



## LJMU Research Online

Zaman, P, Penson, P, Barreto, GE and Sahebkar, A

**Recent advancements in liposome-based strategies for effective drug delivery to the brain.**

<http://researchonline.ljmu.ac.uk/id/eprint/14351/>

### Article

**Citation** (please note it is advisable to refer to the publisher's version if you intend to cite from this work)

**Zaman, P, Penson, P, Barreto, GE and Sahebkar, A (2020) Recent advancements in liposome-based strategies for effective drug delivery to the brain. Current Medicinal Chemistry, 28 (1). ISSN 0929-8673**

LJMU has developed **LJMU Research Online** for users to access the research output of the University more effectively. Copyright © and Moral Rights for the papers on this site are retained by the individual authors and/or other copyright owners. Users may download and/or print one copy of any article(s) in LJMU Research Online to facilitate their private study or for non-commercial research. You may not engage in further distribution of the material or use it for any profit-making activities or any commercial gain.

The version presented here may differ from the published version or from the version of the record. Please see the repository URL above for details on accessing the published version and note that access may require a subscription.

For more information please contact [researchonline@ljmu.ac.uk](mailto:researchonline@ljmu.ac.uk)

<http://researchonline.ljmu.ac.uk/>

1           **Recent advancements in liposome-based strategies for effective drug delivery to the brain**

2  
3           Parvin Zaman<sup>1,2</sup>, Peter E. Penson<sup>3</sup>, George E. Barreto<sup>4</sup>, Amirhossein Sahebkar<sup>5,6,7</sup>

4  
5  
6           <sup>1</sup>*Department of Medical Biotechnology, Faculty of Medicine, Mashhad University of Medical Sciences,*  
7           *Mashhad, Iran*

8           <sup>2</sup>*Nanotechnology Research Center, Pharmaceutical Technology Institute, Mashhad University of Medical*  
9           *Sciences, Mashhad, Iran*

10          <sup>3</sup>*School of Pharmacy and Biomolecular Sciences, Liverpool John Moores University, Liverpool, UK.*

11          <sup>4</sup>*Department of Biological Sciences, University of Limerick, Limerick, Ireland*

12          <sup>5</sup>*Neurogenic Inflammation Research Center, Mashhad University of Medical Sciences, Mashhad, Iran*

13          <sup>6</sup>*Biotechnology Research Center, Pharmaceutical Technology Institute, Mashhad University of Medical*  
14          *Sciences, Mashhad, Iran*

15          <sup>7</sup>*School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran*

16  
17  
18          \***Corresponding author:**

19          *Amirhossein Sahebkar, PharmD, PhD, Department of Medical Biotechnology, School of Medicine,*  
20          *Mashhad University of Medical Sciences, Mashhad, Iran, P.O. Box: 91779-48564, Iran. Tel:*  
21          *985118002288; Fax: 985118002287; E-mail: [sahebkar@mums.ac.ir](mailto:sahebkar@mums.ac.ir); [amir\\_saheb2000@yahoo.com](mailto:amir_saheb2000@yahoo.com)*

22  
23          **Running title:** Liposomes in brain drug delivery

30 **Abbreviations**

31 NP, nanoparticles

32 polyethylene glycol, PEG

33 D-a-tocopherol polyethylene glycol 1000 succinate, TPGS

34 Cell-penetrating peptides, CPPs

35 transferrin receptor, TfR

36 5-fluorouracil, 5-FU

37 folic acid, FA

38 paclitaxel, PTX

39 p-Hydroxybenzoic Acid, pHA

40 tripeptide motif arginine-glycine-aspartic acid, RGD

41 cyclic RGD, c(RGDyK)

42

43

44

45

46

47

48

49

50

51

## 52 **Introduction**

53 Disorders of the central nervous system (CNS) and tumors of the brain are challenging to treat, and they  
54 rank amongst the most common causes of death worldwide. [1]. In recent years, many attempts have  
55 been made to develop drugs and therapeutic agents for disorders of the brain and CNS. However,  
56 researches programmes aimed at the discovery and development of drugs for brain disorders have had  
57 very poor success compared with those in other therapeutic areas [2]. The major obstacles encountered  
58 in the development of drugs for the treatment of CNS disorders are the complexity of the brain and the  
59 impermeability of the blood-brain barrier (BBB). The BBB serves to protect the brain from damage  
60 caused by drugs and chemicals by selectively allowing small, lipid-soluble molecules to pass through the  
61 endothelial cell membrane while preventing the transfer of most drugs, peptides, large molecules,  
62 pathogens and toxins [3]. Therefore, the potential therapeutic advantages of drugs designed to act on the  
63 CNS has not yet been fully realised. Development of efficient technologies to deliver drugs across the  
64 BBB remains the major challenge to the effective treatment of CNS disorders [4]. Hence, it is necessary  
65 to develop suitable strategies to overcome these difficulties and thus to permit improved drug delivery  
66 into the brain.

67 Various approaches have been used to enable drugs to permeate the BBB and to reach the brain.  
68 Nanocarriers are a promising technology in this respect. Multiple types of nanocarriers with a range of  
69 sizes and physicochemical properties have been used to target therapeutic agents to the brain. These  
70 include polymeric nanoparticles, carbon nanotubes, liposomes, and inorganic nanoparticles. Of these,  
71 liposomes have been most extensively investigated as potential drug delivery agents. Liposomes are  
72 considered to be the most efficient drug delivery system in a range of diseases. Liposomes are vesicles  
73 made up of one or more spherical lipid bilayer structures. Typically, a lipophilic (hydrophobic)  
74 phospholipid bilayer surrounds an internal aqueous compartment. Liposomes are biodegradable,  
75 biocompatible, safe and nontoxic and can be used to carry both hydrophilic and hydrophobic drug  
76 molecules. They are frequently used for numerous practical applications due to their non-immunogenic  
77 nature and their ability to maintain sustained drug release in biological systems [5]. This review discusses  
78 recent development and new strategies related to liposome technologies designed to deliver therapeutic  
79 agents to the brain and, and possible applications of liposomes in the treatment of CNS disorders.

## 80 **Challenges to CNS-targeted drug delivery**

81 The BBB plays a key regulatory role in the proper functioning of the brain by acting as a permeability  
82 barrier in its blood vessels. The selective permeability of the BBB protects the brain against harmful  
83 stimuli and toxic agents and homeostasis to take place, maintaining the conditions for the complex  
84 functions of the neurons in the brain. The BBB is a complex system consisting of endothelial cells,  
85 pericytes, astrocytes, microglia and neurons [6]. The cerebral endothelial cells of the brain are considerably  
86 different from cells in other parts of the body in that they are connected by tight junctions at their margins.  
87 This prevents paracellular diffusional of aqueous agents between the adjacent endothelial cells or *via*  
88 transcellular pathway [7]. Pericytes surround the endothelium and together with the basal lamina, and  
89 astrocytic end-feet, contribute to the organization of the BBB and form the neurovascular unit (Figure 1)  
90 [6]. The endothelial cells of the BBB have an essential function in the transport of ions and other polar  
91 solutes between the blood and the extracellular fluid of the CNS [8]. Multiple mechanisms are involved  
92 in the transcellular transport pathway, ranging from simple passive diffusion to more complex receptor-  
93 mediated transport and transcytosis. Passive diffusion depends upon the physicochemical characteristics  
94 of the substance such as size, molecular weight, lipophilicity and the surface charge of the molecule [9].  
95 A wide range of substances such as small lipophilic molecules, O<sub>2</sub> and CO<sub>2</sub> can diffuse into the BBB by  
96 passive diffusion along concentration gradients. Others materials such as nutrients, polar molecules,  
97 proteins and peptides cannot diffuse through cell membranes and therefore require specific transporters  
98 such as efflux transporters (e.g., P-glycoprotein (P-gp)), glucose transporter-1 (GLUT-1) and insulin  
99 transporter (Figure 1B) for transportation across BBB in either of directions [9]. By resulting in the efflux  
100 of different substances from the brain, these transporter proteins play a prominent role in the barrier to  
101 drug delivery. For example, P-gp results in the efflux of a large number of lipophilic drugs and cationic  
102 substances from the brain and prevents the uptake anticancer agents in brain tumors [10].

103 Because of the structural complexity of the BBB, it presents considerable physiological challenges to the  
104 delivery of drugs to the brain. The BBB adapts to the needs of the CNS, responds to physiological  
105 changes, and is affected by various pathological conditions. Disorders of the BBB can promote disease  
106 [9], and the BBB loses its normal function in a range of conditions including brain tumours, stroke and  
107 neurodegenerative diseases. Therefore, to treat CNS disorders, it is necessary to develop an efficient  
108 delivery system for the delivery of drugs into the brain without compromising the other functions of the  
109 BBB.

## 110 **Nanotechnology for brain drug delivery**

111 Previous efforts to treat neurodegenerative disorders through the delivery of drugs to the brain have  
112 encountered the problem of CNS drug delivery. Recent breakthroughs in BBB research provide new  
113 strategies and approaches to solving these issues. Among different approaches, nanoparticles technology  
114 have been attracted much attention and is rapidly advancing. For this purpose, a wide range of  
115 nanoparticles have been examined for their potential to enable the delivery of drugs to the brain. These  
116 include polymeric nanoparticles, liposomes, dendrimers, carbon nanotubes, gold nanoparticles and  
117 polymeric micelles [11, 12]. In general, nanoparticles (NPs) have several advantages such as the  
118 possibility of multi-functionalization, the ability to carry drug without altering their effects, specific  
119 targeting NPs to the enhancement of BBB crossing, control of drug release and modification of the  
120 pharmacokinetics of the drug. When NPs are used to deliver drug to the brain, it is the physiochemical  
121 properties of the NP which dictate the passage into the brain. The physiochemical properties of the drug  
122 enclosed in the NP are not important in this context. [11].

123 Moreover, NPs with a particle size  $< 200$  nm could penetrate into tumor tissues through enhanced  
124 permeability and retention (EPR) effect. In general, NPs concentrate in tumors and have low systemic  
125 concentrations. It has recently been shown that NPs provide more opportunities for drug delivery to brain  
126 tissues by enhancing the duration over which the drug circulates in the blood [1, 11]. Here, we focus on  
127 liposomes as one of most promising NPs for brain drug targeting and delivery. In recent years, the  
128 liposomes have attracted much attention and been extensively used as important vehicles with which to  
129 transport of drugs into the brain [12, 13]. This review focuses on recent advancements in liposome –  
130 based strategies to enable drugs to cross the BBB in the management of pathologies of the brain and  
131 CNS.

### 132 **Liposomes as Drug Delivery Vehicles to the Brain**

133 Liposomes are vesicles made up of one or more spherical lipid bilayer structures. Typically, a lipophilic  
134 phospholipid bilayer surrounds an internal aqueous compartment. Liposomes are preferred over almost  
135 any other drug-carrier system due to their similar morphology to cellular membranes, and their unique  
136 ability to carry a variety of lipophilic, hydrophobic or amphipathic drugs in a single formulation. The  
137 aqueous compartment is predominantly used to encapsulate hydrophilic agents, whereas lipophilic  
138 molecules can be adsorbed on the hydrophobic bilayer. Furthermore, liposomes have several advantages  
139 including biocompatibility and biodegradability, safety and low toxicity, drug-targeted delivery and  
140 controlled drug release [14]. As mentioned above, several mechanisms are involved in transcellular  
141 pathway different material across the BBB that include passive diffusion (nonspecific endocytosis) and

142 active transporting (by binding to specific receptors on the surface of BBB cells). In the case of passive  
143 diffusion, the physicochemical properties of the NPs have a major role in penetration of the BBB. The  
144 size and surface properties of the liposomes play important roles in passive diffusion. Hence, alterations  
145 in the surface features such as charge and coating may influence their ability to cross biological barriers  
146 [15]. For example, it seems that cationic liposomes undergo endocytosis across the BBB cells easily  
147 owing to electrostatic interactions between positive charges on the liposomes and the negatively charged  
148 surface of BBB cells. The interaction triggers the cell internalization processes. Coating and surface  
149 functionalization of liposomes with polyethylene glycol (PEG) and other polymers may increase their  
150 circulation time in blood, preventing fast clearance through the reticuloendothelial system and improving  
151 transport of molecules to the brain. In passive diffusion, liposomes could enter the brain through passive  
152 influx and release the encapsulated drug to the target site. However, although passively targeting of  
153 liposomes is the most common method that used in clinical therapy, it suffers from several limitations,  
154 such as nonspecific uptake, low EPR effect within the brain, uncontrolled release and the crossing of  
155 BBB barriers [12]. Therefore, to enable successful delivery of liposomes to the brain, various surface  
156 modification have been made to improve and enhance their effectiveness in circumventing the barrier  
157 properties of the BBB to maintain a higher concentration of drugs inside the brain in a controlled manner  
158 [12, 13].

### 159 **Surface modification of liposomes**

160 Liposomes can transport encapsulated drug specifically (actively) or nonspecifically (passively) into  
161 cells. In the passive route, the phospholipid bilayer of the liposome exterior fuses with the phospholipid  
162 bilayer of the plasma membrane and thus the contents of the liposome enters the cytoplasm.  
163 Alternatively, the liposome can be destabilized by certain cell membrane components when adsorbed on  
164 the surface resulting in the release of the drug which then enters the cell by micropinocytosis. Liposomes  
165 can also undergo specific or nonspecific endocytosis [16]. A variety of modifications has been made to  
166 liposomes to improve the bioavailability of drugs in various regions of the brain. One of the most  
167 common strategies, is to use a variety of molecules as surface biologically active ligands (proteins,  
168 peptides and antibodies) that bind to receptors present on the surface of BBB cells and facilitate the  
169 translocation *via* receptor-mediated transcytosis or other transfection methods [12, 17]. In this section,  
170 we summarize and assess the functional roles of various modifications made to the surface of the  
171 liposomes to improve the bioavailability and concentration of drugs in the CNS.

### 172 **Passive delivery of liposomes to BBB**

173 The development of stealth liposomes, such as liposomes coated with biocompatible polymers (e.g.  
174 PEG), is an advancement in liposomal formulation which extends the duration over which liposomes  
175 circulate in the blood. This is achieved through reduced mononuclear phagocytosis and recognition by  
176 opsonins, consequently slowing down the clearance of liposomes. This helps to improve the targeting  
177 efficiency and activity of molecules that encapsulated in the liposomal formulations [18]. Vijakumar  
178 and colleagues used the passive brain targeting ability of PEGylated liposomes to enable the delivery of  
179 resveratrol to glioma tumors. Their in-vivo biodistribution study revealed that drugs loaded in PEGylated  
180 liposomes persist for longer in the circulation and accumulate more readily in the CNS than non-  
181 PEGylated liposomes [19]. In another study by the same group, D- $\alpha$ -tocopherol polyethylene glycol  
182 1000 succinate (TPGS), a PEGylated vitamin E, was used to coat liposomes to increase the circulation  
183 time in the bloodstream and to enable the passive targeting of resveratrol to the brain. The TPGS coated  
184 liposomes were evaluated *in vitro* and *in vivo*. *In vitro* experiments using C6 glioma cell showed that  
185 TPGS coated liposomes have excellent cellular internalization. Additionally, a biodistribution study in  
186 rats revealed an increase in the amount of resveratrol in the brain when delivered by the liposomes as a  
187 result of passive brain targeting [20].

188 A study conducted by Muthu and coworkers indicated that TPGS coated liposomes loaded with docetaxel  
189 have a higher cellular uptake and cytotoxicity in C6 glioma cells compared to conventional (non-coated)  
190 and the PEG-coated liposomes [21]. Recently, verapamil and riluzole-containing PEGylated liposomes  
191 have been developed for the treatment of amyotrophic lateral sclerosis (ALS) to overcome limitations in  
192 the transport of riluzole across the BBB [22]. Using conventional formulations, the deposition of riluzole  
193 in the brain is inhibited by the efflux transporter P-gp at the BBB, this leads to treatment failure. Yang et  
194 al. developed a liposomal co-delivery system containing riluzole and verapamil (a P-gp inhibitor) for  
195 efficient transport of riluzole to brain cells. These liposomes were able to transport encapsulated drug  
196 into brain endothelial cells through endocytotic pathways. As a result, verapamil was able to suppress  
197 the P-gp efflux protein and reduce the efflux of riluzole, leading to increased concentrations of riluzole  
198 in brain cells. An in-vitro study on bEnd.3 and C8D1A astrocyte cells indicated that treatments with  
199 liposomes have a potential inhibitory effect on P-gp and decreased riluzole efflux. Hence, it seems that  
200 in this model of BBB function, the delivery of drugs to the brain can be improved by liposomes [22].  
201 These studies revealed that liposomes coated with biocompatible polymers facilitate improved delivery  
202 of biomacromolecules to the brain, and prolong their circulation to allow passive targeting.

### 203 **Active transport of liposomes to BBB**

## 204 **Cell-penetrating peptide modified liposomes**

205 Active targeting or targeted delivery using specific ligands is a novel and attractive technology that can  
206 greatly improve the potential of drug delivery to the specific site, thereby requiring a considerably  
207 reduced dose and resulting in fewer adverse effects of the drug.. To date, various ligands have been  
208 evaluated as nanocarriers for active targeting of the brain t. Here, we discuss some of the important  
209 targeting ligands that have been explored for for active targeting to the brain

210 Cell-penetrating peptides (CPPs) are short-chain amphipathic peptides which facilitate the transport of a  
211 wide variety of compounds such as peptides, proteins, oligonucleotides and drugs across cell membranes  
212 and into cells [23]. A variety of CPPs have been identified, these include natural CPPs, such as  
213 transactivator of transcription (TAT) from human immunodeficiency virus (HIV-1) and synthetic CPPs,  
214 such as mastoparan and transportan which are used extensively to deliver compounds into cells [24].  
215 Numerous studies have shown that surface modification of liposomes with CPPs could improve the  
216 delivery of drugs to the brain. CPP facilitates the binding and internalization of CPP-liposomes to  
217 endothelial cell membranes, improves endosomal escape and increases the cellular delivery of liposomal  
218 cargo. The uptake of CPPs is mediated through endocytic pathways, but its exact mechanism is still under  
219 debate. Possible mediators include clathrin-mediated endocytosis and micropinocytosis and non-  
220 endocytic pathways, [25].

221 TAT (AYGRKKRRQRRR) is one of the most common cell-penetrating peptides that is used to decorate  
222 the surface of nanoparticles such as liposomes to improve the efficient intracellular delivery of liposomal  
223 cargo [26]. It has been reported that liposomes modified with TAT can deliver drugs into cells efficiently  
224 *via* a receptor-independent and transporter-independent pathway [27]. In a recent study Qin et al. used  
225 TAT to decorate the surface of doxorubicin-loaded liposome for delivery to brain glioma. The potential  
226 of TAT-modified liposomes too be used to deliver drugs to the CNS was explored using brain capillary  
227 endothelial cells (BCECs) and C6 glioma cells. The investigators demonstrated that TAT played an  
228 important role in the trans- endothelial and cellular uptake process in an *in vitro* model of the BBB, and  
229 that cellular uptake of the doxorubicin-loaded liposome was improved by TAT. An in-vivo  
230 biodistribution study in the brain revealed that the doxorubicin-TAT liposome more accumulated in the  
231 brain and the concentrations of doxorubicin in the brain of doxorubicin-TAT liposomes were found in  
232 greater abundance in the brain than unmodified liposomes. Additionally, the cardiac concentrations of  
233 doxorubicin in the group treated with the doxorubicin-TAT liposome were much lower than in the groups  
234 treated with the unmodified liposome and free doxorubicin. Thus the TAT liposome had the potential to

235 reduce the cardiotoxic effects of doxorubicin, The survival study on brain glioma-bearing rat  
236 demonstrated that animals treated with TAT-modified liposome survived for substantially longer than  
237 those in other groups [28].

238 In addition to improving the penetrative capacity on liposomes into mammalian cells, some CPPs are  
239 able to facilitate targeted delivery to specific subcellular structures such as the cytoplasm, cell nucleus,  
240 mitochondria and lysosomes [29]. Asparagines-Glycine-Arginine (NGR), a peptide that contains a  
241 vascular homing motif has been used to modify drug-loaded liposomes in order to increase their  
242 penetration into and accumulation in tumor tissues. NGR peptide was able to target  
243 CD13/aminopeptidase N, which is over-expressed on the endothelial cells of glioma, resulting in  
244 improved tumour-targeting efficiency and anti-tumor effect [30]. The peptide iNGR (CRNGRGPDC)  
245 containings three motifs including a tumor vascular antigen CD13 targeting motif, a protease recognition  
246 site and tissue penetration motif. Hence, iNGR was able to specifically recognize tumor vascular antigen  
247 CD13, penetrate into tumor vessels and reach deep tumor parenchyma through specific interaction with  
248 the receptor NRP-1 which is overexpressed on the tumor vessels and glioblastoma cells [31]. Recently,  
249 liposomes conjugated with iNGR peptide have been developed for the purpose of targeting the tumor  
250 vasculature and to penetrate across tumor blood vessels in the treatment of glioblastoma. In one study, it  
251 was demonstrated that iNGR-modified liposomes resulted in a remarkable enhancement of the cellular  
252 uptake of drug by U87MG cells and HUVECs compared to unmodified liposomes. Also, *in vivo* imaging  
253 in mice bearing glioblastoma demonstrated that these liposomes effectively accumulated at the site of  
254 the tumor and could penetrate into tumor blood vessels and tissues. Moreover, iNGR-modified  
255 doxorubicin liposomes have a greater cytotoxic effect on the tumor - more than that of unmodified  
256 liposomes and the survival time was significantly increased in an animal model of glioblastoma.  
257 Therefore, it is apparent that the modification of liposomes with the iNGR peptide enhances the  
258 penetration of liposomes into tumors and is therefore a potentially interesting means to improve  
259 anticancer therapies [32].

## 260 **Receptor-mediated transportation**

### 261 **Transferrin receptor-mediated transcytosis**

262 Many receptors are overexpressed on the BBB. These include receptors for transferrin (Tf), insulin and  
263 low-density lipoprotein protein. When these receptors interact with their specific target ligands, the  
264 receptor-ligand interaction promotes transport of the ligand into the cell. Thus, the surface of liposomes

265 could be functionalized with receptor ligands, to mediate their cellular internalization *via* BBB [33]. In  
266 recent years, liposome functionalized with ligands have been successfully used in the delivery of drug-  
267 loaded liposomes to the brain. The transferrin receptor (TfR) is a transmembrane glycoprotein that is  
268 highly expressed on the surface of brain endothelial cells and cancer cells and is involved in the  
269 transportation of iron to the brain, by receptor-mediated endocytosis [34]. TfR is the most commonly  
270 evaluated receptor in BBB targeted delivery. Transferrin is an iron-binding serum glycoprotein and it is  
271 the most specific protein that is widely used as a TfR ligand. It improves the targeting of therapeutic  
272 cargo across the BBB and increases the accumulation of drug in the brain [35]. Tf is an 80 kDa  
273 glycoprotein, it has an isoelectric point of 5.5, hence it exhibits negative charge in a solution with a pH  
274 of 7.4. It confers a negative charge on liposomes modified with Tf as compared with that of unmodified  
275 liposomes. Transferrin modified liposomes have been studied for their potential to deliver therapeutic  
276 agents to the brain. It has been demonstrated that these types of functionalized liposome (Tf-modified  
277 liposomes) have a higher affinity for brain capillary endothelial cells and significantly enhanced  
278 liposomal cargo delivery to the brain than unmodified liposomes [36, 37]. Recently, liposomal  
279 resveratrol (a natural polyphenol with anti-cancer effects), was modified with transferrin (TF) to produce  
280 Tf- resveratrol- liposomes for the purpose of drug delivery to the brain. The Tf-modified liposomes  
281 showed a significantly greater accumulation in cancer cells compared to normal human astrocytes,  
282 possibly due to overexpression of TfRs in cancer cells. Tf- resveratrol- liposomes induced significantly  
283 greater apoptosis and cell cycle arrest, in U-87 glioblastoma cells compared to free drug and drug-loaded  
284 liposome. Furthermore, in an *in vivo* study, it was demonstrated that mice treated with Tf- resveratrol-  
285 liposome had smaller tumors and prolonged survival compared to free drug and non-targeted liposome.  
286 Biodistribution studies indicated that PEGylated resveratrol-liposome and Tf- resveratrol-liposome  
287 accumulate to a greater extent in the tumors, hence, it appears that passive targeting, the EPR effect and  
288 receptor mediated transcytosis may be involved in mediating the accumulation of resveratrol-liposomes  
289 at the tumor site [38].

290 Moreover, Lopalco et al. indicated that transferrin-functionalized dopamine-loaded liposomes could be  
291 successfully transferred across an in-vitro model of the BBB [39]. Song and colleagues developed TF-  
292 modified liposomes to transport vincristine and tetrandrine across the BBB. They demonstrated that TF-  
293 modified encapsulated drug liposomes increased cellular uptake of drug across the BBB and induced a  
294 greater cytotoxic effects on C6 cells. Furthermore, TF-modified vincristine and tetrandrine liposomes,  
295 vasculogenic mimicry (VM) channels were significantly inhibited, cancer cell invasion was suppressed  
296 and the expression of apoptotic proteins were significantly increased. In glioma-bearing mice, treatment

297 with TF-modified vincristine and tetrandrine liposomes was associated with longer median survival time  
298 than the other groups [40]. In summary, all studies clearly demonstrated that transferrin is very useful  
299 ligand that can be used for the transport of NPs across BBB by receptor-mediated transcytosis

300

### 301 **Multi-ligand functionalized liposomes**

302 The use of multiple ligands and surface-active agents is another promising approach to enhance the  
303 efficacy of drug targeting nanocarriers. This approach could overcome several drawbacks such as  
304 receptor saturation and lysosomal degradation during endocytic uptake and thereby provide a feasible  
305 approach to yield enhanced therapeutic results. Using this approach, liposomes have been modified with  
306 more than one active ligand capable of binding to specific receptors in the BBB in order to enhance the  
307 efficiency of drug delivery [41].

### 308 **CPP-TF dual functionalized liposome**

309 An example of a dual functionalized liposome is the use of both CPPs and transferrin on the surface of  
310 the liposome. Binding and translocation of CPP-coated liposomes occurs as a result of the positive charge  
311 of CPPs and the interaction of CPP-coated liposomes with negatively charged endothelial cell  
312 membranes. The presence of transferrin on the liposomes facilitates transport *via* receptor-mediated  
313 translocation and improved penetrative effect of CPPs [42]. Lakkadwala et al. developed dual  
314 functionalized liposomes to enhance the delivery of chemotherapeutic agents across the BBB for the  
315 treatment of glioma. They modified the surface of liposomes with transferrin to target receptors, and the  
316 cell penetrating peptide PFVYLI (PFV) to enhance cell penetration [43]. In another study this group  
317 modified the surface of liposomes with transferrin and two CPPs (TAT and QLVPM) to enhance cell  
318 penetration [44]. They used the modified liposomes to promote the translocation of doxorubicin (Dox)  
319 and erlotinib (an epidermal growth factor receptor inhibitor) across the BBB in an *in vitro* glioblastoma  
320 tumor model. Tf- CPPs modified liposomes demonstrated relatively high cellular uptake and high  
321 concentrations of Dox and erlotinib in glioblastoma tumor cells. Additionally, Tf- CPPs modified  
322 liposomes enhanced tumor cell death and antitumor efficacy in an in-vitro brain tumor model [43, 44].  
323 Recently, bifunctional liposomes containing Tf mediated receptor targeting and poly-L-arginine (PR) as  
324 a CPP were produced with the intention of delivering genes to brain. The bi-functional liposomes were  
325 more readily taken up by brain endothelial cells and had a higher transfection efficacy in primary culture  
326 of glial than the Tf liposomes. Additionally, bi-functional liposomes exhibited considerably enhanced

327 cell penetration in an in-vitro BBB model [42]. Using both *in vitro* and *in vivo* methods, Sharma et al.  
328 investigated multi-functionalized liposome modified with CPPs-TAT, Penetratin and Mastoparan on the  
329 transport of doxorubicin encapsulating transferrin liposomes into brain endothelial cells. This study  
330 demonstrated that the dual functionalized (CPP-Tf) liposomes were more efficiently transported across  
331 cell membranes as compared to single ligands (including Tf or CPP-liposomes). Tf-TAT, Tf-Penetratin  
332 liposomes demonstrated efficient delivery of doxorubicin across the brain endothelial barrier in an in-  
333 vitro model of brain tumor. Tf-Penetratin liposomes demonstrated greater cellular uptake and transport  
334 of doxorubicin *in vivo* and *in vitro* in comparison to Tf-TAT liposomes due to higher cationic charge of  
335 penetratin. Mastoparan peptides improved cellular uptake of Tf-liposomes *in vitro* and have a minimum  
336 endothelial transcytosis owing to lower cationic charge. It was also demonstrated that Tf-Mastoparan  
337 liposomes have a higher cytotoxicity and hemolytic activity and faster clearance, therefore leading to  
338 lower transport of doxorubicin *in vivo* and *in vitro* in comparison to other Tf CPP liposomes. Tf-  
339 mastoparan liposomes have a greater uptake by liver, spleen and lungs and therefore, have an easier  
340 availability for transport to brain [45].

341 Recently, the effect of dual-functionalized liposomes conjugated with the CPP peptide, penetratin and  
342 TF was investigated to enhance the transport of 5-fluorouracil (5-FU), across the BBB into tumor cells.  
343 It was reported that the co-modification of liposomes with Tf and penetratin improved the cellular uptake  
344 of the liposomes in U87 glioblastoma cells and a monolayer of bEnd.3 cells. The investigators suggested  
345 that the cationic charge of penetratin could reduce the negative charged on Tf and thereby facilitate the  
346 binding and internalization of liposomes. In addition, 5-FU-loaded dual-functionalized liposomes was  
347 able to induce significantly higher apoptosis in U87 cells and were associated with enhanced transport  
348 across the brain endothelial barrier. Additionally, Tf-penetratin modified liposomes loaded with 5-FU  
349 were able to undergo endocytosis, thereby delivering 5-FU to tumor cells with greater efficiency than  
350 single ligand liposomal formulations in an *in vitro* brain tumor model. Therefore, is believed that a  
351 combination of Tf and penetratin have a synergistic effect in enhancing the uptake of liposomes across  
352 the BBB and that this may play key role in delivery of drug and induction of excellent anti-tumor efficacy  
353 in brain cancer cells [46].

354 Recent studies by Liu et al. reported that liposomes functionalized with Tf and arginine-rich residues as  
355 CPP sequences had a strong targeting efficacy on brain microvascular endothelial cell and brain glioma  
356 C6 cell uptake. This conferred a significant advantage for liposomal crossing across the BBB and entry  
357 into C6 glioma cells. Additionally, it has been shown that Tf-CPP decorated liposomes were able to

358 successfully escape from the endosomal compartment of C6 glioma cells to release the liposomal  
359 contents into the cytosol [47]. Recently, Zong et al. have developed dual-targeting doxorubicin liposomes  
360 (T7-TAT-liposomes) conjugated with cell-penetrating peptide (TAT) and peptide T7 (HAIYPRH), a  
361 unique targeting agent with high affinity for TfR, to transport drugs across the BBB, and to penetrate  
362 brain glioma. Their results indicated that T7-TAT-liposomes markedly enhanced *in vitro* cellular uptake  
363 and drug delivery compared with DOX liposomes. An *in vivo* study showed that T7-TAT-liposomes could  
364 cross the BBB and importantly penetrate the tumor and selectively deliver drug to glioma regions.  
365 Transport of liposomes across the BBB was markedly increased when they were decorated with both  
366 TAT and T7. Therefore, T7-TAT can act as an effective brain targeting ligand [48].

367 It has been reported that several receptors such as transferrin receptor, epidermal growth factor receptor  
368 insulin receptor, integrins and low-density lipoprotein receptor are overexpressed on brain tumor cells  
369 specially cancerous glioma cells [49]. Thus, dual targeting strategies could be used for the delivery of  
370 drugs specifically to brain tumors. Zong et al. used co-modified liposomes decorated with specific ligand  
371 T7 and nonspecific peptide TAT in order to enhance the BBB penetration, and then to increase the  
372 penetration efficiency in glioma tumor cells. *In vitro* cellular uptake in C6 and bEnd.3 cells and a BBB  
373 model indicated that the cellular uptake of T7-TAT-liposomes was significantly higher than those of T7-  
374 liposome, TAT-liposome and PEGylated liposomes. Furthermore, the hemolytic study showed that the  
375 outer PEG on the liposomal surface could shield TAT and reduce the hemolytic toxicity of the latter.  
376 Hence, the internalizing efficiency of T7-TAT-liposomes demonstrates that the ligands T7 and TAT have  
377 a synergistic effect on the cellular uptake in a concentration-dependent manner and improve the cell  
378 penetration of liposomes. When T7 peptides are attached to the TfR, TAT peptide close to the surface of  
379 cell membrane they promote the cellular delivery liposomal cargo to the glioma cell. The *in vivo*  
380 biodistribution results showed that the accumulated of T7-TAT-liposome and the concentrations of  
381 doxorubicin in the brain was higher than all other liposomal formulations four hours after administration.  
382 Moreover, the hearts of the group treated with T7-TAT-liposomal loaded doxorubicin had lower  
383 concentrations of doxorubicin at four hours compared with other groups. Collectively, the above  
384 evidence indicates that T7-TAT-liposomal delivery system could effectively increase cellular uptake,  
385 transport across the brain, and enable the targeting of brain glioma tumor whilst minimizing the  
386 cardiotoxicity of doxorubicin [50].

### 387 **Folate receptor-mediated transcytosis**

388 Recently, liposomes modified with acid-cleavable (pH-sensitive) folic acid (FA) and dNP2 peptide have  
389 been used for the delivery of drug to the brain. dNP2 is a safe and humanized blood–brain barrier  
390 penetrating peptide [51]. FA may act by binding to the folate receptor (FR) on the BBB and enhancing  
391 transport across the BBB by receptor-mediated transcytosis [52]. Li et al. design paclitaxel (PTX) loaded  
392 liposomes co-modified with FA and dNP2 for efficient delivery to the brain metastasis caused by breast  
393 cancer. It is thought that the acid-cleavable FA drug-loaded liposomes accumulated at tumor site *via* the  
394 interaction of FA and folate receptor. The dNP2 peptide enhanced liposome uptake into tumor cells.  
395 Penetration studies using an *in vitro* BBB model indicated that the uptake of FA-dNP2 liposome by  
396 bEnd.3 cells was higher than single ligand modified liposomes (FA- liposome, dNP2 liposome).  
397 Therefore, FA and dNP2 have synergistic effect on the transportation across the bEnd.3 and were able to  
398 improve the delivery of PTX to orthotopic breast cancer and its metastatic sites in the brain [53]. In  
399 another study, Li et al. used PTX loaded liposomes co-modified with FA and dNP2 to improve the  
400 efficiency of penetration across the BBB and the targeting of glioma. The result indicated that co-  
401 modification PTX loaded liposome with FA and dNP2 has a synergistic effect on the targeting of FR-  
402 positive C6 cells. In addition, pH sensitive FA exhibited sensitive cleavage of FA at pH 6.8 and enhanced  
403 the effect of dNP2 and elevated the cellular uptake compared to non-cleavable FA and single modified  
404 liposomes. An *in vivo* study indicated that the dual modified liposomes displayed enhanced BBB  
405 transportation effects, greater accumulation in orthotopic glioma resulting in an improved therapy of  
406 tumors in a mouse model of glioma. The dual modified liposomes loaded with PTX had excellent  
407 penetration into tumor cells resulting in greater cytotoxicity and extended survival in these mice [54].

#### 408 **RGD modified liposome**

409 The cell adhesion molecules including integrins are crucial for cell adhesion, migration, signalling and  
410 viability of most cells. These molecules are particularly overexpressed on cancer cells such as melanomas and  
411 glioblastoma. Thus, ligands that recognize specific integrin molecules are excellent candidates to target  
412 tumor cells [55]. In this regard, tripeptide motif arginine-glycine-aspartic acid (RGD) has been identified  
413 to have high affinity for integrins, particular for the  $\alpha\beta3$  integrin that is highly over-expressed on many  
414 cancer cells. To date, RGD sequence along with other molecules has been extensively used for targeted  
415 drug delivery to cancer cells, especially in brain tumor cells [55]. A study conducted by Qin et al.  
416 demonstrated that liposome modified by RGD and TF effectively target C6 and b.End.3 cell lines and  
417 significantly increased uptake and penetration into tumor cells. RGD/TF modified liposomes markedly  
418 increased the accumulation and distribution of liposomes in the brain *in vivo*. Additionally, PTX loaded

419 liposomes co-modified with RGD/TF more efficiently induced anti-proliferative activity against C6 cells  
420 and 3D tumor spheroids [56].

421 Belhadj et al. developed multi-functionalized liposome modified with cyclic RGD (c(RGDyK)) and p-  
422 Hydroxybenzoic Acid (pHA) to improve the efficiency of drug delivery and glioblastoma treatment.  
423 They used c(RGDyK) that could bind to integrin  $\alpha v \beta 3$  on the BBB and a small molecule ligand p-pHA  
424 which could bind to dopamine receptors (an attractive target, because of their abundant expression on  
425 the BBB) and increase cellular uptake through the pHA-dopamine special binding pathway. An *in vitro*  
426 study indicated that c(RGDyK)/pHA-liposomes could target glioblastoma cells and U87, bEnd.3 and  
427 HUVECs and increase cellular uptake efficiency. Furthermore, doxorubicin-loaded c(RGDyK)/pHA  
428 liposomes were able to penetrate into the tumor spheroids and increase the cytotoxicity of doxorubicin,  
429 thus inducing enhanced growth inhibitory effect on glioblastoma cells. *In vivo* work also demonstrated  
430 that the c(RGDyK)/pHA modified liposomes have a higher targeting ability and enhanced accumulation  
431 and distribution within the tumor resulting in a longer duration of survival than any other treatment  
432 groups. Therefore, liposomes modification with c(RGDyK)/pHA enhanced anti-glioma efficacy drug  
433 such as doxorubicin for treatment of brain disorder through facilitate the accumulation and transferring  
434 more liposomes, hence showed significantly better anti-brain tumor effect in the tumor-bearing animal  
435 [57].

436 Peptide 22 (NH<sub>2</sub>-C<sub>6</sub>-(cMPRLRGC)-NH<sub>2</sub>), is a specific ligand for Low-density lipoprotein receptors  
437 (LDLR) which are overexpressed on the BBB and glioma cells. Recently, Peptide 22 along with the  
438 ligand cRGD was used for the surface modification of liposomes (c(RGDfK)/Pep-22 liposome) and the  
439 ability of these liposomes were evaluated for facilitating drug delivery across BBB, BBTB and for their  
440 ability to target tumor cells and neovasculature. An *in vitro* study showed that cellular uptake of ligand  
441 decorated liposome c(RGDfK)/Pep-22 on BCECs, HUVECs and U87 cells was significantly higher than  
442 other prepared liposomes. The study further verified the importance of c(RGDfK)/Pep-22-liposomes for  
443 brain targeting and indicated that these liposomes accumulated to a greater extent in brain tumor tissue  
444 than single ligand modified liposomes. Therefore, it seems that c(RGDfK) and Peptide-22 have  
445 synergistic roles for the liposomal delivery across the BBB. Also, c(RGDfK)/Pep-22 liposome loaded  
446 with doxorubicin confers the longest median survival time in treated mice and inhibits the growth of  
447 glioma [58]. One of the major problems relating to the use of cRGD-modified nanocarriers is that these  
448 nanocarriers are mainly accumulated around the tumor site, rather than entering the tumor parenchyma  
449 [59]. To improve the BBB penetration of cRGD-modified nanocarriers across the BBB and into the tumor

450 parenchyma, Shi et al. used a multifunctional peptide TR, a tandem peptide consisting of cRGD and  
451 histidine-rich TH peptide. TH peptide possesses the capacity of 'proton sponge effect' and pH-responsive  
452 cell penetration, hence was able to enhance nanoparticle penetration into the core of tumor. Hence,  
453 cRGD-modified nanocarriers were able to target the integrin  $\alpha\beta3$  and also, increase the ability of  
454 nanocarrier penetration at tumor sites [60]. Shi et al. used liposomes modified by TR peptide to enhance  
455 the transport efficacy across the BBB. They indicated that PTX-loaded liposomes modified with TR  
456 peptide have a very high affinity for integrin  $\alpha\beta3$  and improved BBB penetration and therapeutic  
457 efficacy in a glioma model. Therefore, it seems that TR peptide plays a key role in the transportation of  
458 PTX-loaded liposome to the brain. An *in vitro* study has shown that PTX-TR-liposome exhibited the  
459 greatest anti proliferative effects against C6 glioma cells and brain cancer stem cells (CSCs) when  
460 compared with PEG- and RGD-modified liposomes. Also, this formulation was able to effectively  
461 destroy the glioma vasculogenic mimicry (VM) channels [60].

#### 462 **Glucose mediated transporter**

463 Glucose transporter 1 (GLUT1) is one of the major carrier-mediated transporter system that is abundant  
464 on the surface of endothelial cells and glioma cells in the brain. GLUT1 is responsible for transporting  
465 glucose from the blood into the extracellular space of the brain. Glucose is an essential nutritional  
466 substance for brain function but could be exploited as a carrier for brain targeting drugs. GLUT1 is  
467 therefore a promising and efficient transportation carrier to facilitate the delivery of drugs to the brain  
468 [61]. Recently, liposomes modified with glucose have been for this purpose [61, 62]. For example, Xie  
469 et al. demonstrated that PEGylated liposomes modified by glucose possess the potential of brain targeting  
470 and exhibited an enhanced efficiency for brain delivery [63]. In another study, Qin et al. used a glucose-  
471 mediated liposome as a brain delivery system. Their data indicated that glucose-mediated liposomes  
472 were able to transport drugs across the BBB and that this approach significantly enhanced drug  
473 accumulation in the brain [64]. In a recent investigation, Peng et al. developed a novel dual brain-  
474 targeting glucose-vitamin C (Glu-Vc) modified liposome to enable the efficient delivery of paclitaxel  
475 (PTX) to the brain. A cellular uptake assay on GLUT1- and SVCT2-overexpressed C6 cells indicated  
476 that Glu-Vc-liposome have a higher rate of uptake in comparison to unmodified and singly-modified  
477 liposomes. Also, the Glu-Vc modified liposomes showed higher targeting ability *in vivo* and exhibited  
478 maximum accumulation of drug-loaded liposomes at tumor sites [65]. Recent evidence suggests that  
479 substances with similar structures to glucose including 2-deoxy glucose, galactose, mannose, and glucose  
480 analogs are able to pass through the BBB *via* glucose mediated transporters [66]. Because of the affinity

481 of GLUTs for mannose, liposome decorated with mannose derivates have been used as a recognition  
482 marker for brain targeting and studies have indicated that mannose modification of liposomes plays a  
483 major role in the transport of liposomes across the BBB [67-69]. Previous work conducted by Hao et  
484 al. demonstrated that P-aminophenyl- $\alpha$ -d-mannopyranoside (MAN) modification of liposomes was able  
485 to cellular uptake in C6 glioma cells *in vitro* and to promote penetration through the BBB into brain and  
486 accumulation in the intracerebral regions such as cerebellum and cerebral cortex [70]. Later, Du et al.  
487 found that MAN-modified liposome may enter the brain through GLUT1 and GLUT3 transporter  
488 pathway. They showed that MAN may mediate the transport of the MAN modified liposomes across  
489 BBB through GLUT1 and GLUT3 [71]. Moreover, Ying et al. developed dual-targeting daunorubicin-  
490 loaded liposomes by conjugating with MAN and TF to improve the transport of drug across the BBB and  
491 into glioma. MAN-TF targeting daunorubicin liposomes significantly increased cellular uptake by C6  
492 glioma cells and exhibited the strongest dual-targeting effects and transportation efficacy across the BBB  
493 model compared with non-targeted liposomes and liposomes targeted with either MAN or TF. Also, an  
494 *in vivo* study showed that tumor-bearing rats treated with dual-targeting daunorubicin liposomes have a  
495 higher median survival time and were able to evidently reduce the volume of tumor competed to free  
496 daunorubicin and other control groups [69]. It has also been reported that liposomes which had been  
497 modified with MAV and cell penetrating peptides such as penetratin (Pen) or rabies virus glycoprotein  
498 (RGV) on the surface, promote selective and enhanced delivery to the brain [72, 73]. Based on the  
499 reported studies and the rationale for using GLUT1 targeting ligands for brain-targeted delivery of  
500 nanoparticles, it seem that liposomes modified with glucose and MAN are promising vehicles for  
501 delivery of cargoes to the brain.

502

### 503 **Immunoliposomes**

504 Surface functionalization of liposomes by antibody (immunoliposomes) is an exciting potential approach  
505 to allow targeted delivery of drugs and diagnostic agents to specific tissues [74]. OX26 and RI7217 are  
506 a well-known monoclonal antibody (mAb) with high affinity for rat and mouse transferrin receptor  
507 respectively and are able to cross the BBB by transferrin receptor-mediated transcytosis [75, 76].  
508 Huwyler et al. developed PEG-liposomes conjugated with OX26 mAb for targeted drug delivery to brain.  
509 They indicated that OX26 PEGylated liposomes are capable of successfully transferring daunomycin  
510 into the rat brain [74]. Recently the effect of OX26 immunoliposomes were investigated for their ability  
511 to bind to BCECs and thereby to transport substances to the brain. This study demonstrated that OX26

512 decorated liposomes enhanced the ability of binding to BCECs through an active endocytotic uptake  
513 mechanism and increase immunoliposome accumulation in the BCECs of the BBB [77]. Kong and  
514 colleagues used PEGylated liposomes conjugated with OX26 mAb as carriers of dopamine in animal  
515 model of Parkinson's disease (PD). They indicated that the uptake of dopamine-loaded PEGylated  
516 OX26-immunoliposome in the brain in a rat model of PD is higher than encapsulated dopamine-  
517 PEGylated liposomes and dopamine alone. It was also demonstrated that the brain distribution of  
518 PEGylated OX26-immunoliposome was significantly greater than dopamine-PEGylated liposomes  
519 which is due to the effective role of OX26 mAb in binding to the transferrin receptor of the brain capillary  
520 endothelium that leading to increased efficient and specific delivery of liposome to brain tissue [78].  
521 Dual PEGylated immunoliposomes, composed of OX26 and anti- $\alpha$ -synuclein LB509 antibodies, were  
522 developed by Loureiro et al. to enhanced drug delivery to brain in PD. The study indicated that these  
523 immunoliposomes were able to target the BBB through TF receptors and  $\alpha$ -synuclein protein (aneuronal  
524 protein that is associated with Parkinson's disease) and effectively enhanced the transport of drugs across  
525 the BBB [79]. Recently, Gregori et al. employed a novel approach by using MYBE/4C1 antihuman TfR  
526 mAb for the surface functionalization of liposomes. They demonstrated that functionalization with  
527 MYBE/4C1 mAb improved the passage of doxorubicin-loaded liposomes in an *in vitro* BBB model [80].  
528 CD133, is a 120 kDa transmembrane single-chain transmembrane glycoprotein which is expressed in  
529 cancer stem cells such as glioblastoma stem cells (GSCs) [81]. Recently, immunoliposomes modified  
530 with CD133 have been used as a targeting ligand to GSC. In this study, dual-modified immunoliposomes  
531 conjugating with angiopep-2 and CD133 antibody were used for the targeting of GSC [82]. Angiopep-2  
532 (TFFYGGSRGKRNNFKTEEY) is a peptide derivative of the Kunitz domain with good BBB  
533 penetration. Angiopep-2 extensively used to target the low-density lipoprotein receptor related protein 1  
534 (LRP1) which is expressed both in the BBB and on glioblastoma cells [83]. Kim et al. indicated that  
535 dual targeting immunoliposome modified by angiopep-2 and CD133 loaded with temozolomide (TMZ)  
536 (Dual-LP-TMZ) increased cytotoxicity and apoptosis against U87MG GSCs *in vitro* compared to free  
537 TMZ and non-targeted liposomes. *In vitro* experiments indicated that the mice treated with Dual-LP-  
538 TMZ exhibited lower tumor size, and highest median survival time (MST) and increased life span (ILS)  
539 compared to free TMZ and non-targeted liposomes [82]. In summary, the available evidence  
540 demonstrates that that antibody as a specific targeting ligand provides a high targeting affinity with  
541 receptors and significantly enhances the efficiency of drug delivery to the brain.

## 542 **Cationic liposomes**

543 In recent years, cationic liposomes have been developed as a potential brain drug delivery vehicle. This  
544 type of liposome is negatively charged at physiological pH. Therefore, these liposomes are able to attach  
545 to the molecules that are positively charged at physiological pH *via* electrostatic interaction [84]. Chen  
546 and colleagues, developed a lactoferrin-modified procationic liposome as a potential brain drug delivery  
547 vector. They used Cholest-5-en-3-ol-(3)-(2-((4-((carboxymethyl) dithio]-1- iminobutyl) amino) ethyl)  
548 carbamate (CHETA, C<sub>36</sub>H<sub>61</sub>N<sub>3</sub>O<sub>4</sub>S<sub>2</sub>), (a cholesterol derivative), to prepare the procationic liposomes  
549 [85, 86]. Lactoferrin which is a cationic iron-binding glycoprotein belonging to the transferrin family  
550 was used as a targeting ligand for delivery of drug to the brain. Lactoferrin was able to attach to the  
551 lactoferrin receptor, which is highly expressed on the surface of brain endothelial cells. Receptor-  
552 mediated transcytosis across the BBB was thereby enhanced [87]. The cationic liposomes modified with  
553 lactoferrin confer two important features on these delivery systems. First, lactoferrin has a positive charge  
554 at physiological pH, therefore, is able to be easily absorbed onto the negatively charged surface of the  
555 procationic liposome *via* electrostatic interaction. Secondly, high-affinity binding of lactoferrin to the  
556 lactoferrin receptors on brain cells leads to improved delivery of drug to the brain [86, 88]. The  
557 experiments conducted by Chen et al. indicated that procationic liposome modified with lactoferrin  
558 served as brain specific targeting ligands and showed improved performance in the uptake efficiency and  
559 cytotoxicity in primary brain capillary endothelial cells. They also have a greater ability to cross BBB *in*  
560 *vitro* compared to conventional and cationic liposomes [85]. In another study, Chen et al. studied the  
561 therapeutic effects of doxorubicin-loaded procationic liposomes for glioma treatments. Their results  
562 show that these modified liposomes improved the uptake efficiency in BCECs and C6 cells and could  
563 effectively inhibit the growth of C6 *in vitro*. In *in vivo* models, survival time was longer compared with  
564 other DOX formulations [89]. Moreover, several studies have demonstrated that fusogenic liposomes  
565 composed of pH sensitive and cationic liposomes (such as neutral lipid  
566 dioleoylphosphatidylethanolamine (DOPE) combined with the cationic lipid 1, 2-dioleoyl-3-  
567 trimethylammoniumpropane (DOTAP)) enhance cellular cytoplasmic delivery [90, 91]. Recently, it has  
568 been demonstrated that fusogenic liposomes effectively enhance cytoplasmic delivery of their cargos to  
569 bEnd.3 cells [73]. Therefore, it seems that the presence of cationic lipid in liposomal formulations  
570 improves cellular cytoplasmic delivery by inducing membrane fusion via electrostatic interactions with  
571 the cell membranes.

## 572 **Future perspectives**

573 Liposome-based strategies are one of the most promising approaches to facilitate the delivery of drugs  
574 to the brain. To date, a number of studies have been performed using liposomal carrier systems, but many  
575 of them have so far been limited to preclinical studies extensive further investigation, particularly for  
576 toxicity, is necessary prior to clinical use, to enable this technique to be widely employed in a range of  
577 CNS and brain disorders. In addition, the clinical success of liposomal therapies will require an  
578 interdisciplinary group of researchers with expertise in liposome technology, neuroscience, oncology,  
579 pharmacology and medical imaging. The nanoliposomes need to be less than 100 nm in diameter to  
580 enable them to cross the BBB deliver drug to the brain. Many of drugs that are used in the treatment of  
581 brain cancer (including glioblastoma) are highly cytotoxic. In order to reduce the toxicity of these agents,  
582 they must be specifically targeted to the affected site to overcome the side effects of non-specific binding.  
583 Therefore, the formulation of nanoparticles must be optimized to meet these needs. Extensive research  
584 investment in this field is justified by the high market price that successful agents would attract.

585 Furthermore, most of the research has been conducted on brain tumors, and reports on other CNS  
586 disorders are relatively rare, and require further investigation. However, a major limitation of current  
587 liposomal brain cancer therapies is the low ability and inhomogeneous distribution of liposome  
588 therapeutics to penetrate the BBB, to accumulate in the tumor region and to enter the tumor mass. This  
589 problem is not unique to liposomal drug delivery to the brain, but is a common problem limiting the  
590 effectiveness of all types of therapeutic agents, including other nanoparticle-based drug delivery  
591 systems. Therefore, different strategies should be considered to improve the intratumoral distribution of  
592 liposome therapeutics. One problem is the rapid clearance from the circulation by the reticuloendothelial  
593 system (RES) organs, an issue which has been partially resolved by modification of the size and shape  
594 of particles and pegylation of the liposomal formulation. Furthermore, targeted drug delivery by specific  
595 ligands offers a significant advantage by promoting more efficient delivery of therapeutic compounds to  
596 specific cells or tissue of the body and minimizing the exposure of non-target tissues to the drug.  
597 Additionally, the results of several studies suggest that intratumoral administration can be increase tumor  
598 liposome concentrations and improve the accumulation and distribution of liposomes within the tumor.  
599 Increased understanding of the BBB, the blood-cerebrospinal fluid barrier, the mechanisms of drug  
600 movement within the CNS, tumor biology and macromolecular structure and nanoparticle transport  
601 properties, may lead to advances in technology, and further therapeutic gains for drug delivery to the  
602 brain in the near future.

## 603 **Conclusions**

604 The treatment of central nervous system (CNS) disorders remains challenging due to the functions of the  
605 BBB, which impedes the delivery of many therapeutic drugs to the brain. Therefore, development of  
606 novel therapeutic strategies for drug delivery to the brain tissue and treatment of neurological disorders  
607 is a major prerequisite for the clinical application of many drugs. The use of nanotechnology-based drug  
608 delivery systems such as liposomes has great potential to improve the therapy of a range of neurological  
609 disorders. Liposomes are promising carriers for drug delivery to the CNS and offer various advantages  
610 for drug delivery over other nanocarrier systems since they are easy to prepare and are highly  
611 biodegradable and biocompatible. Moreover, liposomes can minimize the side effects of drugs, decrease  
612 required drug dose, increase drug half-life, enable controlled drug release and enhance penetration across  
613 the BBB. Moreover, passive or active targeting of drugs to brain regions is achievable using surface  
614 modification of liposomes and by creating liposomes covalently coupled with specific ligands (such as  
615 TF, FA) and coating their surface with certain hydrophilic polymers such as PEG (**Table 1**). A wide  
616 variety of liposomal formulations with a range of structural modifications and features have been used  
617 to enhance the delivery of drugs to the CNS. Such approaches are extremely promising, however at  
618 present the quantity of drug that can be delivered to the brain by these mechanisms is small in comparison  
619 with the delivery of free (non-liposomal) drugs to other organs and tissues. Extensive work is required  
620 to improve our understanding of the mechanisms which manage the transportation of drug loaded  
621 liposomes to the brain and to investigate the clinical efficacy and safety of these preparations in patients.

622

623

624

625

626

627

628

## 629 **References**

- 630 1. Dong, X., *Current strategies for brain drug delivery*. *Theranostics*, 2018. **8**(6): p. 1481.  
631 2. Pankevich, D.E., et al., *Improving and accelerating drug development for nervous system disorders*.  
632 *Neuron*, 2014. **84**(3): p. 546-553.

- 633 3. Zhou, Y., et al., *Crossing the blood-brain barrier with nanoparticles*. Journal of controlled release, 2017.
- 634 4. Connor, J. and L. Huang, *pH-sensitive immunoliposomes as an efficient and target-specific carrier for*
- 635 *antitumor drugs*. Cancer research, 1986. **46**(7): p. 3431-3435.
- 636 5. ElBayoumi, T.A. and V.P. Torchilin, *Current trends in liposome research*, in *Liposomes*. 2010, Springer.
- 637 p. 1-27.
- 638 6. Abbott, N.J., et al., *Structure and function of the blood–brain barrier*. Neurobiology of disease, 2010.
- 639 **37**(1): p. 13-25.
- 640 7. Weiss, N., et al., *The blood-brain barrier in brain homeostasis and neurological diseases*. Biochimica et
- 641 Biophysica Acta (BBA)-Biomembranes, 2009. **1788**(4): p. 842-857.
- 642 8. Guerra, M., J. Blázquez, and E. Rodríguez, *Blood–brain barrier and foetal-onset hydrocephalus, with a*
- 643 *view on potential novel treatments beyond managing CSF flow*. Fluids and Barriers of the CNS, 2017.
- 644 **14**(1): p. 19.
- 645 9. Banks, W.A., *From blood–brain barrier to blood–brain interface: new opportunities for CNS drug*
- 646 *delivery*. Nature reviews Drug discovery, 2016. **15**(4): p. 275.
- 647 10. Khan, A.R., et al., *Recent progress of drug nanoformulations targeting to brain*. Journal of Controlled
- 648 Release, 2018.
- 649 11. Masserini, M., *Nanoparticles for brain drug delivery*. ISRN biochemistry, 2013. **2013**.
- 650 12. Vieira, D.B. and L.F. Gamarra, *Getting into the brain: liposome-based strategies for effective drug*
- 651 *delivery across the blood–brain barrier*. International journal of nanomedicine, 2016. **11**: p. 5381.
- 652 13. Agrawal, M., et al., *Recent advancements in liposomes targeting strategies to cross blood-brain barrier*
- 653 *(BBB) for the treatment of Alzheimer's disease*. Journal of Controlled Release, 2017. **260**: p. 61-77.
- 654 14. Bozzuto, G. and A. Molinari, *Liposomes as nanomedical devices*. International journal of nanomedicine,
- 655 2015. **10**: p. 975.
- 656 15. Leonor Pinzon-Daza, M., et al., *Nanoparticle-and liposome-carried drugs: new strategies for active*
- 657 *targeting and drug delivery across blood-brain barrier*. Current drug metabolism, 2013. **14**(6): p. 625-
- 658 640.
- 659 16. Torchilin, V.P., *Recent advances with liposomes as pharmaceutical carriers*. Nature reviews Drug
- 660 discovery, 2005. **4**(2): p. 145.
- 661 17. Noble, G.T., et al., *Ligand-targeted liposome design: challenges and fundamental considerations*. Trends
- 662 in biotechnology, 2014. **32**(1): p. 32-45.
- 663 18. Immordino, M.L., F. Dosio, and L. Cattel, *Stealth liposomes: review of the basic science, rationale, and*
- 664 *clinical applications, existing and potential*. International journal of nanomedicine, 2006. **1**(3): p. 297.
- 665 19. Vijayakumar, M.R., et al., *Trans resveratrol loaded DSPE PEG 2000 coated liposomes: An evidence for*
- 666 *prolonged systemic circulation and passive brain targeting*. Journal of Drug Delivery Science and
- 667 Technology, 2016. **33**: p. 125-135.
- 668 20. Vijayakumar, M.R., et al., *Pharmacokinetics, biodistribution, in vitro cytotoxicity and biocompatibility of*
- 669 *Vitamin E TPGS coated trans resveratrol liposomes*. Colloids and Surfaces B: Biointerfaces, 2016. **145**:
- 670 p. 479-491.
- 671 21. Muthu, M.S., et al., *Vitamin E TPGS coated liposomes enhanced cellular uptake and cytotoxicity of*
- 672 *docetaxel in brain cancer cells*. International journal of pharmaceutics, 2011. **421**(2): p. 332-340.
- 673 22. Yang, T., et al., *Verapamil and riluzole cocktail liposomes overcome pharmacoresistance by inhibiting P-*
- 674 *glycoprotein in brain endothelial and astrocyte cells: A potent approach to treat amyotrophic lateral*
- 675 *sclerosis*. European Journal of Pharmaceutical Sciences, 2018. **120**: p. 30-39.
- 676 23. Bolhassani, A., *Potential efficacy of cell-penetrating peptides for nucleic acid and drug delivery in cancer*.
- 677 Biochimica et Biophysica Acta (BBA)-Reviews on Cancer, 2011. **1816**(2): p. 232-246.
- 678 24. Borrelli, A., et al., *Cell penetrating peptides as molecular carriers for anti-cancer agents*. Molecules,
- 679 2018. **23**(2): p. 295.
- 680 25. Madani, F., et al., *Mechanisms of cellular uptake of cell-penetrating peptides*. Journal of biophysics, 2011.
- 681 **2011**.
- 682 26. Gao, H., et al., *Cell-penetrating peptide-based intelligent liposomal systems for enhanced drug delivery*.
- 683 Current pharmaceutical biotechnology, 2014. **15**(3): p. 210-219.

- 684 27. Torchilin, V.P., et al., *TAT peptide on the surface of liposomes affords their efficient intracellular delivery* even at low temperature and in the presence of metabolic inhibitors. Proceedings of the National Academy of Sciences, 2001. **98**(15): p. 8786-8791.
- 685
- 686
- 687 28. Qin, Y., et al., *Liposome formulated with TAT-modified cholesterol for improving brain delivery and* therapeutic efficacy on brain glioma in animals. International journal of pharmaceutics, 2011. **420**(2): p. 304-312.
- 688
- 689
- 690 29. Koren, E. and V.P. Torchilin, *Cell-penetrating peptides: breaking through to the other side*. Trends in molecular medicine, 2012. **18**(7): p. 385-393.
- 691
- 692 30. Negussie, A.H., et al., *Synthesis and in vitro evaluation of cyclic NGR peptide targeted thermally sensitive liposome*. Journal of Controlled Release, 2010. **143**(2): p. 265-273.
- 693
- 694 31. Ahmad, A., et al., *Development of liposomal formulation for delivering anticancer drug to breast cancer stem-cell-like cells and its pharmacokinetics in an animal model*. Molecular pharmaceutics, 2016. **13**(3): p. 1081-1088.
- 695
- 696
- 697 32. Zhou, J.-e., et al., *iNGR-Modified Liposomes for Tumor Vascular Targeting and Tumor Tissue Penetrating Delivery in the Treatment of Glioblastoma*. Molecular pharmaceutics, 2017. **14**(5): p. 1811-1820.
- 698
- 699 33. Gao, H., *Progress and perspectives on targeting nanoparticles for brain drug delivery*. Acta Pharmaceutica Sinica B, 2016. **6**(4): p. 268-286.
- 700
- 701 34. Prabhakar, K., et al., *Brain delivery of transferrin coupled indinavir submicron lipid emulsions—Pharmacokinetics and tissue distribution*. Colloids and Surfaces B: Biointerfaces, 2011. **86**(2): p. 305-313.
- 702
- 703
- 704 35. Georgieva, J., D. Hoekstra, and I. Zuhorn, *Smuggling drugs into the brain: an overview of ligands targeting transcytosis for drug delivery across the blood–brain barrier*. Pharmaceutics, 2014. **6**(4): p. 557-583.
- 705
- 706
- 707 36. Sharma, G., et al., *The role of cell-penetrating peptide and transferrin on enhanced delivery of drug to brain*. International journal of molecular sciences, 2016. **17**(6): p. 806.
- 708
- 709 37. Gan, C.W. and S.-S. Feng, *Transferrin-conjugated nanoparticles of poly (lactide)-D- $\alpha$ -tocopheryl polyethylene glycol succinate diblock copolymer for targeted drug delivery across the blood–brain barrier*. Biomaterials, 2010. **31**(30): p. 7748-7757.
- 710
- 711
- 712 38. Jhaveri, A., et al., *Transferrin-targeted, resveratrol-loaded liposomes for the treatment of glioblastoma*. Journal of Controlled Release, 2018. **277**: p. 89-101.
- 713
- 714 39. Lopalco, A., et al., *Transferrin Functionalized Liposomes Loading Dopamine HCl: Development and Permeability Studies across an In Vitro Model of Human Blood–Brain Barrier*. Nanomaterials, 2018. **8**(3): p. 178.
- 715
- 716
- 717 40. Song, X.-l., et al., *Targeting vincristine plus tetrandrine liposomes modified with DSPE-PEG2000-transferrin in treatment of brain glioma*. European Journal of Pharmaceutical Sciences, 2017. **96**: p. 129-140.
- 718
- 719
- 720 41. Kibria, G., et al., *Dual-ligand modification of PEGylated liposomes shows better cell selectivity and efficient gene delivery*. Journal of controlled release, 2011. **153**(2): p. 141-148.
- 721
- 722 42. Sharma, G., et al., *Grafting of cell-penetrating peptide to receptor-targeted liposomes improves their transfection efficiency and transport across blood–brain barrier model*. Journal of pharmaceutical sciences, 2012. **101**(7): p. 2468-2478.
- 723
- 724
- 725 43. Lakkadwala, S. and J. Singh, *Co-delivery of doxorubicin and erlotinib through liposomal nanoparticles for glioblastoma tumor regression using an in vitro brain tumor model*. Colloids and Surfaces B: Biointerfaces, 2019. **173**: p. 27-35.
- 726
- 727
- 728 44. Lakkadwala, S., et al., *Biodistribution of TAT or QLPVM coupled to receptor targeted liposomes for delivery of anticancer therapeutics to brain in vitro and in vivo*. Nanomedicine: Nanotechnology, Biology and Medicine, 2020. **23**: p. 102112.
- 729
- 730
- 731 45. Sharma, G., et al., *Influence of short-chain cell-penetrating peptides on transport of doxorubicin encapsulating receptor-targeted liposomes across brain endothelial barrier*. Pharmaceutical research, 2014. **31**(5): p. 1194-1209.
- 732
- 733
- 734 46. Lakkadwala, S. and J. Singh, *Dual functionalized 5-fluorouracil liposomes as highly efficient nanomedicine for glioblastoma treatment as assessed in an in vitro brain tumor model*. Journal of pharmaceutical sciences, 2018. **107**(11): p. 2902-2913.
- 735
- 736

- 737 47. Liu, C., et al., *A dual-mediated liposomal drug delivery system targeting the brain: rational construction,*  
738 *integrity evaluation across the blood–brain barrier, and the transporting mechanism to glioma cells.*  
739 *International journal of nanomedicine*, 2017. **12**: p. 2407.
- 740 48. Zong, T., et al., *Synergistic dual-ligand doxorubicin liposomes improve targeting and therapeutic efficacy*  
741 *of brain glioma in animals.* *Molecular pharmaceutics*, 2014. **11**(7): p. 2346-2357.
- 742 49. Gao, H., Z. Pang, and X. Jiang, *Targeted delivery of nano-therapeutics for major disorders of the central*  
743 *nervous system.* *Pharmaceutical research*, 2013. **30**(10): p. 2485-2498.
- 744 50. Zong, T., et al., *Enhanced glioma targeting and penetration by dual-targeting liposome co-modified with*  
745 *T7 and TAT.* *Journal of pharmaceutical sciences*, 2014. **103**(12): p. 3891-3901.
- 746 51. Lim, S., et al., *dNP2 is a blood–brain barrier-permeable peptide enabling ctCTLA-4 protein delivery to*  
747 *ameliorate experimental autoimmune encephalomyelitis.* *Nature communications*, 2015. **6**: p. 8244.
- 748 52. Wu, D. and W.M. Pardridge, *Blood-brain barrier transport of reduced folic acid.* *Pharmaceutical research*,  
749 1999. **16**(3): p. 415-419.
- 750 53. Li, M., et al., *Synergistic tumor microenvironment targeting and blood–brain barrier penetration via a*  
751 *pH-responsive dual-ligand strategy for enhanced breast cancer and brain metastasis therapy.*  
752 *Nanomedicine: Nanotechnology, Biology and Medicine*, 2018. **14**(6): p. 1833-1843.
- 753 54. Yang, Z., et al., *Structural basis of ligand binding modes at the neuropeptide Y Y1 receptor.* *Nature*, 2018.  
754 **556**(7702): p. 520-524.
- 755 55. Marelli, U.K., et al., *Tumor targeting via integrin ligands.* *Frontiers in oncology*, 2013. **3**: p. 222.
- 756 56. Qin, L., et al., *A dual-targeting liposome conjugated with transferrin and arginine -glycine -aspartic acid*  
757 *peptide for glioma -targeting therapy.* *Oncology letters*, 2014. **8**(5): p. 2000-2006.
- 758 57. Belhadj, Z., et al., *Multifunctional targeted liposomal drug delivery for efficient glioblastoma treatment.*  
759 *Oncotarget*, 2017. **8**(40): p. 66889.
- 760 58. Chen, C., et al., *Peptide-22 and cyclic RGD functionalized liposomes for glioma targeting drug delivery*  
761 *overcoming BBB and BBTB.* *ACS applied materials & interfaces*, 2017. **9**(7): p. 5864-5873.
- 762 59. Wang, K., et al., *Tumor penetrability and anti-angiogenesis using iRGD-mediated delivery of*  
763 *doxorubicin-polymer conjugates.* *Biomaterials*, 2014. **35**(30): p. 8735-8747.
- 764 60. Shi, K., et al., *Liposomes combined an integrin  $\alpha\beta 3$ -specific vector with pH-responsible cell-penetrating*  
765 *property for highly effective antiglioma therapy through the blood–brain barrier.* *ACS applied materials*  
766 *& interfaces*, 2015. **7**(38): p. 21442-21454.
- 767 61. Zhao, Y., et al., *GLUT1-mediated venlafaxine-thiamine disulfide system-glucose conjugates with “lock-*  
768 *in” function for central nervous system delivery.* *Chemical biology & drug design*, 2018. **91**(3): p. 707-  
769 716.
- 770 62. Chen, Q., et al., *Synthesis, in vitro and in vivo characterization of glycosyl derivatives of ibuprofen as*  
771 *novel prodrugs for brain drug delivery.* *Journal of drug targeting*, 2009. **17**(4): p. 318-328.
- 772 63. Xie, F., et al., *Investigation of glucose-modified liposomes using polyethylene glycols with different chain*  
773 *lengths as the linkers for brain targeting.* *International journal of nanomedicine*, 2012. **7**: p. 163.
- 774 64. Qin, Y., et al., *In vitro and in vivo investigation of glucose-mediated brain-targeting liposomes.* *Journal*  
775 *of drug targeting*, 2010. **18**(7): p. 536-549.
- 776 65. Peng, Y., et al., *Dual-targeting for brain-specific liposomes drug delivery system: Synthesis and*  
777 *preliminary evaluation.* *Bioorganic & medicinal chemistry*, 2018. **26**(16): p. 4677-4686.
- 778 66. Pardridge, W.M., *Transport of small molecules through the blood-brain barrier: biology and*  
779 *methodology.* *Advanced drug delivery reviews*, 1995. **15**(1-3): p. 5-36.
- 780 67. Umezawa, F. and Y. Eto, *Liposome targeting to mouse brain: mannose as a recognition marker.*  
781 *Biochemical and biophysical research communications*, 1988. **153**(3): p. 1038-1044.
- 782 68. Sarkar, S. and N. Das, *Mannosylated liposomal flavonoid in combating age-related ischemia–reperfusion*  
783 *induced oxidative damage in rat brain.* *Mechanisms of ageing and development*, 2006. **127**(4): p. 391-  
784 397.
- 785 69. Ying, X., et al., *Dual-targeting daunorubicin liposomes improve the therapeutic efficacy of brain glioma*  
786 *in animals.* *Journal of Controlled Release*, 2010. **141**(2): p. 183-192.

- 787 70. Hao, Z.-f., et al., *Liposomes modified with P-aminophenyl- $\alpha$ -d-mannopyranoside: a carrier for targeting*  
788 *cerebral functional regions in mice*. European Journal of Pharmaceutics and Biopharmaceutics, 2013.  
789 **84**(3): p. 505-516.
- 790 71. Du, D., et al., *The role of glucose transporters in the distribution of p-aminophenyl- $\alpha$ -d-mannopyranoside*  
791 *modified liposomes within mice brain*. Journal of Controlled Release, 2014. **182**: p. 99-110.
- 792 72. Arora, S., D. Sharma, and J. Singh, *GLUT-1: An Effective Target To Deliver Brain-Derived Neurotrophic*  
793 *Factor Gene Across the Blood Brain Barrier*. ACS Chemical Neuroscience, 2020. **11**(11): p. 1620-1633.
- 794 73. Farid, M., et al., *Cell membrane fusing liposomes for cytoplasmic delivery in brain endothelial cells*.  
795 *Colloids and Surfaces B: Biointerfaces*, 2020. **194**: p. 111193.
- 796 74. Huwyler, J., D. Wu, and W.M. Pardridge, *Brain drug delivery of small molecules using immunoliposomes*.  
797 *Proceedings of the National Academy of Sciences*, 1996. **93**(24): p. 14164-14169.
- 798 75. Jefferies, W., et al., *Analysis of lymphopoietic stem cells with a monoclonal antibody to the rat transferrin*  
799 *receptor*. Immunology, 1985. **54**(2): p. 333.
- 800 76. Kang, S., et al., *Muscone/RI7217 co-modified upward messenger DTX liposomes enhanced permeability*  
801 *of blood-brain barrier and targeting glioma*. Theranostics, 2020. **10**(10): p. 4308.
- 802 77. Johnsen, K.B., et al., *Targeting transferrin receptors at the blood-brain barrier improves the uptake of*  
803 *immunoliposomes and subsequent cargo transport into the brain parenchyma*. Scientific reports, 2017.  
804 **7**(1): p. 10396.
- 805 78. Kang, Y.S., et al., *Use of PEGylated Immunoliposomes to Deliver Dopamine Across the Blood-Brain*  
806 *Barrier in a Rat Model of Parkinson's Disease*. CNS neuroscience & therapeutics, 2016. **22**(10): p. 817-  
807 823.
- 808 79. Loureiro, J.A., et al., *Immunoliposomes doubly targeted to transferrin receptor and to  $\alpha$ -synuclein*. Future  
809 *science OA*, 2015. **1**(4).
- 810 80. Gregori, M., et al., *Novel antitransferrin receptor antibodies improve the blood-brain barrier crossing*  
811 *efficacy of immunoliposomes*. Journal of pharmaceutical sciences, 2016. **105**(1): p. 276-283.
- 812 81. Grosse-Gehling, P., et al., *CD133 as a biomarker for putative cancer stem cells in solid tumours:*  
813 *limitations, problems and challenges*. The Journal of pathology, 2013. **229**(3): p. 355-378.
- 814 82. Kim, J.S., D.H. Shin, and J.-S. Kim, *Dual-targeting immunoliposomes using angiopep-2 and CD133*  
815 *antibody for glioblastoma stem cells*. Journal of Controlled Release, 2018. **269**: p. 245-257.
- 816 83. Tian, X., et al., *LRP-1-mediated intracellular antibody delivery to the Central Nervous System*. Scientific  
817 *reports*, 2015. **5**: p. 11990.
- 818 84. Zhong, Z.-R., et al., *Preparation and characterization of a novel nonviral gene transfer system:*  
819 *procationic-liposome-protamine-DNA complexes*. Drug delivery, 2007. **14**(3): p. 177-183.
- 820 85. Chen, H., et al., *Lactoferrin-modified procationic liposomes as a novel drug carrier for brain delivery*.  
821 *European Journal of Pharmaceutical Sciences*, 2010. **40**(2): p. 94-102.
- 822 86. Zhong, Z.-R., et al., *Characteristics comparison before and after lyophilization of transferrin modified*  
823 *procationic-liposome-protamine-DNA complexes (Tf-PLPD)*. Archives of pharmacal research, 2007.  
824 **30**(1): p. 102.
- 825 87. Huang, R.-q., et al., *Characterization of lactoferrin receptor in brain endothelial capillary cells and mouse*  
826 *brain*. Journal of biomedical science, 2007. **14**(1): p. 121-128.
- 827 88. Suzuki, Y., V. Lopez, and B. Lönnerdal, *Lactoferrin*. Cellular and Molecular Life Sciences, 2005. **62**(22):  
828 p. 2560.
- 829 89. Chen, H., et al., *Lactoferrin modified doxorubicin-loaded procationic liposomes for the treatment of*  
830 *gliomas*. European Journal of Pharmaceutical Sciences, 2011. **44**(1-2): p. 164-173.
- 831 90. Kube, S., et al., *Fusogenic liposomes as nanocarriers for the delivery of intracellular proteins*. Langmuir,  
832 2017. **33**(4): p. 1051-1059.
- 833 91. Kunisawa, J., et al., *Fusogenic liposome delivers encapsulated nanoparticles for cytosolic controlled gene*  
834 *release*. Journal of controlled release, 2005. **105**(3): p. 344-353.
- 835 92. dos Santos Rodrigues, B., T. Kanekiyo, and J. Singh, *In Vitro and In Vivo characterization of CPP and*  
836 *transferrin modified liposomes encapsulating pDNA*. Nanomedicine: Nanotechnology, Biology and  
837 *Medicine*, 2020: p. 102225.

838 **Figure Legend**

839 **Figure 1.** A) Structure of the neurovascular unit. Pericytes surround brain microvascular endothelial  
840 cells and together with the basal lamina and astrocytic end-feet, they contribute to the organization of the  
841 BBB and form the neurovascular unit. B) Schematic representation of the different mechanisms of  
842 transport of molecules across the blood-brain barrier. Paracellular pathway: very small hydrophilic  
843 molecules penetrate the BBB through the tight junctions. Transcellular pathway (diffusion): small  
844 lipophilic molecules can diffuse across the endothelial cells passively. Transport proteins pathway:  
845 specific molecules such as amino acids, glucose and nucleosides could be non-covalently binding to the  
846 protein transporters on one side of the membrane and released on the other side. Receptor-mediated  
847 transcytosis: larger molecules such as insulin, transferrin and low-density lipoprotein (LDL) are  
848 transported through specific receptors. Adsorptive mediated transcytosis: cationic drug could be  
849 electrostatically attracted anionic sites present on the cell membrane and increases its uptake by  
850 adsorptive mediated transcytosis or endocytosis. Efflux Pumps: these pumps are responsible for drug  
851 expulsion from the brain.

852

853

854

**Table 1.** Effects of different liposomal preparations in penetrating the brain tissue.

Surface modification	Study type	<i>In vitro</i> Cell type	Targeting ligand	Delivered Drug	Function	Ref.
PEGylation	<i>In vivo</i>	-	-	Resveratrol	PEGylated liposomes have a longer systemic circulation time and more extensive accumulation in the brain.	[19]
	<i>In vitro</i>	bEnd.3 <sup>1</sup> /astrocytes C8D1A cells	-	Riluzole	More extensive accumulation of the drug in the brain.	[22]
	<i>In vivo</i>					
TPGS coating	<i>In vitro</i> / <i>In vivo</i>	C6 glioma cell	-	Resveratrol	TPGS coated liposomes have excellent cellular internalization, and more extensive accumulation in brain.	[20]
	<i>In vitro</i>	C6 glioma cell	-	Docetaxel	More extensive cellular uptake and cytotoxicity in C6 glioma cells.	[21]
CPP	<i>In vitro</i> / <i>In vivo</i>	BCECs/ C6 glioma cells	TAT	Doxorubicin	More extensive cellular uptake and accumulation of drug in brain, Less cardiotoxicity.	[28]
	<i>In vitro</i> / <i>In vivo</i>	U87MG cells/ HUVECs	iNGR	Doxorubicin	More extensive cellular uptake and accumulation of the drug in the brain, Increased survival time in an animal model.	[32]
TF	<i>In vitro</i> / <i>In vivo</i>	U87 glioblastoma cell line	TF <sup>2</sup>	Resveratrol	More extensive cellular uptake/ induced significantly greater apoptosis and cell cycle arrest and accumulation of drug in tumor. Increased survival time in an animal model.	[38]
	<i>In vitro</i>	hCMEC/D3	TF	Dopamine	successfully transferred across <i>in vitro</i> model of the BBB	[39]
	<i>In vitro</i> / <i>In vivo</i>	C6 cell	TF	Vincristine/ Tetrandrine	More extensive cellular uptake/ inhibiting the cancer cell invasion and VM channels/ more extensive accumulate in brain tumor site	[40]
Tf-CPP	<i>In vitro</i>	U87 glioblastoma cell line	Tf- PFVYLI	Doxorubicin and Erlotinib	More extensive cellular uptake, incurring drug concentration in tumor cells inside, enhanced tumor cell death and antitumor efficacy in glioblastoma tumor cells	[43]
	<i>In vitro</i>	Primary glial cell, bEnd.3	Tf- PR <sup>3</sup>	No	More extensive cellular uptake and transfection, improved cell penetration	[42]
	<i>In vitro</i> / <i>In vivo</i>	Daoy medulloblastoma, U87 glioblastoma, bEnd.3	Tf-TAT, Tf-Penetratin, Tf-Mastoparan	Doxorubicin	Tf-Penetratin liposomes have an efficient cellular uptake, more extensive translocation of doxorubicin,	[45]
	<i>In vitro</i> / <i>In vivo</i>	U87 glioblastoma, bEnd.3	TF-penetratin	5-fluorouracil	More extensive cellular uptake, induced significantly more extensive apoptosis in U87 cell. Induction of excellent anti-tumor efficacy in brain cancer cells.	[46]

	<i>In vitro</i>	C6 glioma cell	TF-arginine-rich residues	No	More extensive cellular uptake, successful escape from endosomal compartment of glioma C6 cells	[47]
	<i>In vitro/ In vivo</i>	C6 glioma cell, bEnd.3	T7 peptide <sup>4</sup> -TAT	Doxorubicin	More extensive cellular uptake, Increased brain targeting efficacy	[48]
	<i>In vitro/ In vivo</i>	U87, bEnd.3 and glial cells	TF-QLPVM, TF-TAT	Doxorubicin, erlotinib	More extensive cellular uptake, Increased brain targeting efficacy	[44]
	<i>In vitro/ In vivo</i>	bEn.d3, primary neuronal, glial cells	PFVYLI, R9F2	pDNA	Enhanced <i>in vitro</i> transfection efficacy, superior ability to translocate <i>in vitro</i> and <i>in vivo</i> BBB	[92]
FA-CPP	<i>In vitro/ In vivo</i>	bEnd.3 cells	FA <sup>5</sup> -dNP2 <sup>6</sup>	Paclitaxel	Enhanced BBB transportation effect, more extensive accumulation of drug in tumor cells	[53]
	<i>In vitro/ In vivo</i>	C6 glioma cell	FA-dNP2	Paclitaxel	Enhanced BBB transportation effect, more extensive accumulation of drug in tumor cell.	[54]
RGD	<i>In vitro/ In vivo</i>	C6, b.End.3 cell	TF-RGD <sup>7</sup>	Paclitaxel	More extensive cellular uptake, highest brain distribution,	[56]
	<i>In vitro/ In vivo</i>	U87, b.End.3 cell, HUVECs <sup>8</sup>	c(RGDyK)/pHA <sup>9</sup>	Doxorubicin	More extensive cellular uptake, increased cytotoxicity of doxorubicin and induced the strongest inhibitory effect on glioblastoma cell growth <i>in vitro</i> and <i>in vivo</i>	[57]
	<i>In vitro/ In vivo</i>	BCECs <sup>10</sup> , HUVECs and U87 cells	c(RGDfK)/Pep-22	Doxorubicin	More extensive cellular uptake <i>in vitro</i> , more extensive distribution in brain tumor, longest median survival time in treated mice and inhibiting growth of glioma	[58]
	<i>In vitro/ In vivo</i>	C6 glioma cells	cRGD and histidine-rich TH peptide	paclitaxe	Greater affinity for integrin $\alpha v \beta 3$ , more extensive abilities for transferring liposomes across the BBB, improving therapeutic efficacy in brain glioma-bearing animals	[60]
Glucose	<i>In vitro/ In vivo</i>	BCECs	Glucose	No	Greater potential for brain targeting and strongest brain delivery efficacy.	[63]
	<i>In vitro/ In vivo</i>	BCECs	Glucose	No	Greater potential for transport of drug across the BBB, increased accumulation of drug in the brain	[64]
	<i>In vitro/ In vivo</i>	C6 cells	Glucose-vitamin C	Paclitaxel	Greater <i>in vivo</i> targeting ability, exhibiting maximum accumulation of drug-loaded liposomes at tumor sites	[61]
	<i>In vitro/ In vivo</i>	C6 glioma cells	MAN <sup>11</sup>	No	Greater cellular uptake, promoting penetration through the BBB into the brain, accumulation in the intracerebral regions	[70, 71]
	<i>In vitro/ In vivo</i>	C6 glioma cells	MAN/ TF	Daunorubicin	More extensive cellular uptake, greater median survival time	[69]

	<i>In vitro/ In vivo</i>	b.End.3 cell	MAN- RGV <sup>12</sup> , MAN- Pen <sup>13</sup>	ApoE2 encoding plasmid DNA (pApoE2)	Improved transport and transfection of ApoE2 gene across the <i>in vitro</i> and <i>in vivo</i> BBB model	[73]
	<i>In vitro/ In vivo</i>	b.End.3 cell, primary glial, primary neuronal cells	MAN-RGV, MAN- Pen	BDNF <sup>14</sup> encoding plasmid (pBDNF)	Higher transfection efficacy, more extensive cellular uptake	[72]
Immunoliposome	<i>In vivo</i>	-	OX26 mAb	Daunomycin	Delivery of daunomycin to the rat brain	[74]
	<i>In vitro</i>	BCECs	OX26 mAb	No	Enhanced ability of binding to BCECs, increasing immunoliposome accumulation in the BCECs	[77]
	<i>In vivo</i>	-	OX26 mAb	Dopamine	Increased cellular uptake, increased delivery of liposome to brain tissue	[78]
	<i>In vitro</i>	hCMEC/D3	OX26-LB509 Ab	EGCG <sup>15</sup>	Increased transport of drugs across the BBB	[79]
	<i>In vitro</i>	hCMEC/D3	MYBE/4C1	Doxorubicin	Increased passage of doxorubicin-loaded liposome cross an <i>in vitro</i> BBB model	[80]
	<i>In vitro/ In vivo</i>	U87	CD133- angiopep-2	Temozolomide	Increased <i>in vitro</i> cytotoxicity and apoptosis, Exhibiting smaller tumor size, and higher median survival time	[82]
	<i>In vitro/ In vivo</i>	hCMEC/D3, U87-MG cells	R17217 mAb	Docetaxel	Enhanced <i>in vitro</i> cellular uptake, increased <i>in vitro</i> penetration across BBB model, improved <i>in vivo</i> brain targeting, enhanced the efficacy of drug delivery in animal model	[76]
Cationic liposome	<i>In vitro/ In vivo</i>	BCECs, C6	lactoferrin	Doxorubicin	Improved cellular uptake, inhibiting the growth of C6 <i>in vitro</i> and enhancing survival time <i>in vivo</i> animal models	[85, 89]
	<i>In vitro</i>	b.End.3 cell	MAN/ RGV/Pen	No	Fusogenic liposomes enhanced cytoplasmic delivery of cargos and reduced endocytosis	[73]

<sup>1</sup> brain capillary endothelial cells (bEnd.3), <sup>2</sup>transferrin (Tf), <sup>3</sup> poly-L-arginine (PR), <sup>4</sup>T7 peptide (HAIYPRH), <sup>5</sup>folic acid (FA), <sup>6</sup> a safe and humanized blood–brain barrier penetrating peptide, <sup>7</sup>arginine-glycine-aspartic acid (RGD), <sup>8</sup>human umbilical vein endothelial cells (HUVECs), <sup>9</sup> p-Hydroxybenzoic Acid (pHA), <sup>10</sup>brain capillary endothelial cells, <sup>11</sup>P-aminophenyl- $\alpha$ -d-mannopyranoside (MAN), <sup>12</sup>rabies virus glycoprotein peptide (RGV), <sup>13</sup>penetratin (Pen), <sup>14</sup> Brain-derived neurotrophic factor (BDNF), <sup>15</sup>flavonoid epigallocatechin-3-gallate (EGCG),