modest imaging agent and is affected by vascular surface area, blood flow, and extracellular volume. A therapeutic carrier may vary significantly in dimensions, charge, protein affinity, and interactions with endothelial cells. Strategies targeting the blood-brain barrier (BBB) must therefore be assessed using direct assessments of intact therapeutic cargo, rather than relying solely on inferences from magnetic resonance imaging enhancement [86-89].

Manufacturing, GMP and Regulatory Considerations
Product Definition and Regulatory Starting Point

The clinical application of dendritic cell-derived exosomes (DEX) necessitates the transformation of a diverse biological secretion into a consistently produced therapeutic product with established identity, purity, potency, safety, and stability. This shift is especially difficult due to the fact that DEX are not singular molecular entities. They consist of populations of membrane-bound particles whose size, composition, and biological activity are affected by the origin of dendritic cells, their maturation state, culture circumstances, antigen-loading strategy, genetic modification, isolation technique, formulation, and storage conditions. Consequently, the regulatory definition of the product should precede the creation of the process. The developer must ascertain if the active substance comprises purified extracellular vesicles, an extracellular-vesicle-enriched secretome, a specific immunoaffinity-selected DEX subpopulation, or DEX combined with a therapeutic nucleic acid, recombinant protein, synthetic nanoparticle, or medical device. These medicines may significantly vary in regulatory classification, essential quality features, and production controls, despite all being colloquially referred to be "exosome therapies." The designation "exosome" should not be determined exclusively by particle size or the identification of CD9, CD63, CD81, TSG101, or ALIX. Within the MISEV2023 paradigm, evidence of endosomal origin is necessary for conclusive biogenetic classification. In the absence of this data, words like "dendritic cell-derived extracellular vesicles" or "small extracellular vesicle-enriched preparation" offer a more precise characterization of the investigational product. Product nomenclature in regulatory filings must accurately represent the established composition and manufacturing method, rather than an inferred vesicle origin. The proposed mechanism of action is a fundamental aspect of classification. A DEX preparation may function via peptide–MHC-dependent antigen presentation, the transfer of regulatory RNA, surface-associated cytokine activity, or a combination of these methods. The sponsor must ascertain the primary mechanism of action and differentiate it from ancillary or secondary functions. This differentiation impacts product categorization, potency assay formulation, non-clinical evaluation, and the organization of clinical trial paperwork [90-93].

Conclusion

Future DEX-based GBM therapy will likely progress towards individualized, multifunctional vesicles that integrate neoantigen presentation, blood-brain barrier targeting, and molecular cargo delivery. Multi-omics and artificial intelligence may facilitate the identification of actionable tumor antigens, immunosuppressive pathways, and patient subgroups most likely to derive benefit. Combination strategies seem particularly promising. Engineered DEXs may be used with checkpoint inhibitors, radiation, temozolomide, tumor-treating fields, oncolytic viruses, CAR-T cells, or personalized vaccinations. Such combinations should be systematically formulated based on processes of immune suppression and resistance rather than being added empirically.Primary objectives encompass comprehensive EV characterisation, enhanced loading control, dependable BBB targeting, potency assays correlated with mechanisms of action, scalable GMP production, and transparent reporting. These criteria will ascertain whether DEXs remain experimental instruments or evolve into therapeutically significant medicines. Engineering dendritic cells and their exosomes for gene therapy in glioblastoma is a biologically appealing and technologically advancing therapeutic paradigm. DEXs have the potential to integrate antigen presentation, immunological regulation, targeted delivery, and gene therapy in a cell-free system. The discipline must maintain scientific prudence. GBM exhibits significant heterogeneity and immunosuppressive characteristics, while DEX products are intricate, heterogeneous biological formulations.

Consequently, therapeutic assertions must be substantiated by comprehensive characterization, functional potency assessments, orthotopic models, and clear clinical trial methodologies. DEX-based gene therapy should presently be regarded as a promising research approach rather than a validated medicine. The ongoing convergence of immunology, extracellular vesicle biology, nanomedicine, gene editing, good production Practice (GMP) production, and neuro-oncology will shape its translational future.

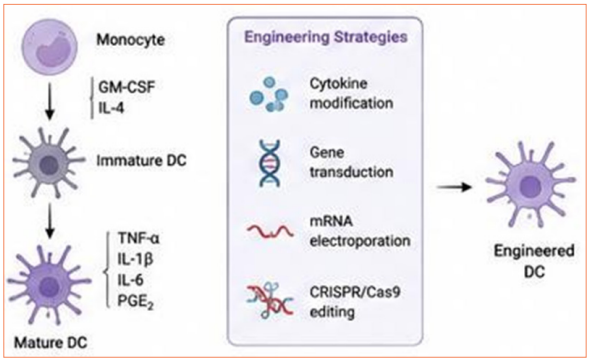

Figure 1: Dendritic Cell Differentiation and Engineering Workflow

Peripheral blood-derived monocytes are differentiated into immature dendritic cells (DCs) in the presence of granulocyte–macrophage colony-stimulating factor (GM-CSF) and interleukin-4 (IL-4).

Subsequent stimulation with a maturation cocktail containing tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), interleukin-6 (IL-6), and prostaglandin E2 (PGE2) promotes the

acquisition of a mature antigen-presenting phenotype. Dendritic cells may then be functionally or genetically modified through cytokine modulation, gene transduction, mRNA electroporation, or CRISPR/Cas9-mediated genome editing. These approaches aim to generate engineered DCs with enhanced antigen presentation, immunomodulatory activity, therapeutic cargo expression, and dendritic cell-derived exosome production for glioblastoma-directed immunotherapy and gene-delivery applications.

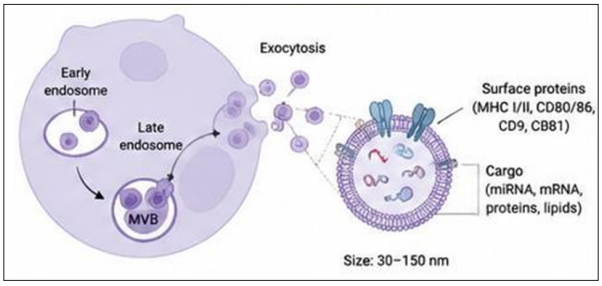

Figure 2: Biogenesis and Molecular Composition of Dendritic Cell-Derived Exosomes (DEX)

DEX biogenesis begins with endocytic membrane invagination and the formation of early endosomes, which subsequently mature into late endosomes and multivesicular bodies (MVBs). Inward budding of the endosomal membrane generates intraluminal vesicles that are released into the extracellular environment following fusion of MVBs with the plasma membrane. The resulting nanoscale vesicles, typically ranging from approximately 30 to 150 nm, retain selected components of their parental dendritic cells, including major histocompatibility complex class I and II molecules, costimulatory proteins such as CD80 and CD86, and tetraspanins including CD9 and CD81. Their intravesicular cargo may contain microRNAs, messenger RNAs, proteins and lipids, enabling DEX to participate in antigen presentation, intercellular communication, immune modulation and therapeutic cargo delivery.

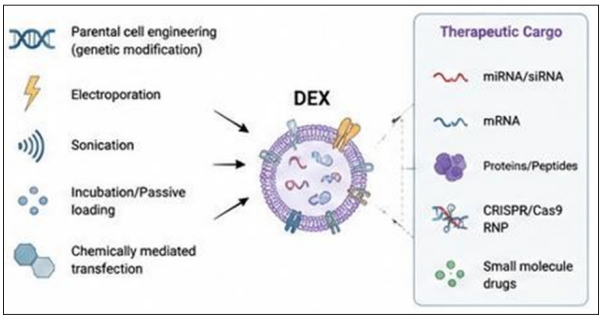

Figure 3: Cargo-loading strategies for dendritic cell-derived exosomes (DEX)

Therapeutic cargo may be incorporated into DEX through either endogenous or exogenous engineering approaches. Endogenous loading is achieved by genetically modifying parental dendritic cells, thereby promoting the expression and subsequent vesicular incorporation of selected nucleic acids or proteins during exosome biogenesis. Exogenous loading methods include electroporation, sonication, passive incubation and chemically mediated transfection, each of which differs in loading efficiency, cargo compatibility and potential effects on vesicle integrity. These approaches enable DEX to transport diverse therapeutic agents, including miRNA, siRNA, mRNA, proteins, peptides, CRISPR/Cas9 ribonucleoprotein complexes and small-molecule drugs. Selection of the loading strategy should therefore consider cargo size, molecular stability, preservation of DEX membrane structure and the biological activity of the final engineered product.

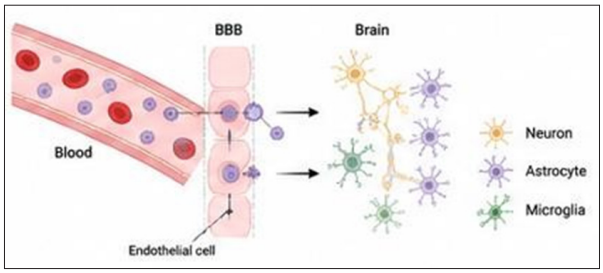

Figure 4: Transport of dendritic cell-derived exosomes across the blood–brain barrier

Following systemic administration, DEX interact with brain microvascular endothelial cells and may cross the blood–brain barrier through receptor-mediated transcytosis, adsorptive uptake or other vesicular transport mechanisms. After reaching the brain compartment, DEX can potentially be internalized by neurons, astrocytes and microglia, thereby enabling the transfer of therapeutic nucleic acids, proteins and immunomodulatory molecules to different cellular components of the central nervous system. However, BBB passage is not an intrinsic or uniform property of all DEX preparations; transport efficiency is influenced by vesicle size, surface composition, targeting-ligand expression, administration route and the physiological or pathological condition of the barrier. In glioblastoma, the heterogeneous permeability of the blood–tumor barrier further determines regional DEX distribution and therapeutic cargo delivery.

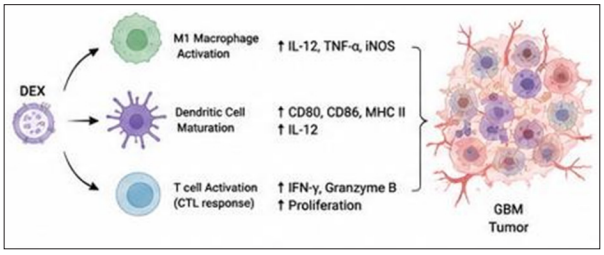

Figure 5: Immunomodulatory functions of dendritic cell-derived exosomes within the glioblastoma microenvironment

DEX may modulate antitumor immunity through coordinated effects on myeloid cells, dendritic cells and cytotoxic T lymphocytes. DEX-mediated stimulation of tumor-associated macrophages can promote a pro-inflammatory phenotype characterized by increased expression of interleukin-12, tumor necrosis factor-α and inducible nitric oxide synthase. They may also enhance dendritic-cell maturation and antigen-presenting capacity by increasing CD80, CD86 and major histocompatibility complex class II expression, together with interleukin-12 production. These effects can support cytotoxic T-cell activation, proliferation and effector function, including interferon-γ and granzyme B production, thereby promoting immune-mediated recognition and elimination of glioblastoma cells. The M1 macrophage designation shown in the figure

represents a simplified functional model, since glioblastoma-associated myeloid populations exhibit broader and more heterogeneous transcriptional states in vivo.

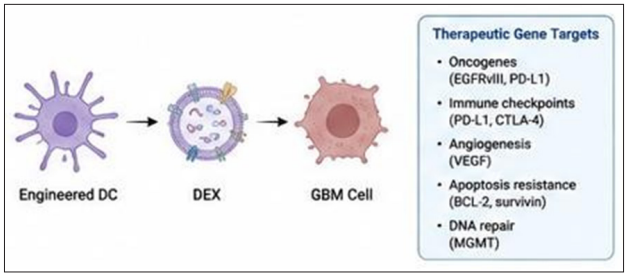

Figure 6: DEX-based gene-therapy strategies targeting glioblastoma cells

Genetically or functionally engineered dendritic cells can generate DEX loaded with therapeutic nucleic acids, gene-editing components, proteins or other regulatory molecules. Following delivery to glioblastoma cells, engineered DEX may modulate multiple tumor-promoting pathways, including oncogenic signaling mediated by EGFR and EGFRvIII; immune-checkpoint pathways such as PD-1/PD-L1 and CTLA-4; VEGF-dependent angiogenesis; apoptosis-resistance mechanisms involving BCL-2 and survivin; and DNA-repair activity mediated by O6-methylguanine-DNA methyltransferase (MGMT). Depending on the selected cargo, these approaches may suppress target-gene expression, restore tumor-suppressive functions, enhance treatment sensitivity or promote antitumor immune recognition. Because GBM exhibits marked molecular heterogeneity and cellular-state plasticity, simultaneous or patient-specific targeting of multiple pathways may be required to achieve durable therapeutic activity.

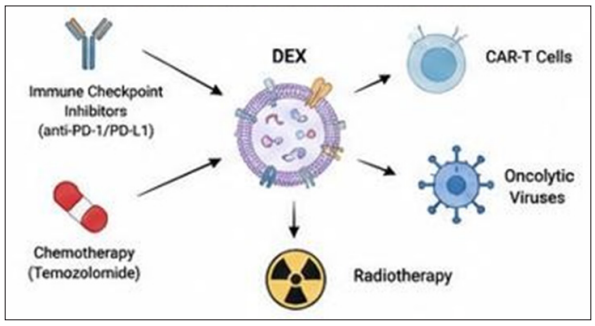

Figure 7: Combination strategies integrating dendritic cell-derived exosomes with established and emerging glioblastoma therapies

DEX may be incorporated into multimodal treatment strategies involving immune-checkpoint inhibitors, temozolomide, radiotherapy, CAR-T cells and oncolytic viruses. Checkpoint blockade targeting the PD-1/PD-L1 axis may support the activity of DEX-induced tumor-reactive T cells by reducing inhibitory signaling, whereas temozolomide and radiotherapy can increase tumor-cell injury and antigen release, potentially enhancing DEX-mediated antigen presentation. Engineered DEX may also complement CAR-T-cell therapy by modifying the immunosuppressive tumor microenvironment, improving antigen availability or delivering immunostimulatory cargo. Oncolytic viruses may further promote immunogenic tumor-cell death and local inflammatory signaling, thereby supporting DEX-dependent activation of innate and adaptive immune responses. However, the efficacy of these combinations depends on treatment sequence, dose, lymphocyte availability, corticosteroid exposure and the molecular heterogeneity of glioblastoma, and most DEX-based combination approaches remain at the preclinical or early translational stage.

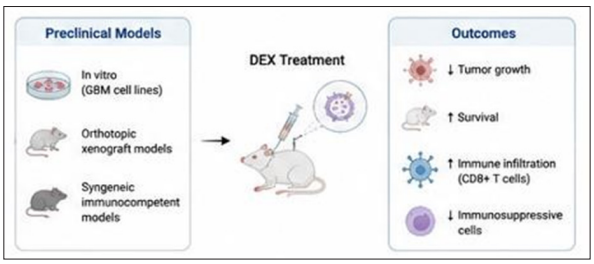

Figure 8: Preclinical evaluation and reported outcomes of DEX-based therapies in glioblastoma

Dendritic cell-derived exosome therapies are primarily evaluated using in vitro GBM cell-line systems, orthotopic xenograft models and syngeneic immunocompetent animal models. These complementary platforms allow assessment of direct tumor-cell effects, intracranial biodistribution, immune-system interactions and treatment-associated toxicity. In preclinical studies, DEX administration has been associated with reduced tumor growth, prolonged survival, increased infiltration or activation of CD8-positive T cells and decreased accumulation or activity of immunosuppressive cell populations within the tumor microenvironment. However, these outcomes depend strongly on the DEX source, cargo, engineering method, administration route and experimental model. Clinical evidence in human GBM remains limited; therefore, the therapeutic effects illustrated here should primarily be interpreted as preclinical findings rather than established clinical efficacy.

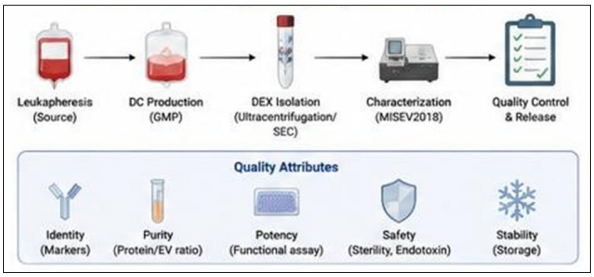

Figure 9: Good Manufacturing Practice-compliant production and quality-control workflow for dendritic cell-derived exosomes

The manufacturing process begins with the collection of peripheral blood mononuclear cells by leukapheresis, followed by the isolation of monocytes and their differentiation into dendritic cells under GMP-controlled culture conditions. DEX are subsequently recovered from dendritic-cell-conditioned medium using validated isolation and purification methods, such as ultracentrifugation, tangential-flow filtration or size-exclusion chromatography. The purified product is then characterized using complementary particle, molecular and functional assays before quality-control review and batch release. Critical quality attributes include product identity based on extracellular-vesicle and dendritic-cell-associated markers, purity assessed through protein-to-particle ratios and contaminant testing, biological potency demonstrated by mechanism-relevant functional assays, microbiological safety including sterility and endotoxin testing,

and stability under the proposed storage and transport conditions. These controls are required to ensure batch consistency, traceability and suitability for clinical administration.

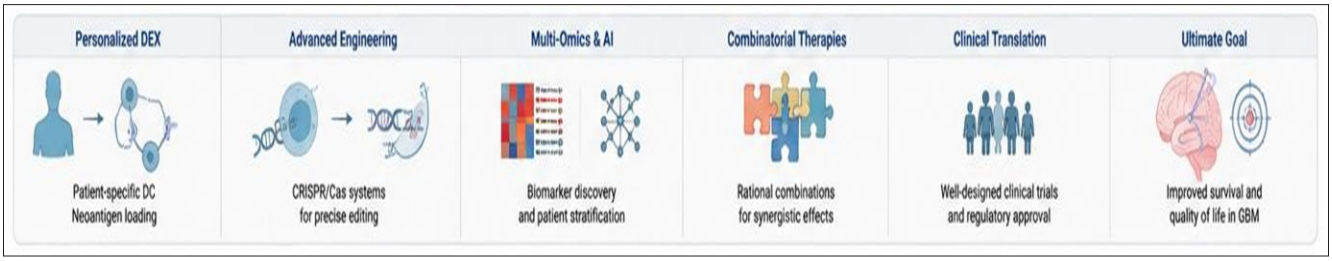

Figure 10: Future perspectives and translational roadmap for dendritic cell-derived exosome-based therapies in glioblastoma

Future development of DEX-based therapies will likely depend on the integration of personalized manufacturing, advanced molecular engineering, multi-omics-guided patient stratification and rational combination treatments.

Patient-specific dendritic cells may be loaded with individualized tumor antigens or neoantigens to generate DEX products tailored to the molecular and immunological characteristics of each tumor. Advanced engineering approaches, including CRISPR/Cas-based systems, may enable precise modification of therapeutic cargo, targeting ligands and immunoregulatory functions. Genomic, transcriptomic, proteomic, metabolomic and spatial data, supported by artificial-intelligence-based analytical tools, may facilitate biomarker discovery, treatment-response prediction and patient selection. DEX may also be combined with radiotherapy, chemotherapy, immune-checkpoint blockade, cellular therapies or oncolytic viruses to achieve complementary or synergistic antitumor effects. Clinical translation will require standardized manufacturing, validated potency assays, biomarker-driven trial designs and adequately controlled clinical studies to establish safety and therapeutic efficacy. The ultimate objective is to improve survival, preserve neurological function and enhance quality of life in patients with glioblastoma.

Figure Resources

All scientific illustrations presented in Figures 1–10 were conceptually designed and originally created by Alper Demirezen using BioRender.com. The figures were prepared specifically for this manuscript to visually summarize dendritic cell engineering, dendritic cell-derived exosome biology, therapeutic cargo loading, blood–brain barrier transport, immunomodulatory mechanisms, gene-therapy applications, combination strategies, preclinical evaluation, GMP manufacturing and future clinical translation.

Acknowledgements

The authors gratefully acknowledge the use of BioRender.com in the preparation of the scientific illustrations presented in this review. All schematic illustrations included in Figures 1–10 were conceptually designed and created by Alper Demirezen specifically for this manuscript. The figures were developed to summarize the biological characteristics of dendritic cells and dendritic cell-derived exosomes, engineering and therapeutic cargo-loading strategies, blood–brain barrier targeting, immunomodulatory mechanisms, gene-therapy applications, combination approaches, preclinical evaluation, GMP-compliant manufacturing, and the future clinical translation of DEX-based therapies for glioblastoma. No professional medical writing or commercial editorial assistance was used in the preparation of this manuscript.

Table 1: Therapeutic Rationale for DEX-Based Gene Therapy in GBM

Barrier in GBM	DEX-based opportunity	Key validation need
Tumor heterogeneity	Personalized antigen and multi-cargo loading	Neoantigen and cargo identity testing
Immunosuppressive microenvironment	DC-like antigen presentation and immune stimulation	T-cell activation and cytokine potency assays
BBB restriction	Ligand-directed or endogenous vesicle transport	Orthotopic biodistribution studies
Therapy resistance	siRNA/miRNA/ mRNA/CRISPR cargo delivery	Functional target modulation

Table 2: Representative Gene Cargo Options

Cargo	Therapeutic aim	Major challenge
siRNA	Silence oncogenic or immunosuppressive transcripts	Efficient loading and off-target control
miRNA	Restore regulatory networks or inhibit oncomiRs	Pleiotropic effects
mRNA	Transient expression of antigens/cytokines/proteins	Stability and translation efficiency
CRISPR/Cas	Genome editing of defined targets	Delivery, off-targets, safety

Table 3: Preclinical Evidence Framework for Future Studies

Required Element	Recommended Approach	Reason
Model	Orthotopic immunocompetent glioma model when possible	Captures BBB and immune context
EV characterization	MISEV-aligned particle, marker, purity and morphology assays	Improves reproducibility
Biodistribution	Brain/tumor and off-target organ uptake	Supports delivery claims

Efficacy	Tumor burden, survival, immune phenotyping	Connects mechanism and outcome

Table 4: GMP Quality Attributes for DEX Manufacturing

Quality Attribute	Purpose
Identity markers	Confirm EV and DEX-associated profile
Particle size/concentration	Batch consistency
Sterility/endotoxin/mycoplasma	Patient safety
Cargo loading and stability	Dose and potency control
Potency assay	Mechanism-linked biological activity
Documentation and traceability	Regulatory compliance

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