

Tailored nanocarriers and bioconjugates for combating glioblastoma and other brain tumors

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ABSTRACT

Worldwide, the incidence of primary brain tumors is on the rise. Unfortunately, noninvasive drug therapy is hampered by poor access of most drugs to the brain due to the insurmountable blood-brain barrier (BBB). Nanotechnology holds great promise for noninvasive therapy of severe brain diseases. Furthermore, recent bioconjugation strategies have enabled the invasion of the BBB via tailored-designed bioconjugates either with targeting moieties or alterations in the physicochemical and/or the pharmacokinetic parameters of central nervous system (CNS) active pharmaceutical ingredients. Multifunctional systems and new entities are being developed to target brain cells and tumor cells to resist the progression of brain tumors. Direct conjugation of an FDA-approved drug with a targeting moiety, diagnostic moiety, or pharmacokinetic-modifying moiety represents another current approach in combating brain tumors and metastases. Finally, genetic engineering, stem cells, and vaccinations are innovative nontraditional approaches described in different patents for the management of brain tumors and metastases. This review summarizes the recent technologies and patent applications in the past five years for the noninvasive treatment of glioblastoma and other brain tumors. Till now, there has been no optimal strategy to deliver therapeutic agents to the CNS for the treatment of brain tumors and metastases. Intensive research efforts are ongoing to bring novel CNS delivery systems to potential clinical application.

Key words: Glioblastoma; brain delivery; blood-brain barrier; nanotechnology; novel treatment

INTRODUCTION

The central nervous system (CNS) was first described in the Edwin Smith papyrus about 3,600 years ago.^[1,2] Tumors and cancer were described in this papyrus, as well as in the Ebers papyrus, dating back to 1,300 BC.^[1-3] Hippocrates, the father of Western Medicine, was the first to use the terminology

“*karkinos*,” a Greek word for “crab/cancer,” because he noted that these tumors had tentacles reminiscent of the legs of a crab.^[4]

According to GLOBOCAN 2012, the number of new cases diagnosed with brain tumors were 256,000 for both sexes, out of 14.1 million total cancer cases.^[5] The incidence of brain tumors is higher in men than in women.^[5] The highest incidence rates

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How to cite this article: ELAmrawy F, Othman A, Adkins C, Helmy A, Nounou M. Tailored nanocarriers and bioconjugates for combating glioblastoma and other brain tumors. *J Cancer Metastasis Treat* 2016;2:112-22.

Received: 17-10-2015; **Accepted:** 15-12-2015.

Access this article online

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Website:
www.jcmtjournal.com

DOI:
10.20517/2394-4722.2015.78

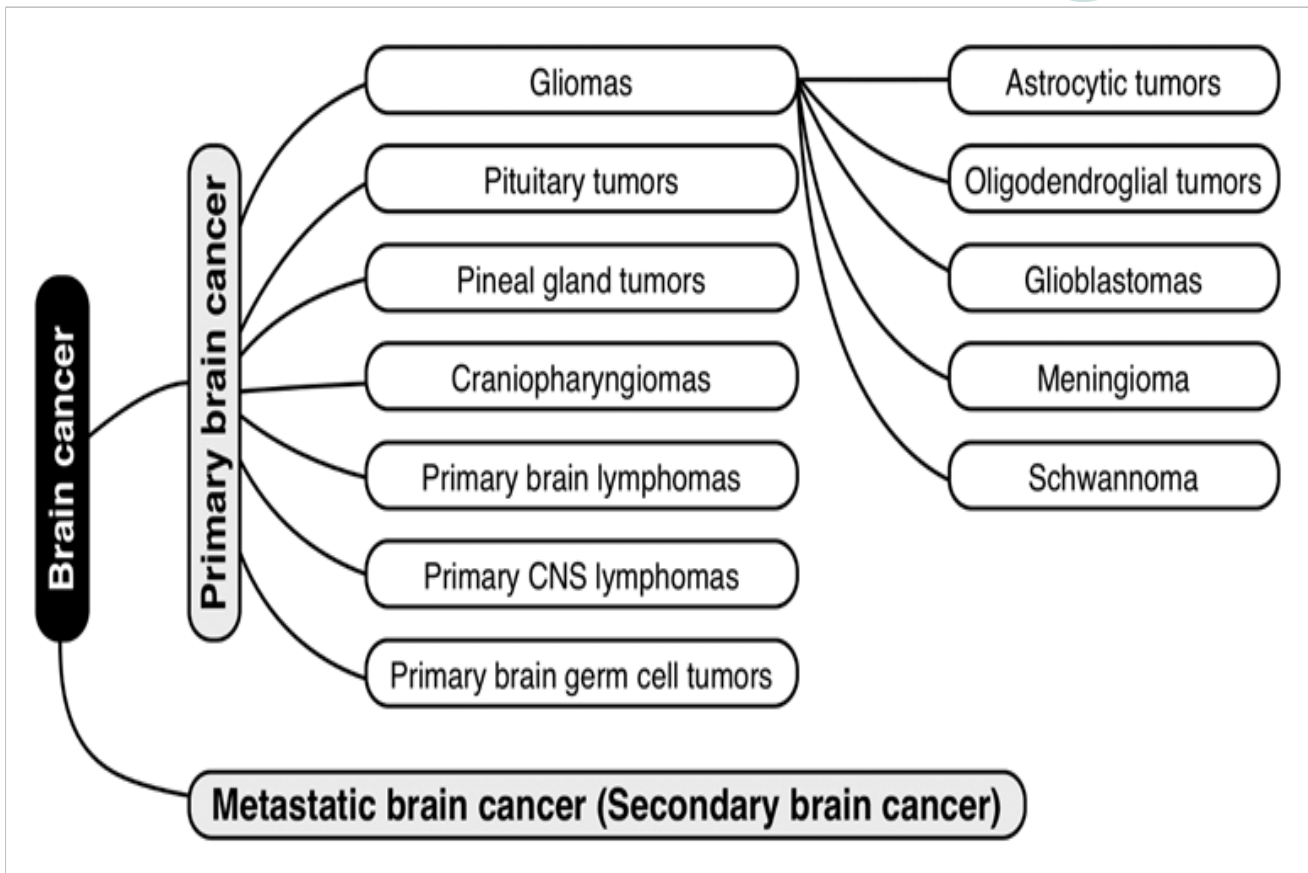


Figure 1: Most common types of brain tumors^[6-8]

occur in people between 65 and 79 years of age.^[5]

This review provides an overview of the management of primary brain tumors, especially glioblastoma multiforme. The huge surge in the development of novel strategies for management of primary brain tumors in the past 5 years will be demonstrated in this review article via recent published patents. Table 1 enumerates patents on brain drug delivery and treatment of brain tumors between 2010 and 2015 [supplement material Table 1]. This part of the review will focus on recent patents and studies using nanoparticles and bioconjugates in brain tumor treatment and diagnosis.

TYPES OF BRAIN TUMORS

Primary brain tumors originate within brain tissue. They are classified according to the type of originating tissue [Figure 1]. The most common primary brain tumors are gliomas, pituitary adenomas, and vestibular and primitive neuroectodermal tumors.^[6,7] Gliomas are tumors that begin in the glial tissue. Gliomas include glioblastomas, astrocytomas, schwannomas, oligodendrogliomas, and others.^[8]

The most common malignant brain tumor is glioblastoma multiforme (GBM, 81% of malignant CNS tumors), which is usually associated with poor prognosis.^[9-11] GBM is classified as a subtype of astrocytoma. GBM is classified as grade IV/V according to the WHO.^[11] With regard to treatment, GBM and

grade III brain tumors are managed similarly.

Any intracranial tumor, regardless of the degree of malignancy, can potentially invade or displace critical brain areas, resulting in neurologic compromises.^[12] The most common complications are seizures, peritumoral edema, venous thromboembolism, fatigue, and cognitive dysfunction.^[11-13]

GBM, is usually described in two different clinical forms, primary and secondary.^[14] Primary GBM is the most common form (about 95%); it typically arises de novo, within 3-6 months,

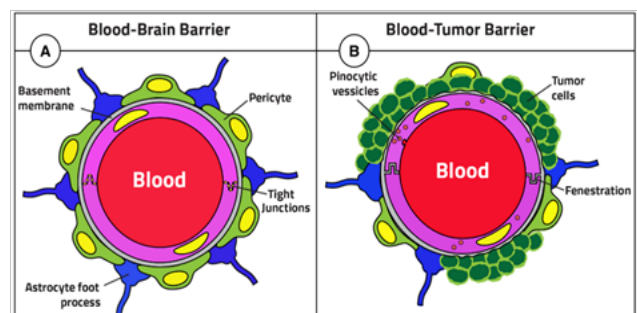


Figure 2: Diagram illustrating the difference between BBB and BBTB. BBB: blood brain barrier; BBTB: blood brain tumor barrier

in older patients. On the other hand, secondary GBM arises from prior low-grade astrocytomas over 10-15 years in younger patients.^[13] Both types respond similarly to treatment.^[13]

THE BLOOD-BRAIN BARRIER: THE BRAIN'S PROTECTION SYSTEM

The blood-brain barrier (BBB) represents a diffusion barrier system that protects the brain. BBB maintains the brain's homeostasis by controlling the influx of blood components into the brain.^[15-17] The BBB is mainly formed by brain capillary endothelial cells (BCEC), in addition to other cell types such as pericytes, astrocytes, and neuronal cells that play an important role in its function.^[17] BCEC's tight junction prevents paracellular transport of small and large water-soluble compounds from the circulation to the brain, except for some very small or gaseous molecules such as water and carbon dioxide [Figure 2].^[15,17-20]

In addition to physical barriers, several functional barriers contribute to the restrictive nature of BBB, creating major obstacles to effective drug delivery into the CNS.^[21] Besides tight junctions, a group of efflux transporters [such as P-glycoprotein (Pgp), breast cancer resistance protein, and multidrug resistance-associated proteins] are expressed on the brain tissue and collectively cause rapid efflux of large groups of lipophilic drugs from the CNS.^[22,23] Also, the presence of numerous degradative enzymes in the BBB creates another functional barrier.^[17,24,25]

The functioning and organization of the BBB can be altered under pathological conditions, such as in the case of tumors. In such a case, the barrier is called the blood-brain tumor barrier (BBTB).^[19] In low-grade gliomas, BBTB resembles BBB, while in high-grade gliomas, BBTB becomes disrupted and "leaky," characterized by major alterations of the normal vascular function manifested by contrast-enhanced MRI by Dhermain *et al.*^[19,26] However, the magnitude of this disruption is unlikely sufficient to allow drug penetration in therapeutically meaningful quantities, and thus BBTB remains a major obstacle for brain drug delivery.^[27,28]

BRAIN DRUG DELIVERY

Although BBB is difficult to bypass, inventions in the area of brain delivery in the last five years have shown promising progress and well-established techniques. There are two general strategies adopted to facilitate crossing the blood-brain barrier: invasive techniques and noninvasive techniques.^[29] Invasive techniques rely primarily on disrupting the BBB's integrity by direct intracranial drug delivery through intracerebroventricular, intracerebral, or intrathecal administration, use of osmotic pumps, or biochemical means.^[29] All these approaches are severely limited by poor distribution into brain parenchyma.^[30]

Noninvasive methods include drug modification through transformation of the drug into lipophilic analogues or prodrugs or through chemical drug delivery, carrier-mediated drug delivery, receptor/vector-mediated drug delivery, and intranasal drug delivery.^[29,31] The noninvasive techniques depend on either pharmacologic strategies (lipid-based systems), or physiologic-based strategies (nutrient or receptor-mediated systems).^[31] These techniques will be the focus of the

next sections of this review.

Receptor mediated transcytosis

Receptor-mediated transcytosis facilitates trans-BBB transport of various macromolecules after initial binding of a targeting ligand to a receptor expressed on the brain endothelial cells.^[32,33] Transferrin receptor (TfR), insulin receptor, low-density lipoprotein receptor (LDLr), acetylcholine receptor, glutathione transporter, and diphtheria toxin receptor are examples of receptors of interest.^[34] Several ligands have been studied and utilized to shuttle nanoparticles, antibodies, and drugs across the BBB and into the brain cells.^[35] For instance, the LDL receptor family can be targeted via aprotinin, ApoE3 mimetic, angiopep-2, and p97 (melanotransferrin).^[36-38]

Angiopep-2, a 19-amino-acid peptide, is one of the promising vectors designed to target the LDLr-related protein to mediate transcytosis across the BBB.^[39] Angiochem Inc., in partnership with Geron Inc., developed ANG1005 (also known as GRN 1005), an Angiopep-2-PTX conjugate for treating primary (glioblastoma) and metastatic brain tumors. ANG1005 showed promise in many preclinical studies and was well tolerated in phase I clinical studies.^[32,40] However, phase II clinical trials utilizing ANG1005 are either terminated or ongoing but not actively recruiting participants, and Geron has announced that it discontinued development of GRN1005 (NCT014880583, NCT01967810, NCT02048059).^[41] Other Angiopep drug conjugates include ANG1007 (angiopep-2-doxorubicin),^[42] ANG1009 (angiopep-2-dimethylglycine etoposide), and ANG4043 (angiopep 2-trastuzumab). ANG4043 is a novel brain-penetrant peptide-mAb conjugate that is effective against HER2-positive intracranial tumors in mice, an angiopep anti-HER 2 mab conjugate. Applications of angiopep as brain targeting moiety are still under intensive research.^[43-47]

Pieter Gaillard, in a patent for "to-BBB technologies BV," suggested delivery of drugs to cells and across the blood-brain barrier by targeting them to endogenous internalizing uptake receptors for glutathione on the capillaries of the brain, without modifying or disrupting the normal function of the neuroprotective BBB.^[48] In another set of patents, Gaillard and his to-BBB technologies BV group used diphtheria toxin receptor ligand to control the blood-brain barrier vascular permeability and deliver lipopolysaccharide-sensitive nucleic acids and polypeptides across the BBB.^[49,50]

Dickerson *et al.*^[51] developed agents that modulate calcitonin-gene related peptide (CGRP) signaling. This represents a novel target for cancer, particularly glioma and breast cancer, since CGRP stimulates cell replication and growth. In another patent, Furness *et al.*^[52] invented a method for detecting calcitonin receptor in brain cells of the subject; this method can be used for therapeutic, diagnostic, and prognostic purposes.

Due to the increased expression of the transferrin receptor in brain glioma, it is one of the most extensively studied targets for receptor-mediated transcytosis (RMT).^[53] Cedars-Sinai

Medical Center owned two patents on using anti-TfR antibodies conjugated to polycefin-LLL to cross BBB.^[54] In the second patent, Patil *et al.*^[55] prepared polycefin-LLL nanonjugates that could be loaded with temozolomide (TMZ) in its hydrazide form and modified with PEG.

A promising approach to enhance brain delivery is to inhibit efflux transporters by modulating their expression and/or activity.^[56,57] Clinical trial data of third-generation inhibitors (ariquidar, zosuquidar and elacridar) are awaited for possible clinical application of this treatment approach.^[58] Other naturally occurring compounds such as curcumin,^[59] quercetin,^[60,61] and kaempferol are being studied and modified for use in brain cancer therapy to overcome the problem of multidrug resistance (MDR).^[62] Barthomeuf *et al.*^[22] studied the use of curcuminoid compounds to enhance the clinical efficacy of docetaxel for the treatment of cancers including GBM. The group proposes that, in addition to reducing Pgp transport, curcumin may reduce HIF-1-dependent and HIF-1-independent angiogenesis, which in turn would inhibit tumor progression, angiogenesis, and induction of resistance.^[22] Banks *et al.*^[63] provided a method to inhibit the function of RNA- and DNA-encoding efflux transporters among other blood-brain barrier proteins using antisense compounds. The patent suggests that inhibition of Pgp expression would allow increased accumulation of chemotherapeutic drugs in the CNS and thus improve therapeutic clinical outcomes. In another patent, McChesney *et al.* used a group of taxane analogues that stabilize tubulin dimers or microtubules at G2-M during mitosis but are not substrates for MDR proteins.^[64]

The physiologic approach to target brain tumors takes advantage of endogenous receptors that are highly expressed at the BBB.^[30,31] Unfortunately, almost all the receptors are nearly nonspecific as indicated by percentage dose reaching the brain following administration compared to percentage reaching other organs such as the liver, spleen or lung.^[30] To avoid such nonselective patterns, Tosi *et al.*^[65] used double-targeting ligands to provide added targeting benefit and minimize nonselectivity. The targeting ligands used by Tosi *et al.*^[65] were sialic acid and glycopeptides. The targeting ligands were covalently conjugated to PLGA nanoparticles (SA-g-7-Np).^[65]

Nanocarriers for brain drug delivery

Nano-based delivery systems have seized increased attention from formulators, as indicated by recent patents and studies [supplement material Table 1]. This can be attributed to their unique ability to deliver to therapeutic and diagnostic moieties.^[66-72] Nanocarriers are unique because of their small size (typically sub 200 nm).^[73] Nanoparticles are easily tailored in their structure and properties.^[73] They also can carry active therapeutic or diagnostic moieties of heterogeneous physicochemical properties, and their release pattern can be controlled.^[73]

A representation of possible NP structure(s) is shown in Figure 3A. NPs can be formulated from different materials including polymers, lipids, organometallic compounds, and viruses.^[74]

However, mostly amphiphilic molecule-formed liposomes and polymeric nanoparticles (chemical species having a “polar” head group and “hydrophobic” tails) have been extensively exploited for brain drug delivery.^[73,75] Long circulation time of the delivery system can be achieved by conjugating the nanoparticles with polyethylene glycol (PEG) (“PEGylation”).^[66,67] The PEG-coated nanoparticles can escape the mononuclear phagocytic system and circulate in the body for a longer time, increasing the chance of reaching the target and thereby enhancing the effect of the loaded drug.^[66,67] The effect and benefits of PEGylation are discussed later.

Unfortunately, nanoparticles can carry some serious adverse effects.^[76] Adverse effects of nanoparticles depend on individual factors such as genetics, existing disease conditions, exposure, nanoparticle chemistry, size, shape, agglomeration state, and electromagnetic properties.^[76] The key to understanding the toxicity of nanoparticles is their size.^[76] Nanoparticles are smaller than mammalian cells and cellular organelles, which allows them to penetrate these biological structures and disrupt their normal function.^[76] Examples of toxic effects include tissue inflammation and altered cellular redox balance toward oxidation, causing abnormal function or cell death.^[76]

Polymeric nanoparticles

Polymeric micelles are formed from amphiphilic block copolymers forming a core/shell nanostructure. In aqueous media, the hydrophilic heads are arranged to the outside and the hydrophobic tails to the inside to stabilize the structure, which is suitable for IV injections^[77]. Delivery of docetaxel for the treatment of brain tumors by cyclic arginine-glycine-aspartic acid (RGD)-tagged polymeric micelles was developed by Li *et al.*^[78] The authors found that RGD has affinity to bind to integrin receptor, which is overexpressed in glioblastoma tissues.^[78]

Krebs invented a novel biodegradable hydrogel polymer comprising chitin and poly(lactic-co-glycolic acid) for delivery of therapeutic agents to brain tumors.^[79] The biodegradable hydrogel detailed in Krebs’ patent would allow release of anti-VEGF to the periphery of the resected tumor site in a localized manner, with stable release rate over a sustained period. The pH-sensitive polymers which release the drug in an acidic microenvironment of solid tumors and endosomes, were the focus of a patent by Bae *et al.*^[80] Targeting ligands, such as folate, can also be attached to the mixed micelles for enhancing drug delivery into brain cells.^[56]

Zhou *et al.*^[81] in a recent patent, developed small, less aggregable brain-penetrating polymeric nanoparticles that can be loaded with drugs. In another patent, Wu *et al.*^[82] used polymethacrylic acid grafted starch (PMAA-g-St) nanoparticles containing polysorbate moieties that can target the polymer to brain tissues. Hyper-branched polymer of polyglycerol-amine (PG-NH₂) was demonstrated to accumulate in the tumor environment due to the enhanced permeability and retention effect (EPR), as described in a patent by Yerushalmi *et al.*^[83]

Tour *et al.*^[84] devised poly(ethylene glycolated) Hydrophilic Carbon Clusters Antibody Drug Enhancement System (HADES), in which nanovectors are coupled with an active agent and one of the agents that target glioma surface antigens, such as Interleukin 13 receptor (IL-13R), epidermal growth factor receptor (EGFR), and Gglial fibrillary acidic protein (GFAP).

Lipid-based nanoparticles

Liposomes are the first generation of nanoparticulate drug delivery systems and consist of one or more vesicular bilayers (lamellae) composed of amphiphilic lipids, delimiting an internal aqueous compartment.^[85] The most advantageous features of liposomes are their ability to incorporate and deliver large amounts of drugs and the possibility of decorating their surface with various ligands.^[86]

Chlorotoxin-modified, doxorubicin-loaded liposomes were described by Xiang *et al.*^[87] to target chloride channel-mediated brain gliomas. Also, Li *et al.*^[88] suggested that chemotherapy using functional targeting of paclitaxel via artemether liposomes could provide a novel strategy for treating invasive brain glioma. Chen *et al.*^[89] studied lactoferrin-modified, doxorubicin-loaded procationic liposomes and showed that the system offers effective therapeutic potential for gliomas. Cationic liposomes were described in a patent by Migliore *et al.*^[90] to provide a novel, noninvasive strategy for nasal delivery of neuroactive proteins to the brain for treatment of central nervous system disorders. In another patent by Munson *et al.*^[91] PEGylated uni-lamellar vesicle liposomes were described that were appropriately sized and formulated to cross the blood-brain barrier to deliver imipramine. To overcome toxicity associated with high peak drug concentration, Redelmeier and Luz used a non-PEGylated liposomal composition comprising at least one saturated neutral phospholipid and at least one saturated anionic phospholipid encapsulating a therapeutic or diagnostic agent.^[92]

Solid lipid nanoparticles

Solid lipid nanoparticles (SLN) are stable lipid-based nanocarriers with a solid hydrophobic lipid core in which the drug can be dissolved or dispersed.^[93,94] They are made of biocompatible lipids such as triglycerides, fatty acids, or waxes.^[93,94]

Nanoparticles containing brain-derived lipids may be transported into the brain via specific receptors for these lipids. Panyam and Chavanpatil designed nanoparticles composed of a brain lipid (phospholipid), a supplemental lipid (long chain saturated or unsaturated fatty acids, stearic acid, palmitic acid, linolic acid, or linoleic acid) and a PEG-conjugated lipid (dist earoylphosphatidylethanolamine-polyethylene glycol).^[95] This nanoparticle system can deliver a drug or therapeutic compound to the brain.^[95]

Jin *et al.*^[96] used solid lipid nanoparticles made of lipids extracted from deproteinated lipoproteins and enriched with cationic cholesteryl hydrochloride and phosphatidyl-ethanolamine. The authors, after intravenous administration of such cationic NPs

for the delivery of siRNA to inhibit c-Met expression, were able to suppress the tumor growth without evident signs of systemic toxicity in an orthotopic xenograft tumor mouse model of glioblastoma.^[96]

Singh *et al.*^[97] studied lactoferrin-bioconjugated solid lipid nanoparticles as a new drug delivery system for potential brain targeting. Lactoferrin was conjugated to the surface of SLN using carbodimide coupling. SLN surface-conjugated with lactoferrin-encapsulating docetaxel maintained its complete activity and conserved its mechanism of action as characterized by cell viability and apoptosis studies.^[97]

PEGylated-liposomal formulation for enhanced pharmacokinetics (Stealth® technology)

PEGylated liposomal doxorubicin (PLD; Caelyx™, Doxil®) represents the first commercial liposomal formulation for passive cancer management with enhanced efficacy and reduced toxicity profile.^[98] PLD is superior to the conventional doxorubicin preparation, showing reduced cardiotoxicity and prolonged activity due to stealth properties imparted by its polyethylene glycol PEG layer. Despite PLD smart passive properties in targeting cancer, its long circulation half-life and its ability to escape the reticuloendothelial system (RES) defense mechanism, it fails to manage brain tumors because of the BBB enhanced protective features.^[50]

For the PLD to cross BBB, glutathione-PEGylated liposomal doxorubicin (2B3-101) is being investigated. Based on the patent owned by BBB Therapeutics BV (formerly, to-BBB technologies), glutathione-based drug delivery system can target brain tissues by receptor-mediated transcytosis.^[50] According to the preclinical studies, 2B3-101 showed a 5-fold enhanced doxorubicin brain delivery versus PLD (Doxil®).^[99] The company held a phase I/IIa clinical study in patients with solid tumors and brain metastases or recurrent malignant glioma.^[100]

Nektar develops new drug candidates by utilizing its proprietary 3D 4-armed branched PEGylation and advanced polymer conjugate technologies to modify the chemical structure of various active pharmaceutical ingredients. It is a PEGylation technology supplier to a number of pharmaceutical companies including Affymax Inc., Amegen Inc., Merck and Co. Inc., Pfizer Inc., and UCB Pharma.^[101] Nektar Therapeutics is currently investigating the use of etirinotecan pegol (NKTR-102) for treating brain tumors.^[101,102] Furthermore, Nektar Therapeutics is conducting a phase II pilot study of NKTR-102 in patients with recurrence of high-grade glioma after bevacizumab therapy.^[102]

Bioconjugates delivery systems

The main aim of bioconjugation is to form a stable, biologically cleavable covalent link between two molecules, at least one of which is a biomolecule [Figure 3B].^[103] Bioconjugation is a form of functionalization of nanoparticles, which aims to increase stability, protect a drug from proteolysis, or enhance the targeting properties of the delivery system.^[77,103] In spite of the historic fact that bioconjugates are older than nanoparticles,

research is increasingly being diverted back to it.^[103] Factors that may encourage this resurgence of interest could include its ease of synthesis, high scale-up yield, ease of bench-to-bedside transformation, ease of formulation, and final formulation stability.^[103] Bioconjugation reactions are generally categorized by the general reactivity or the functional group involved in the conjugation process, such as amine reactions, thiol reactions, carboxylate reactions, hydroxyl reactions, aldehyde and ketone reactions, active hydrogen reactions, photochemical reactions, and cyclo-addition reactions.^[103] The design of a useful bioconjugate will depend mainly on its use, purpose, and the desired properties needed.^[104] Thus, one could choose a suitable molecule and a proper cross-linker to form the bioconjugate.^[104] The key to forming a successful bioconjugate is choosing the suitable crosslinker between the molecules.^[103]

As in any delivery system, bioconjugates are usually tailor-designed to provide the function of interest. The active drug entity can be linked to a diagnostic agent, targeting moiety, pharmacokinetics-modifying agent such as PEG, bioresponsive or stimuli-sensitive agent, an aptamer, or an antibody. Furthermore, the choice of the proper linker can impart new functions and smart characteristics to the bioconjugate system [Figure 3].

A bioconjugate was patented by Bacha *et al.*^[105] that may compromise a chimeric peptide of the structure of Formula (D-III): A-NH(CH₂)₂S-S-B (cleavable linkage), avidin-biotin-agent complex, PEG layer, and a fusion protein for targeting the brain tumor. Another bioconjugate formulation, developed by Jefferies *et al.*^[38] comprised a BBB-transport moiety linked to an antibody or therapeutic Fc-fusion polypeptide. Jefferies *et al.*^[38] modified Fc regions to facilitate the delivery of therapeutic and/or diagnostic polypeptides across the BBB and thereby treat and/or diagnose conditions associated with the CNS, including cancer.

A patent entitled “Anti-EGFR antibody drug conjugate formulations” by Tschoepe *et al.*^[106] discussed a staple formulation including: an anti-EGFR antibody or antigen-binding portion thereof conjugated to an auristatin, a sugar,

a surfactant, and histidine. In their patent Adair *et al.*^[107] described nonaggregating resorbable calcium phosphosilicate nanoparticles bioconjugated to targeting molecules that are specific for brain cells. The targeting moieties used by Adair *et al.*^[107] included antibodies, peptides, ligands, and/or receptors having sulfhydryl-group. Hutchison invented p97-antibody conjugates and related compositions that could be used in the treatment of cancers such as Her2/neu-expressing and Her1/EGFR-expressing cancers to inhibit, prevent, or delay the metastasis of an antibody-resistant cancer.^[108]

Kang *et al.*^[109] hypothesized that modification of calreticulin (CRT) peptide to poly(ethylene glycol)-poly(L-lactic-co-glycolic acid) (PEG-PLGA) nanoparticles would mediate drug transport across the BBB and enable deep penetration to the interior of the glioma by functionally mimicking iron. Their study proved that CRT-NP significantly improved the therapeutic efficacy of paclitaxel for the treatment of gliomas.^[109]

Toxins: targeting agents and a potential treatment

Disintegrins, a group of snake venom toxins, have the potential to block cancer cell migration and invasion by interaction with integrins.^[110] Contortrostatin, a snake venom disintegrin, was proven to inhibit tumor growth and angiogenesis and to prolong survival in a rodent glioma model by Pyrko *et al.*^[111] Similarly, scorpion venoms has been used in targeting brain tumors, in tumor painting, and in cell sensitization to chemotherapy.^[112-114] Chlorotoxin (CTX) is a promising tool for glioma management.^[112,115-118]

Chlorotoxin binds to metallomatrix proteins-2 and a glioma-specific chloride channel.^[119] CTX is a highly diffusible peptide that can cross the BBB or the BBTB with, to date, no evident signs of toxicity for normal human cells.^[110] Coated iron superoxide particles conjugated to CTX may be used as a MRI contrast agent as well as for delivering therapeutic agents (e.g. O6-benzylguanine and siRNA) to glioma cells.^[120-122] Other toxins such as BLZ-100 are being investigated.^[123,124]

Physically facilitated brain drug-delivery

Advanced physically manipulated systems can be used to treat diseases and allow controlled dosage of drugs. Physical manipulation can be achieved via ultrasound, electric, magnetic, or photonic-emission technologies.^[125] Davalos *et al.*^[126] applied pulsed electric fields into brain tissue of an animal to cause temporary disruption of the BBB. There are examples of using electromagnetic field pulses to induce the permeability of the BBB. Qiu *et al.*^[127] showed that electromagnetic pulses alter BBB permeability via regulating protein kinase C signaling and translocation of tight junction's protein ZO-1.

Kievit *et al.*^[122] attached chlorotoxin to an iron oxide magnetic nanoparticle (MNP) core using a short PEG linker. Similarly, *in vivo* experiments by Braun *et al.*^[128] have shown the effects of MNPs within a magnetic field on glioma cells lasting up to 100 min postexposure. A patent by Akhtari and Engel used functionalized MNP that comprise a moiety that provides

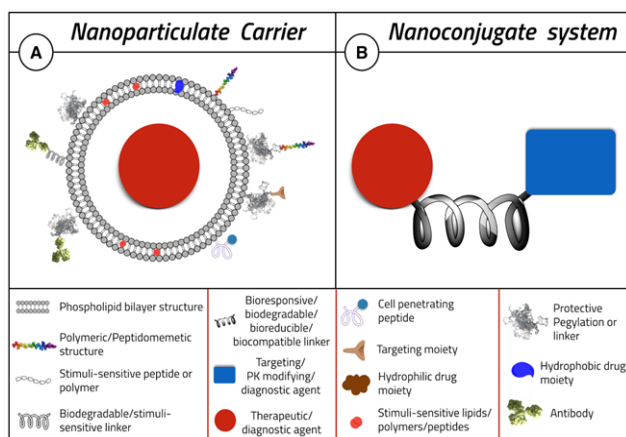


Figure 3: Diagrammatic sketch for nanoparticulate and nanoconjugate systems design strategies

selective association with cancer cells for the treatment and diagnosis of brain tumors.^[129]

Yang and David formulated magnetic iron oxide nanoparticles (MIONs) coated with a molecule that is noncovalently associated with a brain-targeting molecule. The coated MIONs comprise an anti-tumor agent linked to a cell-penetrating peptide.^[130] MIONs are oriented at the site of the brain tumor with an external magnetic field.^[130] In a patent by Dixit *et al.*^[131] gold nanoparticles conjugated with peptides against both EGFR and TfR and loaded with the photosensitizer phthalocyanine 4 have been designed and characterized. Laser was then applied to activate the photosensitizer, causing subsequent cell death.^[131]

On the other hand, nonthermal techniques to reversibly open BBB have been studied. One of these techniques is using ultrasound in the presence of microbubbles (MB).^[132,133] MB work by resonating in an ultrasound beam, rapidly contracting and expanding in response to the pressure changes of the sound wave.^[134] Inertial cavitation and destruction of microbubbles are capable of producing strong mechanical stress to enhance the permeability of the surrounding tissues and further increase the extravasation of drugs into the cytoplasm or interstitial cells.^[135] Chen *et al.*^[136] studied MB-carrying TGFβ1 inhibitor combined with ultrasound sonication to induce BBB/BBB disruption and enhance drug delivery. Pulsed-mode ultrasound exposure therapy was recently shown to enhance the antitumor effect of an EGFR-targeting chemotherapeutic drug facilitating glioma treatment.^[137]

NUCLEIC ACID TECHNOLOGIES

MicroRNA

MicroRNAs (miRNAs) are endogenous RNAs composed of about 22 nucleotides. The miRNAs can play important regulatory roles in animals and plants by targeting mRNAs for cleavage or translational repression.^[138,139] Currently, about 2% of known human genes encode microRNAs.^[140] A growing body of evidence shows that miRNAs are one of the key players in cell differentiation and growth, mobility, and apoptosis.^[141-143] Most microRNAs in animals are thought to function by inhibition of effective mRNA translation of target genes through imperfect base pairing with the 3-untranslated region of target mRNAs.^[138,140]

MiRNAs are appealing therapeutic targets and potential biomarkers of GBMs.^[141-143] Chan *et al.*^[144] were the first to investigate the functional properties of a single miRNA in GBM cell lines. They discovered that high expression of miR-21 is a common feature of GBM.^[144] In GBM, 15 types of miRNAs are the most studied (miR-7, miR-10b, miR-15b, miR-17, miR-21, miR-23a, miR-25, miR-124, miR-128a, miR-128b, miR-132, miR-137, miR-195, miR-221 and miR-222).^[145] In a patent by Park *et al.*^[146] hypoxia-induced angiogenesis-associated diseases including cancers was suggested to be treated by miRNA-125.

Aptamers

Aptamers are nonbiological oligonucleotides that can bind

to protein targets.^[147] Aptamers can be used for therapeutic purposes in the same way as monoclonal antibodies.^[147] However, unlike traditional methods for producing monoclonal antibodies, no organisms are required for the *in vitro* selection of oligonucleotides.^[147] For this reason, aptamers avoid the immunogenicity of antibodies while maintaining all their properties.^[147] However, there still remain largely unknown pharmacokinetic properties which make them harder to develop than any given therapeutic antibody.^[147]

Aptamers, consisting of a single-stranded nucleic acid having 100 nucleotides or less that specifically bind to tumor-initiating cancer cells, were developed and described by Rich *et al.*^[148] The aptamer specifically binds to tumor-initiating cells of GBM.^[148] Aptamers were the targeting agent of choice for a patent by Bloembergen *et al.*^[149] where they used an aptamer-biopolymer-active agent conjugate system for the treatment of cancer.

CONCLUSIONS AND FUTURE DIRECTIONS

The development cycle of new therapeutic drug entities for brain and CNS costs from \$500 million to \$1.5 billion to get to market. Such huge expense could be directly attributed to drugs failing late in clinical trials or during the post-market follow-up (Phase IV).^[150] In spite of the advances in drug discovery technologies and high-throughput screening techniques, the development cycle of new therapeutic entities is still costly and lengthy. It is challenging to ensure efficacy and safety throughout the four phases of clinical trials.^[151,152]

To overcome these problems and alleviate some of the costs associated with new drug entity letdown, pharmaceutical formulators spend effort modifying and reinventing therapeutic and diagnostic agents, giving them new characteristics with enhanced safety and efficacy profiles. The use of novel nano-sized drug delivery systems (nanoDDS) is a major approach in such reinvention process. The nanoDDS can provide methods for targeting and releasing large quantities of therapeutic agents in exact, well-defined organs or tissues. Furthermore, they can easily be tailored, decorated, and modified via various agents such as stimuli-sensitive moieties, targeting agents, pharmacokinetics-modifying mediators, diagnostic agents, cell-penetrating peptides, protective PEGylation layer, or antibodies. Such modifying moieties can provide novel functions and better efficacy or safety profiles to current therapeutic agents. Furthermore, most nanoDDSs provide both hydrophobic and hydrophilic environments, facilitating better drug solubility and enhanced physicochemical characteristics.^[153]

Despite their advantages, nanoDDS suffer from many problems such as stability issues, formulation scale-up difficulties, and short shelf life. Developing novel complexes and sophisticated systems that could never reach the market due to high cost, inability of scaling-up the system, or instability of the final formulation is a major problem. Major process and formulation development concerns exist with respect to the scale-up process of complex nanoparticulate carriers. To overcome

these problems, pharmaceutical formulators started to divert their effort from nanoDDS to simple bioconjugate techniques to directly attach old problematic active pharmaceutical agents such as stimuli-sensitive moieties, targeting agents, pharmacokinetics-modifying mediators, diagnostic agents, cell-penetrating peptides, protective PEGylation layer, or antibodies. Active pharmaceutical ingredients can be directly conjugated to antibodies against specific cell-type markers to create a hybrid smart molecule that is able to direct the active molecule to the disease tissue specifically. Consequently, many patents currently focus on simple bioconjugate structures that are easily synthesized with high yield, reduced cost, and high stability of the final formulation. This could provide a practical direction for the development of novel management tools and therapeutics for brain cancer for researchers worldwide, paving the road to affordable, scalable, stable, efficient, and safe management strategies.

All such techniques and technologies were illustrated in the recent patents analyses discussing brain drug delivery during 2010 to 2015. Despite such efforts, the development of brain drug delivery carrier system is still costly and troublesome in its transformation from bench to bedside. Such systems require huge effort in their *in vivo*, *in vitro* testing and clinical trials. Most of the research funding in academia for brain delivery research comes from investing companies. Most of the companies investing in this field are small startups such as to-BBB and BiOasis Therapeutics. If such industrial startups fail to develop a promising moiety or carrier for brain drug delivery, their existence is usually jeopardized.^[154,155] An integrated "bench-to-clinic" approach, realized through a structural collaboration between industry and academia, would strongly promote the development of brain tumor-targeted nanomedicines towards effective and safe clinical application.^[156]

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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