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Nanocarriers Call the Last Shot in the Treatment of Brain Cancers

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Abstract

Our brain is protected by physio-biological barriers. The blood–brain barrier (BBB) main mechanism of protection relates to the abundance of tight junctions (TJs) and efflux pumps. Although BBB is crucial for healthy brain protection against toxins, it also leads to failure in a devastating disease like brain cancer. Recently, nanocarriers have been shown to pass through the BBB and improve patients' survival rates, thus becoming promising treatment strategies. Among nanocarriers, inorganic nanocarriers, solid lipid nanoparticles, liposomes, polymers, micelles, and dendrimers have reached clinical trials after delivering promising results in preclinical investigations. The size of these nanocarriers is between 10 and 1000 nm and is modified by surface attachment of proteins, peptides, antibodies, or surfactants. Multiple research groups have reported transcellular entrance as the main mechanism allowing for these nanocarriers to cross BBB. Transport proteins and transcellular lipophilic pathways exist in BBB for small and lipophilic molecules. Nanocarriers cannot enter via the paracellular route, which is limited to water-soluble agents due to the TJs and their small pore size. There are currently several nanocarriers in clinical trials for the treatment of brain cancer. This article reviews challenges as well as fitting attributes of nanocarriers for brain tumor treatment in preclinical and clinical studies.

Keywords

nanocarrier, brain drug delivery, brain cancer, blood–brain barrier, nanomedicine

Introduction

Structural or functional impairments to the brain or spinal cord including trauma, infection, inflammation, tumors, degeneration, and autoimmune disorders are classified as central nervous system (CNS) disorders.[1,2] These conditions can lead to serious cognition and physiological impairments and may prove fatal in certain cases.[3] Brain tumors have a high fatality rate and can seriously affect and devastate lives. Despite many improvements in treatment protocols, drug delivery remains a major challenge, and treatment options are limited. The most malignant brain tumors begin with genetic mutations that impair and dysregulate cell function and division.[4–6] Brain tumors are classified as malignant and benign, and further subcategorized as primary and secondary. Whereas primary tumors are caused by the division of brain cells only, secondary tumors develop from the metastasis of other organs to the brain and are also hardly treatable. Primary tumors unlike secondary types start and involve the brain cells. The glioblastoma multiform (GBM), with a treatment-refractory disorder with a patients' short lifespan, cognitive impairments, and high mortality rate,

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is one of the most common primary brain tumors.[7–11] The patient's survival percentage with grade IV glioblastoma is reported to be 4.5% for 5 years by World Health Organization (WHO). Metastatic brain tumor as the secondary tumor type has almost 6 months overall survival. The median survival was at its highest with surgery which was followed by radiation therapy.[12–14]

The surgical resection plus radiotherapy, as well as adjuvant chemotherapy frequently, has been comprised by the treatment guidelines (Figure 1).[15–17] With the lack of clear tumor margins, precise anatomical location, and biases that exist during the surgery, chemotherapy has been one of the most effective approaches. Despite recent progress in the development of effective chemotherapy agents and efforts on improved drug formulations, the tumor's molecular heterogeneity has made the treatment process and the resulting outcomes more complicated.[18] The delivery of chemotherapy agents to the brain tissue faces the challenge of crossing the anatomical and physiological barrier, the blood–brain barrier (BBB) being the most important one. The BBB is a highly selective barrier (hardly permeable) that protects the brain cells against blood-circulating agents such as pathogens and toxins. Many approaches to overcoming BBB have been suggested in the literature. One of the promising applied approaches lies in the nanotechnology.[19–21]

Nanotechnology, particularly nano-drug delivery systems, is emerging as promising tools in the cancer diagnostics and therapeutics.[22,23] Based on the drug-loading methods and nanoparticle surface, size, and zeta potential modifications, the effector molecules can be encapsulated, adsorbed, or attached to the nano-drug delivery system. To date, the most successful nanocarriers based on clinical trials are inorganic nanoparticles (such as metal, metal oxide, carbon, and silica particles), liposomes, micelles, dendrimers, and polymers. Assuming that blood capillaries and cells range between 6 to 9 and 10 to 20 μm, respectively, almost all types of nano-drug delivery systems can reach and deliver therapeutics into organs and cells. Specifically targeting the BBB receptors by surface-functionalized nano-drug delivery systems makes them ideal candidates for the brain cancer drug delivery.[24–27]

This study aims to discuss the challenges regarding brain tumor treatment and to review the current application of nanotechnology in preclinical and clinical studies for this disease category. With careful examination of the literature, the physiological and biological aspects of the BBB were summarized along with the nano-drug delivery strategies that have been reviewed in detail based on the nanostructures. Finally, the majority of all ongoing nano-drug delivery systems which have made their way through the clinical trials were reviewed as the realistic perspective of nanomedicine for brain cancer drug delivery systems.

Blood-Brain Barrier

In 1885, Ehrlich[28] and his colleagues demonstrated the presence of barriers between CNS and the periphery using brain parenchyma staining via intravenous injection. One of Ehrlich's students, Edwin Goldmann completed Ehrlich's dye experiments by directly administrating trypan blue directly into the cerebrospinal fluid (CSF).[29] The term BBB generally refers to the distinct characteristics of continuous nonfenestrated brain microvasculature.[30] This unique feature is the consequence of physical barrier properties, molecular barrier properties, as well as specific transporters.[31–34] The cells, molecules, and ions entrance are suppressed by the protective role of the BBB. Furthermore, it is almost an impermeable barrier for drugs. Even though the BBB makes uniform coverage between the brain parenchyma and blood vessels interface, the circumventricular organs link the CNS and peripheral blood vessels.[35] Blood vessels are made up of 2 cell types: endothelial cells (ECs) that build up blood vessels, and mural cells including vascular smooth muscle cells and pericytes (PCs), which are placed on the outer layer of the ECs. While ECs are primarily responsible for the BBB characteristics, the function and maintenance of the BBB depend on interactions between ECs, PCs, and astrocytes (Figure 2).

Simple squamous epithelial cells are the bricks of the blood vessel walls. CNS microvascular ECs are 39% thinner than muscle ECs and also there is a 200 nm diameter between the luminal and abluminal surface.[36] The main role of ECs is to limit the entry of cells, molecules, and ions to the brain. The EC tight junctions not only block the paracellular pathway but also vesicle-mediated transcellular flux too.[36–39] As a result of paracellular and transcellular restrictions, blood–brain transportation is highly controlled.[40–42] Higher amounts of mitochondria and very low levels of leukocyte adhesion molecules are other specific limiting features.

The PCs incompletely cover the abluminal microvascular side that is attached to the vascular basement membrane.[43] PC cells with their contractile proteins control the capillary diameter.[44] In comparison with other tissues like muscles, in the CNS PCs provide the most coverage. The EC-to-PC content ratio is between 1:1 and 1:3 in CNS microvascular versus 100:1 in muscles.[45]

Astrocytes are polarized glial cells that cover the entire vessel's tube.[46] They not only connect neuronal cells with the blood vessels but also reflect the neuronal signals on the microvessels' blood flow. This includes contraction/dilation regulation of the vascular smooth muscle cells next to capillaries and arterioles, respectively.[32]

The main CNS endothelial cell transporters are efflux and nutrient types.[34] Efflux transporters, including MRPs, Mdr1, and BCRP take advantage of ATP hydrolysis to actively transport different biological membranes. Nutrient transporters facilitate the transportation of nutrients against their concentration gradient. Most of these belong to the family of solute carrier transporters, including slc16a1 (lactate, pyruvate), slc2a1 (glucose), slc7a5 (neutral amino acids, L-DOPA), and slc7a1 (cationic amino acids).[47–49]

Nanotechnology Approaches to Overcome Brain Drug Delivery Challenges

Most of the small lipophilic molecules, less than 400 to 500 Da, can pass through the BBB.[50] Of the greater than 7000 drugs for

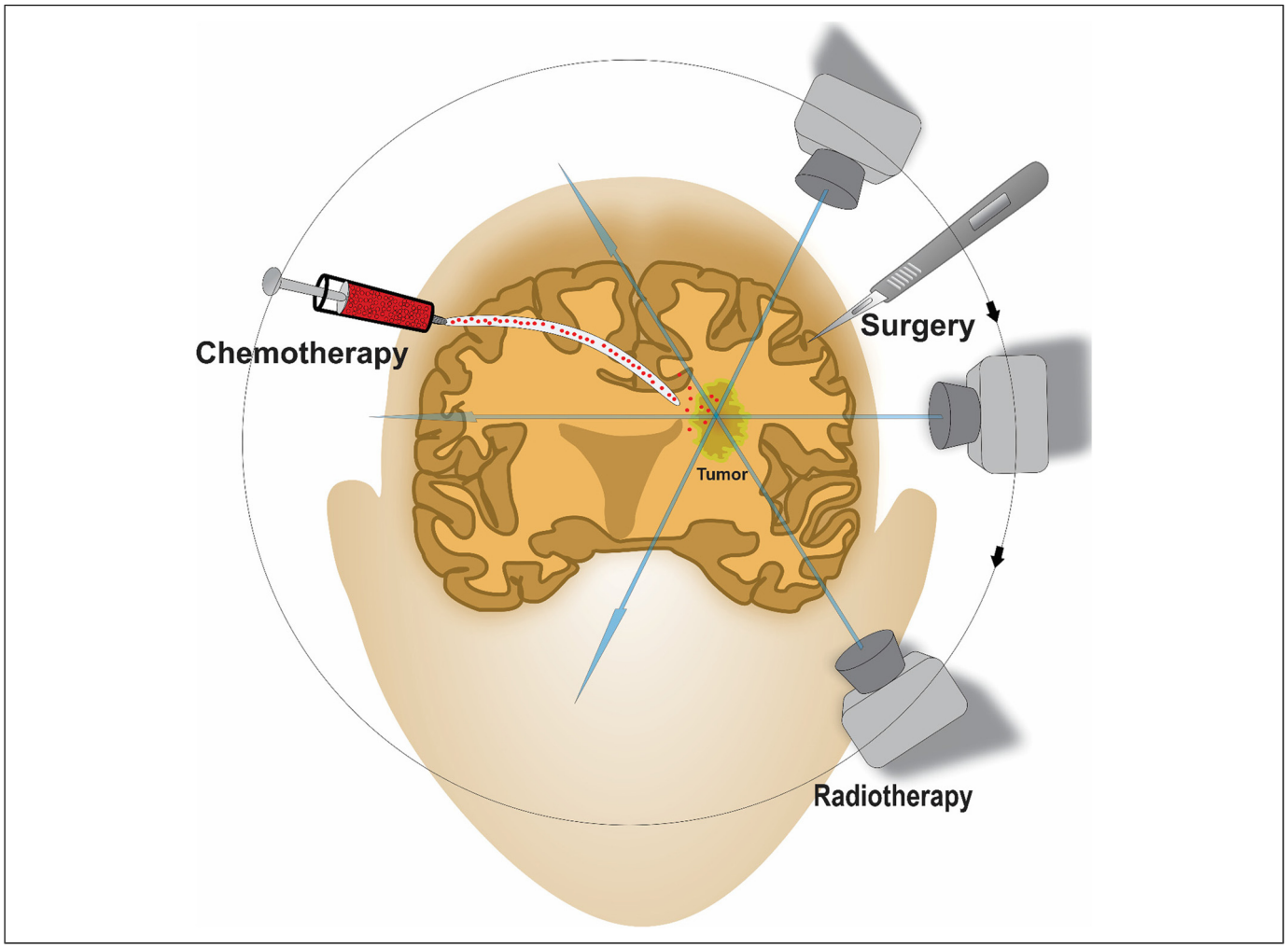

Figure 1. Current methods in the treatment of brain tumors. These methods are including surgical resection plus radiotherapy, as well as adjuvant chemotherapy.

insomnia, depression, and schizophrenia that were assessed in comprehensive medicinal chemistry (CMC) database study,[51] only 5% reached the CNS and averaged 357 Da. In another study, 12% of drugs were activated upon entering the CNS while only 1% of them were of use for nonpsychotic disorders (neurosis). Antibiotics, antineoplastics, and neuropeptides are common examples of compounds with limited transfer rates through the BBB.[52] To that end, the objective for many brain drug delivery systems is concerned with targeting the BBB receptors.[35,53]

Generally, invasive and noninvasive methods are 2 major interventions to allow passage through BBB. The invasive procedures occur with transient disruption of BBB by chemical, biological, and physical stimuli. These methods are expensive and have proven to be highly risky. As a result, they are not preferable for the brain drug delivery enhancement.[54–57] In contrast, noninvasive methods have proven more effective at providing a relatively harmless drug-to-brain delivery system. Furthermore, a blood-to-brain strategy that improves the BBB permeability and facilitates drug-carrier conjugation minimizes the mentioned drawbacks.[58,59] It has been demonstrated that the transcytosis mechanism by the BBB cells supports active drug transportation due to the sizable presence of mitochondria. Various nanocarriers have been thought to be able to overcome the BBB, potentiating drug delivery to ischemic lesions and various tumors in CNS. Many research and review papers have aimed to comprehensively examine the effectiveness of brain drug delivery systems.[60–62]

Generally, the paracellular pathway is considered the common route of entry for small hydrophilic molecules, and the transcellular pathway is the preferable route for the transport of small nutritional or therapeutic compounds.[63–65] Unfortunately, due to the physiologic limitations of the BBB, both pathways apply to a small selection of compounds. Other more feasible and commonly used modes of transportation include a carrier or receptor-mediated transcytosis. Briefly, the endosomal formation following carrier conformational change due to the concentration gradient elucidates pathways. These established pathways to get through the BBB are illustrated in Figure 3.[66–71]

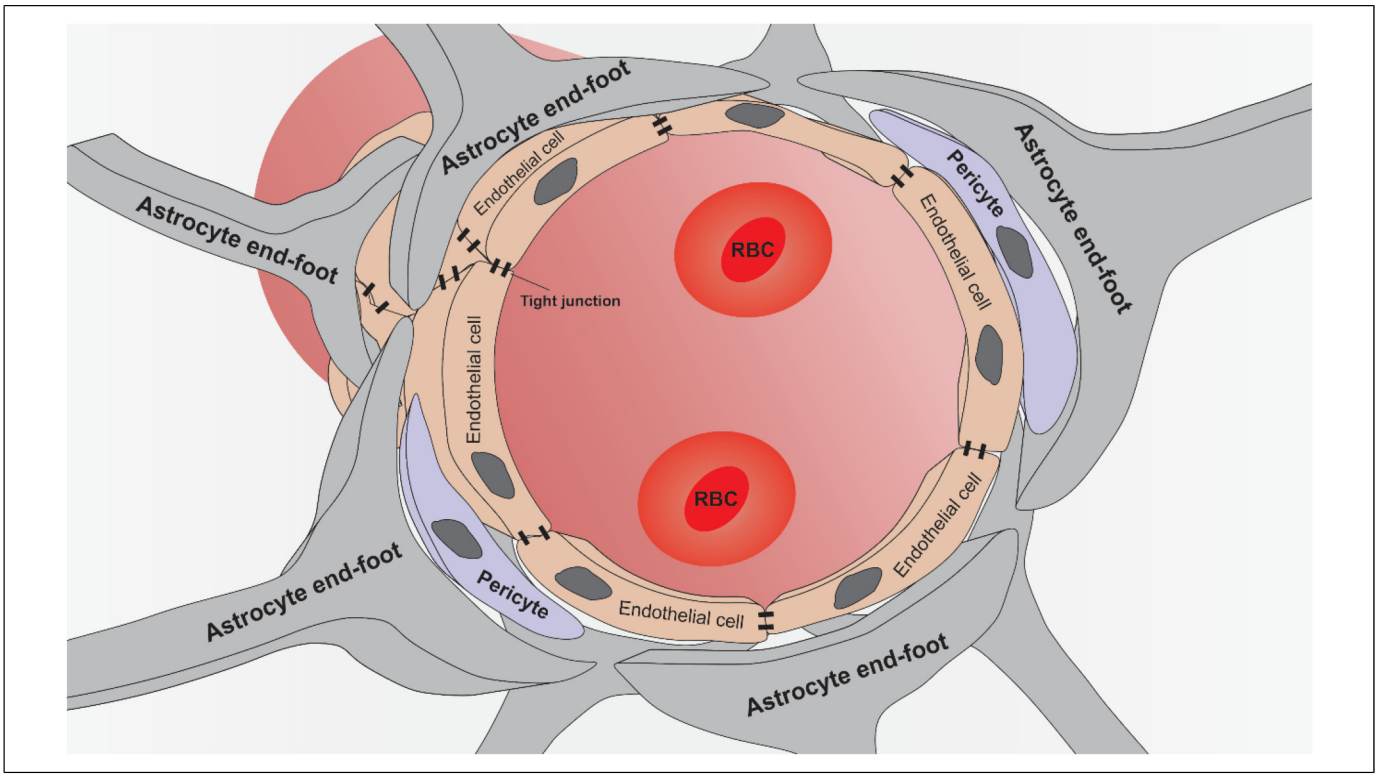

Figure 2. Schematic representation of the BBB. Endothelial cells are made which are tightly attached via tight junctions. Blood vessels, and mural cells including vascular smooth muscle cells and PCs, are placed on the outer layer. The PCs have incompletely covered the abluminal microvascular side that is attached to the vascular basement membrane. Astrocytes are polarized glial cell types, cover the entire vessel's tube.
Abbreviations: BBB, blood–brain barrier; PCs, pericytes.

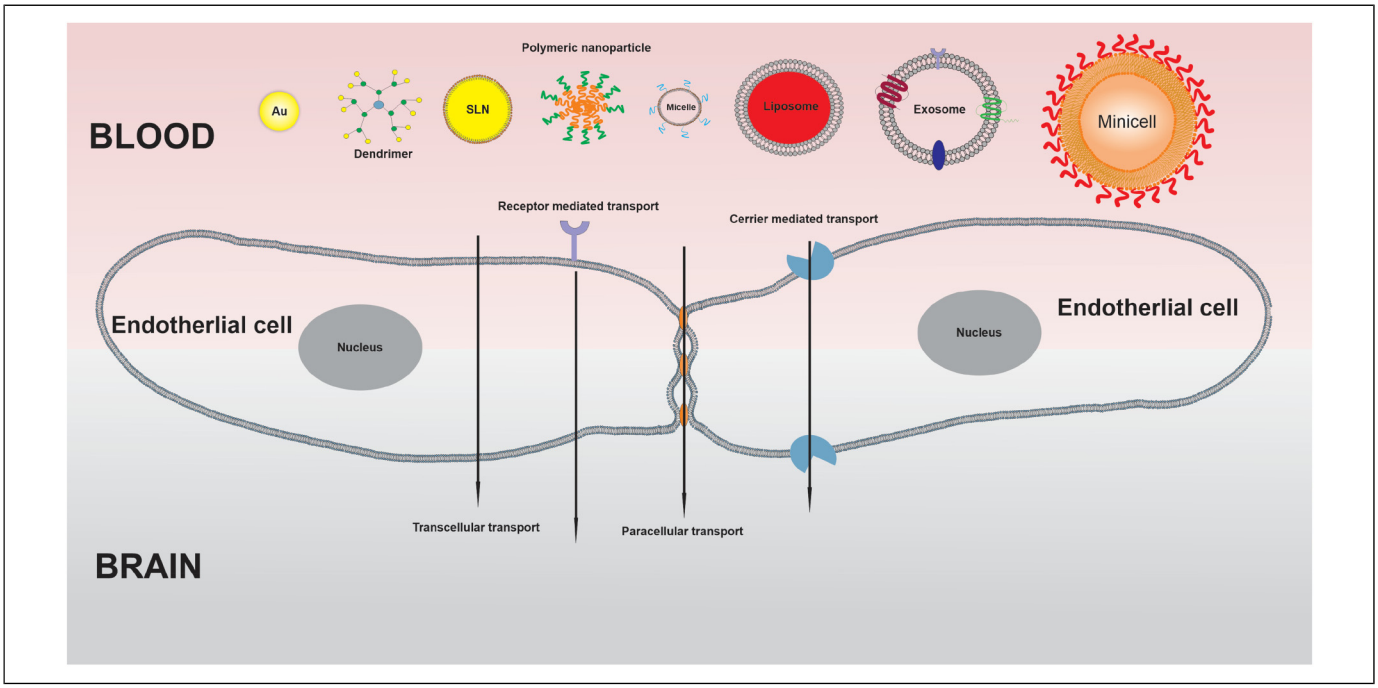

Figure 3. Nanocarrier and brain delivery. Various types of nanocarriers including inorganic nanoparticles, dendrimers, SLNs, polymeric nanoparticles, micelles, exosomes, minicells, and liposomes encounter 4 types of transport mechanisms including transcellular, receptor-mediated, paracellular, and carrier-mediated transport to pass through BBB.
Abbreviations: BBB, blood–brain barrier; SLNs, solid lipid nanoparticles.

Nanotechnology Platforms for Brain Cancer Drug Delivery

Inorganic Nanomaterials. These types of nanomaterials based on silica, carbon, metal, and metal oxide particles are widely used in imaging techniques. Stabilized size and monodispersed formation in the bloodstream, high surface area, and for this reason, ease of functionalizing are just a few positive aspects of inorganic nanomaterials.[72–75] Silica mesoporous nanoparticles, carbon nanotubes with an ultrahigh surface area, gold and iron oxide nanoparticles, especially superparamagnetic iron oxide nanoparticles (SPIONs), are typical inorganic nanoparticle examples (Table 1). In addition to chemical modification by PEG, lactoferrin, cationic serum albumin, poly(isobutylene-alt-maleic anhydride [PMA])s[73,76,77] can be used as chemical modifications. These improve the hydrophilicity and decrease both blood aggregations and the reticuloendothelial system (RES) clearance. Additionally, physical approaches like magnetism as a novel drug delivery mechanism have been used to facilitate passing through BBB.[73] Furthermore, it has been demonstrated that external magnetic forces can effectively cross SPIONs through the BBB.[73,78]

Although numerous investigations have found inorganic nanoparticles to be efficient enough to pass through the BBB, hard degradability and its following toxicities, and undesirable drug delivery, have excluded them from tangible clinical studies.[79]

Solid Lipid Nanoparticles (SLNs). SLN refers to nanodispersions ranging from 10 to 1000 nm of biocompatible lipids including fatty acids (eg, stearic acid), triglycerides (eg, tristearin), waxes (eg, cetyl palmitate), and steroids (eg, cholesterol) that are stabilized with surfactants[80–82] (Table 1). A combination of surfactants with the hydrophilic–lipophilic balance (HLB) values less than 12, such as Poloxamer 188 and Pluronic®F68, are used in the SLN structure.[81,83] The core of SLN is made of solid lipids, which makes them ideal for hydrophobic drug loading. SLNs have attracted growing attention as potential nano-based anticancer drug delivery formulations for gliomas and glioblastoma.[84,85] Similar to other nanocarriers, the surface of SLNs is modified and functionalized via the attachment of various targeting ligands including proteins, peptides, small molecules, and antibodies. This results in increased antitumor activities and reduced adverse effects by targeting specific receptor-mediated endocytosis.[85–88] Along with the aforementioned advantages, prolonged retention time in the serum and brain can be increased by improving the hydrophilicity of SLNs via PEGylation.

Biocompatibility, biodegradability, and surface modifications are the main advantages of SLNs. However, they can be easily eliminated by the RES from the blood due to their lipophilicity, which can present a potential challenge.[89]

Polymeric Nanoparticles. Polymeric nanoparticles including nanospheres and nanocapsules are thermodynamically stable structures made of natural or synthetic polymers, with a range of sizes between 1 and 1000 nm.[90] As a result of considering an optimum nanoformulation that is biodegradable for over a few days, the nondegradable formulations are excluded including quantum dots, nonorganic nanocarriers (silica and metal particles), and needle-shaped carbon nanotubes.[91,92] Consequently, 3 types of nanostructured materials are considered including polylactic acid (PLA) or its copolymer poly lactide-co-glycolide acid (PLGA), poly butyl cyanoacrylate acid (PBCA) or poly iso hexyl cyanoacrylate acid (PIHCA), and human serum albumin (HSA).[93] It has been reported that 80% of PBCAs are degraded 24 h after IV injection. Therefore, poly alkyl cyanoacrylates with approximately 2000 to 3000 Da molecular weights have the fastest degradability among polymers. Higher poly alkyl cyanoacrylates toxicity rates were observed with the slowest or fastest degradability rates. The intermediate rates showed lower toxicities.[94,95] Interestingly, a formulation of PBCA has achieved clinical trial phase III and now is purchased by the trade name of Livatag® (doxorubicin Transdrug™). Livatag® is the product of PBCA and Dox HCl attachment, which increases the PBCA's molecular weight and substantially improves hepatocarcinoma drug delivery.[92,96] The enzymatic cleavage by lipases and esterases is thought to be the prevailing degradation mechanism for PLGA and cyanoacrylates, respectively.[97–99] Furthermore, albumin nanoparticles are degraded within 3 days in macrophages.[100] Natural polymers are prospective candidates for brain drug delivery over synthetics due to the lessened toxicity, improved biodegradability, and lowered costs. Chitosan with a linear structure and randomly distributed N-acetyl-D-glucosamine (acetylated unit) and β-(1→4)-linked D-glucosamine (deacetylated unit) units are the product of chitin extraction from shrimp shells. As a stabilized, biodegradable, and biocompatible formulation with lower toxicity among natural polymers, it can be prepared by simple techniques.[101,102] As the hydrophobic structures have increased permeability, chitosan-based nanoformulations have been modified to enhance their hydrophobicity and thus their ability to penetrate the BBB. Wang et al[103] have shown that trimethylated chitosan loading on the surface of PLGA has enhanced brain uptake. Some polymeric surfactants have been used as the BBB's permeation enhancer such as polysorbate 20, 40, 60, and 80[104,105] and also poloxamer 188 in contrast with the polymers such as poloxamine 908, Cremophor®, and Brij®35.[106–108] Moreover, PEGylated or the so-called stealth nanoparticles are characterized by lower liver uptake and better blood circulation time and tissue distribution.[109,110] The elevated brain concentrations of nanopolymers in tumor-bearing animals versus nontumoral animals indicate that diseases such as brain cancer considerably increase the delivery of nanoformulations to target sites (Table 1).[92,111] Although PEGylation has improved many aspects of nano-drug delivery, it is not sufficient for an optimum brain delivery system.[92]

Several studies have revealed the substantial effect of the adsorbed drug on the nanoparticle's charge. In some, tumor accumulation reduction has been referred to as ionic interactions especially the therapeutic agents' positive charge which

Table 1. Preclinical Studies. Nanocarriers That Were Used in Preclinical Studies for Brain Delivery.

Name and materials	Advantages	Limitations	References
Inorganic and are based on silica, carbon, metal, and metal oxide, for example, silica mesoporous nanoparticles, carbon nanotubes, gold nanoparticles, iron oxide nanoparticles especially SPIONs	• Stabilize size, • Monodispersed formulation • High surface area, • Ease of functionalizing, • Physical drug delivery systems like magnetism	• Low hydrophilicity • High blood clearance by RES • Hard degradability • Undesirable drug delivery	[293]
Solid lipid nanoparticles (SLN) and are made of lipids and stabilized by surfactants	• 10-1000 nm size • Biocompatible • Biodegradable • High loading efficiency • Functionalized by targeting	• Not suitable for hydrophilic drugs • High clearance by RES	[294,295]
Polymeric nanoparticles made from natural or synthetic polymers for example, poly(alky cyanoacrylates), poly(lactic acid), human serum albumin (HAS), and chitosan	• 1-1000 nm • Stable • Biodegradable • Controlled degradation rate • Functionalized by targeting	• Catabolites and degradation rate should be examined before clinical use because of adverse immunological responses	[296,297]
Dendrimers mainly are based on PAMAM, PPI, or PLL	• Structural functional groups • Many reaction sites • Dual targeting • Cationic dendrimers (gene delivery) • Endosome destruction (Gene delivery) • Uniform size distribution • High drug loading capacity	• Complexity • Multi-step synthesis • Toxicities and safety • High clearance by RES	[132,298,299]
Micelles are based on amphiphilic block copolymers, a hydrophobic core, and a hydrophilic surface	• 10-100nm • Drug delivery of both lipophilic and hydrophilic compounds • Stability and long blood circulation time • EPR mechanism • Easy and reproducible formulation • Sterilization by simple filtration • Evading the RES	• Low stability • Premature drug release • Immunogenicity • Dissociate below CMC	[300,301]
Exosomes are natural extracellular nanovesicles	• 30-100 nm • Natural biocompatibility • Stability • Controllable intercellular interactions • Not immunogenic • No toxicity	• Lack of standardized exosome separation and purification criteria • Uncertain mechanism in cancer • Heterogeneity • Release modifications	[171,172,175–177]
Minicells are bacterially derived nanoparticles	• 100-300 nm • Multiple targeting ligands for targeting • Biocompatible • Increased encapsulation efficiency • Less drug leakage	• Stability • Release profile • Immunogenicity • Organ toxicity • Further evaluations	[184,188,189,198–200]
Liposomes are based on phospholipids	• 25-1000 nm • Delivery of various molecules: MLVs for extended drug release, LUVs for vaccine and gene delivery, SUV for drug delivery through the endothelial cell layer • Targeted drug delivery • Both hydrophilic and hydrophobic drug delivery • High encapsulation efficiency • Biocompatible • Biodegradable • pH-sensitive formulations • Thermosensitive formulations • Dual targeting • EPR mechanism	• Low circulation time without surface modification • Difficulties in sterilization • Poor reproducibility in terms of size • Limited control over drug release • A small variety of surface functional groups • Poor stability	[208,230,237,302]

causes higher reticuloendothelial system accumulation.[112–114] The polymeric nanoparticles are penetration enhancers with the ability to link with a peptide as a targeting ligand which improves brain drug delivery.[115] Apolipoprotein targeting ligands have been used on the surface of polymer-based nanocarriers to target the low-density lipoprotein receptor and scavenger receptors on the BBB.[116] Additionally, a few studies have investigated targeting the transferrin receptors with anti-transferrin or transferrin antibodies (OX26 or R17217).[117] Likewise, the insulin receptors have been targeted with just 100 μm amounts of insulin receptor antibody which improves nanopolymer's drug delivery.[118]

Optimistically, polymeric nanocarriers can reach the clinic for brain cancer treatment only if they and their catabolites are biodegradable, and less toxic and immunogenic.[89] Unfortunately, as none of the BBB's receptors are specific or ubiquitously expressed, the adverse effects might be a limiting factor.[92]

Dendrimers. Dendrimers are monodisperse, symmetric, and spherical compounds with a chemical core. They are classified based on their molecular weight. Their properties are mostly determined by their surface functional groups.[119–121] The particle size growth starts from a nucleation site in the center of dendrimers, from where many consecutive branches develop. Consequently, hundreds of reaction sites are available on the surface of the particles. Poly-amidoamine (PAMAM), poly-L-lysine (PLL), and polypropylene imine (PPI) are the most common types of dendrimers that have been applied for drug delivery. They can be loaded by treatment agents and targeted as novel drug delivery systems.[122,123] Another advantage of these types of formulations is dual targeting by different agents.[124]

In one study, modified dendrimers via Serine–Arginine–Leucine (SRL) peptide were used as gene delivery systems for the brain. It was shown that SRL peptide was bound to endothelial cell membrane receptors on the BBB and lipoprotein receptor-related protein (LRP). Consequently, this enhanced the dendrimers uptake by brain parenchyma tissue.[125] Furthermore, transferrin and wheat-germ agglutinin (WGA) dual targeting on the PEGylated 7 to 10 nm dendrimers can serve as brain tumor therapeutic agents because of well penetration and accumulation.[126]

Cationic dendrimers electrostatically bind to the negatively charged genes for gene delivery applications. The reason why gene delivery through the dendrimers seems to be so promising is this mechanism in which they destroy endosome storage in the cytoplasmic environment.[127,128] Furthermore, exogenous genes, microRNA (mRNA), and small interfering RNA (siRNA) delivery to tumor sites have been shown in a few studies.[129]

In conclusion, dendrimers have specific advantages such as uniform size distribution, high drug loading capacity, multiple targeting ligand conjugations, and high stability.[130,131] In contrast, complex synthesis and formulation development, toxicity, and safety issues (especially the amino-functional groups) have restricted the clinical application of dendrimers (Table 1). Furthermore, their positive amino groups can interact with the blood cells which are negatively charged and structurally disrupt and erode cells. This can lead to hematologic toxicities and nano-drug eliminations. Even though the dendrimer cationic groups cause toxicity, their chemical modifications generally minimize these effects.[132]

Micelles. Spheroidal nanomicelles are made by the aggregation of amphiphilic block copolymers in an aqueous environment. In micelles, the hydrophobic core and hydrophilic surface have provided low soluble drug loading, modification, and conjugation for targeted brain drug delivery.[133] The hydrophilic outer layer prolongs both blood circulation and stability. Micelles range between 10 and 100 nm, which is ideal for the enhanced permeation and retention (EPR) mechanism at the tumor site.[130] These nano-drug delivery systems are prioritized over other types of novel drug delivery approaches due to simple, stable, and reproducible formulations, as well as simple filtration techniques for sterilization. Their small size and hydrophilic shell help them evade the RES and consequently have a longer circulation time. Therefore, nanomicelles are the potential candidates for the treatment of brain cancer. However, the potential of immunogenicity, premature drug release, poor stability, and lack of appropriate methods for formulation scale-up have limited their application. In addition to the aforementioned disadvantages, the dissociation of micelles at concentrations lower than the critical micelle concentration (CMC) is one of the most significant challenges.[134–136]

Exosomes. Natural nanovesicles with a diameter of 30 to 100 nm have gotten a lot of attention for potential drug delivery applications because of their ability to carry targeted ligands on their surface. They can escape the immune system entrapments due to their production from body cells, resulting in better blood circulation, biodegradability, and biocompatibility.[65,137,138] They are also considered intriguing nano-drug delivery systems due to their inherent aptitude for passing across biological membranes and barriers including the BBB without affecting its integrity. Additionally, some investigations have reported glioma-secreted exosomes circulating in the blood, which indicates their capacity to cross the BBB.[139–143] The abovementioned characteristics have made them promising candidates for delivery for brain cancer treatment. Many studies have utilized exosomes for the delivery of nucleic acids, proteins, and small molecules.[144,145] Many studies have been carried out to take advantage of cutting-edge exosome research, including RNA therapeutics. Exosomes containing mRNA inhibited and reduced vasculogenic mimicry, migration, and angiogenesis, leading to glioma tumor suppression.[146,147] The administration of brain endothelial cell-derived exosomes that were loaded with siRNA was used to treat brain cancer in another investigation. Despite their limited cell absorption, siRNAs have shown intriguing therapeutic promise. The nano-sized exosomes were successful in delivering siRNAs for the treatment of brain diseases.[148–150] Interestingly, the main

mechanism by which exosomes acquired the ability to cross through the BBB was unraveled in vitro. The findings suggested active transcytosis under the impression of inflammation factors (eg, TNF-α).[151–153] For simultaneous glioma imaging and treatment study, the neuropilin-1-targeted exosomes were co-loaded with SPIONs and curcumin by electroporation method. Natural nanostructures were given in vitro and in vivo, and their therapeutic and diagnostic benefits were greatly improved. Potent synergistic anticancer effects were attributed to the effect of SPION-mediated magnetic flow hyperthermia and Curcumin-mediated treatments.[154] Exosomes have also been employed to decrease brain malignancies in some innovative and exciting studies. Chaperone-rich cell lysates (CRCLs) in particular may play a key role in the development of antitumor vaccinations. Dendritic cells (DCs) are activated by tumor-derived CRCLs, resulting in potential anti-tumor efficacy. DC-derived exosomes were produced in this study using DCs loaded with CRCLs obtained from GL261 glioma cells. They made antitumor T cell immune responses more robust and effective.[155–159] The notion that brain endothelial cell-derived exosomes can transfer anticancer medicine across the BBB for the treatment of brain cancer in a zebrafish model has been tested in new findings on anticancer drug delivery. The findings show that exosomes supplied to tumors reduced tumor growth markers in a significant manner. As a result, brain endothelial-derived exosomes could be a promising new nano-drug delivery system that could be investigated further in the clinical development of brain cancer therapy.[160,161] In a 3D glioblastoma model, exosomes generated from human endometrial stem cells harboring the apoptotic drug atorvastatin, which inhibits cancer growth through a variety of mechanisms, dramatically reduced tumor growth.[162–164] A number of researchers have looked into the possibility and mechanism of exosomal surface changes. Surface adjustments are implemented to exosomal surface proteins and functional groups. The presence of exosomal membrane proteins such as cytoskeletal components (actin, tubulin, etc), intracellular fusion proteins (annexin and RAB), and heat shock proteins have been decoded by proteomic research. MHC class I and II, CD86 proteins, integrins, as well as other proteins were also listed.[165–167] In conclusion, covalent and noncovalent alterations, as well as genetic engineering, are being investigated as techniques to improve exosome efficacy and reduce their drawbacks in the targeted drug delivery.[154,165,168–170]

Considering all the facts, exosomes have natural biocompatibility, greater chemical stability, and the ability to control intercellular interactions when compared to synthesized nanoparticles. They are also regarded as nonimmunogenic, nontoxic, and nonspecific nanocarriers.[171–173] However, there are challenges to exosome use, such as a lack of standardized exosome separation and purification criteria, an uncertain mechanism for exosome uses in cancer treatment, heterogeneity, and difficulty maintaining exosomes. They must be analyzed and adjusted to get a more desirable and controllable release and scalation in clinical scenarios.[174–177]

Minicells. Minicells are anucleated, nano-sized (100-300 nm), neither alive nor dividing cells due to mutation in genes involved in the normal bacterial cell cycle. Ribosomes, peptidoglycans, plasmids, RNA, and bacterial proteins are all kept intact. As a result, they are metabolically active and capable of carrying out cell processes such as mRNA translation, transcription, and translational activities, as well as ATP synthesis.[178–180] Therefore, various minicell-producing bacterial strains (such as *E coli* or others) have been brought into attention or are being researched for possible adoption.[181,182] Chemotherapeutics, si/shRNA, drugs, and chemotherapeutics can all be administered to malignant tissue with pinpoint accuracy. Additionally, multiple targeting ligands, such as bi-specific antibodies, can modify their surface to improve their clinical applications.[183–187] The biocompatibility and biodegradability of these nanocarriers are equivalent to that of other nanocarriers. Furthermore, they appear to be one of the most unique and appealing nano-drug delivery approaches due to increased encapsulation efficiency, overcoming drug leakage, enhancing targeting specificity, and improving treatment agents' therapeutic index.[184,188,189] Many studies are being conducted in order to develop optimal minicell-based drug delivery systems for therapeutic purposes. Minicells that administered the miR-34a greatly increased the temozolomide effects in vivo in a study as adjuvant therapy. MiR-34a regulates signaling pathways implicated in intratumoral heterogeneity and, as a result, temozolomide resistance.[190–192] In another experiment, late-stage brain cancer dogs were treated with EGFR-targeted minicells carrying doxorubicin and coated with BsAbs. The nanoparticles were found in the brain tumor's center and had a high median survival rate. There have been no reports of particular toxicity. A Phase 1 clinical trial employing EGFR-targeted, doxorubicin-loaded minicells for the management of patients with relapsed glioblastoma was designed on this premise.[193–197]

Despite the benefits and advantages of minicell structures, more research is needed to interpret them in the clinic, notably on their stability and release profile, as well as their release mechanism in the tumor microenvironment and intratumoral. According to the experiments that have been reported so far, they had no significant detrimental impacts. Before they are extensively employed, however, their immunogenicity due to bacterial sources must be thoroughly investigated. Additionally, the risk of organ damage from long-term organ accumulations adds to the concerns regarding these novel nanoformulations.[198–200]

Liposomes. Liposomes are attractive vesicles for the delivery of various drugs and compounds such as antibiotics, therapeutic proteins, antineoplastics, and peptides.[201–204] These vesicles, consisting of phospholipids, are spontaneously formed and comprise of single or multiple layers. Liposomes are categorized based on the following classes: (I) multi-lamellar vesicles (MLVs), (II) large unilamellar vesicles (LUVs), and (III) small unilamellar vesicles (SUVs). The sizes of MLVs, LUVs, and

SUVs are approximately 500, 100 to 500, and 25 to 100 nm, respectively. MLVs are commonly used for extended drug release objectives, while SUVs are optimal for drug delivery through the endothelial cells lining the blood vessels and tissue epithelium. LUVs are medium-sized structures for vaccine and gene delivery purposes.[205–207]

Due to the recent improvements in encapsulation efficiency, drug loading, stabilized formulation preparations, decoration for the molecules targeting delivery, and co-delivery, liposomes are promising drug delivery systems.[201,208]

However, some limitations affect the liposomal formula application and restrict its utilization for intended purposes. These particulate systems have high clearance and low blood circulation and are cleared by the RES. The blood circulation time can be improved by decreasing the particle size to less than 100 nm, and liposomal surface PEGylation.[209,210] Additionally, active targeting is accomplished by ligand attachment for a specific receptor (like monoclonal antibody) on the liposomes, preferentially to the end of the PEG moieties as the targeted liposomes have been demonstrated to be much more effective if they are sterically stabilized.[211,212]

Many attempts have been made to reach an optimal liposomal formulation.[213–215] Improving liposomal transportation to the tumor site has been the main objective of several previous studies. Most of these studies examined either cationic or PEGylated liposomes.[216,217] Based on recent studies, the main mechanism for liposomal entrance into the brain remains unclear, however, it has been suggested that tight junction disruption may be the most probable mechanism.[218] Some pH-sensitive cell-penetrating peptides such as TR peptides are surface conjugated to the drug-loaded liposomes and were developed and utilized for glioma treatment.[214] This type of nanoformulation was shown to enhance the drug efficacy toward gliomas, both in vitro and in vivo. Moreover, adriamycin (ADM)-encapsulated thermosensitive liposomes enhanced Dox delivery to the brain and prolonged the survival of glioma-bearing mice.[219]

Receptor-mediated endocytosis is a general mechanism of cells to import particles. The endocytosis mechanism has been targeted by nanosystems especially liposomes. For instance, the transferrin receptor is over-expressed on the brain capillary endothelial cells.[220–222] Therefore, the efficacy of dual-targeted doxorubicin (Dox) liposomes conjugated concomitantly to folate and transferrin was investigated in a study. This dual-targeting Dox liposome enhanced the therapeutic efficacy of Dox toward gliomas and reduced the off-target side effects as compared to Dox solution.[223] In another study, the TF-specific targeting ligand and TAT, a nonspecific cell-penetrating peptide (a small positive charged variant derived from trans-activating transcription activator peptide of HIV) were attached to the paclitaxel and Dox containing liposomes (TF/TAT-LP). They exhibited an effective antitumor activity against gliomas and enhanced the median survival time of glioma-bearing mice.[213]

Moreover, targeting is an approach that enhances the liposomal formulations' therapeutic and antitumor effects. It is ascribed to their median particle size and an extent to the homogeny particle size distribution. The EPR effect is the main mechanism for liposomal accumulation and accordingly, 100 nm liposomes can merely reach specific regions.[224–228] In one study, the noninvasive focused ultrasound method accompanied bubble liposome (with 55-299 nm diameter range) injection into the circulatory system. The results showed that smaller liposomes serve as more effective drug delivery systems than larger ones.[229,230] In another study, cationic liposomes were used to transfect tumor cells with an interferon gene-expressing vector (plasmid), which resulted in tumor regression.[231,232] Likewise, antisense epidermal growth factor was entrapped into cationic liposomes and tested in human malignant glioma cell lines.[233] The possible cationic liposomal cell uptake mechanism can be explained by negatively charged phospholipid head group interactions with the positively charged liposomes (absorption) and therefore is called adsorptive mediated transcytosis.[234,235] In this regard, cationic bovine serum albumin as a positive ligand has shown increased cell absorption and enhanced drug delivery through the aforementioned mechanism.[224,236]

The difficulties in surface conjugation and small number of functional groups can be the main liposomal drawbacks (Table 1). Moreover, other disadvantages exist, such as poor and low reproducibility of nanoparticle size, stability, and insoluble agent loading. Optimum sterilization and uncontrolled drug release are other challenges associated with the liposomes.[89,130,237,238]

Nanotechnology Clinical Approaches for Brain Cancer Drug Delivery

ONZEALD™. Sponsored by Lawrence Recht, Nektar Therapeutics has designed a polymeric version of irinotecan as the first long-acting topoisomerase I inhibitor. Irinotecan molecules are attached via an ester bond to the PEG polymer. Carboxylesterase and other enzymes react with the ester bond and cause the release of irinotecan and consequently, producing 7-ethyl-10-hydroxy-camptothecin or SN38 as the active metabolite. SN38 attacks DNA and causes its damage through topoisomerase inhibition. The main objective of the etirinotecan pegol (NKTR-102 or Onzeald) design is to eliminate or attenuate the irinotecan side effects and also improve its efficacy by drug distribution modifications. In preclinical studies, it demonstrated a 300-fold tumor concentration in comparison with a first-generation topo I-inhibitor. The NKTR is larger than normal vessel pores, helping it reach the tumor microenvironment by enhanced permeation and retention. Onzeald efficacy has been assessed in ovarian, breast, brain, colorectal, and lung cancer. The pharmacokinetic characteristics of the ONZEALD metabolite (SN38) differ significantly from others by providing maximal tumor exposure of the active drug (Table 2). For example, the C_{max} has been reduced 5 to 10 times and also the half-life has been increased to almost 50 days. The dose of 145 mg/m^2 ONZEALD in phase II clinical

trial almost equals the dose of 350 mg/m^2 irinotecan. The protracted exposure between continuous dosing cycles and lower C_{max} has improved the therapeutic effects of the treatments and clinical outcomes. Its open-label, single-arm 2016 phase II clinical trial had completed the ONZEALD's efficacy in bevacizumab-resistant high-grade gliomas.[239–241]

NU-0129. NU-0129 is a class of gene regulation spherical nucleic acid (SNA) nanostructures having well-orientated siRNA oligonucleotides finely designed and synthesized at their surface. Such SNAs are made up of siRNA oligonucleotides constructed on gold (Au) nanoparticle centers with oligo ethylene glycol (OEG) or PEG (OEG/PEG) on their surface to improve stability and circulatory half-life.[242,243] Based on the earlier investigations, using Au as the SNA heart allows for accurate spatial analysis of Au distribution in cells and tumors using inductively coupled plasma mass spectrometry (ICP-MS), X-ray fluorescence microscopy (XFM), and silver histopathology staining.[244–247] For the treatment of glioblastoma, the siRNA gold nanoparticles target the Bcl-2-like protein 12 (BCL2L12) domains. The tumor cells die as a result of the inhibition of BCL2L12 expression. In glioblastoma, BCL2L12 is hypothesized to be upregulated in a tumor-promoting direction. It inhibits the activation of effector caspase-3 and caspase-7. It can also bind wild-type p53 and its mutants, destabilizing them and preventing p53 from attaching to target gene promoters.[246,248–250] In order to test safety, pharmacokinetics, and intratumoral SNA nanoconjugate accumulation, the very first phase 0 clinical trial involving the systemic microdose delivery of NU-0129 in adults with recurrent glioblastoma was done. As a consequence, it was discovered to be safe and well tolerated by endothelial, immunological, and tumor cells, and it was linked to lower target protein levels in patients.[243 251]

Liposomal Rhenium-186 (186Re). Radiation is an essential part of brain cancer treatment, but due to the toxicity of high doses, its usage is limited. Rhenium-186 (186Re) is a diagnostic imaging rhenium isotope that is chemically similar to technetium-99m (99mTc) and is a reactor-produced isotope with great potential for medical therapy only after successful delivery (Table 2). It has a 90-hour half-life with a 2 mm tissue path length.[252] Its low tissue penetration has provided higher administration doses with the least toxicity. Localized radiation at the tumor site is achievable through the 100 nm liposomal formulations of the 186Re. Its applicability in the failed glioblastoma treatment procedures and accumulation at the tumor site is because of the EPR effect.[253–255]

2B3-101. The phase I/IIa clinical study for 2B3-101 or glutathione (GSH) PEGylated liposomal Dox in patients with glioma and breast cancer brain metastases has concluded. The G-technology employed to create this ideal nanostructure is established on the GSH identified transporter on BBB endothelial (Km of 6 mM). It is generally considered safe and utilizes micromolar glutathione targeting molecules on PEGylated nanoliposomal dosage forms.[256–259] The brain-to-blood ratio of doxorubicin was 4.8 times greater upon injection of 2B3-101 than generic PEGylated nanoliposomal Dox in discoveries. As a result, the brain's doxorubicin concentration rises without compromising the BBB's integrity.[260,261] It greatly slowed tumor growth when compared to nontargeted PEGylated liposomal Dox.[262,263]

PEGylated Liposomal Dox (Doxil®). Dox refers to a wide range of effective chemotherapeutics for the most aggressive malignancies such as glioblastoma. However, its effectiveness in vivo is under question due to the poor penetration as the result of the BBB. Its CSF and brain tissue concentrations have dramatically increased in tumor models after being sterically stabilized. A PEGylated liposomal Dox formulation with or without another chemotherapeutic agent like temozolomide not only has enhanced drug delivery to the brain but also case series and two-phase II studies concerning recurrent glioblastoma have demonstrated modest promising results.[264]

EGFR (V)-EDV-Dox. The EnGeneIC EDVTM technology-based EGFR (V)-EDV-Dox is a 400 nm Dox-loaded bacterial minicell that utilizes bispecific antibodies to function as a targeted therapy in cancer treatment (Table 2). In pigs and dogs, they were well tolerated with modest and temporary toxicity and inflammation, according to earlier investigations.[193,265] The minicells go from the bloodstream to the tumor microenvironment, where they assault the tumor cells' surface and release Dox. Furthermore, remnant EDV bodies in the tumor microenvironment that were unable to infiltrate malignant cells signal the immune system to the tumor site, counteracting the tumor's immunosuppression. Overall, these microcells polarize M1 macrophages and engage NK cells at the same time, resulting in a Th1 cytokine response with powerful anticancer activity. Upon that, dendritic cell maturation and antigen presentation proceeds, leading to tumor-specific CD8+ T cells and durable tumor remission.[193,266–269]

Discussion

Brain drug delivery systems have significantly advanced over the past few years with current research progressing the field every further. The latest advanced biological and physicochemical properties of the nanocarriers have taken them to higher levels. Furthermore, they have enhanced blood circulation and bioavailability. Not only do they provide a productive functionalized surface for a variety of molecules, but also they can be modified for controlled release over time. However, translating brain tumor treatment into clinical trials encounter unique barriers, largely in part due to the CNS biological barriers such as the BBB. Reduced tumor accumulation seems to be another obstacle for such delivery systems to reach the clinic. Optimizing the physicochemical parameters may overcome the disadvantages of novel nanoformulation. Shape, size, functionality, and surface charge have been modified in a variety of studies in the field of brain drug delivery research.[270–273]

Table 2. Nanocarrier-based Clinical Trials.

Name	Nanocarrier	Properties	Clinical phase	References
Ozeald (NKTR-102)	PEGylated polymeric irinotecan, etirinotecan pegol	• Limited side effects of irinotecan • Improved efficacy, a 300-fold increase in tumor concentration • EPR mechanism • Evaluated in breast, ovarian, colorectal, brain, and lung cancer • Constant exposure of the tumor to the active drug due to reduced C_{max} and increased half-life	Phase 2 for Anaplastic Astrocytomas Anaplastic Oligodendrogliomas Glioblastomas (GBM) is completed	239–241
NU-0129	Spherical nucleic acid (SNA) gold nanoparticle formulation composed of small interfering RNAs (siRNAs) targeting BCL2L12 gene	• Inhibiting the expression of BCL2L12 by NU-0129 induces tumor cell apoptosis	Early Phase 1 for gliosarcoma and Recurrent glioblastoma is active	243,251
186RNL	186Rhenium Nanoliposomes	• 100 nm • The half-life of 90 h • Limited penetration which limits toxicity of other forms of radiation • The liposomal formulation helps to retain within the tumor • EPR mechanism	Phase 1 Phase 2 for glioma which is recruiting	252–255
2B3-101	Glutathione PEGylated liposomal Dox	• ~110 nm • Targeted drug delivery of Dox • Optimal distribution to the brain • Targeting glutathione transporters on the surface of BBB	Phase 1 Phase 2 for brain metastases Lung cancer Breast cancer Melanoma Malignant glioma is completed and Phase 2 for meningeal carcinomatosis is unknown	262,263
Doxil®	PEGylated Liposomal Dox	• ~100-110 nm • Improved tissue and CSF concentrations	Phase 1 Phase 2 for glioblastoma is completed	264
EGFR (V)-EDV-Dox	BsAb-targeted, payload-packaged EDV nanocells	• 400 nm • EPR mechanism • BsAb binds to the tumor cell-surface receptor which causes the release of Dox within the cancer cell • EDVs are derived from bacteria and cause immunostimulating • Bypass the immunosuppression caused by the tumor	Phase 1 for glioblastoma astrocytoma, Grade IV which is recruiting	266,267

Designing a triggered release, as well as stimuli-responsive formulations, are among the most novel fields of drug delivery studies.[130,274,275] Nonetheless, only a handful of studies have attempted for cancer treatment examining stimuli-responsive systems. Temperature, pH, magnetic field, enzymes, oxidative stress, etc are triggering signals that have been frequently used for cancer treatment. The advantages of employing intrinsic environmental features in the tumor site in comparison with normal tissues for improved and efficient stimuli-responsive systems have been comprehensively discussed in the literature.[130] Furthermore, external stimuli such as light, heat, and magnetic fields are other options for controlled release. Liu et al[276] have obtained physicochemical sensitive (pH, temperature, etc) nanopolymers with both anticancer effects and brain tumor magnetic resonance imaging. Their application for both diagnosis and treatment procedures has been positively approved. The concept of these multifunctional nanosystems demonstrates the future of nanocarriers with vast room for growth and advancements in the field.[277,278] Nonetheless, the aforementioned nanoparticles' potential systemic toxicity and neurotoxicity in the clinic should be considered.

Despite numerous efforts to successfully improve the use of nanocarriers in different areas of clinical research, there remain challenges that need to be addressed. Investigating immortalized brain endothelial cell models for the BBB penetration assessment and testing the nanocarriers' efficiency to get through the BBB with the minimum cellular damaging effects are considered as examples of challenging aspects of this area. The high cost of these cell lines, such as hCMEC/D3, bEND3, and RBE4, limited availability and accessibility, and susceptibility to media and cultural contaminations have widely affected in vitro studies.[279,280] However, they have been used more frequently in comparison with astrocyte- or PC-derived cell lines in BBB studies.[279,281] Preclinical in vivo studies in the field of brain cancer is often hindered by the difficulty of modeling the biological heterogeneity that is observed between humans and mouse models, which are additional challenges for brain cancer.[282–284] Through advancements in pathological imaging methods and factoring in higher animal-to-human translational success rates, the in vivo complications may be reduced. The design of clinical trials is faced with difficulty in group classifications given the heterogeneity of tumor and the affected cell types. Furthermore, additional prognostic factors such as the low number of participants in each group may lead to a lack of statistical power to detect significant differences between control and therapeutic arms.[285,286]

Although more research and development are needed for effective nanocarriers with optimal clinical kinetics, they show promise as a suitable method of delivery for brain cancer treatment based on recent clinical studies.

Concluding Remarks, and Future Perspectives

Nanomedicine provides innovative opportunities in the development of tissue-specific targeted therapeutics, and imaging agents for brain tumor management. We have reviewed the challenges and advantages of several development efforts using nanocarriers for the treatment of brain tumors. Here we highlight the importance of further investigations for the development of effective treatments using nanotechnology for monitoring and treatment. This will facilitate the extremely effective development of nanomedicine in brain cancer disease. Taken together, the main goals of the upcoming brain cancer researches should be not only about having a higher survival rate, but also the patient's quality of life and especially the burden of treatment morbidities. Therefore, other challenges in brain cancer development may take the nanomedicine drug development under, which a few will be discussed.

First, we found an unmet need in coordinating a multifaceted team of specialists like researchers, neurologists, surgeons, neuropathologists, and other health professionals that will cause less suffering for those who burden the disease. Moreover, clarifying, organizing, and improving the fund and support in cancer investigations and research is required to make small communities of brain cancer research and investigations more developed.

Second, considering prioritized molecular and genetic tumor detection can lead to precise diagnosis and patient stratification and consequently move us toward specific and efficient drug development that more than likely will promote and facilitate current challenging brain cancer medications. This is in line with broadening tailored individualized therapy areas.

Finally, a clinical trial center in this field mostly cannot by itself recruit a considerable statistically powerful number of patients to run the study. Exploiting and extending more clinical trial centers can be a solution plus donating more grants for the brain cancer research portfolio.[287–292]

Thus, even though nanomedicine is a crucial milestone in brain cancer treatment but it requires a better understanding of other essential key elements for further reliable advancements.

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