

## Therapeutic Approaches for Treatment of Gliomas

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### ABSTRACT

Although tremendous research efforts have been made, cancer remains one of the leading causes of death worldwide. Glioblastoma multiforme (GBM) is the most aggressive primary malignant brain tumor due to its highly heterogeneity and resistance to standard treatment approaches. Poor survival rate in gliomas patients related to the presence of blood-brain barrier (BBB) which represents potent obstacle against a wide range of drugs including most anticancer agents. Many novel strategies have been developed to improve glioma prognosis and treatment by designing delivery systems for targeting therapies that specifically attack cancer cells without causing damage to surrounding healthy brain tissue. These targeted therapies are often promising, but with limited progress upon clinical applications. In this review, we highlighted the recent novel techniques for malignant glioma treatment, including cell encapsulation technique, gene therapy, nanotechnology, stem cell-based therapy, immunotherapy, and targeted therapy.

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**Key words:** Glioma, Brain tumor, Targeted treatment, Cell-based therapy, Gene therapy, Nanotechnology, Cancer vaccine

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### INTRODUCTION

Cancer is a fatal disease of uncontrolled cell growth of genetically altered cells which can be developed in almost all tissues. One of the most critical and challenging tumors is that of the central nervous system (CNS) which arises in the tissues of the brain and spinal cord. According to the World Health Organization, three major types of brain tumors are characterized and classified into gliomas: astrocytomas, oligodendrogliomas, and oligo-astrocytomas. Each year, more than 20 million people are diagnosed with cancer and one million die from this disease despite most recent available remedies<sup>[1]</sup>. Patients diagnosed with brain tumors have the lowest survival rates within five years after diagnosis<sup>[2-4]</sup>. Astrocytoma is the most frequent type of brain tumors. It is developed in glial cell type. It constitutes about 50% ~ 60% of primary brain tumors. And glioblastoma multiforme (GBM), the grade IV astrocytoma, is the most common and aggressive type of these primary malignant brain tumors<sup>[3,5,6]</sup>. The invasive behavior, rapid and aggressive regression of GBM results from cellular heterogeneity. Besides, GBM appears to be resistant to conventional treatment like chemo and radiotherapies<sup>[2]</sup>. Glial tumor cells are highly infiltrating. They can disperse within normal brain tissue leading to tumor recurrence. Residual brain tumor cells resist conventional adjuvant remedies by intrinsic factors like protection from alkylating agents, and extrinsic factors like selective properties of the blood-brain barrier (BBB)<sup>[4]</sup>. These anticancer techniques aimed at controlling tumor growth rather than trying to cure it<sup>[1]</sup>.

A unique challenge in brain cancer treatment is the blood-brain barrier (BBB). BBB-associated selectivity is controlled by the endothelium of capillary perfusion to the brain<sup>[7]</sup>. The concept of this unique membranous barrier that separates the blood and brain was developed hundred years ago, after the evidence that most organs can be stained by intravenously injected dye, excluding the brain as well as spinal cord<sup>[8]</sup>. BBB is important for the roles of the central nervous system (CNS)<sup>[9]</sup>. The protective and selective characteristics of the BBB results from tight junctions between capillary endothelial cells formed through cell adhesion molecules<sup>[8]</sup>. The BBB represents a powerful hindrance against large number of drugs including most anticancer compounds, peptides, and nucleic acids. Therefore, this barrier restricts the access of effective remedies of many acute and life threatening diseases such as brain cancer. The relative impermeability in order to constrain the access of molecules and cells between blood and brain is giving a natural protection against circulating toxic or infectious factors. However, to overcome and

cross the BBB, assertive research efforts have lately aimed to develop new approaches to efficiently deliver drug targeting brain tumors<sup>[8]</sup>. In this review, we review and discuss the conventional therapies as well as some new strategies for brain tumors treatment.

## CONVENTIONAL THERAPIES IN GLIOMAS

Gliomas are normally resistant to conventional therapies, including aggressive surgical removal of tumor mass, chemotherapy and radiotherapy. Poor survival rate and inefficiency of conventional treatment to eradicate gliomas is due to their highly invasive nature since tumor cells disperse within normal brain parenchyma and typically cause tumor recurrence at the surgical site<sup>[4,5,10]</sup>. The infiltrative nature of glioma cells permits some cell migration into long distances from the original tumor mass. Several factors result in GBM resistance to the therapies. They are (1) poor ability to deliver anti-cancer drugs to the brain across BBB<sup>[11]</sup>, (2) because of their low molecular weight, chemotherapeutic agents do not maintain efficient concentration within GBM due to short blood half-life<sup>[6]</sup>, (3) the expression of multidrug resistance genes is considered an important factor in developing resistance<sup>[12]</sup>, and (4) continual repopulation of gliomas from cancer stem cells allows prolonged cell survival<sup>[13]</sup>. Therefore, the cancer stem cells play a major role in tumor aggressiveness and therapy resistance.

### Surgery

Glioma surgery aims to establish a tissue sample for a pathologic diagnosis, alleviate tumor mass and eliminate the influence on the surrounding brain. Hence, surgery improves neurological symptoms, provides possibility of achieving complete resection, and provides time for other remedies such as irradiation and chemotherapy to be performed<sup>[14,15]</sup>. Several techniques are in use to improve glioma surgery. Some are designed to conserve functional integrity including preoperative MRI diffusion tensor imaging. Others are designed to maximize glioma removal such as intraoperative MRI and fluorescence light microscopy<sup>[16]</sup>. Furthermore, intraoperative electrostimulation mapping, which serves both aims, is increasingly used by neurosurgeons to improve the advantage/risk ratio of surgery<sup>[17]</sup>. Intraoperative stimulation mapping has been proven to be a gold standard in neurosurgery in patients with glioma for the avoidance of postoperative neurological worsening. Brain mapping techniques have been utilized to provide clinical and radiological finding for preoperative programming and enable the visualization of functional area and their proximity to the tumor sites. Preoperative information determines the ability of not causing injury in trajectory of tumor excising<sup>[14]</sup>. Intraoperative stimulation mapping for cortical and subcortical mapping is a credible and powerful approach to facilitate greater extent of surgical debulking with an impact on survival and minimize associated morbidity profile even when gliomas are located within or close adjacent functional pathways. Particularly, the importance of mapping motor and language pathway is well accomplished for secured resection of intrinsic tumors<sup>[18]</sup>. However, neuronavigation is known to be associated with intraoperative brain shifts. To solve this problem, intraoperative MRI has been introduced to continuously update imaging data with compensation for the brain shift<sup>[14]</sup>. Historically, biopsy has been utilized for tumor placed closed to or deep within eloquent territory whereas stereotactic biopsy is adapted for deep-seated tumors. Thus, resection is usually saved for superficial lesions. Moreover, craniotomy and resection have been used for patient with considerable mass effect which leads to enhanced intracranial pressure or permanent functional deterioration<sup>[18]</sup>.

### Chemotherapy

Chemotherapy has gained a significant function as primary treatment for progressive pediatric low grade glioma (LGG). Several promising studies have demonstrated that the safety and efficiency of using chemotherapy as an alternative therapy for LGG due to the concerns about toxicity of radiotherapy in young children. Radiotherapy is known for its late effects on neurocognitive, endocrine function and growth. It may also enhance malignant transformation and recurrence. Therefore, there is an intensive interest regarding repeated chemotherapy for progressive LGG, especially in young children to delay or replace radiotherapy<sup>[19,20]</sup>. Chemotherapy has been mostly used as a single agent approach, by either alkylating or platinum- based chemotherapies<sup>[19]</sup>. Recently, combined therapies over prolonged periods were applied to address the biology of tumors<sup>[19,21,22]</sup>, such as receiving carboplatin and vincristine (CV) or thioguanine, procarbazine, lomustine, and vincristine (TPCV)<sup>[14]</sup>. It was found by meta- analyses that adjuvant chemotherapy could be beneficial, and this was indicated by a significant increase in the survival rate for particular time intervals. Patient with anaplastic astrocytomas can take the advantage more than GBM patients<sup>[21]</sup>. The best outcomes of adjuvant chemotherapy are achieved with a nitrosurea based- regimens either carmustine (BCNU) or a combination of procarbazine and lomustin (CCNU) besides vincristine, known as PCV-3 therapy<sup>[15]</sup>.

In chemotherapy, temozolomide (TMZ) represents a class of second generation imidazotetrazine prodrugs<sup>[2]</sup>. TMZ undergoes spontaneous hydrolysis under physiologic pH into highly reactive methylating agent, methyl- triazenyl imidazole carboxamide (MTIC)<sup>[15,23]</sup>. TMZ acts as an alkylating agent with antitumor activity against malignant glioma<sup>[21]</sup>. *In vitro*, TMZ has shown schedule- dependent activity against broad spectrum of tumor types such as sarcoma, lymphoma, and melanoma<sup>[22]</sup>. TMZ gains its antitumor activity in CNS tumors due to its small molecular weight and lipophilic characters which enables it to cross the BBB<sup>[24]</sup>. It also has shown distribution to all tissues, relatively low toxicity. It displays 100% bioavailability within 1-2 hr when taken orally and has been reported to pose antineoplastic activity in relapsed high grade glioma (HGG) and mycosis fungoides. This mild- to moderate- cytotoxicity which is predictable and easy to be managed has made TMZ a valuable potential in treating and improving the quality of life of patient with glioma<sup>[22]</sup>. On the other hand, the enzyme methylguanine methyl transferase (MGMT) is responsible for repairing mechanism such as chlorethylation or methylation damage stimulated by nitrosurea and TMZ. Therefore, characterization of molecular markers such as MGMT has shown to be predictive marker for treatment response since the expression of MGMT can be silenced by methylation of the CpG island in the promoter region<sup>[14,25]</sup>. Therefore, suppressed MGMT gene results in lacking the full ability to repair the chemotherapy- induced DNA damage and consequently tumor reduction<sup>[26]</sup>. This was associated with better survival in all patient with GBM, especially those who are treated by RT plus TMZ<sup>[25]</sup>. Although both TMZ and dacarbazine (DTIC) are structurally and functionally related, and both are hydrolyzed to the same active compound MTIC, TMZ has a major potential benefits over DTIC in that TMZ is able to penetrate the CNS thus affects CNS tumor metastases. Furthermore, DTIC is only metabolized in the liver whereas TMZ does not require hepatic metabolism for activation due to its ability to cross CNS<sup>[22]</sup>. Compared to other chemotherapeutic agents such as nitrosureas, platinum compound, and procarbazine, TMZ does not lead to cross- linking of DNA strands. Therefore, it has less cytotoxicity to the hematopoietic progenitor cells in the bone

marrow than the other compounds<sup>[26]</sup>.

Chemotherapy is also used as an alternative choice of treatment if tumor continues progression after surgery or radiation<sup>[19]</sup>. In 2007, See and Gilbert demonstrated the significance of using chemotherapy combined with RT. According to a study conducted on randomized patients with GBM, there was a considerable increase in survival rate of 26.5% at 2 years compared to 10% in the combined RT with MTZ group and the RT alone group respectively<sup>[25]</sup>. Depends on the outcomes of clinical studies, TMZ has been approved by European Union for the treatment of patient with recurrent GBM after standard therapy<sup>[22]</sup>. Moreover, TMZ received approval from FDA for the treatment of refractory and relapsed anaplastic astrocytoma (AA) in adult<sup>[24]</sup>. Furthermore, understanding the mechanisms of tumor angiogenesis have had an immense impact on targeting recurrent GBM by applying bevacizumab (BV)<sup>[27]</sup>. BV, also called Avestin, is a humanized monoclonal that was accelerated to be approved from FDA in May 2009. It acts as an antiangiogenic agent by targeting the formation of new blood vessels or angiogenesis which contribute to a considerable development in the treatment of this fatal disease<sup>[28]</sup>. GBM are highly vascularized tumors that highly utilize vascular endothelial growth factor (VEGF) for new vascular supply which is required for further tumor expansion and aggressiveness<sup>[29]</sup>. By targeting the tumor neovascularization, it is possible to bypass the poor drugs penetration across the BBB to reach their target. Moreover, it was evident experimentally and clinically that antiangiogenic agents can minimize vasogenic edema and patients' demand for corticosteroids, which is considerably associated with morbidity<sup>[27]</sup>. Addition of BV to RT/TMZ first line therapy is endurable without obvious unexpected toxicities, except for potentially increased frequency of arterial and venous thromboembolism. Increased median progression-free survival (PFS), but prolongation of overall survival is still unclear. Therefore, further clinical studies are required to determine patients who will benefit from the incorporation of BV into upfront treatment of GBM<sup>[29]</sup>.

### Radiotherapy

Radiotherapy is always applied in the treatment of malignant glioma. The addition of radiotherapy and surgical resection is more beneficial compared to surgical therapy alone<sup>[14,15]</sup>. Also, postoperative radiotherapy combined with TMZ has considered being the mainstay of care for patient with newly diagnosed GBM, according to the outcome gained from a large European- Canadian phase III trial. This phase III trial indicates prolong median survival of one year after applying radiotherapy alone to 14.6 months after applying combined therapy RT plus TMZ with survival rate of 1.9% versus 9.8%, respectively<sup>[30]</sup>. Radiation therapy is recommended when the complete surgical resection is not possible with tolerable morbidity<sup>[31]</sup>, and when symptomatic patients display progressive relapsed glioma<sup>[30]</sup>. External beam radiation is advantageous for selected patients. However, it only occasionally results in prolonged survival<sup>[21]</sup>. This so called "limited-field" approach of irradiation is applied to the majority of gliomas (greater than 90%) because the relapse at the primary site, the junction of the primary site, and the surrounding brain<sup>[15]</sup>. Due to this recurrence pattern, brachytherapy, stereotactic radiosurgery (SRS) or stereotactic radiotherapy (SRT) techniques were applied. These techniques included the delivery of high dose of radiation by the tumor volume and minimizing the radiation dose to the surrounding normal brain tissues<sup>[21]</sup>. A woman diagnosed with optic glioma was treated with radiotherapy which has contributed to the improvement of ten years PFS, and overall survival. Modern RT techniques were used that permits high specificity in delivering

radiation therapy including fractionated stereotactic radiotherapy (FSRT) combined with image guided radiation therapy (IGRT). Therefore, these modern techniques are more beneficial than classical ones because it provides the opportunity to reduce the amount of normal tissue within the high dose volume<sup>[31]</sup>.

## DRAWBACKS OF CONVENTIONAL THERAPIES

Cancer patients receiving chemotherapy suffer from unwanted side effects. Many chemotherapy drugs induce mutation that causes abnormal changes in the DNA. They also cause skin irritations, hair loss, weakness, anemia, and loss of appetite. Chemotherapy is toxic, and it should only be utilized when it is verified to be effective. Therefore, side effects occur when the chemotherapy damages normal and healthy cells which maintain the body's function and image<sup>[32]</sup>. Radiotherapy has similar side effects as chemotherapy and a number of studies have shown that radiation can kill oligodendrocytes, stem cells populating the subventricular zone, and progenitor cells of the dentate gyrus of the hippocampus in rodent models<sup>[33]</sup>. Therefore, there is an urgent need to develop novel therapeutic approaches that would both specifically targeting tumor cells and repairing brain damaged tissues<sup>[33]</sup>. We describe some approaches in brain tumor treatment in the following sections.

## MANIPULATING TUMOR ACIDIFICATION

Controlling of the extra and/or intracellular pH of tumors may have significant prospects as anticancer remedy<sup>[34]</sup>. Acidification of extracellular space plays an important role in cancer invasiveness or what is called acid-mediated invasion<sup>[35,36]</sup>. Otto Heinrich Warburg, the Nobel Prize winner, found that malignant tumors unlike normal cells use glycolytic metabolic pathway, wasteful glycolytic conversion of glucose into lactate, even in the presence of sufficient oxygen tension, this phenomenon is called aerobic glycolysis, the Warburg effect<sup>[5]</sup> which is less efficient than oxidative phosphorylation to generate energy (ATP)<sup>[37]</sup>, or what is called mitochondrial respiration<sup>[34]</sup>. Therefore cancer cells are able to survive and proliferate in the acidic microenvironment that developed as a result of (a) tumor production of lactic acid by anaerobic glycolysis in tumor sites which are hypoxic, and (b) aerobic glycolysis or Warburg effect<sup>[34,35]</sup>. Tumor cells keep their high proliferating rate in this hostile microenvironment by continuous removal of accumulating protons produced as a result of high lactic acid production to avoid acidification of intracellular pH, so glycolytically produced acid must be expelled by tumor cells via a number of proton transporter<sup>[37]</sup>, such as V-ATPase<sup>[36]</sup>, the Na<sup>+</sup>/H<sup>+</sup> exchanger (NHE)<sup>[1]</sup>, the carbonic anhydrases<sup>9[37]</sup>. These hyper active proton pumps contribute to disturbance of pH gradients that enhance cancer phenotype<sup>[1]</sup>. Therefore, blockage or inhibition of the membrane ion pumps tends to reduce intracellular pH. Few drugs have been used clinically as proton pump inhibitors (PPIs) like omeprazole, esomeprazole. For example, PPIs can covalently inhibit the V-ATPase when activated by mildly acidic environment<sup>[34]</sup>. The anticancer role of PPIs was first identified in human B-cell tumors. These inhibitors enhance pro- apoptotic action in different B-cell tumor- derived cell lines. PPIs can be tumor- specific agents as they are activated in the acidic extracellular pH, which is a characteristic of cancer cells<sup>[1]</sup>. Another strategy is to suppress cancer metastasis achieved by oral administration of high doses of systemic buffer such as sodium bicarbonate<sup>[35]</sup> or trisodium citrate or even special diet of low to moderate protein content and full of potassium enriched juices, fruits, and vegetables. These substances are able to inhibit

cancer aggressiveness by reducing extracellular acidification<sup>[34]</sup>.

## CELL ENCAPSULATION TECHNOLOGY AS A THERAPEUTIC APPROACH FOR BRAIN TUMORS

In 1964, T.M.S. Chang suggested the concept of using ultrathin polymer membrane microcapsules for the immunoisolation of implanted cells and introduced the term 'artificial cells' to define the idea of bioencapsulation<sup>[38]</sup>. Cell encapsulation system, by which viable cells entrapped within a non-degradable, selectively permeable membrane to be isolated from host cells and immune response, shows promising method for long-term delivery of therapeutic factors including powerful cancer remedies<sup>[39]</sup>. Encapsulation technique can be classified as either macro or microencapsulation<sup>[33,40,41]</sup>.

### Macroencapsulation approach

In the 1940s, macroencapsulation was developed by Algire who implanted cells into diffusion chambers and it works as arteriovenous (AV) shunts which are connected to blood vessels enabling blood flow through the lumen of these synthetic blood vessels. Drug-containing cells are immobilized on the surface of the AV shunts and they are within the diffusion area of influence of the blood vessel surrounded by a membrane<sup>[40,41]</sup>. Macroencapsulation devices can be removed easily in case of undesired side effects or graft failure. However, it is unfavorable application because of their physical properties related to their large size, especially their surface-to-volume ratio; it also results in membrane breakage and limited cell loading capacity<sup>[42]</sup>. In addition, due to the large sizes of macroencapsulation devices, they trigger an inflammatory response in host tissue, and this leads to limited use of this technique as therapeutic release system<sup>[41]</sup>.

### Microencapsulation approach

Microencapsulation has many considerable advantages in comparison to macroencapsulation<sup>[40]</sup>, including small size, larger surface-to-volume ratio. It can be synthesized easily from different types of stable and biocompatible polymers. Furthermore, it can be easily implanted and retrieved<sup>[42]</sup>. In this aspect, genetically modified cells that produce recombinant proteins may affect the tumor microenvironment. The encapsulated cells that are protected from immune rejection have been implanted to target tumor cells. Genetically engineered cells can be immobilized in polymers. Currently, various polymers have been utilized for immunoisolation. Alginate had the lowest cytotoxic effect and the optimal cell attachment properties<sup>[40]</sup>. Cells embedded in alginate beads meet all required considerations which include the capsule should be semi-permeable to nutrients, oxygen, and the manufactured drug, while antibodies and immune cells are excluded. Also, biocompatibility is considered between the encapsulated cells and the implantation site. Moreover, the capsule should be mechanically and chemically stable<sup>[38]</sup>. Biodistribution is another index for the success of therapeutic applications. For cancer therapy, implanted cells which can be encapsulated for immunoisolation may comprise (1) Cells that secrete chemotherapeutic drugs targeted tumor tissues<sup>[41]</sup>, or cells that code for enzymes that are locally activated a non-toxic prodrug to a cytotoxic drug at the tumor site<sup>[10]</sup>. (2) Cells uploaded with anti-angiogenic factors such as angiostatin and endostatin<sup>[33,40,41]</sup>, and also anti-vascular endothelial growth factor (VEGF) which inhibits the overexpressed VEGF, a prominent growth factor which function an important role in tumor progress<sup>[43]</sup>. (3) Cells that release

cytokines that trigger host's immune response against tumors such as TNF- $\alpha$ <sup>[10,41]</sup>.

## GENE THERAPEUTIC APPROACHES

The use of gene delivery for brain cancer therapy is another promising strategy as it involves in situ delivery of the required genes that drive the therapy to tumor cells. Hence, it is important to design a vector that delivers a specific gene to tumor tissues effectively<sup>[12,44]</sup>. Some strategies of these vectors have been developed to deliver therapeutic transgenes for cancer treatment including prodrug- activating genes, conditional cytotoxic approach<sup>[3]</sup>, or 'suicide' genes that enhance local cytotoxicity of anticancer drugs<sup>[45]</sup>. For instance, tumor cells were transduced with herpes simplex virus thymidine kinase (HSV-tk) gene which activates systemically delivered ganciclovir (GCV)<sup>[10,44]</sup>. Cellular kinases convert GCV- monophosphate into triphosphate form, which leads to DNA polymerase inhibition and binding to the DNA of proliferated cells. This results in inability of cell proliferation, promotes cell apoptosis, and cell death of transduced cells<sup>[4]</sup>. Alternatively, genes that code for immunostimulatory molecules to initiate antitumor immune responses<sup>[44,45]</sup> and to enhancing the immunologic memory against tumors<sup>[3]</sup> are employed for tumor therapy as well. For instance, cytokines play an important role as immunoregulators, and they show positive effects in experimental animal tumor models. It is significant to use gene therapy since the direct administration of cytokines is restricted by their possible cytotoxic effect and their short half life time. This approach has been applied successfully in experimental brain cancer patterns<sup>[10]</sup>. The best studied cytokines include IL-2, IL-4, IL-12, granulocyte-macrophage colony stimulating factor (GM-CSF)<sup>[44]</sup>, IFN- $\alpha$ , IFN- $\beta$ , IFN- $\gamma$ , and TNF- $\alpha$ . In addition to stimulate an immune response, these molecules can promote programmed cell death and also act as anti-angiogenic agents<sup>[10]</sup>. Another strategy for gene therapy is to use RNA interference (RNAi) to block glioma pro-survival pathways and to silence oncogenic genes<sup>[3,10]</sup>. RNAi technique utilizes antisense oligonucleotides to inhibit gene expression at translational level by binding specifically to certain mRNA sequences<sup>[3]</sup>. For example, inhibition of overexpressed epidermal growth factor receptor (EGFR) which is responsible for the propagation of 90% of GBM and inhibition of transforming growth factor  $\beta$  II (TGF $\beta$ 2) expression by RNAi, can lead to suppression of glioma growth<sup>[11,44]</sup>. Furthermore, genes that code for anti-angiogenic factors can be another therapeutic gene. Endostatin, as angiogenic molecule, is one of the most important factors required for brain tumor development and progression<sup>[44]</sup>. Endostatin was delivered via viral vector which injected intra-arterially and promoted survival time by more than 47% in brain cancer rats<sup>[10]</sup>.

Gene transfer can be accomplished by the utilization of various vectors which can be divided into three major groups: viral, non-viral, and cell-based vectors<sup>[4]</sup>. Retroviruses, adenoviruses, and herpes simplex are the best and widely used vectors against brain cancers<sup>[10,44]</sup>. Viral vectors can be subdivided into replication-deficient<sup>[10]</sup>, replication-competent or oncolytic viruses<sup>[44]</sup>, and oncolytic viruses associated with a therapeutic gene<sup>[45]</sup>. Bacteria have shown an important role as efficient vehicles of cellular-based gene delivery, especially to hypoxic areas of tumor, and also to produce a potent immune response<sup>[44]</sup>. Recently, the use of neural stem cells for the delivery of prodrug- converting enzyme has been developed for a new cell-based delivery carrier<sup>[10]</sup>. Non-viral vectors involve naked DNA and liposomes which enter the target cancer cells by endocytosis<sup>[44]</sup>.



## APPLICATION OF NANOTECHNOLOGY TO BRAIN CANCER TREATMENT

Nanoparticles (NPs) can be defined as solid particles at nano scale. Their size ranges from 10-1000 nm. Nanotechnology-based cancer therapies have shown wide application in medicine such as screening, diagnosis, and treatment of cancer. Therapeutic agents are dissolved, restrained, encapsulated, or bounded to the matrix of nanoparticles<sup>[46]</sup>. Due to their small size, NPs can easily interact with biomolecules located on the cell surface or inside the cells. Furthermore, small size of NPs enables them to penetrate cancerous tissues deeply in specific manner, resulting in enhanced tissue-specific drug delivery<sup>[47]</sup>. Hence, nanoparticles offer broad spectrum applications in the treatment of tumors. They can either function as drug delivery system or promote cytotoxicity to cancer cells<sup>[3]</sup>. It is important to use nanomaterials-based therapy technique since it has several significant advantages including the improvement of poorly water-soluble drugs, prolonged circulation half-time by minimizing immunogenicity<sup>[48]</sup>, sustained or controlled release rather than frequent administration of drug<sup>[47]</sup>, and preferential accumulation of NPs at the site of disease as a result of passive targeting via enhanced permeability and retention effect by passing through fenestrations of tumor's blood vessels which are more permeable than normal ones because of their deformity<sup>[6,48,49]</sup>. Moreover, NPs concentrate within tumor mass via active targeting specific surface ligands. Although NP-mediated drug delivery can reduce systemic side effects<sup>[48]</sup>, a drug delivery NP must be stable in the circulation for adequate period of time, may be for days, to reach their desired targets<sup>[6]</sup>. After their parenteral administration, NPs are easily recognized by plasma proteins called opsonins. These opsonins include reticulo-endothelial system (RES) cells which bind onto surface of NPs through a process called opsonization that function as a bridge between NPs and phagocytes. Delivering this drug-carrier system to phagocytic cells leads to its rapid clearance from blood and hence alters the drug biodistribution profile<sup>[6,46]</sup>. For above reasons, it is essential to develop NPs for medical applications by surface coating them with hydrophilic molecules such as polyethylene glycol (PEG), polyethylene oxide, polysorbate 80, and poloxamine<sup>[6,46-49]</sup>. Studies show that PEG is designed to enhance pharmacokinetics and biodistribution of NPs-associated drugs for lowering clearance rate and reducing uptake by opsonins<sup>[48,49]</sup>. PEG- surface coated NPs or stealth-shielding on the surface of these drug carrier systems are referred to as PEGylation, a process of either covalent or non-covalent binding or adsorption of PEG onto NPs<sup>[6]</sup>.

Two major classes of NPs are used in clinical trials include liposomes and polymer-drug conjugates. Other NP modalities like dendrimer, nanoemulsions, inorganic<sup>[47,48]</sup>, gold, and ceramic NPs have also utilized as therapeutic carrier systems<sup>[48]</sup>. Gold nanoparticle-mediated hyperthermia is based on the systemically administration of the gold nanoparticles and their accumulation in tumor tissue. The tumor is then exposed to a heated environment through external inducer like near infrared (NIR) laser light, radiowaves, or magnetic field. Thus, NIR- absorbing gold nanoparticles like gold-silica nanoshells, gold nanocages, and gold nanorods can kill tumor tissues by heating both *in vitro* and *in vivo*. This type of cancer therapy has demonstrated an immense impact in cancer treatment<sup>[3,49]</sup>. Importantly, the amount of scattered light is proportional to the absorbed size of tumor site. Moreover, magnetic targeted hyperthermia relay on their specific inorganic characteristics by using metallic nanoparticles which turns electromagnetic energy into heat<sup>[49,50]</sup>. Direct cytotoxicity can be enhanced by targeted delivery of chemotherapeutic agents specifically to GBM. Madhankumar *et al.* illustrated that specific

delivery can be occurred by the specific binding of liposome-based anticancer agents with IL-13R $\alpha$ 2 receptors which overexpressed by GBM but not by normal counterparts. IL-13-conjugated liposome is an efficient and promising approach for direct killing of cancerous lesions<sup>[51]</sup>. Liposomes have been utilized as therapeutic drugs carriers due to their good biocompatibility, easy preparation, low toxicity, and commercial availability<sup>[6]</sup>. This combination between nanoparticle and anticancer drug can reduce systemic cytotoxicity and side effects<sup>[51]</sup>.

## STEM CELL-BASED THERAPIES

In the 1960s, stem cells were discovered by Drs. Ernest McCulloch, James Till, and professors at the University of Toronto and the Ontario Cancer Institute. These cells have unique properties such as the ability to divide and self-renew over long period of time; they are unspecialized; and they can differentiate into more than multiple types of cells, a feature known as plasticity<sup>[52]</sup>. It has been anticipated that over 100 million Americans can get benefits from using stem cell in the targeting therapy including cancer treatment<sup>[53]</sup>. Among organs that harboring adult stem cells, neural stem cell and neural progenitor cell populations (NSC/NPC) of the central nerve system are found from brain early development to adult stage. These cells are responsible for the maintenance of neurogenesis and gliogenesis in the developing and adult brain<sup>[54]</sup>. In general, there are three sources of human stem cells that can be used for neurological disorders treatment: NSCs, embryonic stem cells (ESCs), and bone marrow<sup>[53]</sup>. NSCs are characterized by their unique migratory ability and targeting glioma<sup>[3]</sup>, in addition to self-renewal capacity and multipotency<sup>[53,55]</sup>. In the adult brain, neurogenesis is primarily occurring in two precise regions: the dentate gyrus of the hippocampus and the subventricular zone (SVZ) of the cerebral cortex. NSCs localize in a specific microenvironment known as the stem cell niche<sup>[54]</sup>. The complexities of treatment of the brain tumors is due to the unique neuroanatomical location of the injuries next to special neurovascular structures<sup>[56]</sup> and number of lesion region(s) which can be either focal or multifocal<sup>[57]</sup>, as well as cellular heterogeneity that includes cells expressing neural/progenitor stem cells, astroglial, and neuronal markers<sup>[3,54]</sup>. Furthermore, the highly diffuse invasiveness of tumor cells and their resistance to conventional therapies result in rapid and aggressive recurrence<sup>[56,58]</sup>. While as NSC selectively migrates to tumor site, it is considered as the best candidate for the treatment of brain tumors either for injured tissue replacement or as drug delivery system *in vivo*<sup>[12,56,59]</sup>. Because of the selective migratory potential, NSC can be engrafted into injured CNS within distinct areas. After NSCs implantation, the migrated cells are incorporated into the local neural site of injury and accompanied by their regular gene expression. Recent evidence has indicated that the implanted exogenous NSCs may affect the surrounding niche by enhancing protection and renewal of host neural pathways<sup>[56]</sup>. Damaged tissue repairing can be promoted by recruitment of endogenous stem cells or lineage-committed precursor cells. Experimental stimulation of some lesions was accompanied by maximizing proliferation of stem cells of the subventricular zone and homing of newly formed cells into the site of injury<sup>[59]</sup>. Moreover, the recruitment of endogenous stem cells and their ability of self-renewal are associated with the formation of new neurons and oligodendrocytes that become normally integrated into the CNS parenchyma<sup>[59]</sup>. Transplantation of unmodified NSCs leads to extend survival of animals with experimentally induced tumors<sup>[56]</sup>. The preferential migration and homing of stem cells to brain tumor sites is enhanced by factors secreted by gliomas and cells in the surrounding niche, and this can play a role in delivering anti-tumor

agents to gliomas<sup>[12]</sup>. In addition, NSCs that are genetically modified to produce IL-4 enable them to promote tumor remission. Using NSC treatment, the life time was prolonged in glioma mice<sup>[59]</sup>.

On the other hand, genetically modified NSCs are considered to be a desirable option for delivering anti-cancer remedies because of NSC's glioma-tropism<sup>[12]</sup>. Such modified cells can display either direct cytolytic molecules like TNF-related apoptosis inducing ligand (TRAIL)<sup>[3]</sup> or suicide genes which regulate the enzymatic conversion of non-toxic prodrug into activated oncolytic agents<sup>[3,55]</sup>. A variety of prodrug/enzyme systems have been developed by using NSCs as an enzyme delivery vehicle for brain tumors, such as cytosine deaminase and HSV- thymidine kinase<sup>[3,12]</sup>. There are other two types of stem cells except NSC have been also used in the treatment of brain tumors<sup>[12]</sup>. Mesenchymal and embryonic stem cells (MSCs and ESCs) have been suggested for cell transplantation. MSCs derived from bone marrow have attractive properties such as providing degree of immune tolerance. This may help reduce host rejection to transplanted cells. ESCs have also been used to produce neural precursors by their pluripotent potential with a degree of immune privilege<sup>[53]</sup>. The route of cell administration depends on the site and number of CNS lesions<sup>[57]</sup>. The concept of intranasal delivery of NSCs may be a convenient and noninvasive alternative approach for stem cell-based therapies since the direct intracerebral administration is an invasive approach with low engraftment efficacy<sup>[58]</sup>.

## IMMUNOTHERAPY FOR TREATMENT OF MALIGNANT BRAIN TUMORS

### The immune system

The immune system is designed to function in the specific recognition and to eliminate foreign pathogen from the body within minutes of infection. The patterns of response can be classified into innate response and adaptive response. The innate immunity comprises monocytes and tissue macrophages such as microglia, granulocytes, natural killer cells (NK), and antigen presenting cells such as dendritic cells (DC). These cells are capable of initiating rapid and nonspecific immune response as a result of responding to signals from damaged tissues or infection through recognition of surface pathogen-associated molecular patterns (PAMP) such as mannose and lipopolysaccharides as well as heat-shock proteins derived from tumor cells. Inflammatory mediators and cytokines are involved in the immune response<sup>[60]</sup>. On the other hand, adaptive immune response including highly specific, lymphocyte-directed response which developed through four distinct stages: recognition and activation, clonal expansion, and effector function, and memory. This type of response is either humeral (antibody- mediated immune response) secreted by differentiated B-cells (antibody producing plasma cells) or cell-mediated immune response achieved by CD4<sup>+</sup> helper T-cells or CD8<sup>+</sup> cytotoxic T-cells (CTL)<sup>[61,62]</sup>.

### Immune privilege in the brain

The brain is considered an immune-privileged organ to which the immune system has limited access due to the lack of lymphatic draining system and its separation from circulation by BBB<sup>[60]</sup>. The concept of immune privilege in brain has been supported by the successful engraftment of allogeneic tissue grafts placed within the brain of experimental models<sup>[63]</sup>. In addition, many autopsy studies have revealed the absence of naïve T-cells in the brain due to their inability to pass through BBB, suggesting the lack of major machinery important to initiate an immune response in the brain. Nevertheless, there is growing evidence that supports the presence of

multiple immune pathways in the brain<sup>[60]</sup>. First, local microglial cells, which can process and present tumor antigens, function as major antigen presenting cells (APCs) in the brain. These cells express phenotypic and functional features of both DC and macrophage<sup>[64-66]</sup>. Second, activated lymphocytes can infiltrate into a tumor and initiate anti-brain tumor immune response<sup>[63,67]</sup>. These tumor-infiltrative lymphocytes (TILs) are associated with long survival in patient with GBM. Finally, *in vitro* expansion of TILs revealed the existence of tumor antigen-specific lymphocyte, indicating that an effective adaptive immune response can be occurred<sup>[66]</sup>.

### Tumor mediated immune suppression

Patients with GBM display a profound immune suppression induced by gliomas production of immune inhibitory factors. Such immune suppression is mediated by cytokines and growth factors such as interleukin-10 (IL-10), transforming growth factor- $\beta$  (TGF- $\beta$ ), prostaglandin- E2<sup>[3]</sup>, macrophage chemoattractive protein (MCP-1), IL-6<sup>[65]</sup>, as well as VEGF. Those inhibitory factors can cause lymphopenia, downregulated lymphocyte protein expression, and impaired antibody yield<sup>[64]</sup>. Therefore, it is important to develop effective strategies that specifically combat these suppressive effects and to find effective approaches to deliver the immunologic effector molecules to the brain tumors<sup>[3,64]</sup>. Immunotherapy can be divided into active immunotherapy which imply the use of tumor vaccines in which the patients can be immunized by tumor cells themselves or characterized tumor antigens, and passive immunotherapy in which the tumor-specific effector cells are prepared *in vitro* and transferred to the patients either systemically or intracranially<sup>[64,68]</sup>.

### Immunotherapy

The immune system has various levels of control to ensure the appropriate balance between immune stimulation and immune suppression. Over two decades the basics of this regulation have become comprehensible. The knowledge may provide opportunities for a precise immune response against tumors<sup>[65]</sup>. For example, tumor- associated antigens expressed by gliomas and their ability to artificially stimulate class I and II MHC- restricted antigen presentation have contributed to manipulate the immune response as a target of immunotherapy<sup>[67]</sup>. T-cells play an important role in tumor vaccine therapy because of their ability to specifically activate and proliferate against tumor cells and also generate a memory mechanism<sup>[62]</sup>. Major histocompatibility complex (MHC) class I-tumorigenic peptide complex which is able to activate CD8<sup>+</sup> T-cells has been utilized in the preparation of tumor vaccines<sup>[61,69]</sup>. Moreover, CD4<sup>+</sup> T-cells are involved in the elimination of tumors that are resistant to CD8<sup>+</sup> mediated response. CD4<sup>+</sup> cells cooperated with NK cells to perform this effector function. This observation of anti-tumor activity by CD4<sup>+</sup> cells is independent on the expression of MHC molecules on tumor cells, suggesting the possibility to recognize poorly MHC-expressing tumor cells by designing approaches to stimulate CD4<sup>+</sup> T-cells response against tumor antigens<sup>[65]</sup>. Thus, an attractive strategy is to use DCs to stimulate an immune response against tumors by loading of tumor antigens on the autologous DCs which are isolated from the patient by leukapheresis followed by re-administration of these cells into the patient. During the incubation period, autologous DCs are activated by a mixture of recombinant cytokines or immunostimulatory molecules<sup>[65]</sup>.

Furthermore, it is possible to utilize irradiated autologous whole cancer cells<sup>[68]</sup>. Gliomas not only display a variety of tumor antigens, but also are able to present these antigens to T cells. During *in vitro* and *in vivo* stimuli, such as exposure to IFN- $\gamma$ , it was found

that gliomas are able to express low levels of class I MHC. Hence, glioma showed ability to present tumor-associated antigens to CTLs by the class I MHC pathway<sup>[67]</sup>. Moreover, phase I studies of subcutaneous vaccinations using synthetic peptides against glioma-specific antigen epitopes have been conducted. The ultimate goals of vaccination were safety and to obtain CD8<sup>+</sup> T-cells sensitization against targeted glioma-specific antigen. It was found that patient with newly diagnosed high-risk LGG demonstrated better vaccine-responsiveness than recurrent patient represented by a positive tendency for IFN- $\gamma$  ELISPOT responses as well as median PFS<sup>[70]</sup>. Another approach involves *ex-vivo* activation and proliferation of tumor-specific CTLs by peptide-pulsed DCs and to stimulate potent CTLs immune response *in vitro* by using mRNA-transfected DCs<sup>[3]</sup>. Transfection of DCs with mRNA encoding certain tumor proteins is simple and efficient<sup>[71]</sup>. Furthermore, specific targeting of overexpressed determinant in GBM such as epidermal growth factor variant III (EGFRvIII) by DC vaccines. The overexpression of those surface receptors which are commonly mutated in malignancies results in uncontrolled cell growth<sup>[3]</sup>. Moreover, monoclonal antibodies can be directed towards the gliomas by targeting EGFRvIII<sup>[11]</sup>. For instance, Nimotuzumab and Cetuximab/Erbitux are the two antibodies used currently<sup>[66]</sup>. Another cytotoxic approach involves the blocking of early angiogenesis by targeting of tumor endothelial cells which display distinct surface markers that are not found on normal tissues. Anti-angiogenic activity can be achieved by the adoptive transfer of T-cells that specifically recognize tumor endothelial cells and leads to destruction of tumors by cutting off the blood supply to the growing tumor<sup>[64]</sup>. Anti-angiogenic activity can also be reached by means of anti-VEGF pathways such as using Avastin antibody that competitively binds to VEGF receptors and thus inhibits the cytokine binding and simulation of these receptors<sup>[66]</sup>.

## TARGETED THERAPY

The long-term usage of anti-cancer agents leads to lethal destruction for normal cells in addition to the cancerous cells due to their systemic cytotoxicity and limited specificity. Therefore, development of novel anti-cancer drugs with minimized toxicity and targeted delivery approaches for the treatment of GBM are highly desired. Accomplishment of targeted therapy requires preferential binding to tumor cells<sup>[6]</sup>. To overcome the drawbacks of chemotherapy, many strategies can be used such as enzyme-prodrug therapy which confers a highly localized drug at the site of choice. Well-characterized enzyme-prodrug combination involves the use of herpes simplex viral vector-thymidine kinase/ganciclovir (HSV-TK/GCV). It achieves *in situ* delivery of effector enzyme by encapsulation<sup>[41]</sup>. Inhibiting angiogenesis in glioma is another targeted treatment. This approach is based on the fact that glioma stem cells are regulated by producing VEGF and glioma tissues receive nutrient and oxygen via vascular niche. VEGF and stromal-derived factor-1 are two angiogenic agents that stimulate angiogenesis. Hence, utilizing anti-angiogenic therapy is believed to have the potential to overcome GBM resistance to conventional therapy<sup>[54]</sup>. Besides, VEGF contributes to BBB destruction in glioma, results in increase in infiltration of interstitial fluid and the formation of intracerebral edema. This formed edema reduces the delivery efficacy of medication to glioma cells. Moreover, hypoxia plays a role in the sensitivity of gliomas to chemotherapy and radiotherapy. Hence, it was proposed to use angiogenic inhibitors to reduce brain edema, and to promote drug delivery and increase radio-sensitization. Anti-cancer treatment can be achieved by using single inhibitor or a combination of inhibitors and also a combination of inhibitors with chemotherapeutic agents<sup>[6]</sup>.

Taken together, many efforts have been made for brain tumor treatment even though GBM is still incurable. Clinical trials have been conducted based on targeted drug delivery strategy and can be found elsewhere<sup>[29,72-74]</sup>. As a direction for further development of effective treatment of brain tumors, eliminating cancer stem cells from the tumor is likely the key in order to control recurrence of brain tumors. CD133 antigen has been considered as a putative stem cell marker in malignant brain tumor<sup>[75]</sup>. Thus, targeting this cancer stem cell marker could impact the survival of GBM patients. Mammalian target of rapamycin (mTOR) is another GBM cancer stem cell marker that can be used in a targeted therapy<sup>[76]</sup>. Overall, identifying biomarkers for targeted drug delivery which can overcome BBB would improve patient response to therapy and enhance survival of brain tumor patients.

## CONFLICT OF INTERESTS

There are no conflicts of interest with regard to the present study.

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