REVIEW ARTICLE

Role of Nanostructures and Immunotherapies in Management of Glioblastoma Multiforme: Current Perspectives and Challenges

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Abstract

Glioblastoma Multiforme (GBM), a highly infiltrative grade IV primary malignant brain tumor, is characterized by poor prognosis which ultimately leads to a high mortality rate. Various restrictions such as inadequate penetration of the drug through the blood-brain barrier, active moieties falling short in achieving accumulation at tumor site, and short circulation half-life make current conventional chemotherapy insufficient in delivering the drug molecules successfully. In an attempt to overcome current modalities, nanotechnology has emerged as an alternative novel therapy for the management of GBM. Nano-therapies such as liposomes, hybrid vesicles, dendrimers, nanogels, nanorods, and nanowires have been developed for the effective treatment and diagnosis of GBM. Nano-therapies have been proven superior to conventional drug delivery due to less toxicity, higher biocompatibility, and enhanced site-specific targeting. In addition, vaccines and immunotherapy have become a promising strategy for efficacious treatment of GBM. This review provides a brief detail about numerous aspects of nano-therapies mediated treatment of GBM, associated risk, and advancement in the field of enhanced brain tumor targeting. The appraisal deals with the triggering factors responsible for GBM and treatment with various immunotherapies and vaccines and elaborates the significant progress that has been made in the arena of nanoparticles, immunotherapies, and vaccines which are implemented for combatting GBM.

Key words: Glioblastoma multiforme, Immunotherapies, Molecular pathology, Nanoparticles, Vaccines

INTRODUCTION

Glioblastoma Multiforme (GBM) is a heterogeneous, highly infiltrative grade IV primary malignant brain tumor that affects the central nervous system (CNS). It presents a poor prognosis indicating high mortality with an incidence rate of 3–5/100,000 each year.[1] The median survival rate is 12–18 months and long-term survival time of 5 years for only 35% of diagnosed patients.[2] As a consequence of treatment limitations, elderly age of onset, diffusive nature of the tumor, rapid progression, and limited knowledge of the tumor pathophysiology.[3] Previously conducted studies suggest radiation and genetic variations as primary risk factors for the development of GBM. The variation in clinical symptoms is correlated to the site of the tumor in the brain and can be verified through advanced imaging techniques that may be invasive or non-invasive.[4] Studies reveal that GBM is composed of various cell types (mature and glioblastoma stem cells [GSCs]) according to their origin and epigenetic conversions and are classified into three major subtypes within the same tumor, that is, mesenchymal, proneural, and classical.[5] This genetic drift gives rise to self-renewing, tumorigenic GSCs that facilitate tumor initiation and treatment resistance.[2]

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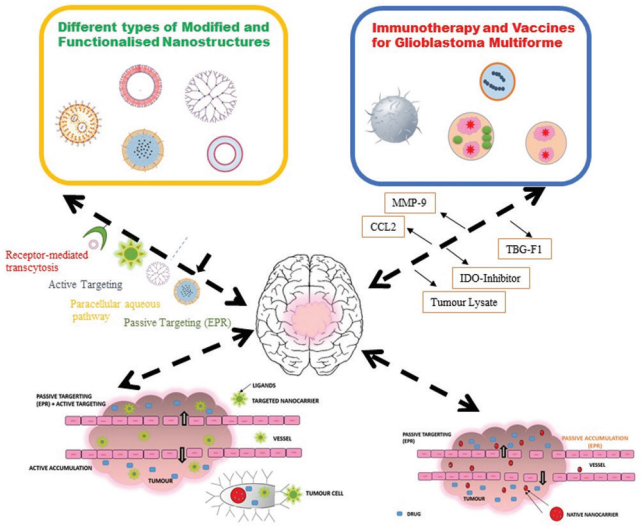
Received: 02-10-2021
Revised: 05-12-2021
Accepted: 14-12-2021

GRAPHICAL ABSTRACT



The existing standard therapy includes surgical tumor resection with subsequent radiotherapy and chemotherapy with Temozolomide (TMZ). However, complete surgical resection is dependent on the site, grade, and morphology of the tumor and is usually difficult due to its infiltrative nature. Incidences of relapse within 2–3 cm of the primary tumor margin have been reported in up to 80% of the cases.[1] Around 50% of patients don't respond to TMZ due to methyl guanine methyl transferase (MGMT) overexpression, a characteristic GBM DNA repair mechanism that annuls effects of TMZ.[6] The residue cells of the tumor post-surgery, when subjected to combination therapy, exert a cytotoxic effect on surrounding healthy tissues that severely impairs the quality of life and exhibit significant dose-related toxicity resulting in an increased risk of bone marrow suppression; followed by radio- and chemoresistance rendering the treatment ineffective.[7] Another factor is the reduced diffusivity of chemotherapeutic agents due to the highly selective blood-brain barrier (BBB) that prevents them from reaching the tumor site.[8]

Present standard anti-cancer treatment is almost ineffective against glioblastomas due to the presence of series of barriers that thwart them from reaching the tumor cells. These barriers include BBB that is selective for the transport of drug moieties in tumor cells, macrophages that engulf anti-GBM drugs, and non-specific targeting mechanisms that possess challenges in the therapeutic management of the disease.[9] Thus, there is an urgent demand for a novel approach for therapeutics and the management of GBM. Nanoparticles (NPs) have been utilized for passive targeting drugs into intracranial tumors to facilitate drug delivery across BBB as studies show that NPs can homogenously distribute better in tumor mass in comparison to drugs in solution form.[4] The use of nano-formulations has been proposed as a novel alternative as they primarily enhance tumor cell targeting by various pathways including boosting drug diffusion through the BBB, enhancing permeability and retention effect for Specific tumor cell targeting, accumulating anti-GBM drugs in tumor cells through convection-enhanced delivery into tumor site.[8]

Nano-formulations are also able to intensify the efficacy of drugs through various anti-tumor mechanisms such as encouraging cellular internalization of chemo/gene therapeutic drugs in tumor mass, radio-sensitization, destroying angiogenic vasculature, and activation of anti-tumor immune-mediated response (e.g., T cells, NK cells) and deactivation of pro-tumor cells (e.g., regulatory T [T_{reg}] cells). It also produces radical oxygen species and illuminates tumor margin that makes visualization easy for resection and facilitates restoration of the apoptotic pathway. The utilization of nanocarriers increases the bioavailability and half-life of the drug with a simultaneous reduction in side effects by enhanced specificity.[10]

The immunosuppressive nature of GBM plays a key role in initiating antitumor immune responses,[11] the GBM cells enhance the secretion of immunosuppressive factors like programmed cell death 1 ligand and indolamine 2,3-dioxygenase (IDO) which restricts the exhibition of antigens.[12] The macrophages which are linked with gliomas conceal interleukin (IL)-10 and transforming growth factor β (TGF-β),[13] which lower downs the activities of immune cells[14] and further the T_{reg} cells present in the microenvironment of glioma initiate immunosuppression by the mechanism of cytotoxic T lymphocytes draining and ultimately causing destruction of the tumor cells directly.[15] Hence, immunotherapeutic modalities for the management of GBM ranging from Checkpoint biomarker and inhibition, Cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4 or CD152), Programmed death axis, programmed death-1 (PD-1)/programmed death ligand 1 (PD-L1), and combinational therapies may serve as an advanced treatment regimen for overcoming GBM with high BBB penetrability.

Another novel approach for the treatment of GBM is vaccination which includes vaccines targeting epidermal growth factor receptor (EGFR) variant III, and the use of either immunogenic peptides or tumor lysates to stimulate autologous dendritic cells. Some of the strategies have reached phase III trials while some are in the early clinical trial phase like multi-peptide vaccines such as IMA-950, cytomegalovirus-derived peptides, or tumor-derived peptides such as heat shock protein (HSP)-96 peptide complexes and the Arg132His mutant form of isocitrate dehydrogenase.[16] Although proof of efficacy is not yet available for any of the glioma-specific vaccines and immunotherapies currently in clinical development, the addition of immune checkpoint inhibitors or other approaches that boost immune responses in vaccinated patients might ultimately be able to demonstrate that active immunotherapy and vaccines can control the growth of human GBM neoplasms.

In this review, we explicate the current management therapies of GBM, including the current perspectives and challenges of nanostructured formulations comprising *in vitro* and *in vivo* studies that have reported promising results for drug-loaded NPs targeted to GBM, and novel approaches such as immunotherapies and vaccines are been discussed.

MOLECULAR PATHOLOGY OF GBM

GBM typically originates from the lineage of glial cells (usually astrocytes) and tumor develops in the subcortical white matter of the cerebral hemisphere located in the cortical and temporal regions. Crucial histopathological features of GBM include increased vascularity, focal necrosis, persistent edema, and rapid cellular proliferation.[17] Initial studies on pathogenesis of GBM revealed biologically related alterations in three fundamental pathways: (a) receptor tyrosine kinase/RAS/phosphatidylinositol 3 kinase (PI3K) signaling (altered in 88% of GBMs); (b) p53 signaling (altered in 87% of GBMs); and (c) RB signaling (altered in 78% of GBMs).[18] Correlating the alterations reported in these core pathways to different epigenetic and molecular subtypes of GBM indicated coordinated combinations that exhibited diverse molecular subtypes, a factor that influences therapeutic outcome and tumor sensitivity to given therapy.[19]

GBM arises from genetic alterations and atypical regulation of the growth factor signaling pathway. Pathogenesis of GBM is mediated through augmentation of EGFR, Mesenchymal Epithelial Transition, Platelet-derived growth factor receptor A, MDM4, MDM2, CCND2 and Phosphatidylinositol-4,5-biphosphate-3-kinase A, etc. In addition, omission of cyclin-dependent kinase inhibitor 2A/B, CDKN2C, PTEN, retinoblastoma 1 (RB1), and NFKB1A is also observed. Somatic mutations of variable frequencies of p53, PTEN, neurofibromatosis type 1, RB1, isocitrate dehydrogenase (IDH)1, and IDH2 along with EGFR variant III (EGFRvIII) were reported.[20] These mutations give rise to elevated tyrosine kinase receptor followed by activation of RAS and PI3K pathways. Development and persistence into a high-grade GBM are further related to RB1 inactivation and HDM2 gene hyperactivity. Primary glioblastomas are usually more prevalent in patients over 50 years of age and are characterized specifically by EGFR amplification, loss of heterozygosity of chromosome 10q, deletion of phosphatase, tensin homolog on chromosome 10 PTEN, and omission of p16. On the contrary, secondary GBM occurs mostly in young patients as low-grade aplastic astrocytoma and later develops into GBM over the years. Mutation in p53, overexpression of PDGFR, and abnormality in p16 and Rb are its key characteristics.[21] These anomalies show a significant effect on growth factor-mediated cell signaling pathways and encourage uncontrolled cell proliferation, inhibiting apoptosis and stimulating angiogenesis.[22]

NANOSTRUCTURED DRUG DELIVERY FOR MANAGEMENT OF GBM

Nanostructured drug delivery systems have proven to be a promising tool to augment the drug uptake to the brain to a larger extend, the attributes of nano-formulations like enhanced loading of therapeutic agents and membranes functionalized with multiple ligands making them target/tumor specific, these properties allow the drug pathway through BBB and making the drug bioavailable at the site.[23] The various physicochemical properties of nanoformulations such as particle size, surface charge, hydrophobicity, and coating material have a greater impact on the active targeting process and also mediates the interface between the particles with the cell membrane along the passage through various biological barriers.[24] The tumor specificity of NPs, fluid dynamics, and particle uptake is greatly influenced by the shape and size of the nanoformulations and distribution of NPs in the bloodstream rest on the surface charge, literature reflects that positively charged particles can efficiently improve the tumor targeting and additionally replaced by neutrally charged NPs which extravagate quicker into the tumor tissue.[25,26] In recent years, nanoformulations have gained substantial importance for the treatment of various CNS diseases and have been explored to deliver chemotherapeutic drugs to various parts of the brain, for the same various nanoformulations consisting of different polymers, lipids, and targeting moieties are investigated, Figure 1 depicts applications of various NPs in the treatment of GBM.[27] Various modifications in the formulation of NPs during last some years have increased the loading, encapsulation efficiency and release profile of drug from the delivery system, further efforts are been made for improvements in the stealth capabilities of NPs to protect them from agglutination with various proteins in the blood and overcoming form clearing through the reticuloendothelial system (RES). Figure 1 graphically shows the presentations of different NPs for management of GBM and their possible targeting mechanisms. Surface-modified NPs having attachments of ligands and various targeting moieties facilitates imaging of brain tumors NPs.[28]

Liposomes

Liposomes for treatment of glioblastoma somehow are impaired due to two major intrinsic setbacks of nanosystems, like limited blood circulation due to favoring of RES which leads to sub-lethal tumor distribution of drugs and secondarily the tumor penetration of drug due to the existence of several biological and pathological barriers including the dense extracellular matrix and the elevated interstitial fluid pressure.[29] Various improvizations are being made in the liposomes used for the treatment of glioblastoma for improving the delivery of chemotherapeutics to the brain and targeting glioma such as receptor-, transporter-, or adsorption-mediated drug delivery according to different transport mechanisms. Some of these strategies involve modifying

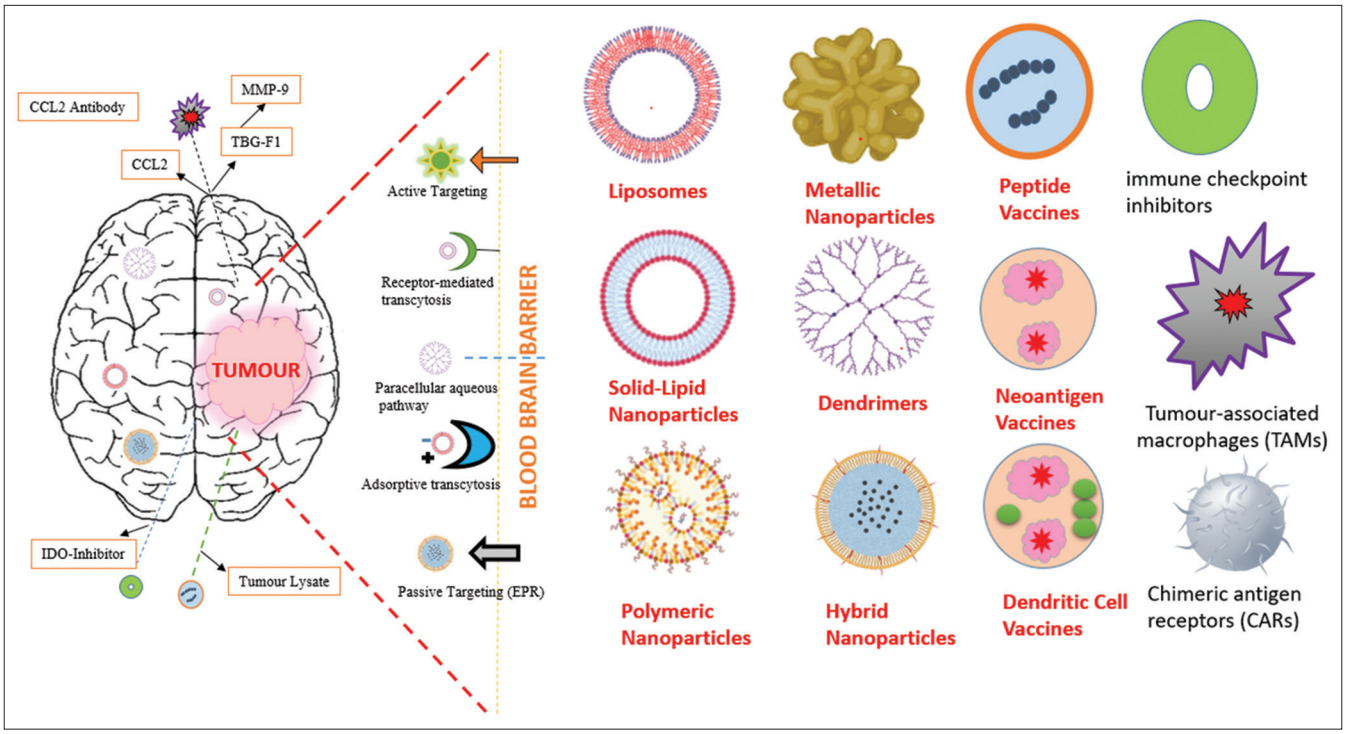


Figure 1: Display of various nanoparticles, immunotherapeutic agents, and vaccines for successful management of Glioblastoma Multiforme and their possible mechanism behind effectively crossing the blood-brain barrier

the surface of liposomes with polymer-based coatings, such as PEG, that increase circulation time in the organism and prevent opsonization, or/and with antibodies, aptamers, or peptides that promote active targeting or stimuli-triggered release.[29]

Belhadj *et al*. structured multifunctional liposomal delivery system for targeting glioma cells comprising RGDyK/pHALS modified with cyclic RGD (c(RGDyK)) and p-hydroxybenzoic acid (pHA) in which c(RGDyK) targets integrin αvβ3 overexpressed on the BBTB and glioma cells and pHA targets dopamine receptors on the BBB. Encapsulation of this both the ligands in modified liposomes gave higher accumulation at the site of tumor cells and tissues in the brain by favoring the transport through BBB. These ligands were actively linked on the surface of PEGylated liposomes and enhanced the uptake of doxorubicin (DOX). Hence, the cellular uptake of formulated liposomes when estimated through confocal microscopy and flow cytometry gave promising results by higher uptake in glioma cells, brain capillary endothelial cells, and umbilical vein endothelial cells when compared with single peptide and RGDyK and pHA ligands.[30]

Grafals-Ruiz *et al*. formulated gold-liposomes comprising targeting peptides apolipoprotein E (ApoE) and rabies virus glycoprotein (RVG) on the surface and encapsulating oligonucleotide miRNA inhibitors leading to form spherical nucleic acids. Evaluation studies suggested that the gold liposomes were effectively up taken by the GBM cells and highly profuse miRNA (miR-92b) in these cells. In addition, surface modification with ApoE and RVG provided a higher ability to cross the resistance provided by BBB and amplified reach to tumor site and making optimal nanocarrier for f RNAi-based treatments against GBM malignancies.[31]

Solid-lipid NPs (SLN's)

SLN's are the nanocarriers mainly comprising the matrix of lipids like CompritolR 888 ATO, PrecirolR ATO5, cetyl alcohol, tripalmitin, trimyristin/DynasanR 114, tristearin/Dynasan 118, stearic acid, glyceryl monostearate and cetyl palmitate which is usually solid at room temperature and stabilized with the use of various surfactants. SLN's and NLC's have numerous advantages like physical stability which enables greater protection to the encapsulated drug, further modification of surface provides active targeting to the site of tumor and release of drug at a controlled manner, the unique structural attributes of nanostructures provides enhanced biocompatibility which facilitates avoid easy seepage from the RES.[32,33] Grillone *et al*. showed the targeting of Nutlin loaded superparamagnetic NPs matrixed in solid lipid nanocarrier toward the U-87 MG glioblastoma cells, which showed good colloidal stability with enhanced BBB crossing ability and gave superior pro-apoptotic activity toward glioblastoma cells concerning the free drug.[34]

Polymer-lipid hybrid NPs

Polymer-lipid hybrid NPs are mainly composed of three functional units; firstly, a polymer core which is responsible

for encapsulating the drug, then an inner lipid layer that surrounds the internal polymer core and plays a key role in offering high biocompatibility and assures the retention of drug in the core. The outer layer lipid-polymer layer enhances the blood circulation time and steric stabilization.[35] Further modification on the outer surface of the hybrid materials with various antibodies and similar targeting molecules would increase the specificity of the nanocarriers toward the tumor site by active targeting.[36] Hence, the numerous benefits of hybrid nanosystems had made them useful in the application of various diagnosis and treatment agents, siRNA for gene therapy, diagnostic imaging agents.[37] Yang *et al*. targeted lipid polymer hybrid NPs loaded with clustered regularly interspaced short palindromic repeat (CRISPR)-associated protein 9 (CRISPR/Cas9) plasmids to O6-methylguanine-DNA methyltransferase (MGMT) drug-resistance gene to TMZ commodified with cRGD peptide which targets the overexpressed integrin αvβ3 receptors in tumor cells ($LPHNs_{pCas9/MGMT}$-cRGD) and evaluated the targeting efficiency of genes. The pathway for targeting was assisted by Microbubbles consisting of $LPHNs_{pCas9/MGMT}$-cRGD lipid polymer hybrid NPs under influence of Focused ultrasound (FUS) irradiation. The results suggested that the described formulation possibly guards pCas9/MGMT against enzyme degradation, $LPHNs_{pCas9/MGMT}$-cRGD lipid polymer hybrid NPs mediated the transfection of pCas9/MGMT to downregulate the expression of MGMT, resulting in an increased sensitivity of GBM cells to TMZ. FUS enhanced the BBB permeability and accumulation of NPs, simultaneously gave higher inhibited tumor growth, and prolonged survival of tumor-bearing mice, with a high level of biosafety.[38] Agrawal *et al*. encapsulated paclitaxel in folic acid targeted lipid polymer hybrid NPs and were subjected to evaluation for cell uptake and cytotoxicity in T98G tumor cells. The formulated NPs gave higher in vitro inhibitory effect, cell apoptosis and cellular uptake, and enhanced pharmacokinetic and drug biodistribution attributes. The *in vivo* and *in vitro* studies proposed that paclitaxel and RGDfK loaded folic acid surface-modified lipid polymer hybrid NPs have the increased ability to cross BBB and target the integrin receptor After treatment of Balb/C mice with the said formulation, the median survival time of animals was significantly increased when compared to the free drug and control groups. Hence, this dual targeting technique of brain- and tumor-targeting ligands gave a promising treatment for GBM with reduced drug toxicity, distribution to the non-target sites.[39]

Nanogels

Nanogels are especially nanostructured nanocarriers that have the capability of invading the smallest capillary vessels enabling them to penetrate tissues through transcellular pathways and availing enhanced drug encapsulation because of interpenetrating network structure. The nano size of nanogels leads to increase cellular uptake, increasing the blood circulation time, and along with hiked bioavailability of the anti-GBM moiety.[40,41] Gao *et al*., formulated a nucleic acid nanogel with an outer coating of virus-mimicking membrane incorporated with miRNA, immunotherapy is one of the most appealing approaches for the treatment of cancers however in combatting glioblastoma with this approach it falls insufficient because of the immunosuppressive tumor microenvironment (TME). The main immune infiltrating cells of TME in GBM are microglia and macrophages which function in reverse, rather than initiating anti-GBM response the immune infiltrating cells switches to tumor-promoting phenotype (M2) and enhances tumor growth, angiogenesis and the release of cytokines causes immunosuppression. Hence, to overcome mentioned issue nucleic acid nanogel coated with virus-mimicking membrane tends to reprogram microglia and macrophages from a pro-invasive M2 phenotype to an antitumor M1 phenotype. The said formulation augments the cellular uptake and targeting of encapsulated miRNA's which enables the exceptional tumor-targeting capability and tumor inhibition due to prolong blood circulation time and the invasive nature of drug delivery makes it a choice for treating GBM.[42]

Dendrimers

Dendrimers are the macromolecular nanocarriers having a central core with surrounding copious division branches integrating a range of functional groups, these branching units are generally termed as Dendron's and are symmetrically arranged around the core which usually is a linear polymer or a small molecule. The size and shape of the dendritic nanostructures can be improvised as per the need for targeting moieties.[43] The most commonly used dendrimer in PAMAM because it avails various benefits like 3D hyper branching, they have monodispersing properties secondarily PAMAM has well-defined molecular weight and enables enhanced entrapment of active molecules. The branches are heavily loaded with high-density amino groups which provide high solubility and increased reactivity.[44] Liaw *et al*. explored the effect of the size of dendrimers on the tumor targeting efficiency in two orthotopic tumor models viz. 9L rat and GL261 mouse models, as the size of a nanocarrier, play a vital role in determining the tumor-targeting efficiency, intratumor distribution, and clearance mechanism. The study reveals the assessment of tumor targeting efficiency of generation of dendrimers ranging from 4 to generation 6 dendrimers and simultaneously increased tumor accumulation (~10-fold greater at 24 hr), tumor specificity (~2–3 fold higher), and tumor retention was noted. The generation 6 dendrimer gave a reduced renal clearance rate which ultimately prolonged the circulation time of the moiety and enables homogenous distribution in the tumor environment with intrinsic targeting of tumor-associated macrophages. The comparative study suggested that generation 6 dendrimers molecules have the potential for improved delivery of immunomodulators with significantly lowering the side effects as compared to generation 4 dendrimers.[45]

Quantum dots (QDs)

QDs are unique nanostructured drug delivery systems having an average size of 2–10 nm and possessing an exclusive optical seminductance and optical electric properties. ODs are supposed to emit a characteristic wavelength that enables their easy detection and absorb photons over a wide range of wavelength.[46] Rajakumari *et al*., synthesized Functionalized Graphene QDs (GQDs) of DOX and evaluated anti-GBM activity on U87 human glioblastoma cells and primary cortical neurons. Two types of Graphene QDs were formulated, that is, non-functionalized GQDs and dimethyl formamide-functionalized GQDs (DMF-GQDs) which gave high biocompatibility and DMF-GQDs showed a toxic effect on both cell lines, respectively. GQDs demonstrated high synergistic efficacy in chemotherapy treatment on U87 human glioblastoma cells because the capability of GQDs surface chemistry enables altering the membrane permeability and increase cell uptake of DOX. Hence, these benefits of GQDs such as increased efficacy, lowering dose requirement, and high safety profiles make its choice for the management of GBM.[47] Wu *et al*. demonstrated c(RGDyk)-modified NPs encapsulating QDs as fluorescence probe for imaging-guided surgical resection of glioma under the auxiliary UTMD. QDs as fluorescent probes were encapsulated into the self-assembled c(RGDyk)-poloxamer-188 polymeric NPs, the fluorescence imaging with QDs-c(RGDyk)NP guided accurate surgical resection of glioma. The QDs-c(RGDyk) NP may be a potential imaging probe for imaging-guided surgery.[48] The study by Lin *et al*. shows how QDs can be used as a tool for future application of gene therapy against brain cancer. These authors developed QDs in combination with siRNA for silencing genes in GBM cell lines using these QDs as safe and effective nanocarriers.[49]

Nanorods and nanowires

Nanorods and nanowires are nanomaterials generally obtained from semiconducting materials or metals and having dimensions between 1 and 100 nm.[50] Gold nanorods possessing low toxicity and hence used as contrast agents in photoacoustic and near-infrared imaging of tumors, photothermal therapy, and gold nanorods also used in early tumor diagnosis.[51] Gonçalves *et al*. synthesized gold nanorods (AuNRs) engineered with modular peptide nestin (NesPEG-AuNRs) for targeting solid tumors originated from human GBM CSC multicellular tumor spheroids (MCTS). The formulation possessed lower toxicity profiles, and proficiently taken up by MCTS, the gathering/diffusion of NesPEG-AuNRs and NIR-irradiation result in photothermally convinced GBM CSC apoptosis and MCTS growth inhibition. In contrast combination of Nestin with gold, nanorods contribute to better tumor accumulation/penetration, and thus in GBM CSC elimination.[52] Nanowires are the nanodiagnostic material having diameters of few nanometers and extended lengths assisting on early diagnosis of tumors, which are generally made up of silicon, germanium, carbon, gold, and copper and used to monitor the brain electrical activity without using a probe.[53] Prepared zinc oxide nanowires conjugated to green fluorescent peptide for imaging U87MG human glioblastoma cells and proposed this system for cancer imaging and therapy.[54]

VACCINES AND IMMUNOTHERAPY FOR COMBATTING GBM

The human brain is an immunologically specialized region; however, it isn't as immunologically advantaged as anticipated before.[55] The brain may emerge as an active harbor for tumors, on which systemic chemotherapies are rather responsive. These immunological loopholes in the CNS are laid through different stratagems and GBM happens to be a well-explored subject for demonstration of these shortcomings. Glioblastomas can suppress immune activation in the tumor microenvironment and systematically. Extended exposure to tumor glioma cells changes the typical antitumor response that induces immunosuppression. In addition, the microglia and glioma cells within GBM release cogent T-cell growth inhibitors, downregulate MHC II and induce Tregs. On the systemic level, the IL-2 deficit signaling in blood lymphocytes, lymphopenia, and overall bone marrow suppression are contributing factors.[56] Taking these factors into consideration, a tailored immunologically advanced therapy can be designed as a novel treatment for GBM. As the understanding of the immune activation and suppression w.r.t the cancer deepens, more pathways will be open for immunologically targeting them. Immunotherapy ensures target specificity, flexibility, and resilience to tackle the challenges the tumor exhibits.[57] Preclinical and clinical data suggest standard immunotherapy alone or with combination chemotherapy shows promising effect.[58] Studies have suggested that effective blockade of IDO, CTLA-4, or PD-L1 in animal models significantly reduced the tumor infiltration of Treg cells and prolonged the survival rate, and proven a promising approach in immunotherapy for GBM.[59]

Checkpoint biomarker and inhibition

Immune checkpoints are surface proteins on T-cells responsible for identification and anchoring to partner protein on tumor cells. Checkpoint inhibitors will restore antitumor response by obstructing receptors that lead to immunosuppression and inhibit cytotoxic T-cells. Two such FDA approved therapy target includes CTLA-4 and PD1/PD-L1 – negative regulatory pathways of T cells.

CTLA-4 or CD152

This inhibitory moiety binds to CD80(/B7.1) and CD86(/B7.2) and blocks the activation of T-cells. It is vastly expressed by regulatory T-cells and is crucial for their inhibitory activity. CTLA-4 blockade initiates T-cell activity that disrupts normal

defense from an autoimmune response.[60] FDA approved ipilimumab, an entirely human-derived IgG antibody against CTLA-4 indicated in unresectable or metastasized melanoma.[56]

Programmed death axis, PD-1/PD-L1

PD-1 is a surface trans-membrane co-inhibitory receptor on cytotoxic T cells important to lower T-cell activity, elevate tolerance and avoid autoimmune activation. PD-L2 is PD1 ligands that are prevailing in antigen-presenting cells. PD-L1 induction in normal cells is usually facilitated by an inflammatory cascade of IFN-γ and TNF-α and expression of PD-L1 is low in brain areas but somehow elevated in GBM.[61]

Combination therapy

The collaborative mechanism of action and the existing clinical data on CTLA-4 and PD-1 suggest that combination therapy may be more effective. Preclinical data indicated the effectiveness of PD-1 inhibition superior to PD-L1 which is superior in action to CTLA-4. Although, the studies suggest promising effects of CTLA-4 in combination with PD-1 in curing 75% of subjects. This therapy was most effective in late-stage, advanced cancers and reduced remission incidences due to its immune memory response. However, combination therapy bears risks when extrapolated to clinical studies.[56]

Vaccines

Vaccination for cancers is not preemptive but focuses on inducing an immune response against the tumor. The vaccine includes a variety of therapeutic approaches such as-

- Direct exposure antigen (peptide/DNA)
- Stimulated patient-derived antigen-presenting cells (dendritic cells)

Peptide vaccines

These vaccines include the administration of tumor-specific antigens like HSPs or EGFRvIII. EGFR expression has a crucial role in tumor generation and is a part of the ErbB family of receptor tyrosine kinases that are associated with downstream pathways such as AKT/PI3K/mTOR and RAS/RAF/MEK). Constant stimulation or mutation of these may contribute to the development of Glioblastoma.[62] A vaccine named rindopepimut (CD110) consists of this peptide sequence that enables a potent immune response against EGFRvIII.[63] Other examples of peptide vaccines include IDHR132H-specific vaccines, HSPPC-96.

Dendritic cell vaccines

These vaccines consist of efficacious APCs that can activate the immune response. Dendritic cells are antigen-presenting cells, express MHC class 1 and 2 moieties. CD14+ monocytes are extracted from the patient's blood mononuclear cell and cultured for up to a week with GM-Colony-stimulating factor (CSF) and IL-4 to allow differentiation into immature dendritic cells.[64] The immature cells are then suspended in a "cytokinin cocktail" consisting of GM-CSF, TNF-α, IL-4, IL-6 for 16-20 hours and allowed to mature. These mature dendritic cells are later packed with tumor antigens (like peptide or tumor lysate). After processing the antigens, the DCs have epitomes mounted on the MHC molecule cell surface and are subjected to the patients through injection.[65]

Chimeric antigen receptor T-cell (CAR-T)

CAR-T therapy consists of manipulated T-cells programmed to kill tumors by targeting surface-specific antigen present on them. CAR-T cells are autologous or allogenic modified T-cell collected from peripheral blood cells of the patient. The extracted T cells are subjected to amplification *in vitro*, genetically modified to present CAR molecules on cell membrane through vectors or electrophoresis. The extracellular region identifies a special tumor related antigen and the intracellular region holds a T-cell activation signal. These CAR-T modified cells are injected into the patient where they destroy cells presenting appropriate tumor antigen.[64,66]

Tumor associated macrophages (TAMs) therapy

TAMs are macrophages found in the tumor microenvironment that promotes tumor development.[67] Previously conducted studies of GBM have cited the contribution of TAMs in the initiation and sustenance of immunosuppression, tumor cell migration, and angiogenesis. TAMs are reliant on the CSF for differentiation and endurance. A study on mouse glioblastoma subjects showed that employing BLZ945 for CSF-1 inhibition may help decline immunosuppressive M2 subtype frequency in TAM denizens, helping increasing survival outcomes and tumor remission.[64]

Viral therapy

The basic mechanism of this therapy includes infecting tumor cells with replicating viruses and destroying them through oncolysis, which in turn will liberate more virus particles in the tumor environment and infect the remaining cancer cells.[64] Oncolytic viral therapy works through various methods most of which are immunogenic and gives rise to Immunogenic cell death (ICD). Throughout ICD, the destructed cancer cell release damage-associated molecular patterns and tumor-associated antigens, together with genomic mutations produced patient-specific neoantigens that activate an active anti-tumor immune response. In addition, they may serve as anticancer vaccines as they are capable of enabling inducing antiviral innate immunity through pathogen-associated molecular patterns that help enhance antigen cross-presentation and consequential adaptive immunity.[68]

CONCLUSION AND PERSPECTIVE

The poor prognosis and very low life expectancy of patients suffering from GBM have raffled researchers to develop a novel therapy for treatment and its management. To overcome the limitations of current conventional therapies, nanotechnology has emerged as an effective tool that enables easy passage of anti-GBM molecules through BBB and helps in overcoming tumor-cell drug resistance. Many nano-formulations like liposomes are under clinical trials for the treatment of GBM; however, there are still some problems associated with Nano therapies such as associated toxicity, size, composition, and efficiency of drug encapsulation. Hence, a deep study and biology of brain tumor and related tumor biomarkers will make ease in development of nano-formulations and will enhance the site targeting by increasing specificity and will ultimately lead to an upsurge in the management of GBM.[69] Immunotherapies are the recent treatment regimens ranging from antibodies to adoptive cell transfers to vaccines to virally-based treatments to immune checkpoint blockade to overcome the local immunosuppression in the brain tissues caused by tumor cells and further BBB avoids the crossing of antibodies.[14] Therefore, nanoformulations, Immunotherapies, and vaccines can become a successful tool in the future for developing a single and combinational comprehensive GBM therapy. In general, designing effective treatments for glioblastoma disease is challenging due to the high tumor heterogeneity, meaning that among different cells present in the tumor, the chosen therapy could be effective for one cell type but ineffective for others. This is why multiple combined approaches are also taken into consideration and are believed to be more successful in eradicating diseased cells. Smart application of NPs in precision medicine will eventually lead to higher percentages of long-term survivors.

ACKNOWLEDGMENTS

The authors are thankful to the Government of India, Ministry of Science and Technology, Department of Science and Technology (DST), India for their financial support through DST-INSPIRE Fellowship (IF 190486) to Sagar Trivedi and express their gratitude to Rashtrsant Tukadoji Maharaj Nagpur University Nagpur, Maharashtra, India.

STATEMENT OF INFORMED CONSENT

All authors give consent for publication.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

STATEMENT OF HUMAN AND ANIMAL RIGHTS

NA.

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Source of Support: Nil. **Conflicts of Interest:** None declared.