Chapter

Potential of Lipid Based Nanodrug Carriers for Targeted Treatment of Glioblastoma: Recent Progress and Challenges Ahead

Bhabani Sankar Satpathy, Binapani Barik, Ladi Alik Kumar and Sangram Biswal

Abstract

Malignant brain tumor at its fourth stage (glioblastoma) is the most dangerous and an unsolved medical challenge till today. Present therapeutic strategies including chemo treatment, radiation along with surgery all together have not succeeded to control the progression of glioblastoma. Challenges in the early detection, unavailability of specific therapeutic strategy and severe cytotoxicity of available chemotherapeutics are the some of the prime causes of treatment failure. Especially presence of blood-brain barrier (BBB) highly limits pharmacological effect of conventional chemotherapy. In lieu of this, lipid based nanodrug carriers (LNCs) have now been evolved with great potential in improving the drug efficacy for the treatment of glioma. Further, LNCs engineered with specific targeting ligand might significantly reduce the dosage regimen, increase specificity, improve bioavailability and reduce off-target distribution. Such modified LNCs possess sufficient ability to cross BBB to deliver the loaded cargo(s) at target location inside the brain; thereby ensuring improved treatment outcome with less side effects than conventional treatment. This review primarily focuses on recent advancements in various engineered LNCs for the treatment of brain cancer. Also, the existing impediments for nanomedicines associated with their effective large scale synthesis or sufficient clinical application have also been highlighted.

Keywords: lipid based nanodrug carriers, glioblastoma, advancements, challenges

1. Introduction

Brain tumor at its malignant stage is the toughest challenge to treat. Glioma is the commonest form of malignant brain tumors and silently progresses to its fourth and most aggressive stage; called gliobalstoma. In fact, modern medical science in spite of cutting age technological advancements is yet to find specific answers for advanced malignant brain tumor.

An uncontrolled growth of cells beyond the cellular regulation inside the brain environment eventually leads to benign and/or malignant cancers [1]. The most common site for the development of tumor inside the brain is glial cells. Further, tumors as per their growth and location inside the brain are further classified from grade I (low grade) to grade IV (highly metastatic) type tumors [2]. Grade I stage of tumor (mostly goes unnoticed) can progress to the malignant stage more often and throws a tough challenge for treatment. Also, secondary metastatic brain tumors can be developed in adults from primary lungs/breast cancer [3]. Among the various grades of brain tumor, grade IV glioma, also called glioblastoma multiforme has been recognized as the severest and highly metastatic type brain tumor [4]. A vast majority of patients across the globe diagnoses with de novo or primary glioblastoma in recent years. Progression of brain tumors are often associated with typical increase in intracranial pressure, altered consciousness, occasional seizures along with severe headaches, vomiting, fever, gastric disturbances etc. [5]. However, these problems are highly variable from patient to patient and thus cannot be generalized prognosis parameters. Thus, primary stage of glioma often goes unnoticed. Aetiological causes related to the development of brain cancer are yet to be unravelled, which further makes the treatment extremely challenging. Classical subtype of glioma is assumed to be associated with amplification of chromosome 7 along with loss of chromosome 10. Coupled with these, over-expression of epidermal growth factor (EGFR) receptor and mutations are other proposed aetiologies of glioblastoma [6]. Mesenchymal glioblastoma has been shown to maintain a higher expression of CH13L1, MET, and genes associated with tumour necrosis factor, nuclear factor-κB, along with deletions of NF1. Mutations in IDH1, TP53 and modification of platelet-derived growth factor receptor A are also associated with secondary glioblastoma or lower-grade gliomas [7]. Though, neural glioblastomas at initial diagnosis shows similar characteristics to normal brain tissue; however, there is overexpression EGFR to several folds than normal.

At present, glioblastoma has been identified as the most complex, metastatic and treatment-resistant type of cancers with alarming prevalence around the globe. In 2020, more than 13,000 Americans have been diagnosed with GBM, which accounts for more than 48 percent of all malignant brain tumor cases [8]. Till now, average length of survival for patients with glioblastoma has been estimated to be only 1 to 1.5 years while the five-year survival rate has been roughly estimated as 6–7% only [9]. Over the past decade, mortality and survival rate of glioblastoma patients has not been improved as such in the developed nations. Even, uncontrollable prevalence of the disease is being witnessed in developing and under-developed countries. India has now become the new epi-centre for all cancer related deaths in recent years among which glioblastoma-related death cases occupies second lead position after breast cancer.

Along with extremely poor prognosis associated with glioblastoma, there is too serious dearth of promising therapeutic options. Much of the available treatment strategies alone or in combinations have been failed measurably over the past years to meet the treatment expectations. Usually, combination of various strategies like surgery, radiation, chemotherapy, non-chemodrug therapy etc. are employed to control the progression of tumor cells to other parts of the brain or to be metastatic [10, 11]. Surgery followed by radiation therapy is applied as the first line of treatment in the initial phases of glioma. Surgery is employed to remove maximum possible mass of tumor tissue from the brain, while radiation therapy is employed

to circumvent tumor mass *via* precise, focused high energy beams [12]. However, in many cases, effective application of surgery and radiation are extremely constrained as majority of brain tumors are usually detected at the advanced stages, i.e., at stage III or at stage IV. Additionally, highly sensitive nature of brain tissue and presence of delicate nervous network across the brain hemispheres with all major control systems of perception, mood, behaviour, cognition etc. further limits surgical procedures and effective radiation therapy [13, 14]. Hence, chemotherapy remains as the inevitable option to check the progression of tumor cells through cytotoxic anticancer drugs. Non-chemotherapeutic drugs are also used during treatment period to control tumor-associated headache/pain and epileptic seizures [15]. However, conventional chemo-drug treatment faces the usual problem just likes other conventional dosage forms such as failure to discriminate in between cancerous tissue and normal healthy tissue or lack of targetability. As a result, off-target biodistribution of cytotoxic anticancer drugs across all vital organs inside the body occurs, which in turn aggravates a wide range of adverse drug effects including alopecia, gastric disturbances, bone marrow depression, heart problems, kidney damage, immunity suppression and many other associated complications in cancer patients [16]. It has now been an accepted fact that the presently available clinical options all together have neither succeeded in extending cancer patient lives just beyond a few extra months nor been able to improve their quality of life after chemo-treatment cycles. In a nutshell, extremely poor prognosis, highly sensitive micro-environment of brain coupled with failure of conventional treatment options has made glioblastoma as a life-threatening disease. At present, it is too one of the most expensive cancers to treat, often leaving patients and families with major financial hardship during the treatments and in turn deteriorates socio-economic burden of the society as well [17]. In the lieu of which, advanced treatment options are being investigated heavily over the past years to improve the treatment outcomes and simultaneously to minimize the dose-related toxic effects on the body.

Moving from the initial treatment options like surgery and radiation, which have their inherent limitations; anti-cancer drug therapy through modified nanocarriers with improved targeting features is being explored as alternative option to improve overall treatment outcomes in cancer patients. In view of the presence of BBB as the major obstacle in brain-drug targeting, especially, lipid based nanocarrier based delivery systems have been recognized as hopeful options in glioblastoma owing to their highly lipophilic, ultra-small size, tuneable surface features. The cytotoxic anti-cancer drugs can be loaded into such nanocarrier vehicles and thus can be effectively surpass the BBB to get into the brain. Additionally, such carriers are now being manipulated at their surface with specific targeting ligands like antibodies, aptamers, small molecules, peptides etc. to enhance their targetability and reduce off-target distribution [18]. These engineered LNCs have been emerged as the prime research area in nanomedicine mediated brain cancer therapy now-a-days.

LNCs have the capability to bypass the BBB without disrupting its normal functionalization [19, 20]. Furthermore, LNCs in lieu of their architectural uniqueness provide requisite criteria of lipophilicity and sustained release of drug from their core/matrix. Attachment of tumor-specific ligands further makes them more specific and helps to mitigate peripheral toxicities [21]. After crossing the BBB, LNCs are endocytosed by endothelial cells and release the drug inside the cell [22]. There is too a growing interest to improve the *in vivo* performance of nanocarriers *via* conjugating them with thiolated and preactivated polymers to efficiently inhibit the P-glycoprotein (P-gp) efflux at brain luminal side [22, 23]. Glioblastoma possesses

a leaky vasculature, and thus may be amenable to LNC-based drug delivery systems that lead to enhanced drug deposition while limiting systemic drug exposure. Various types of LNCs have been investigated over the last decade to enhance therapeutic efficacy of anticancer drugs for the treatment of advanced stage glioma. In the present topic, we want to cover recent advancements in LNCs based drug targeting strategies for glioma. Specifically, we will restrict our discussion mostly on nanoliposomal vesicles and solid-lipid nanocarriers, which have been reported over the recent years for glioma/glioblastoma treatment. Side by side, some lights have been thrown on the challenges faced by such targeted LNCs for their successful clinical translation, regulatory hurdles along with scale-up issues for industrial production.

2. Blood-brain barrier: the prime culprit against effective drug therapy in glioblastoma

Brain, the controlling system of the whole body is undoubtedly the most complex, mysterious structure, which controls a multitude of crucial functions of the body including cognition, information processing, homeostasis, perception, motor control, mood, as well as learning and behaviour [24]. Such important functions are mediated by uncountable nervous networks which are present across the cerebellum. BBB is the main check-gate, which actively protects brain neural tissues from the influx of toxins and other compounds, including therapeutic molecules [25]. In fact, presence of BBB strictly restricts the success of chemotherapy as majority of anticancer drugs fails to permeate sufficiently across BBB, thus results in a sub-therapeutic concentration associated with low clinical outcome.

BBB is characterized by the presence of tight intercellular junctions along with lack of fenestrations. Main components of BBB are tightly placed brain endothelial cells, basal membranes, pericytes embedded in the basal membrane, along with astrocytic end feet [25]. All these structures are so uniquely placed close to each other that they collectively form a strong barrier on the way of every component having higher molecular weight or large size to pass from blood to brain. Only essential components like glucose and essential amino acids can get access inside the brain. Exogenous compounds including drugs having nano-size range or lipophilic property may cross the BBB by passive diffusion. Alternatively, some therapeutics can also cross the BBB through carrier-mediated active transport. Along with the strong barrier system like BBB, the efflux transporter systems present at the luminal side of brain also play crucial role in preventing therapeutic molecules to attain their pharmacological concentration [25, 26].

Similarly, in terms of molecule permeability, it has been found that molecules larger than 400 Da are very unlikely to cross the BBB (especially if highly water soluble) unless a suitable specific transporter is present. However, as mentioned earlier, highly lipophilic molecules tend to have better permeability than neutral or hydrophilic molecules owing to the high lipophilicity of the BBB. Temozolomide is an example of the poorly water-soluble drug with a molecular weight of 194.154 g/mol, which can readily cross the BBB. Similarly drugs like carmustine, lomustine etc. also have reasonable BBB permeation ability owing to their molecular cut-off range and lipophilic nature and have already been recommended for glioma therapy. These, along with few other drugs *viz*. capecitabine, paclitaxel etc. are presently some of the widely used chemotherapy drugs recommended in glioblastoma [27]. However, many lipophilic drugs in their native form/conventional formulation too fail to achieve

required therapeutic concentration at the brain tissue owing to their molecular size, in vivo stability issue, low half-life or affected by efflux transporter systems across BBB. Drugs bound to plasma proteins are also unavailable for crossing the BBB, since most of the proteins require specific transporters for BBB permeation. This phenomenon was demonstrated using Evans blue (an albumin-binding dye), which is completely unable to permeate across the intact BBB [28].

Dose-related adverse reactions are also obvious phenomena with conventional drugs, which further limit their chemo treatment cycle [29, 30]. Hence, it must be taken into account that merely a high degree of lipophilicity or delivery in conventional dosage forms does not either guarantee sufficient availability of drug inside the brain nor ensures its decreased off-target distribution throughout the healthy tissues.

In this context, there always felt an age-old need for an ideal delivery system that has to transport a drug with high efficiency to target brain cells, with minimal healthy tissue toxicity or off-target distribution. To achieve this, delivery of drugs/chemotherapeutics through the LNC based platforms has been attempted over the past few years by the pharma researchers and formulation scientists across the globe.

2.1 Lipid based nanocarriers: effective drug targeting platforms to brain

LCNs have been heavily investigated in recent years to improve the drug delivery at brain tissues owing to their lipophilic nature and ultra-small size. The key features of LNCs primarily involve their desirable size range, surface properties, and also ease of surface manipulation with targeting ligands [31]. The development of a broad range of LCNs with varying size, composition, and functionality has provided a significant resource for nanomedicine based glioblastoma therapy.

However, requirements for LCNs fabrication for effective glioma therapy also depend on tumor characteristics, its location and complicacy. Although LCNs avoid renal clearance preferably within the range of 10–50 nm, but they tend to accumulate heavily in the reticulo-endothelial system (RES), which is also another major setback for their sufficient brain bioavailability. Further, LCNs like other nanodrug carriers below the size of 10 nm possess the risk of higher glomerular filtration followed by renal clearance [32, 33]. All such problems are now being addressed successfully by the advanced formulation technologies, adaptation of cutting age research instruments and effective surface manipulation and employment of novel polymers (natural/synthetic). For example, problem associated with higher RES uptake can be subsided by surface coating/shielding of the LNCs with specific hydrophilic polymers like polyethylene glycol (PEG). Presence of PEG over the surface of LNCs renders hydrophilicity with subsequent reduction in RES uptake and enhancement in plasma half-life [34]. Similarly, by optimizing critical in-process parameters during formulation development such as polymer:drug ratio, amount of drug, sonication time, speed of centrifugation, filtration/separation technique, surface conjugation etc., desired size range of LCNs can be attained (preferably within 10–50 nm) for effective BBB permeation.

Likewise, the off-target bio distribution of the nanodrug carriers can be effectively reduced by surface conjugation with tumor-specific ligands. Several ligands like aptamers, antibodies, small molecules, peptides, sugar moiety etc., can be attached to LCNs to make them more specific with enhanced brain targetability [35]. Such engineered LCNs can effectively reduce healthy tissue toxicity along with chemoresistance of cancer cells, since they promote higher brain uptake of cytotoxic drugs around the tumor area with considerable decrease in drug efflux, thereby enhancing therapeutic outcome as well as (**Figure 1**).

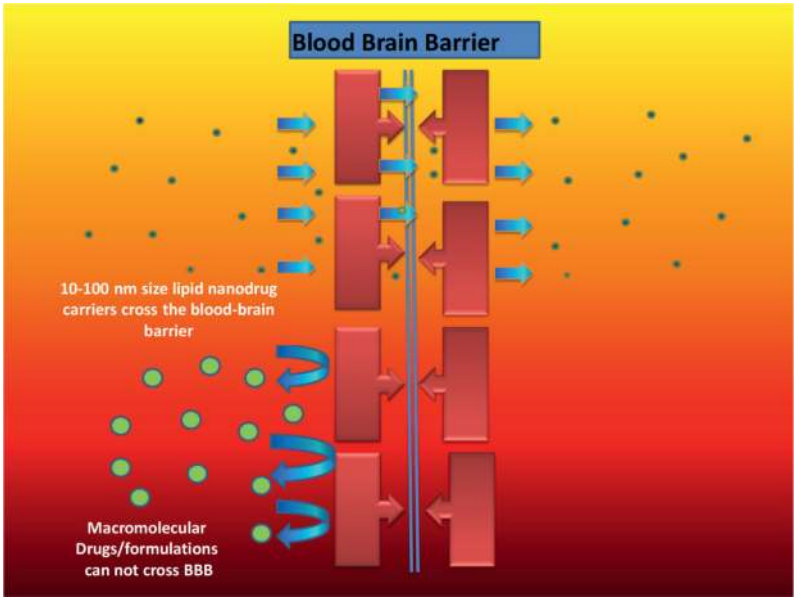

Figure 1.
A representative diagram of blood-brain barrier showing permeation of ultra-small size lipophilic drug carriers, whereas inability of macromolecular drug/ carriers to cross the barrier.

2.2 Types of lipid nanocarriers employed for drug targeting to brain

Lipid based nanocarriers are categorized into mainly three types, *viz*. nanoliposomes, solid-lipid nanoparticles, nanostructured lipid carriers. In our study, we would mostly restrict the discussion on these lipid based nanodrug carriers for glioblastoma therapy, excluding other organic/inorganic nanoparticles or other novel carriers.

2.2.1 Nanoliposomes

This is the first generation of novel drug delivery system, developed in 1960. It is prepared to resemble to the cell membrane compositions mainly by using fats, phospholipids, and cholesterol [36]. Due to its high flexibility, low toxicity, better stability, and biocompatibility, specifically targeting character with highly versatile nature, it has got immense attention in glioblastoma therapy [37, 38].

Liposomes are colloidal nano carriers, comprised in a vesicle. It can be uni-lamellar or multi lamellar i.e. comprising of more than one number of lipid bilayers encapsulating hydrophilic core or aqueous core. Due to unique structural features, both hydrophilic and lipophilic drugs can be delivered through nanoliposomes. By applying various in vitro techniques, the surface of liposomes can be easily modified with surfactants (e.g. tween 80, tween 20) bile salts, or tumor-specific targeting ligands [39, 40]. However, one of the major limitations related to liposome is their earlier uptake by phagocytic cells leading to shorter in circulation half-life. To avoid this PEG is functionalized over the conventional liposomes to keep it safe from the eyes of macrophages and to extend blood circulation profile [41].

2.2.2 Solid lipid nano carrier (SLNs)

This the first generation of solid-lipid based nano carrier was developed in 1991. It is usually spherical in shape having the diameter about 50–100 nm, dispersed in water

or in an aqueous surfactant phase [42]. SLNs have advantages like better stability, low melting point, nontoxic, ease to preparation, higher plasma pharmacokinetics, better bioavailability across BBB, good biocompatibility, bio degradability, very low cytotoxicity along with cost effective method of production [43]. It is an oil in water (o/w) system, in which the oil phase/liquid-lipid is replaced with the solid lipid to make it solid in both room and body temperature. The main ingredients used for the production of SLNs includes monostearates, stearyl alcohol, stearic acid, glycerol, cetyl palmitate etc. including stabilizers like tween 80, poloxamer 188, and dimethyl dioctadecyl ammonium bromine. The variation of ratio occurs in between the range of solid lipid (4:1) to the liquid lipid (1:4), surfactant concentration (0.25 to 6% w/v) to the total lipid concentration (1–30% w/v) [44]. However, it has also got few limitations like moderately drug loading capacity and expulsion of drug due to crystallization during under long-term storage condition.

2.3 Targeting strategies adopted by lipid nanodrug carriers for brain delivery

LNCs with their loaded cargo can be directly targeted to the brain owing to their ultra-small size and lipophilicity, as discussed previously. Since, most of the LNCs constitute phospholipid, sphingo lipid, cholesterol-based structures, they usually possess a cell-mimicking property, for which once get inside the cell, they tend to retain there with subsequent release of loaded cargo. In such cases, no artificial surface manipulation is done, and thus it does not guarantee glioma cell-specific drug targeting also.

Tumor vasculature usually shows abnormal architecture with highly permeable capillaries. Along with that the tumor mass too possesses a poor lymphatic drainage system, which thus allows accumulation of micromolecules having molecular cut-off size ≤40 kDa. LCNs mediated drug targeting actually utilizes this unique feature along with its lipophilic nature to invade inside the tumor tissue. The phenomenon popularly known as the enhanced permeability and retention (EPR) effect is taken as the prime mechanism in passive targeting of nanodrug carriers [45, 46]. Passive method of targeting the chemotherapeutics does not involve targeting to any specific receptor/protein expressed over tumor cell surface. It, thus primarily depends on the size and physicochemical properties of the nanocarriers. The ideal size range to benefit from the EPR effect is usually between 10 and 100 nm. But for successful BBB permeation of LNCs, an average hydrodynamic diameter around 10–50 is now preferably investigated. Outside this range, smaller particles usually clear by the kidney, preventing accumulation within the tumor site, while larger size particles fail to adequately penetrate through the glioma vasculature [46, 47].

In lieu of problems associated with passive targeting, surface engineering of nanocarriers with tumor cell-specific ligands have been investigated widely in past few years. The development of a broad range of LCNs with varying size, composition, and functionality has actually provided a significant revolution in glioblastoma therapy. While, passive targeting utilizes unique internal architecture of tumor tissue to target nano size delivery vehicles, active targeting is primarily based on surface engineering of nanodrug carriers with specific targeting ligands to make them more precise. Though, the leaky tumor vasculature coupled with weak lymphatic drainage of tumor provides a golden opportunity for direct targeting of nanosize drug carriers even without any surface manipulation [48], however, the chances of healthy tissue accumulation still remain there. Thus, surface engineering of LNCs has been emerged as hopeful alternative to decrease drug uptake in normal tissue and to increase accumulation in glioma to elicit better therapeutic outcome.

Active targeting in glioblastoma involves targeting surface membrane proteins that are upregulated in cancer cells [49]. Targeting molecules can be monoclonal antibodies or their fragments, aptamers, small molecules, oligopeptides etc. LNCs attached with surface ligands can be preferably localized to tumor tissue, expressing the associated receptors or antigens and can deliver the loaded drug *via* ligand-receptor interaction [50]. Some ligand receptor interactions also facilitate receptor-mediated endocytosis, which in turn enhances payload delivery inside the tumor cell.

2.4 Major types of targeting ligands in glioblastoma

2.4.1 Monoclonal antibodies (mAb)

Biocompatible mAb has been utilized from a decade as the first line of targeting ligand owing to their highly specific nature in various cancer treatments including malignant brain tumors. Many tumors up-regulate growth factor receptors, such as HER2/ neu in certain breast cancers, which can be targeted with anti-HER2/ neu surface antibodies [51]. Similar mAb mediated targeting strategy has now been investigated for glioblastoma. Though, unlike breast or prostate cancer, the specific receptors/ proteins having higher expression in case of brain tumor are very limited, but some of the recently reported research has provided evidence of improved treatment efficacy with mAb-engineered LNCs in malignant brain tumor as compared to conventional chemo-treatment. One recent example of such mAb is CD 133. This pentaspan transmembrane glycoprotein family member is also known as prominin-1 and has been found closely associated with glioblastoma. Research finding has identified CD133 as a major hallmark of glioblastoma stem cells [52]. Recent reports have further shown that CD133 antigen has elevated expression in glioblastoma, medulloblastomas, along with other brain cancers [53]. Thus, it could serve as a prognostic indicator of tumor recurrence or malignant progression.

2.4.2 Aptamers

Aptamers have recently emerged as effective ligands for their higher specificity, safer in vivo application with lesser chances of immunogenicity. They are basically folded single stranded oligonucleotides (25–100 nucleotides) that bind to specific molecular targets [54]. Aptamer-conjugated nanoparticles *in vitro* have displayed increased cytotoxicity and decreased volume of xenografts compared with non-targeted nanoparticles [55]. Aptamers possess many unique characteristics which make them an ideal imaging and targeting agent for the treatment of glioblastoma. Owing to their higher sensitivity, selective nature, ease of fabrication aptamers are presently lucrative drug-delivery platforms in glioblastoma [56, 57]. Although mAbs have been long history of use as potent therapeutic tool, however, their therapeutic application for glioblastoma including other neurodegenerative diseases has been limited, thanks to the presence of BBB, which checks effective entry of traditional antibodies. As compared to conventional mAbs, aptamers are more stable, smaller size and also easily accessible to chemical modifications. Adverse effects associated with aptamers are also rare. They can be physically/ chemically conjugated to a wide range of probes and therapeutic agents, which make them promising entity for imaging and detection in brain cancer. Successful application of aptamers for the diagnosis or treatment of glioblastoma has been reported in many recent researches. Recent research identified A40s, a novel aptamer that was internalized effectively in GBM stem cells and

successfully delivered miR-34c and anti-miR10b to the stem cell population. The data demonstrated that A40s crossed the BBB to reach the tumor location and selectively attached with the EphA2 receptor, which in turn led to inhibition in tumor growth and reduction in tumor relapse [58].

2.4.3 Folic acid (FA)

FA is essential for DNA synthesis, DNA repair, and methylation of DNA and is therefore necessary for cell survival and proliferation. The human folate receptor (FR), a glycosyl phosphatidyl inositol anchored membrane protein of 38 kDa, which shows high affinity for FA. At present, FR is considered an essential marker component in most of the cancers including glioblastoma. FR expression is very low or almost undetectable in most of the normal cells/tissues, but its expression is much higher in ovarian, breast, brain, lung, colorectal cancers [59]. FR-mediated liposomal delivery has been shown to enhance the antitumor efficacy of doxorubicin both in vitro and in vivo, and to overcome P-glycoprotein-mediated multi-drug resistance. Using folate as a targeting ligand, FR-targeting nanodrug delivery systems have been developed to target in situ glioma tumors [60].

2.4.4 Transferrin (Tf)

Tf receptor has been evolved as another important target for receptor-mediated transcytosis across the BBB. Owing to its higher expression on BBB endothelium, Tf-conjugation to the LNCs could be used as an effective active targeting strategy to enhance therapeutic outcomes in glioblastoma. Tf is basically a single chain iron-transporting glycoprotein that supplies iron into cells via receptor-mediated endocytosis [61]. Though, expression of Tf receptor remains very low in most of the normal tissues but its expression increases drastically in case of brain cancer. The binding affinity of Tf to its receptors on the external surface of tumor endothelial cells has been found 10 to 100 times more than in normal endothelial cells [62]. LNCs can take advantage of this feature through surface conjugation with Tf, which will be then actively transported into the tumor cells. Tf modified liposomes, nanoparticles and dendrimers have been widely investigated in recent years.

2.4.5 Oligopeptides

Oligopeptides are another class of emerging targeting ligands, which are now heavily investigated for glioma-specific drug targeting [63, 64]. The Arg-Gly-Asp (RGD) oligopeptide is a component of the extracellular matrix protein fibronectin, which is involved in the cell adhesion, migration and proliferation [64]. RGD is known to serve as a recognition motif in multiple ligands for several different integrin receptors. RGD-containing peptide can be internalized into cells by integrin-mediated endocytosis.

3. Advancements in lipid nanocarrier based drug delivery research in glioblastoma

LNCs in view of their architectural uniqueness and preferable in vitro characteristics have become leading choice of delivery vehicle in glioblastoma research [33].

Many recent studies have depicted superiority of the LNCs in successful drug targeting to brain as compared to conventional formulations. S D. Hettiarachchi and his co-researchers developed a nano drug formulation of triple conjugated delivery system which included conjugation of two drugs to achieve synergistic effect in glioma. The triple conjugated delivery system comprised of transferrin, epirubicin and temozolomide. The in vitro results showed higher anticancer effect for transferrin conjugated samples. MTT assay depicted dramatically reduced cell viability in case of targeted nanocarriers as compared to non-transferrin conjugated carriers. The triple system of transferrin conjugated samples was significantly more cytotoxic to glioblastoma cell lines and was more effective than their equivalent single agents [65].

Another new strategy reported potentiality of aptamer-based immunoliposomes in modifying PD-1-silencing T cells. PD-1 gene was knocked out from CD8+ T cells using CRISPR/Cas9 system to liberate T cell activity from immunosuppression. The work involved stimulation of PD-1– T cells followed by functional modification of tumor-specific nanoliposomes (hEnd-Apt/CD3-Lipo) to generate FC/PD-1– CTLs. The activation and proliferation of the modified FC/PD-1– CTLs were then measured [66]. The anticancer potential of experimental CTLs against HepG2-tumors was evaluated in xenograft mice. Results indicated that the modification of hEnd-Apt/CD3-Lipo nanocomposites on the FC/PD-1– CTLs had a more substantial synergetic effect in inhibiting tumor growth and prolonging animal survival, rather than other control liposomes [66]. Though, the study was not directed towards glioblastoma therapy, but the active targeting of immunoliposomes towards PD-1 receptor could be taken an attractive strategy for futuristic potential application in glioblastoma. Seeing the over-expression of PD-1 in many brain/CNS disorders including glioma, the outcome of the study could be used as an important input for further research of LNCs based PD-1 targeting to glioblastoma.

The therapeutic potential of hyaluronic acid (HA) as a targeting ligand for glioblastoma was investigated in a study by Stephen L et al. Anticancer effect of HA-conjugated doxorubicin loaded LNCs was reported in cortical astrocytes, MG, and A172 cells. In the study, three different glioblastoma cell lines were employed *viz.* invasive/non-tumorigenic (A172 cells), non-invasive/slightly tumorigenic (U251), and invasive/ highly tumorigenic (U87MG). A 24-hour potency assay demonstrated that the LC_{50} of experimental LNCs on A172 cells was nearly 5 folds lower than the corresponding LC_{50} for the cortical astrocytes and nearly 3 folds lower than that for MG cells [67]. The study thus highlighted potential application of HA in promoting preferential tumor cell uptake, with significant enhancement in chemotherapeutic potency in glioblastoma cells as compared to astrocytes.

Application of monoclonal antibodies as glioma-specific ligands through nanoliposomal vesicular carriers has already been reported. A recent liposomal delivery study has suggested conjugation of CD133 antibodies as a suitable method for targeting glioblastoma [52]. The study reported brain targeted delivery of gemcitabine, a widely used anticancer drug for cancers. However, being a BCS class III category of drug, it has higher water solubility with low permeability. Hence, to meet the challenge of sufficient brain uptake, gemcitabine was loaded in nanoliposome and the surface of the gemcitabine loaded liposome was functionalized with CD 133. The experimental CD 133 modified nanolipsomes was then tested for their in vitro and in vivo performance in glioblastoma cells. The in vitro study showed that conjugation of CD133 significantly enhanced the cytotoxicity of gemcitabine through endocytosis of CD133 surface markers overexpressed on glioblastoma cells [52]. The anti-tumor effect of CD133-modified nanoliposome was 15 times higher than that of free drug.

The formulation also showed enhanced in vivo stability and cytotoxicity through in glioma bearing xenograft models. Moreover, monitoring of body weight changes showed that the use of targeted nanoliposomes significantly reduced the toxicity of gemicitabine.

Compared to single anticancer drug based chemotherapy, a combination of gene and drug therapy is being investigated in recent studies to achieve breakthrough in glioma treatment. It was expected that therapeutic genes and chemical drugs could act on different targeting sites with different mechanisms and could achieve synergistic therapeutic efficacy. The study explored the potential application of angiopep-2 through paclitaxel loaded cationic nanoliposomes. Angiopep-2 possesses the ability to target the low-density lipoprotein receptor-related protein, which is over-expressed on the BBB and glioma cells [68]. In a study, angiopep-2 modified cationic liposome was developed (ANG-CLP) for effective co-delivery of a therapeutic gene and an anticancer drug. The gene encoding the human tumour necrosis factor-related apoptosis-inducing ligand (pEGFP-hTRAIL) was used along with paclitaxel as the drug of choice for targeted delivery to glioma through LNCs. The dual targeting co-delivery system improved cellular uptake and gene expression in U87 MG human glioblastoma cells and also in the infiltrating margin of intracranial U87 MG glioma-bearing models [69]. The dual targeting LNCs selectively induced apoptosis in U87 MG cells while reducing toxicity to BCECs. Results of the pharmacodynamics studies showed that the apoptosis of glioma cells in *in vitro* BBB models and in U87 MG glioma-bearing mice treated by the experimental LNCs was more apparent and widespread than that treated by single medication systems and unmodified co-delivery system. Along with that, the median survival time of brain tumour-bearing mice group treated with angiopep-2-targetd LNCs was 69.5 days, which was significantly longer than that of conventional nanolipsome and standard drug treated groups. The treatment groups received commercial temozolomide showed median survival time of 47 days only [69].

Receptor-mediated endocytosis is one of the major mechanisms which can be effectively employed as active targeting approach to deliver the conventional chemotherapeutic agents to permeate across BBB. The receptors for insulin, transferrin, endothelial growth factors, amino acids, follic acid along with various metabolic nutrients are expressed on BBB, which thus can be taken as an opportunity to modify the surface of nanocarriers with relevant targeting moiety to make them brain specific. Dual-targeting doxorubicin encapsulated nanoliposomes were produced by conjugating the experimental liposomes with both folate and Tf, which were then tested for their effectiveness in glioma model [70]. The nanoliposomes were characterized by particle size, drug entrapment efficiency, and in vitro drug release profile. Drug accumulation, P-gp expression, and drug transport across the BBB in the dual-targeting nanoliposomes were examined by using bEnd3 BBB models. In vivo studies demonstrated that the dual-targeted nanoliposomes could successfully transport doxorubicin across the BBB and mainly distributed in the brain glioma. The anti-tumor effect of the dual-targeting liposome was also found significantly higher as compared to plain liposomes and free drug in terms of increased survival time and decreased tumor volume [70].

From our laboratory, we also carried out few works related to the brain delivery or BBB permeation ability of anticancer drugs through LNCs based strategy. Though our works were mostly based on passive targeting approach where we have mostly utilized the lipophilic nature and nanosize property of our developed liposomal vesicles to target the anticancer drug to brain, but the outcomes of the

work was quite impressive, which has compelled us for their further clinical translational studies. One of the recent studies from our laboratory reported the successful delivery of lomustine in glioma cells via lipid nanovesicular constructs [71]. Experimental LNCs were developed by modified lipid layer hydration technique and evaluated for different *in vitro* characteristics. Anticancer potential of selected lomustine loaded LNCs was tested on C6 glioma cell line *in vitro*. The experimental LNCs were within a size of less than 50 nm along with 8.8% drug loading capacity. Confocal microscopy revealed reasonable internalization of the selected LNCs in C6 cells. Experimental formulations were found more cytotoxic than free lomustine and blank LNCs as depicted from MTT assay. A clear improvement in pharmacokinetic profile both in blood and brain in the experimental mice models was observed for drug loaded LNCs than free drug. The formulations showed negligible haemolysis in mice blood cells, which further justified their safer in vivo applications.

Another similar study by Satapathy et al., reported delivery of docetaxel successfully to the rat brain through DSPE-modified nanoliposomes. In the work, the researchers simply aggravated the passive targeting strategy by utilizing DSPE, a sphingolipid, which has abundant presence the in brain and CNS. In the work, they developed a DSPE incorporated LNCs encapsulating docetaxel and investigated its BBB crossing potential, both qualitatively and quantitatively, in vivo [72]. Pharmacokinetic and biodistribution data showed an enhanced residence time of the docetaxel in the blood and efficient permeation of the drug from the docetaxel loaded LNCs through the BBB, as compared to free drug. The technetium-99 m labeled experimental LNCs effectively crossed the BBB and accumulated in the brain tissue in a time dependant manner as depicted from single photon emission tomography data [72]. At 4 h experimental time period, radiolabelled-LNCs were clearly tracked in the rat brain, whereas the same signal was absent in case of radiolabelled-free drug, which thus clearly confirmed that the sphingolipid modified LNCs possessed the necessary potential for BBB permeation and could be effective for the treatment of glioblastoma. Similar study from another research group in same department revealed successful delivery of docetaxel to rat brain through experimental nanoliposomes. Anti-proliferative effect of the experimental docetaxel loaded LNCs was conducted on C6 rat glioma cells. MTT assay showed that IC_{50} values of docetaxel from experimental nanoliposomes (9.5 ± 0.8 nM) was significantly less in comparison to free-drug (IC_{50} value, 70.8 ± 0.1 nM) and marketed Taxotere (IC_{50} value, 86.5 ± 0.3 nM) [73]. Flow cytometric analysis of C6 glioma cells incubated with fluorescein isothiocyanate (FITC)-labelled docetaxel loaded LNCs indicated about 18 and 23% enhancement of cellular uptake at 0.5 h at 0.5 h and 6 h of treatments in comparison to untreated cells.

Triggered drug delivery now-a-days has been merged as an interesting active targeting option for improved delivery of drugs through nanocarriers for the treatment of glioblastoma. A recent study showed that repeated pulsed high-intensity focused ultrasound can be used to improve the delivery of doxorubicin loaded nanocarriers to brain [74]. Atherosclerotic plaque-specific peptide-1 (AP-1) was used as the targeting ligand over the surface of doxorubicin loaded LNCs to selectively target glioblastoma cells. Compared with the control group, the animals treated with AP-1-conjugated nanoliposomes (5 mg/kg) showed significantly enhanced accumulation of drug at the sonicated tumor site and also a significantly elevated tumor-to-normal brain drug ratio ($p = 0.001$) (**Table 1**).

4. Challenges ahead

It is a fact that nanomedicine has revolutionized the field of medical diagnostics and treatment and significantly improved the therapeutic and pharmacokinetic profile of conventional chemotherapy for effective targeting at brain. However, in spite of all eye-catching progress in nanocarrier based drug targeting, lots of challenges still remain, which in fact need serious insight analysis. Common obstacles with the use of LNCs for successful treatment of glioblastoma yet remain unaddressed largely in the form of the RES uptake, opsonisation, *in vivo* stability etc. [85].

Another issue is the cell/tissue accumulation and toxicity concern of engineered LNCs. Ultra-small size and brain specific delivery through targeting ligands though helpful for increased cellular uptake and diminished off-target toxicity, but accumulation of such engineered nanodrug systems in healthy organ cannot be fully ruled out. Such *in vivo* studies related to the toxicological concern of engineered nanodrug carriers are too highly lacking. Since, the toxic effects upon long-term accumulation of nanodrug carriers largely depend on various physico-chemical factors including shape, size, composition, biocompatibility, route of administration, degradation mechanism, drug-tissue interaction, protein binding etc., these factors thus need to be vividly analysed from case to case basis. The safety and pharmacological effect of engineered LNCs can be influenced by minor variations in multiple parameters and need to be carefully examined in preclinical and clinical studies. Systematic impact analysis of the possible acute/chronic toxicity effects of novel LNCs on humans and environment is the need of the hour.

Oral administration of LNCs is still not a feasible strategy due to stability and liver metabolism issues. Even, after intravenous administration, it is still unclear, how the properties of engineered LNCs change in brain microenvironments, or their effect on complement activation, blood coagulation, etc. Thus, many such important factors related to the *in vivo* behaviour engineered LNCs and their post treatment effect on normal brain cells need thorough investigation.

There is still dearth of ample pre-clinical research outcome of engineered LNCs on glioblastoma. Most of the studies related to glioblastoma are confined to *in vitro* cell line studies. Though experiments on *in vivo* efficacy of LNCs in brain tumor bearing xenograft model is there, but results of such research are highly variable with lack *of in vitro-in vivo* correlation data. Due to reliable *in vitro-in vivo* correlation related studies with variable research outcomes, such engineered LNCs face serious hurdle in clearing requisite regulatory approval for clinical trials [85]. The insufficiency of specific regulatory guidelines for the development, evaluation, *in vivo* testing of engineered LNCs is also another crucial factor in clinical translation. The leading pharma houses or pharma-research and development laboratories are still in confusion, whether to rely on the clinical efficacy of engineered nanodrug carriers for the treatment of glioblastoma on large scale basis. To find a sponsor for clinical trial of engineered nanodrug carriers still remains a tough task.

For anticancer drug loaded LNCs, dose ranges need to be correctly defined along with sufficient blood and brain pharmacokinetics data. Since, clinical testing of nanodrug carriers intended for the treatment of glioblastoma starts from phase II stage, i.e. subsiding phase I clinical trial on healthy volunteers, therefore establishment of proper in vivo safety, pharmacokinetic and dose-range data are highly crucial. In case of in vivo experiments, concerns are also being raised by some formulation scientists and medical experts on the rationality of *in vivo* experiments using xenograft

Lipid nanocarrier based delivery system	Drug/ therapeutic agent	Targeting strategy/ targeting ligand	Research findings	Reference
H-ferritin siRNA conjugated nanoliposome	siRNA	Active targeting/ H-ferritin	H-ferritin siRNA decreased protein expression by 80% within 48 hours. Increased apoptosis in glioma cells *in vitro*	[75]
FTH1 loaded nanoliposome	FTH1 siRNA	Passive targeting	FTH1 down-regulation demonstrated by decreased cell viability, impaired DNA repair and reduced colony formation	[76]
Glutathione PEGylated liposomal Doxorubicin	Doxorubicin	Active targeting/ Glutathione	4.8 fold increase in brain-to-blood ratio of doxorubicin as compared to generic Caelyx® ($p = 0.0016$)	[77]
Dual-functioned nanoliposome	Doxorubicin	Active targeting/ Transferrin and cell-penetrating peptide	Tf/TAT-modified nanoliposomes showed higher anti-proliferative activity against U87 cells and also in orthotropic glioma model *in vivo*.	[78]
OX26/CTX-conjugated liposome	Plasmid DNA	Active targeting/ OX26 and chlorotoxin	The targeted nanoliposome exhibited enhanced therapeutic effects on C6 cells.Dual-targeting effect diminished tumor volumes (18.81 ± 6.15 mm3) and extended median survival time (46 days) in C6 glioma-bearing rats.	[79]
Dual-targeting nanoliposme	Doxorubincin	Active targeting/ folate and transferrin	Dual-targeting liposome demonstrated increased survival time, decreased tumor volume in glioblastoma model	[80]
Folic acid modified nanoliposome	Lidocaine	Active targeting/ Follic acid	Higher uptake of targeted nanoliposomes by U87 cells. Suppressed the motility of U87 glioma cells and stimulated apoptosis.	[81]
Dual-targeting liposome	Paclitaxel	Active targeting/ Transferrin and arginine-glycine-aspartic acid	In vivo imaging demonstrated RGD peptide and transferrin provided the highest brain distribution. Targeted liposomes showed preferential anti-proliferative activity against C6 glioma cells	[82]

Lipid nanocarrier based delivery system	Drug/ therapeutic agent	Targeting strategy/ targeting ligand	Research findings	Reference
Theranostic liposomes	Docetaxel	Active targeting / folate	Higher cellular uptake lower IC_{50} showed for folate-targeted nanoliposomes than non-targeted liposomes and marketed formulation	[83]
Ligand modified nanoliposme	Doxorubicin	Active targeting/ c(RGDfK) and Peptide-22	c(RGDfK) and Peptide-22-modified nanoliposomes increased the internalization in U87 cells. In vivo imaging verified higher brain tumor distribution for targeted nanoliposmes than un-modified liposomes.	[84]

Table 1.
Research outcomes on lipid nanocarrier based drug delivery systems, targeting strategy adopted in metastatic glioma.

mice/rat model bearing brain tumor. As such animal systems are usually athymic or immune-compromised; data derived out of these animal experiments cannot be fully relied on to carry out direct clinical testing on human subjects. In view of the significant anatomical/ physiological differences between immune-compromised laboratory animal model and human subjects in the development and progression of glioblastoma, it has been a point of long argument that whether these animal models could really mimic the human brain micro environment or whether such pre-clinical safety/ dose-range data can be reciprocated in clinical settings. It is a fact that laboratory rodents employed for the study do not suffer from glioblastoma or any other brain/ CNS cancers frequently as normal humans. Furthermore, immune response, cellular reaction, metabolism profile between laboratory animals and human subjects vary significantly differently. In a lay man language the material, which behave nontoxic to animals may show severe toxicity to humans or vice versa. Again till now, exact mechanism behind development/progression of glioblastoma in humans is largely unclear just as other cancer types. We seriously lack sufficient knowledge or well characterized data on specific biochemical factors, diseased conditions or antigens/ proteins responsible for development of glioblastoma. Thus, how much it will be rational to trust on the animal experiment data involving artificial/forcefully develop glioblastoma in nude/athymic animal models. Whether the use of such genetically modified animal models could really serve the purpose of successful clinical translation of LNCs? The budding scientists and medical/pharmacy/clinical professionals have to find specific answer for these unsolved questions in order to convince the manufacturers/sponsors to go ahead for large scale production.

Moving from the regulatory or clinical application problems towards large scale production at industrial scale, there is too lots of challenges remain unaddressed. Many pharmaceutical companies are still hesitant to invest directly in the large scale production of LNCs based delivery platforms. Batch to batch variation, problems with scale up, high cost of raw materials, availability of standardized unique protocol for

manufacturing and testing, stability issues, low drug carrying capacity are some of the major issues associated with LNCs. As a result, maximum research outcomes are confined in academic or small scale research laboratories and cannot able to reach from bench to bed side. To simplify the approval process for LNC based drug delivery system, a closer cooperation among various regulatory agencies is also warranted. Government of various countries too have ample responsibility with regard to develop advanced/simplified protocols that must be genuine, less tedious, yet sufficiently rigorous to address any safety concerns in a timely manner.

5. Conclusion

Glioblastoma still remains an area of unmet medical challenge despite remarkable progress in understanding its genesis and propagation. With advancements in molecular biology, biotechnology and interdisciplinary research horizon covering nanotechnology, computational biology, genetic engineering etc., successful treatment strategies are highly expected in near future. Continuous research by formulation scientists have led to development of novel lipid nanocarrier based formulations, which are showing promise in glioblastoma both *in vitro* and *in vivo* rodent models of the disease. Few of the nanodrug carriers have already seen day light with successful clinical applications in brain cancer patients. However, number of such advanced engineered nanocarrier system at clinical trial stage is still very limited. Stringent regulatory procedure coupled with lack of sponsors/industrial collaborators are being the major hurdles in successful clinical translation of the nanodrug carriers from laboratory to bed side. Active targeting strategies with tumor-specific ligands though emerged as hopeful approach in elevating treatment outcomes and to reduce chemo-induced side effects in glioblastoma, but in reality, lots of challenges are need to be focused. Recent studies have introduced MRI and near infrared imaging to the administration of dual-targeted nanodrug carriers, enabling targeting to be imaged with these new theranostics. Although the engineered LNCs could be plausible option for treating glioblastoma, detailed in depth analysis is highly essential to bring out desired outcomes in patients. In vivo performances of engineered LNCs are yet highly variable and *in vitro-in vivo* correlation data is seriously lacking. Till now, the leading pharma manufactures in India hesitate to go ahead for the large-scale production of targeted nanodrug carriers. Data are also scarce and dissatisfactory for targeted nanomedicnes to show improved clinical outcomes or improved quality of life post treatment in glioblastoma. Despite these daunting facts there is still hope. Personalized cancer planning, advance diagnosis, ample pre-clinical research, continuous research idea exchange between industry and academia are some of the highly focused area, which could finally make this goal a reality. With the growing global trend, the future of modern multimodal, multi-centered treatment approach of LNCs for regular clinical application in glioblastoma looks feasible.

DOI: http://dx.doi.org/10.5772/intechopen.108419

Author details

Bhabani Sankar Satpathy[1*], Binapani Barik[2], Ladi Alik Kumar[2] and Sangram Biswal[1]

1 School of Pharmaceutical Sciences, Siksha 'O' Anusandhan (Deemed to be University), Bhubaneswar, Odisha, India

2 Centurion University of Technology and Management, R. Sitapur, Odisha, India

*Address all correspondence to: bhabanisatapathy@soa.ac.in; bbhabanisatapathy@yahoo.com

References

[1] Aldape K, Brindle KM, Chesler L, Chopra R, Gajjar A, Gilbert MR, et al. Challenges to curing primary brain tumours. Nature Reviews Clinical oncology. 2019;**16**(8):509-520. DOI: 10.1038/s41571-019-0177-5

[2] Gilbert MR, Dignam JJ, Armstrong TS, Wefel JS, Blumenthal DT, Vogelbaum MA, et al. A randomized trial of bevacizumab for newly diagnosed glioblastoma. New England Journal of Medicine. 2014;**370**(8):699-708. DOI: 10.1056/nejmoa1308573

[3] Nimmervoll BV, Boulos N, Bianski B, Dapper J, DeCuypere M, Shelat A, et al. Establishing a preclinical multidisciplinary board for brain tumors: A preclinical multidisciplinary brain tumor board. Clinical Cancer Research. 2018;**24**(7):1654-1666. DOI: 10.1158/1078-0432.ccr-17-2168

[4] Mackay A, Burford A, Carvalho D, Izquierdo E, Fazal-Salom J, Taylor KR, et al. Integrated molecular meta-analysis of 1000 pediatric high-grade and diffuse intrinsic pontine glioma. Cancer Cell. 2017;**32**(4):520-537. DOI: 10.1016/j.ccell.2017.08.017

[5] Zacharakis N, Chinnasamy H, Black M, Xu H, Lu YC, Zheng Z, et al. Immune recognition of somatic mutations leading to complete durable regression in metastatic breast cancer. Nature Medicine. 2018;**24**(6):724-730. DOI: 10.1038/s41591-018-0040-8

[6] Szopa W, Burley TA, Kramer-Marek G, Kaspera W. Diagnostic and therapeutic biomarkers in glioblastoma: Current status and future perspectives. BioMed Research International. 2017;**8013575**:1-14. DOI: 10.1155/2017/8013575

[7] Ohgaki H, Kleihues P. The definition of primary and secondary glioblastoma. Clinical Cancer Research. 2013;**19**:764-772. DOI: 10.1158/1078-0432

[8] National Brain Tumor Society. Glioblastoma Facts & Figures. 2019. Available from: https://braintumor.org/events/glioblastoma-awareness-day/about-glioblastoma

[9] Hanif F, Muzaffar K, Perveen K, Malhi SM, Simjee SU. Glioblastoma multiforme: A review of its epidemiology and pathogenesis through clinical presentation and treatment. Asian Pacific Journal of Cancer Prevention. 2017;**18**(1):3-9. DOI: 10.22034/APJCP.2017.18.1.3

[10] Leone JP, Leone BA. Breast cancer brain metastases: The last frontier. Experimental Hematology & Oncology. 2015;**4**(33):1-10. DOI: 10.1186/s40164-015-0028-8

[11] Urbańska K, Sokołowska J, Szmidt M, Sysa P. Glioblastoma multiforme—An overview. Contemporary Oncology. 2014;**5**:307-312. DOI: 10.5114/wo.2014.40559

[12] Davis ME. Glioblastoma: Overview of disease and treatment. Clinical Journal of Oncology Nursing. 2016;**20**(5):1-8. DOI: 10.1188/16.cjon.s1.2-8

[13] Tamimi AF, Juweid M. Epidemiology and Outcome of Glioblastoma. Brisbane (AU): Codon Publications; 2017. pp. 143-153. DOI: 10.15586/codon.glioblastoma.2017.ch8

[14] Franchino F, Rudà R, Soffietti R. Mechanisms and therapy for cancer metastasis to the brain. Frontiers in Oncology. 2018;**8**:161. DOI: 10.3389/fonc.2018.00161

[15] Fernandes C, Costa A, Osório L, Lago RC, Linhares P, Carvalho B, et al. Current Standards of Care in Glioblastoma Therapy. Exon Publications; 2017. pp. 197-241. DOI: 10.15586/codon.glioblastoma.2017.ch11

[16] Baskar R, Dai J, Wenlong N, Yeo R, Yeoh KW. Biological response of cancer cells to radiation treatment. Frontiers in Molecular Biosciences. 2014;**1**:24. DOI: 10.3389/fmolb.2014.00024

[17] Makale MT, McDonald CR, Hattangadi-Gluth J, Kesari S. Brain irradiation and long-term cognitive disability: Current concepts. Nature reviews. Neurology. 2017;**13**(1):52-64. DOI: 10.1038/nrneurol.2016.185

[18] Zhou J, Rossi JJ. Cell-specific aptamer-mediated targeted drug delivery. Oligonucleotides. 2011;**21**(1): 1-10. DOI: 10.1089/oli.2010.0264

[19] Su S, Kang P. Recent advances in nanocarrier-assisted therapeutics delivery systems. Pharmaceutics. 2020;**12**(9):837. DOI: 10.3390/ pharmaceutics12090837

[20] Yingchoncharoen P, Kalinowski DS, Richardson DR. Lipid-based drug delivery systems in cancer therapy: What is available and what is yet to come. Pharmacological Reviews. 2016;**68**(3):701-787. DOI: 10.1124/ pr.115.012070

[21] Wen H, Jung H, Li X. Drug delivery approaches in addressing clinical pharmacology-related issues: Opportunities and challenges. The AAPS Journal. 2015;**17**(6):1327-1340. DOI: 10.1208/s12248-015-9814-9

[22] Alavijeh MS, Chishty M, Qaiser MZ, Palmer AM. Drug metabolism and pharmacokinetics, the blood-brain barrier, and central nervous system drug discovery. NeuroRx. 2005;**2**(4):554-571. DOI: 10.1602/neurorx.2.4.554

[23] Ventola CL. The nanomedicine revolution: Part 1: Emerging concepts. Pharmacy and Therapeutics. 2012;**9**:512

[24] Zheng M, Tao W, Zou Y, Farokhzad OC, Shi B. Nanotechnology-based strategies for siRNA brain delivery for disease therapy. Trends in Biotechnology. 2018;**36**(5):562-575. DOI: 10.1016/j.tibtech.2018.01.006

[25] Dong X. Current strategies for brain drug delivery. Theranostics. 2018;**8**(6):1481-1493. DOI: 10.7150/ thno.21254

[26] Daniëlle ME, Mark L, Ronald B, Robert CS, Albert DW, Philip S, et al. P-Glycoprotein function at the blood–brain barrier: Effects of age and gender. Molecular Imaging and Biology. 2012;**14**(6):771-776. DOI: 10.1007/ s11307-012-0556-0

[27] Narvekar M, Xue HY, Eoh JY, Wong HL. Nanocarrier for poorly water-soluble anticancer drugs-barriers of translation and solutions. AAPS PharmSciTech. 2014;**15**(4):822-833. DOI: 10.1208/s12249-014-0107-x

[28] Meng-Chih W, Jye-Lin H, Ted WL. Evans blue dye as an indicator of albumin permeability across a brain endothelial cell monolayer in vitro. Neuroreport. 2021;**32**(11):957-964. DOI: 10.1097/ WNR.0000000000001690

[29] Din F, Aman W, Ullah I, Qureshi OS, Mustapha O, Shafique S, et al. Effective use of nanocarriers as drug delivery systems for the treatment of selected tumors. International Journal of Nanomedicine. 2017;**12**:7291-7309. DOI: 10.2147/IJN.S146315

[30] Blanco E, Shen H, Ferrari M. Principles of nanoparticle design for

overcoming biological barriers to drug delivery. Nature Biotechnology. 2015;**33**(9):941-951. DOI: 10.1038/nbt.3330

[31] Manasa MH, Suma P, Srinivas M, Abhishek C, Jayant SG, Satish Rao BS. Multifunctional lipidic nanocarriers for effective therapy of glioblastoma: Recent advances in stimuli-responsive, receptor and subcellular targeted approaches. Journal of Pharmaceutical Investigation. 2022;**52**:49-74. DOI: 10.1007/s40005-021-00548-6

[32] Tu L, Luo Z, Wu YL, Huo S, Liang XJ. Gold-based nanomaterials for the treatment of brain cancer. Cancer Biology & Medicine. 2021;**18**(2):372-387. DOI: 10.20892/j.issn.2095-3941.2020.0524

[33] Patra JK, Das G, Fraceto LF, Campos EV, Rodriguez-Torres MD, Acosta-Torres LS, et al. Nano based drug delivery systems: Recent developments and future prospects. Journal of Nanobiotechnology. 2018;**16**(1):1-33. DOI: 10.1186/s12951-018-0392-8

[34] Jung SS, Qingguo X, Namho K, Justin H, Laura ME. PEGylation as a strategy for improving nanoparticle-based drug and gene delivery. Advanced Drug Delivery Reviews. 2016;**99**(Pt A):28-51. DOI: 10.1016/j.addr.2015.09.012

[35] Rizvi SA, Saleh AM. Applications of nanoparticle systems in drug delivery technology. Saudi Pharmaceutical Journal. 2018;**26**(1):64-70. DOI: 10.1016/j.jsps.2017.10.012

[36] Bozzuto G, Molinari A. Liposomes as nanomedical devices. International Journal of Nanomedicine. 2015;**10**:975. DOI: 10.2147/ijn.s68861

[37] Guimarães D, Cavaco-Paulo A, Nogueira E. Design of liposomes as drug delivery system for therapeutic applications. International Journal of Pharmaceutics. 2021;**601**:120571. DOI: 10.1016/j.ijpharm.2021.120571

[38] Daraee H, Etemadi A, Kouhi M, Alimirzalu S, Akbarzadeh A. Application of liposomes in medicine and drug delivery. Artificial Cells, Nanomedicine, and Biotechnology. 2016;**44**(1):381-391. DOI: 10.3109/21691401.2014.953633

[39] Ahmed KS, Hussein SA, Ali AH, Korma SA, Lipeng Q, Jinghua C. 2019. Liposome: Composition, characterisation, preparation, and recent innovation in clinical applications. Journal of Drug Targeting. 2019;**27**(7):742-761. DOI: 10.1080/1061186x.2018.1527337

[40] Large DE, Abdelmessih RG, Fink EA, Auguste DT. Liposome composition in drug delivery design, synthesis, characterization, and clinical application. Advanced Drug Delivery Reviews. 2021;**176**:113851. DOI: 10.1016/j.addr.2021.113851

[41] Pasut G, Paolino D, Celia C, Mero A, Joseph AS, Wolfram J, et al. Polyethylene glycol (PEG)-dendron phospholipids as innovative constructs for the preparation of super stealth liposomes for anticancer therapy. Journal of Controlled Release. 2015;**199**:106-113. DOI: 10.1016/j.jconrel.2014.12.008

[42] Lingayat VJ, Zarekar NS, Shendge RS. Solid lipid nanoparticles: A review. Nanoscience and Nanotechnology Research. 2017;**2**:67-72

[43] Mu H, Holm R. Solid lipid nanocarriers in drug delivery: Characterization and design. Expert Opinion on Drug Delivery. 2018;**15**(8):771-785. DOI: 10.1080/17425247.2018.1504018

[44] Geszke-Moritz M, Moritz M. Solid lipid nanoparticles as attractive drug vehicles: Composition, properties and therapeutic strategies. Materials Science and Engineering. 2016;**68**:982-994. DOI: 10.1016/j.msec.2016.05.119

[45] Senapati S, Mahanta AK, Kumar S, Maiti P. Controlled drug delivery vehicles for cancer treatment and their performance. Signal Transduction and Targeted Therapy. 2018;**3**(1):1-9. DOI: 10.1038/s41392-017-0004-3

[46] Hoshyar N, Gray S, Han H, Bao G. The effect of nanoparticle size on in vivo pharmacokinetics and cellular interaction. Nanomedicine. 2016;**11**(6):673-692. DOI: 10.2217/nnm.16.5

[47] Longmire M, Choyke PL, Kobayashi H. Clearance properties of nano-sized particles and molecules as imaging agents: considerations and caveats. Nanomedicine (Lond). 2005;**3**(5):703-717. DOI: 10.2217/17435889.3.5.703

[48] Bazak R, Houri M, El Achy S, Hussein W, Refaat T. Passive targeting of nanoparticles to cancer: A comprehensive review of the literature. Molecular and Clinical Oncology. 2014;**2**(6):904-908. DOI: 10.3892/mco.2014.356

[49] Yao Y, Zhou Y, Liu L, Xu Y, Chen Q, Wang Y, et al. Nanoparticle-based drug delivery in cancer therapy and its role in overcoming drug resistance. Frontiers in Molecular Biosciences. 2020;**7**:193. DOI: 10.3389/fmolb.2020.00193

[50] Hirsjarvi S, Passirani C, Benoit JP. Passive and active tumour targeting with nanocarriers. Current Drug Discovery Technologies. 2011;**8**(3):188-196. DOI: 10.2174/157016311796798991

[51] Mukherjee B, Satapathy BS, Mondal M, Dey NS, Maji R. Potentials and challenges of active targeting at the tumor cells by engineered polymeric nanoparticles. Current Pharmaceutical Biotechnology. 2013;**14**(15):1250-1263. DOI: 10.2174/13892010156661406081435235

[52] Shin DH, Xuan S, Kim W-Y, Bae G-U, Kim J-S. CD133 antibody-conjugated immunoliposomes encapsulating gemcitabine for targeting glioblastoma stem cells. Journal of Materials Chemistry B. 2014;**2**:3771-3781. DOI: 10.1039/c4tb00185k

[53] Zeppernick F, Ahmadi R, Campos B, Dictus C, Helmke BM, Becker N, et al. Clinical Cancer Research. 2008;**14**:123-129. DOI: 10.1158/1078-0432.ccr-07-0932

[54] Zhou J, Rossi J. Aptamers as targeted therapeutics: Current potential and challenges. Nature Reviews Drug Discovery. 2017;**16**(3):181-202. DOI: 10.1038/nrd.2016.199

[55] Keefe AD, Pai S, Ellington A. Aptamers as therapeutics. Nature Reviews Drug Discovery. 2010;**9**(7):537-550. DOI: 10.1038/nrd3141

[56] Emma MH, Wei D, Sarah S. Aptamers and glioblastoma: Their potential use for imaging and therapeutic applications. International Journal of Molecular Sciences. 2017;**18**:1-17. DOI: 10.3390/ijms18122576

[57] Ni X, Castanares M, Mukherjee A, Lupold SE. Nucleic acid aptamers: Clinical applications and promising new horizons. Current Medicinal Chemistry. 2011;**18**(27):4206-4214. DOI: 10.2174/092986711797189600

[58] Affinito A, Quintavalle C, Esposito CL, Roscigno G, Vilardo C, Nuzzo S, et al. The discovery of RNA aptamers that selectively bind glioblastoma stem cells. Molecular

Therapy-Nucleic Acids. 2019;**18**:99-109. DOI: 10.1016/j.omtn.2019.08.015

[59] Fernández M, Javaid F, Chudasama V. Advances in targeting the folate receptor in the treatment/imaging of cancers. Chemical Science. 2018;**9**(4):790-810

[60] Chen YC, Chiang CF, Wu SK, et al. Targeting microbubbles-carrying TGFβ1 inhibitor combined with ultrasound sonication induce BBB/BTB disruption to enhance nanomedicine treatment for brain tumors. Journal of Controlled Release. 2015;**11**:53-62. DOI: 10.1016/j.jconrel.2015.05.288

[61] Ediriwickrema A, Saltzman WM. Nanotherapy for cancer: Targeting and multifunctionality in the future of cancer therapies. ACS Biomaterials Science & Engineering. 2015;**1**(2):64-78. DOI: 10.1021/ab500084g

[62] Yu MK, Park J, Jon S. Targeting strategies for multifunctional nanoparticles in cancer imaging and therapy. Theranostics. 2012;**2**(1):3-44. DOI: 10.7150/thno.3463

[63] Lajoie JM, Shusta EV. Targeting receptor-mediated transport for delivery of biologics across the blood-brain barrier. Annual Review of Pharmacology and Toxicology. 2015;**55**:613. DOI: 10.1146/annurev-pharmtox-010814-124852

[64] Pulgar VM. Transcytosis to cross the blood-brain barrier, new advancements and challenges. Frontiers in Neuroscience. 2019;**12**:1019. DOI: 10.3389/fnins.2018.01019

[65] Hettiarachchi SD, Graham RM, Mintz KJ, Zhou Y, Vanni S, Peng Z, et al. Triple conjugated carbon dots as a nano-drug delivery model for glioblastoma brain tumors. Nanoscale. 2019;**11**(13):6192-6205. DOI: 10.1039/c8nr08970a

[66] Xie S, Hou X, Yang W, Shi W, Yang X, Duan S, et al. Endoglin-aptamer-functionalized liposome-equipped PD-1-silenced T cells enhance Antitumoral immunotherapeutic effects. International Journal of Nanomedicine. 2021;**16**:6017-6034. DOI: 10.2147/ijn.s317220

[67] Hayward SL, Wilson CL, Kidamb S. Hyaluronic acid-conjugated liposome nanoparticles for targeted delivery to CD44 overexpressing glioblastoma cells. Oncotarget. 2016;**7**:34158-34171. DOI: 10.18632/oncotarget.8926

[68] Ke W, Shao K, Huang R, Han L, Liu Y, Li J, et al. Gene delivery targeted to the brain using an Angiopep-conjugated polyethyleneglycol-modified polyamidoamine dendrimer. Biomaterials. 2009;**30**(36):697. DOI: 10.1016/j.biomaterials.2009.08.049

[69] Sun X, Pang Z, Ye H, Qiu B, et al. Co-delivery of pEGFP-hTRAIL and paclitaxel to brain glioma mediated by an angiopep-conjugated liposome. Biomaterials. 2011;**33**:916-926. DOI: 10.1016/j.biomaterials.2011.10.035

[70] Gao J-Q, Lv Q, Li L-M, Tang X-J, Li F-Z, Yu-Lan H, et al. Glioma targeting and bloodebrain barrier penetration by dual-targeting doxorubincin liposomes. Biomaterials. 2013;**14**:5628-5639. DOI: 10.1016/j.biomaterials.2013.03.097

[71] Satapathy BS, Kumar LA, Pattnaik G, Barik B. Lomustine incorporated lipid nanostructures demonstrated preferential anticancer properties in C6 glioma cell lines with enhanced pharmacokinetic profile in mice. Acta Chimica Slovenica. 2021;**68**:970-982. DOI: 10.17344/acsi.2021.6977

[72] Satapathy BS, Mukherjee B, Baishya R, Debnath MC, Dey NS, Maji R.

Lipid nanocarrier-based transport of docetaxel across the blood brain barrier. RSC Advances. 2016;**6**:85261-85274. DOI: 10.1039/c6ra16426a

[73] Shaw TK, Mandal D, Dey G, Pal MM. Successful delivery of docetaxel to rat brain using experimentally developed nanoliposome: a treatment strategy for brain tumor. Drug Delivery. 2017;**24**(1):346-357. DOI: 10.1080/10717544.2016.1253798

[74] Yang FY, Teng MC, Lu M, Liang HF, Lee YR, Yen CC, et al. Treating glioblastoma multiforme with selective high-dose liposomal doxorubicin chemotherapy induced by repeated focused ultrasound. International Journal of Nanomedicine. 2012;7:965-974. DOI: 10.2147/IJN.S29229

[75] Liu X, Madhankumar AB, Slagle-Webb B, Sheehan JM, Surguladze N, Connor JR. Heavy chain ferritin siRNA delivered by cationic liposomes increases sensitivity of cancer cells to chemotherapeutic agents. Cancer Research. 2011;**71**(6):2240-2249. DOI: 10.1158/0008-5472.CAN-10-1375

[76] Vagisha R, Achuthamangalam BM, Thomas A, Becky SW, James RC. Liposomal delivery of ferritin heavy chain 1 (FTH1) siRNA in patient xenograft derived glioblastoma initiating cells suggests different sensitivities to radiation and distinct survival mechanisms. PLoS One. 2019;**14**(9):e0221952. DOI: 10.1371/journal. pone.0221952

[77] Thomas B, Reingard R, Werner G, Christina G, Edgar G, Arijit G, et al. Enhanced doxorubicin delivery to the brain administered through glutathione PEGylated liposomal doxorubicin (2B3-101) as compared with generic Caelyx,(®)/Doxil(®)—A cerebral open flow microperfusion pilot study. Journal of Pharmaceutical Sciences. 2014;**103**(7):1945-1948. DOI: 10.1002/jps.23994

[78] Chuanyi Z, Chunyang M, Enqi B, Kun Y, Ruxiang X. Transferrin and cell-penetrating peptide dual-functioned liposome for targeted drug delivery to glioma. International Journal of Clinical and Experimental Medicine. 2015;**8**(2):1658-1668. DOI: 10.3892/ol.2014.2449

[79] Yue PJ, He L, Qiu SW, Li Y, Liao YJ, Li XP, et al. OX26/CTX-conjugated PEGylated liposome as a dual-targeting gene delivery system for brain glioma. Molecular Cancer. 2014;**13**:191. DOI: 10.1186/1476-4598-13-191

[80] Gao JQ, Qing L, Li-Ming L, Jiang T, Fan-Zhu L, Yu-Lan H, et al. Glioma targeting and bloodebrain barrier penetration by dual-targeting doxorubincin liposomes. Biomaterials. 2013;**34**:5628-5639. DOI: 10.1016/j.biomaterials.2013.03.097

[81] Li D, Yang X, Li B, Yang C, Sun J, Yu M, et al. Lidocaine liposome modified with folic acid suppresses the proliferation and motility of glioma cells via targeting the PI3K/AKT pathway. Experimental and Therapeutic Medicine. 2021;**22**(3):1-10. DOI: 10.3892/etm.2021.10457

[82] Qin L, Wang CZ, Fan HJ, Zhang CJ, Zhang HW, Lv MH, et al. A dual-targeting liposome conjugated with transferrin and arginine-glycine-aspartic acid peptide for glioma-targeting therapy. Oncology Letters. 2014;**8**(5):2000-2006. DOI: 10.3892/ol.2014.2449

[83] Muthu MS, Kulkarni SA, Raju A, Feng S-S. Theranostic liposomes of TPGS coating for targeted co-delivery of docetaxel and quantum dots.

Biomaterials. 2012;**33**:3494-3501. DOI: 10.1016/j.biomaterials.2012.01.036

[84] Chen C, Duan Z, Yuan Y, Li R, Pang L, Liang J, et al. Peptide-22 and cyclic RGD functionalized liposomes for glioma targeting drug delivery overcoming BBB and BBTB. ACS Applied Materials & Interfaces. 2017;**9**(7):5864-5873. DOI: 10.1021/acsami.6b15831

[85] Kumar LA, Pattnaik G, Satapathy BS, Swapna S, Mohanty D. Targeting to brain tumor: Nanocarrier-based drug delivery platforms, opportunities, and challenges. Journal of Pharmacy & Bioallied Sciences. 2021;**13**:172-177. DOI: 10.4103/jpbs.JPBS_239_20