



Nanomedicine for targeting brain Neurodegeneration: Critical barriers and circadian rhythm Considerations[☆]

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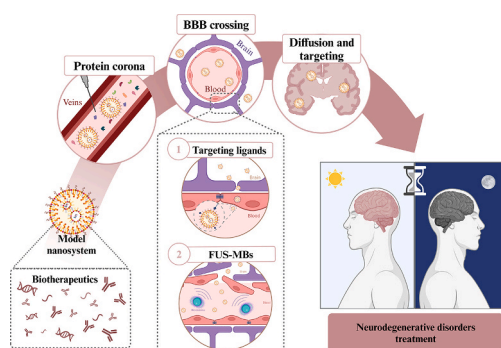
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HIGHLIGHTS

- Nanomedicine holds potential to improve the targeted delivery of biotherapeutics to the brain.
- Biotherapeutics are becoming key treatments for neurodegenerative diseases, such as Alzheimer's disease.
- Tailored nanosystems represent an emerging solution to overcome the biological barriers that hinder brain delivery.
- Surface modifications and transient disruption of the blood-brain barrier are being explored to enhance brain delivery.
- Integrating circadian rhythms with nanotechnology offers a novel approach to optimizing brain-targeted therapies.

GRAPHICAL ABSTRACT



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ABSTRACT

The development of novel therapies for central nervous system (CNS) diseases, particularly neurodegenerative disorders like Alzheimer's disease (AD), is a critical global health priority. Biotherapeutics, such as monoclonal antibodies (mAbs) and RNA-based therapies, have shown potential for treating brain disorders. However, their clinical progress is limited by their difficult access to their brain targets. At the preclinical level, nanotechnology has been shown, to help these molecules overcome the biological barriers that impeded their adequate brain delivery. This review highlights advances in this area and the challenges for the translation to the clinic. Key nanotechnology-based strategies, such as surface modifications utilizing endogenous protein corona, functionalization with targeting ligands, therapeutic ultrasound-mediated microbubble oscillation were particularly analyzed. Additionally, in line with the focus of the Special Issue, this review integrates the concept of chronotherapy, with a focus on AD treatment, highlighting the idea that, by aligning nanoparticle (NP)-based drug delivery with circadian rhythms, it may be possible to improve therapeutic outcomes. Finally, the article analyzes

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current strategies in CNS drug delivery in clinical trials and provides future directions within this frame, notably in the area of AD.

1. Nanomedicine and circadian rhythms of Alzheimer's Disease

CNS diseases represent a significant and growing global health burden with their prevalence increasing due to factors like an aging population and lifestyle changes [1]. Among these disorders, AD is a leading neurodegenerative disorder and the primary cause of dementia, affecting approximately 50 million individuals worldwide [2]. Despite extensive research, advancements in CNS therapeutics remain limited, largely due to challenges in delivering treatments effectively to the brain.

In the context of this special issue, circadian rhythms in brain disorders [3–6], several studies have highlighted the importance of synchronizing drug delivery with circadian rhythms to improve therapeutic outcomes [7]. Building on the known capacity of nanotechnology to modulate the pharmacokinetics and biodistribution of drugs [8–10], it has been hypothesized that NPs could be engineered to synchronize with or exploit circadian rhythms, enhancing therapeutic precision. For example, functionalizing NPs with ligands targeting receptors regulated by circadian cycles could potentially improve specificity in neural tissues [11,12]. This conceptual approach offers interesting opportunities that deserve to be explored.

2. Biopharmaceutical drugs for brain diseases and the potential value of nanotechnology

Biopharmaceutical drugs, such as mAbs, have emerged as effective therapeutic modalities due to their high specificity and potency [13,14]. Notably, several mAbs have received FDA approval for the treatment of mild cognitive impairment or mild dementia associated with Alzheimer's disease, including aducanumab (Aduhelm®), lecanemab (Leqembi®), and donanemab (Kisunla™) [15,16].

Oligonucleotides and messenger RNA-based therapeutics are also gaining attention for treating brain diseases [17,18]. Currently, 10 FDA-approved and 10 in clinical trials oligonucleotides-based drugs are designed to treat CNS disorders [19,20]. In the area of mRNA, four

therapies have been approved for Duchenne muscular dystrophy (DMD) [21] and over 20 clinical trials are underway for CNS disorders, highlighting significant progress in the field [22]. However, challenges remain, as some of these drugs, such as the ASO Qalsody (tofersen), approved in 2023 for amyotrophic lateral sclerosis (ALS), still require intrathecal administration for effective delivery to the CNS [23].

Despite these advancements, there remains substantial room for improvement, particularly in enhancing drug delivery to the brain [24]. In this context, nanomedicine has emerged as a promising tool for the efficient delivery of both therapeutic and imaging agents to the CNS [25,26]. Moreover, nanocarriers can potentially be tailored to target specific brain regions, improving the efficacy of treatments for CNS diseases [14].

However, realizing the potential of nanomedicine for delivering biologics to the brain requires overcoming several challenges. In this review, we critically analyze the different strategies to address these barriers as illustrated in Fig. 1. Additionally, considering the recognized influence of circadian rhythms on the efficacy of specific brain therapies, we examine drug delivery approaches that integrate circadian biology. With this, we aim to contribute to the knowledge required for the rational design and development of nanosystems to deliver biotherapeutics for targeting brain diseases.

Recently, exosomes have also gained attention as promising candidates for targeted therapeutic delivery to the brain. While this is an attractive area of research, comprehensive reviews are already available on the use of exosomes for brain-targeted delivery [27,28].

3. Confronting biological barriers to reach the brain

Achieving effective delivery and targeting of nanocarriers to the brain via systemic administration remains a major challenge in nanomedicine. Although direct brain and cerebrospinal fluid injections can bypass the BBB, there remains a challenge in achieving sufficient diffusion across brain tissue to effectively reach targeted areas [29].

For the systemic administration, the primary biological barriers

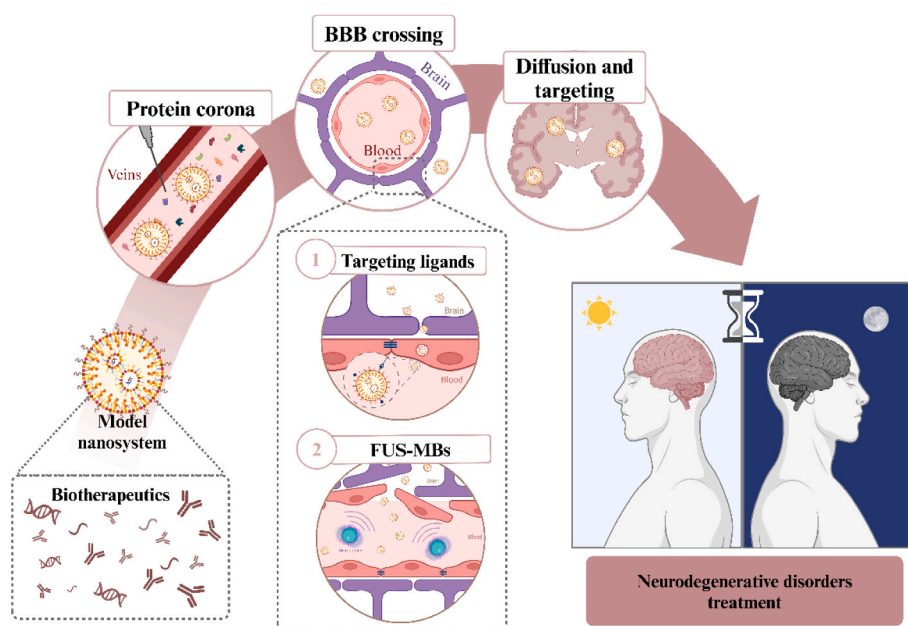


Fig. 1. Overview of the biological barriers that nanosystems need to overcome, including protein corona, BBB and brain parenchyma, to finally reach their target in the brain tissue after intravenous administration.

include: (i) interaction with blood proteins, which can alter their biological fate, (ii) transport across the BBB, (iii) diffusion through the brain parenchyma, and (iv) targeting to the appropriate brain regions and, ultimately, the specific cells.

3.1. Interaction of nanosystems with Plasma proteins

Following intravenous administration, nanocarriers interact with blood proteins, which attach to their surface, resulting in the formation of the so-called “protein corona”. This corona provides the nanocarriers with a “biological identity,” affecting their *in vivo* pharmacokinetics and biodistribution [30–32]. To mitigate this interaction, numerous strategies have been explored. Among them, the incorporation of polyethylene glycol (PEG) on the nanocarrier’s surface has been the most widely adopted [33,34]. In the case of polymeric nanocapsules (NCs), we have observed that the combination of different PEGylated materials with other hydrophilic polymers, i.e. poly- α -glutamic acid (PGA) or hyaluronic acid (HA) on the shell of the NCs, resulted in a reduction of the protein corona. These findings emphasize the importance of considering the protein corona in the rational design of nanocarriers [35,36].

Beyond PEGylation, biomimetic materials have been explored for controlling the protein corona by mimicking natural cell membranes [37]. For example, liposomes coated with exosomes derived from U87-MG cells exhibited a reduced amount of attached proteins, which was ultimately translated into an improved brain distribution in a U87-MG glioblastoma multiforme (GBM) mouse model [38]. Zwitterionic polymers have been also proposed as alternative materials [39,40]. For instance, hyperbranched polycarbonate (HPCB) micelles modified with zwitterionic carboxybetaine (CB) outperformed PEG-modified HPCB micelles in tumor targeting and penetration in an orthotopic GBM-bearing mouse model [41]. In the context of AD, treatment with poly-CB-based NPs was found to improve cognitive and memory function in AD transgenic mice compared to PEG-modified NPs [42], a result that may stimulate subsequent research in this area.

It has also been argued that these stealth molecules may serve as spacers to bind targeting ligands while reducing non-specific binding of serum proteins. However, results reported so far indicated that protein corona formation can still occur, potentially masking these ligands and compromising their interaction. This challenge is often overlooked in the design of targeted nanosystems, yet it has a significant impact on their biodistribution. For example, TAT peptide-modified nanocarriers, failed to achieve an efficient brain transport due to the formation of a protein corona [43]. Similarly, PEGylated silica NPs functionalized with transferrin suffered a significant reduction in their receptor-binding ability [44]. These examples underscore the critical need to consider the effects of the protein corona in the development of targeted NP systems.

Although the field has been largely focused on developing nanocarriers with low protein adsorption, an emerging approach involves the control of the protein corona by attracting the desired proteins [45–47]. For example, based on the expression of apolipoprotein (Apo) receptors on the surface of the BBB and glioma cells, researchers have investigated the deliberate attachment of Apo to the NP surface for decades [48]. For example, phosphatidylcholine (PC)-PLGA NPs engineered to enhance Apo binding, exhibited greater brain accumulation compared to non-modified PLGA NPs (based on DiD fluorescence intensity). This increased accumulation correlated with enhanced recruitment of specific apolipoproteins, including ApoA-I, ApoC-IV, and ApoE [49]. Similarly, liposomes functionalized with short amyloid-beta ($A\beta$) peptides that promote Apo-enriched corona formation increased brain accumulation 45-fold in a GBM mouse model [50]. PEG-PLA micelles functionalized with $A\beta$ peptides also showed higher Apo adsorption compared to non-modified PEG-PLA micelles, with ApoA-I and ApoE being the most abundant. This increased Apo content was associated with a significant reduction in tumor volume in an orthotopic glioma

model [51].

While these findings demonstrate the benefits of Apo adsorption for targeting, it is essential to consider the physiological roles of Apo in lipid and cholesterol metabolism [52]. This must be carefully considered, as their natural roles could influence nanocarriers biodistribution and pharmacokinetics.

Transferrin and transferrin-mimicking targeting ligands, such as antibodies and peptides, have also been widely used to modify the surface of various NPs taking advantage of the high expression of its receptor (TfR) on endothelial cells of the BBB [53]. Recent studies, however, have shifted toward designing NPs that naturally attract endogenous transferrin. In this context, PEGylated NPs modified with the peptide T10, which binds transferrin, achieved a threefold increase in brain uptake in an orthotopic GBM mice model. Nevertheless, the role of specific protein adsorption in these outcomes remains unclear, warranting cautious interpretation of this data [54].

Although it is critical to understand the consequences of protein corona formation in the blood circulation and how this biocorona can be tuned to improve brain accumulation, it is also important to gain an understanding of the processes beyond the BBB. This new information could provide valuable insights for enhancing NP design. In this regard, a combination of direct Stochastic Optical Reconstruction Microscopy (dSTORM) and proteomic analysis was used to investigate the evolution of the protein corona formed on the surface of lipid-based nanocarriers. It was observed that after the interaction with the BBB the components of the “soft corona” were modified with proteins involved in metabolism and transport, such as caveolin-2, becoming predominant [55]. All this underscores the complexity of designing nanosystems for effective BBB penetration and highlights the need to consider protein corona dynamics in future studies.

3.2. Understanding the BBB and defining ways to overcome it

3.2.1. The dynamic nature of the BBB

The BBB is a highly selective permeable membrane that regulates the exchange of materials between the blood and neural tissues [56]. It comprises an extensive network of vascular brain endothelial cells connected by tight and adherent junctions, regulated by astrocytes microglia, and pericytes, and structurally supported by a basement membrane (Fig. 2). This barrier impedes the passive transport of NPs from the bloodstream although some authors argue that very small NPs might have some chances to cross it [24,57].

Maintaining the integrity of the BBB is critical for CNS homeostasis, as its disruption can lead to neuroinflammation and neurodegeneration. Compromised BBB function is a hallmark of several high-incidence neurological disorders, including stroke, Alzheimer’s disease (AD), Parkinson’s disease (PD), amyotrophic lateral sclerosis (ALS), multiple sclerosis (MS), and Huntington’s disease (HD) [58,59].

The characteristics of BBB dysfunction in these neurodegenerative diseases include increased permeability, microbleeds, reduced expression of tight junctions, impaired transporter function, perivascular accumulation of blood-derived products, and degeneration of pericytes and endothelial cells [60]. In AD, for instance, BBB breakdown is evidenced by the accumulation of blood-derived proteins in the brain, and a reduction in the expression of key tight junction proteins [61]. Oxidative stress and inflammation further exacerbate BBB alteration by contributing to endothelial damage and junction degradation [62].

However, BBB breakdown extends beyond endothelial dysfunction, also involving pericyte degeneration, basement membrane alterations, and astrocytic end-feet detachment, which facilitates the infiltration of neurotoxins into the CNS [63]. In AD, vascular dysfunction and BBB breakdown precede amyloid-beta ($A\beta$) accumulation and neuronal injury [64]. Similarly, in PD, BBB alteration and neurovascular abnormalities are linked to the formation of Lewy Bodies inside neurons, and dopaminergic neurodegeneration [65,66], while in MS, the infiltration of immune cells across a compromised BBB is a defining pathological

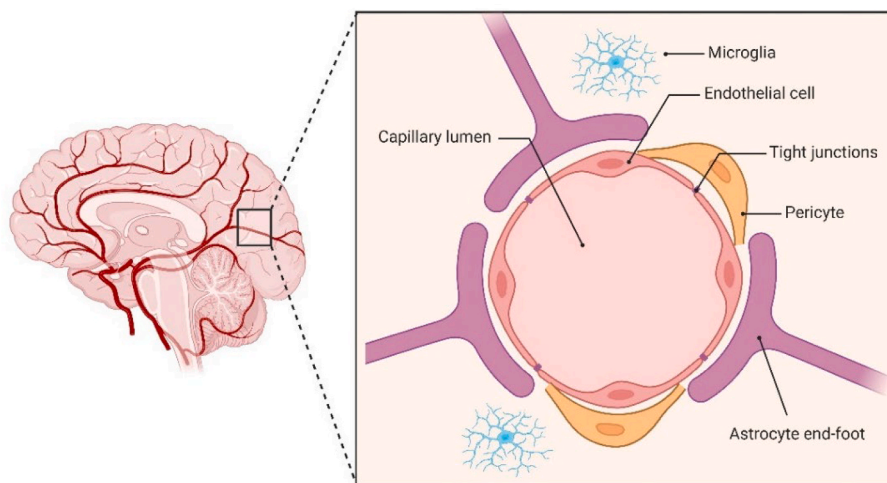


Fig. 2. Schematic representation of the Blood-brain barrier which comprises specialized brain endothelial cells, pericytes, capillary basement membrane, glial cells and astrocyte end-feet.

feature [67].

The alteration of BBB permeability has been suggested as an opportunity for delivering therapeutics. For instance, the increased permeability of the BBB surrounding tumor areas in GBM facilitates drug accumulation in the tumor compared to other brain regions [68]. However, due to alterations in neurovascular physiology and transport mechanisms, as described above, drugs may also be sequestered within pathologically altered brain regions, thus limiting their ability to reach target cells [58,60]. In this regard, even when the BBB is compromised and exhibits increased permeability, it remains a significant obstacle to efficient drug delivery into the brain. Therefore, understanding the regulation of the BBB is crucial for designing effective delivery strategies from the blood to the brain. Studies highlight the dynamic nature of the BBB and how circadian rhythms, and sleep disturbances influence its permeability [69]. Rhythmic BBB permeability and circadian regulation of brain interstitial space modulate molecular passage between the brain and vasculature. This dual influence not only contributes to neurological disease progression but also offers opportunities to optimize drug delivery [70]. In fact, evidence from animal and clinical studies indicates that the pharmacokinetics and effects of drugs can be significantly influenced by circadian timing [71]. Recent studies have shown that brain-targeted drugs should be administered at optimal circadian times to enhance BBB entry and retention, highlighting the importance of timing therapeutic interventions to improve drug delivery efficacy to the brain [72].

On the other hand, an interesting hypothesis proposes that restoring normal circadian rhythms may prevent, slow, or even halt the progression of neurodegenerative diseases [12]. An emerging related approach involves developing novel nanocarriers specifically designed to target key proteins or pathways that regulate circadian rhythms, thereby restoring their normal function. Preclinical studies with small molecules have already begun to explore this concept [73–75]. Hence, it could be envisaged that adequately delivered biological drugs could further enhance the therapeutic output of this strategy.

Despite these insights, the influence of chronobiology on NP uptake and transport into the brain has received little attention. However, this approach could be considered to achieve maximum drug efficacy by taking advantage of the BBB's varying permeability throughout the day.

Another important consideration is the notable difference in receptor expression in brain endothelial cells between a healthy and dysfunctional BBB [68,76].

3.2.2. Advances in functionalizing nanocarriers for efficient BBB transport

The primary strategy to overcome the BBB involves targeting specific receptors expressed on brain endothelial cells, facilitating the passage of biomolecules into the brain. Numerous ligands that selectively bind to these surface receptors have been identified, enabling the transport of nanosystems across the BBB (Table 1) [76–78]. This receptor-mediated transcytosis (RMT) mechanism encompasses the endocytosis of NPs, followed by their exocytosis into the brain parenchyma. Among these receptors, those most extensively studied include LDLR, TfR, nAChRs, scavenger receptor class B type 1, insulin receptor, lactoferrin receptor, and leptin receptor [79].

When attempting RMT, it is important to consider the difference in receptor expression in brain endothelial cells between a healthy and dysfunctional BBB [68,76]. For instance, in the particular case of AD, there is a down-regulation of LDLR and glucose transporter 1 (GLUT1) in the BBB epithelial cells, along with high expression of scavenger receptors A and B in brain endothelial cells, glial cells, and microglia. Another relevant example are acetylcholine receptors (nAChRs) which are upregulated on diseased neuronal cells in neurodegenerative disorders [79].

In addition, the circadian rhythm may play a crucial role in regulating the activity of key BBB transport mechanisms, including tight junctions, ATP-binding cassette (ABC) transporters, and RMT proteins. Understanding these dynamic interactions holds significant potential for optimizing drug delivery strategies [80]. In this regard, several studies have demonstrated circadian fluctuations in ABC transporter activity over a 24-hour period. Specifically, P-glycoprotein (P-gp), a key efflux transporter, exhibits rhythmic variations in its ability to transport substances from the brain back into the bloodstream. Higher efflux activity has been observed during the active phase in *Drosophila* [69], mice [81], and humans [80].

Similarly, leptin receptor follows a circadian pattern, with higher leptin influx into the brain during the active phase compared to the resting phase [82]. However, studies on BBB tight junctions have not yet demonstrated significant oscillations in isolated brain endothelium, despite robust circadian rhythms observed in other organs such as the intestine, heart, and adipose tissue [83]. Future research should explore whether RMT and other BBB processes are similarly regulated by the circadian clock, potentially unveiling new avenues for time-dependent therapeutic strategies.

Additionally, targeting ligands can be used not only to enhance transport across BBB through specific recognition by BBB receptors, but also to selectively target brain cells such as neurons or microglia. For

Table 1

Summary of some relevant nanosystems for brain delivery using targeting ligands.

Receptor	Targeting ligand	Nanocarrier	Degree of functionalization	Relevant outcomes	Disease	Ref	
TfR	T7 peptide	LNPs (protamine, chondroitin sulfate and DSPE-PEG)	5 % mol T7-PEG(2 k)-DSPE/ total PEG	Increased median survival (37 days) encapsulating siEGFR	Glioma	[85]	
		RBC-coated NPs (DSPE-PEG ₂₀₀₀)	4 % T7-PEG(2 k)-DSPE/NPs	Improved brain access in mice based on fluorescence intensity	Glioma	[86]	
	B6 peptide	PEG-PLA NPs	10 % w/w Mal-PEG-PLA/NPs	Enhanced fluorescence in mouse brain treated with B6-NP than with untargeted-NP	AD	[87]	
		HA NPs	3.85 % w/w B6/NPs	Alleviated neuroinflammation and reduced Aβ accumulation	AD	[88]	
	OX26 mAb	PEGylated chitosan NPs	—	Enhanced fluorescence in mouse brain	Cerebral ischemia	[89]	
		PEGylated liposomes	1.9 % w/w mAb/phospholipids	Increased brain drug levels and mean survival time in tumor-bearing rats.	GBM	[90]	
		PEGylated liposomes (DSPE-PEG2000)	1.8 % w/w mAb/phospholipids	Diminished tumor volumes and extended median survival time glioma-bearing rats	Glioma	[91]	
		poly-γ-glutamic acid (γ-PGA)-based NPs	—	Higher brain drug concentration, reduced cerebral infarction and promoted neuronal recovery in diabetic cerebral infarction model rats	Diabetes mellitus/cerebral infarction	[92]	
	LDLR	ANG peptide	PEG-PLGA NPs	8.5 % mol/mol ANG/PEG-PLGA	Increased brain uptake measured by fluorescence	GBM	[93]
			PEGylated nanomicelles	20 % mol/mol ANG/nanomicelles	Higher median survival time	GBM	[94]
PAMAM-PEG NPs			50 % mol/mol ANG/PAMAM	Median survival of ANG-NPs was 61 days compared to 30 days for NPs	GBM	[95]	
POEGMA-PDPA NPs			5 % w/w ANG/POEGMA-PDA	Access to deep brain tissues.	CNS diseases	[96]	
POEGMA-PDPA NPs			5 % w/w ANG/POEGMA-PDA	Improved drug (carnosine) efficacy	Stroke	[97]	
Apo E dipeptide		PEG – DTC – TM – PEI NPs	20 % mol/mol ApoE/NPs	Elevated BBB transcytosis and GBM accumulation	GBM	[98]	
		Methacrylate based PEGylated NPs	10 % mol/mol ApoE/NPs	Increased median survival of mice treated with ApoE-NPs	GBM	[99]	
nAChR		RVG-29 peptide	PAMAM-PEG NPs	12 % w/w RVG/PEG-PAMAM	Increased luciferase expression in the brain	CNS diseases	[100]
			PEG-PLGA NPs	4.3 % w/w RVG-PEG-PLGA/mPEG-PLGA	Increased <i>in vivo</i> fluorescence	PD	[101]
			Chitosan, pluronic F127)	50 % mol/mol RVG/chitosan	Increased brain access	CNS diseases	[102]
	Liposomes (mPEG(2 k)-DSPE)		3.5 % mol/mol RVG/liposome	Higher DiI-labeled liposomes in the striatum and nigra	PD	[103]	
	PLA-PEG-NPs		10 % w/w RVG/NPs	Stronger accumulation of Cy5-labeled NPs into the brain	AD	[104]	
Unknown	TGN peptide	PEG-PCL NPs	0.2 % w/w TGN/NPs	Increased DiR-loaded NPS in the brain tissue	GBM	[105]	
		PEG-PLA NPs	3:1 mol/mol Mal:TGN	Higher DiR-labeled NPs accumulation in the brain	AD	[106]	
	CGN peptide	PEG-PLA NPs	8 % mol/mol CGN/NPs	Increased DiR-loaded NPS in the brain tissue	AD	[107]	
		PEG-PDMAEMA NPs	50 % w/w CGN/NP	Reduction of amyloid plaques	AD	[108,109]	
HIR	Anti-HIR mAb	PLGA NPs	12–21 % w/w mAb/NCs	Enhanced <i>in vitro</i> BBB penetration	AD	[110]	
ICAM	Anti-ICAM mAb	Polystyrene NPs and PEGylated liposomes (DSPE-PEG2000)	>160 mAb per NPs (coupling efficiency: >80 %)	Intra-arterial administration significantly improved uptake in the inflamed brain compared to i.v. injection	inflamed brain	[111]	
PECAM	Anti-PECAM mAb	Lipid NPs (DMG-PEG2000, DSPE-PEG2000)	—	Increased brain delivery after intra-arterial injection into the carotid artery	inflamed brain	[112]	
VCAM	Anti-VCAM mAb	Liposomes (DSPE-PEG2000)	~50 mAbs/liposome	Enhanced fluorescence intensity in the cerebral vasculature of injured mice	intracerebral hemorrhage	[113]	
	Anti-VCAM mAb	Liposomes (DSPE-PEG2000)	~200 mAbs/liposome	Increased fluorescence signal in the brain vasculature of injured mice	inflamed cerebral vasculature	[114]	

Abbreviations: AD Alzheimer's Disease; ANG Angiopep-2; Apo E Apolipoprotein E; BBB Blood-Brain Barrier; CNS Central Nervous System; DOTAP 1,2-Dioleoyl-3-trimethylammonium-propane; DOPE 1,2-Dioleoyl-*sn*-glycero-3-phosphoethanolamine; DPPC 1,2-Dipalmitoyl-*sn*-glycero-3-phosphocholine; DSPE-PEG 1,2-Distearoyl-*sn*-glycero-3-phosphoethanolamine polyethylene glycol; GBM Glioblastoma Multiforme; HA Hyaluronic Acid; HIR Human Insulin Receptor; ICAM Intercellular Adhesion Molecule; LDLR Low-Density Lipoprotein Receptor; Mal Maleimide; mAb Monoclonal Antibody; NP Nanoparticle; PAMAM Polyamidoamine; PC Phosphorylcholine; PD Parkinson's Disease; PECAM Platelet Endothelial Cell Adhesion Molecule; PEG Polyethylene Glycol; PEG-PCL Poly(ethylene glycol)-block-poly(caprolactone); PEG – P(DTC – TMC) – PEI Poly(ethylene glycol)-b-poly(dithiolane trimethylene carbonate-co-trimethylene carbonate)-b-polyethylenimine triblock copolymer; PEG-PLA Polyethylene Glycol-Polylactic Acid; PDMAEMA Poly(dimethylaminoethyl methacrylate); PLA Polylactic Acid; PLGA Polylactic-co-glycolic Acid; PMPC-PDPA Poly[2-(methacryloyloxy)ethyl phosphorylcholine]-poly(2-diisopropylaminoethyl methacrylate); POEGMA-PDPA Poly[oligo(ethylene glycol) methacrylate]-poly(2-diisopropylaminoethyl methacrylate); RBC Red Blood Cell; RVG Rabies Virus Glycoprotein; siEGFR Small Interfering RNA against Epidermal Growth Factor Receptor; siRNA Small Interfering RNA; TfR Transferrin Receptor; VCAM Vascular Cell Adhesion Molecule.

instance, the Tet-1 peptide targets specifically neurons, while the CD11b antibody selectively binds to microglia cells [84]. Although this approach shows potential, it falls outside the scope of this particular section.

3.2.2.1. Peptide-Functionalized nanosystems. Although most nanocarrier functionalization efforts have not specifically aimed to transport biological drugs, we consider it important to briefly discuss some promising approaches utilizing peptide-targeting ligands, while recommending more extensive reviews on the subject [115–118]. The concept of BBB

targeting peptides was advanced by Pardridge et al. [119]. Since then, various peptides have been investigated for their potential to enhance nanocarrier transport across the BBB, among them T7 and B6 (derived from the binding domain of transferrin) [120], angiopep-2, rabies virus glycoprotein fragments (RVG) and ApoE peptides, among others.

TfR ligands have been extensively studied for decades, primarily in the contexts of GBM [121,122] and Parkinson's disease (PD) [123]. Despite this, the effectiveness of transferrin as a targeting ligand has been suboptimal, likely due to the widespread expression of Tf receptors in non-target organs such as the liver, spleen, and kidneys, leading to nonspecific uptake by these tissues [124]. To address this issue, a novel peptide, T7 (HAIYPRH), was designed to target a distinct recognition site on the TfR, thereby minimizing competition with endogenous transferrin [125–128]. T7-based targeting has been utilized in various glioma-targeted drug delivery systems, including T7-PEG-PLGA micelles (10 % w/w T7/PEG-PLGA) [129] and LPC NPs modified with DSPE-PEG-T7 (5 % molar ratio of total PEG) [85]. Similarly, red blood cell-membrane-coated NPs, composed of PEG-PLGA and DSPE-PEG(2 k)-T7 (at molar ratios from 0.5 % to 6 %), showed significant brain uptake in glioma-bearing mice [86]. Furthermore, the *retro-inverse* peptide T7 (^DT7), synthesized to enhance serum stability and binding affinity to TfR, presents a promising alternative, although its efficacy in nanocarrier transport remains unexplored [130–132].

Another TfR-targeting peptide, B6 (CGHKAKGPRK), developed via phage display, has shown potential in the treatment of AD in mice models [87,88,133]. For example, B6-PEG-PLA NPs loaded with the neuroprotective peptide NAP crossed the BBB in an AD mouse model through clathrin-mediated transcytosis [87]. Similarly, B6-NPs (composed of 3.85 % w/w B6/NPs) incorporating polyphenols and hyaluronic acid, demonstrated significant brain accumulation in mouse models [88]. Both T7 and B6 are short peptides exhibiting high specificity and low cytotoxicity, making them attractive alternatives to transferrin for functionalizing brain-targeted nanocarriers.

Angiopep-2 (ANG) (TFFYGGSRGKRNNFKTEEY), an LDLR-targeting peptide, has gained significant attention due to its favorable BBB transport properties [93–97,134–137]. ANG conjugated to paclitaxel advanced to Phase II clinical trials in 2014 for treating recurrent brain metastases in breast cancer (NCT02048059) [138] and entered phase III trials in 2023 (NCT03613181) [27]. Recent studies have focused on optimizing ANG density on NP surfaces to enhance BBB permeability. For instance, lipid nanocomposites composed of PC and ApoA-I, containing 5 wt% ANG, showed optimal penetration efficiency in an AD mouse model [139]. Another study evaluated ANG-ligand densities (0–12 % molar fraction) in poly[oligo(ethylene glycol)methacrylate]-poly [2(diisopropylamino methacrylate)] (POEGMA-PDPA) polyplexes and found that 1.2 % molar fraction of peptide-modified copolymers was the optimal functionalization [140]. Interestingly, three LDLRs, i.e., LDL receptor (LDLR) and LDLR-related proteins 1 and 2 (LRP1 and LRP2), are over-expressed on GBM cells, which renders LDLRs an ideal target for GBM therapy [98].

In comparative studies, polymeric NPs functionalized with either ANG or B6 peptides (each at 2 % molar density) showed differential uptake across normal, AD, and tumor BBB mouse models. ANG-functionalized NPs demonstrated enhanced uptake in healthy and tumor BBB models, while their uptake was reduced in the AD model, reflecting differences in receptor expression [134]. Other LDLR-targeting peptides, such as ApoE-derived peptides, have shown similar potential. For instance, **ApoE dipeptide** [(LRKLRKRL)₂] at 20 % mol density achieved superior accumulation in orthotopic GBM mice models compared to ANG-functionalized counterparts, likely due to ApoE's affinity for multiple receptors [98,99]. Another promising LDLR-targeting peptide derived from ApoE, **CN-105** (Ac-VSRRR-amide), is currently in Phase II clinical trials for improving outcomes in intracerebral hemorrhage (NCT03168581), acute supratentorial intracerebral hemorrhage, and postoperative cognitive decline (NCT03802396) [141,142].

Nicotinic acetylcholine receptors (nAChRs) also represent a viable

target for BBB transport, as they are overexpressed in AD, PD, and certain brain tumor models [143]. The **rabies virus glycoprotein (RVG)**, which mediates the neurotropism of the rabies virus, interacts with nAChRs [144]. The RVG-29 segment (YTIWMPENPRPGTPC-DIFTNSRGKRASNG), which includes key neuro-binding regions, demonstrates affinity not only for nAChRs but also for γ -aminobutyric acid (GABA)(b) receptors [145]. RVG-29 has thus gained attraction as a targeting ligand for functionalizing nanocarriers, including NPs (e.g., 12 % w/w RVG/PEG(3.4)-PAMAM) [100], NCs (50 % molar RVG/chitosan) [102], liposomes (3.5 % molar RVG/liposome) [103], and exosomes [146–149]. It has also shown utility in combination with gadolinium for MRI detection of amyloid plaques (10 % w/w RVG/NPs) [104] and for delivering siRNA to the brain, both with and without carriers [147,150]. RVG can specifically bind to the nAChR on neuronal cells thus enabling the crossing of the BBB and the entry into neuronal cells [151].

Additional peptides identified through phage display have also shown potential for AD treatment. For example, **TGN** (TGNKALHPHNG), identified by Li et al. [152], has exhibited good results in both AD models [106,152–154] and glioma models in mice and rats [105,155,156]. TGN-functionalized PEG-PLA NPs provided the highest *in vivo* uptake in an AD mouse model for 3:1 Mal-TGN molar ration (from 7:1 to 1:1 Mal-TGN) [106]. The *retro-inverse* isomer of TGN, known as CGN (d-CGNHPHLAKYNGT), has also been used to deliver drugs, including siRNA, to the brain [108,109]. Although the exact BBB transport mechanism for TGN and CGN remains unclear, the authors observed that lysosome-mediated internalization was implicated [107].

Fusion peptides represent a promising advancement, as they combine distinct functionalities to enhance BBB penetration and cellular targeting. For instance, Q. Guo et al. designed a fusion peptide (TPL) derived from TGN and Tet1, which facilitated superior BBB penetration and neuronal targeting when conjugated to PEG-PLA NPs (30 % w/w TPL-PEG-PLA/PEG-PLA) in AD model mice [157]. While current multifunctional strategies combine two peptides for a dual-target approach [94,128,135,154,158], peptide fusion technology represents a potential strategy for developing novel multi-functional ligands with enhanced effects.

In conclusion, this section has highlighted some promising peptide ligands targeting BBB receptors, including TfR, LDLR, and nAChR. Despite the promising preclinical data, no peptide-functionalized nanocarriers have yet reached clinical application, underscoring the need for continued research in this field.

3.2.2.2. Antibody-Functionalized nanosystems. The use of mAbs for BBB-targeting has gained substantial attention since the 1980 s due to their high receptor specificity and the potential for targeted modifications that minimize competition with endogenous ligands, a frequent limitation of peptide-based targeting ligands [91,159].

In this context, TfR has been a primary target for mAb-based BBB delivery, with mAbs engineered to bind distinct receptor domains to avoid interference with endogenous transferrin [91]. Anti-TfR fusion constructs with various therapeutic agents have shown significant promise for CNS treatments. For instance, an anti-TfR antibody conjugated to ASOs has demonstrated efficient BBB delivery and sustained RNA target knockdown in both mice and primates [160]. Clinical translation of this approach is underway, with several trials exploring anti-TfR mAbs-based BBB delivery systems. In AD, trontinemab, a “brain shuttle” that combines an anti-A β mAb with an anti-TfR-derived fragment, is currently in phase Ib/IIa trial (NCT04639050). Several companies have also advanced anti-TfR technologies to clinical stages, introducing novel fusion constructs. For example, anti-TfR molecules fused to therapeutic entities have reached clinical trials for a variety of disease indications, i.e. dementia and mucopolysaccharidosis II [161,162]. Collectively, these developments underscore the potential of anti-TfR mAb-based strategies to enhance BBB penetration, opening new avenues for CNS-targeted therapies, including AD.

Nanosystems functionalized with anti-TfR mAb, such as OX26, 8D3, and R17-217 [163], have shown promising results in rodents. For instance, Alonso and colleagues demonstrated that PEGylated chitosan nanospheres functionalized with OX26 mAb significantly enhanced BBB transport in mice [89]. Similarly, OX26-decorated PEGylated liposomes achieved improved drug delivery in glioma-bearing rats compared to untargeted counterparts [90,91]. In another study it was shown that poly- γ -glutamic acid (γ -PGA)-based NPs functionalized with OX26 mAb exhibited greater brain accumulation in diabetic cerebral infarction model rats, outperforming free drug administration [92]. However, the expression of TfR in off-target organs raises concerns about potential adverse effects, emphasizing the need for detailed biodistribution studies to evaluate these strategies fully [164].

From a translational perspective, SynerGene Therapeutics' SGT-53, a liposome encapsulating p53 cDNA and targeted with anti-TfR single-chain antibody fragments, has advanced to Phase II clinical trials for GBM treatment in combination with temozolomide (NCT02340156), highlighting the potential of this approach.

While studies involving anti-human insulin receptor (HIR) mAbs in nanosystems remain inconclusive [110], nanobodies targeting BBB receptors, such as TfR and HIR, have been preliminarily explored in rodents [165]. For example, nanobodies targeting the TfR, coupled with neurotensin, facilitated brain delivery via RMT, successfully inducing hypothermia in mice after peripheral administration [166]. This represents a promising, yet under-explored, avenue for future research.

Recent advances have also focused on targeting endothelial cell markers such as ICAM, PECAM, and VCAM to restore BBB function under pathological conditions, including stroke and traumatic brain injury [111]. Nanosystems functionalized with anti-ICAM and anti-PECAM antibodies demonstrated significant brain accumulation following intra-arterial administration in mice. However, intravenous administration resulted in preferential lung accumulation, emphasizing the critical role of the administration route [111,112]. VCAM-targeted liposomes achieved superior brain uptake in inflammatory mouse models compared to anti-TfR and anti-ICAM-modified liposomes [113,114]. Notably, biodistribution studies involving anti-CAM mAbs utilized nuclear imaging, providing highly reliable and quantitative findings [167].

However, the results presented in this section should be interpreted with caution, as most studies lack of accurate determination of the degree of functionalization and often rely on indirect estimations. For example, Marcos-Contreras et al. estimated functionalization by adding ^{125}I -labeled mAb at 10 % (mol:mol) [111], assuming similar conjugation behaviour to that of unlabeled antibodies, which could lead to over-estimation values, including reports of over 150 antibodies per NP [111,114]. Similarly, data on brain uptake is frequently limited or inconsistently reported.

In conclusion, mAbs, particularly anti-TfR mAbs, have shown significant potential in enhancing brain drug delivery. The emerging use of nanobodies may represent the next frontier in this field. Nonetheless, further research is essential to facilitate the clinical translation of functionalized nanocarriers.

3.2.2.3. Key pending Issues on functionalized nanosystems. Despite four decades of research on functionalized nanocarriers, progress has been limited by insufficient characterization, particularly regarding their degree of functionalization, which is critical for efficient targeting. Current findings suggest that increased ligand density does not necessarily correlate with improved brain accumulation [168]. Therefore, a thorough characterization and identification of optimal functionalization must be adapted for each specific nanocarrier [163,164,169].

Another pending issue is the one associated to *in vivo* studies, based on the use of fluorescent markers. In this sense, more reliable techniques, such as nuclear imaging and the quantification of drug concentrations in brain tissues, are required. With the data reported so far, it

is hard to determine the efficiency of BBB crossing.

Although chemical conjugation is the most common strategy for attaching targeting ligands to nanocarriers due to its strength and stability, it also presents certain challenges. Among these methods, maleimide-thiol conjugation is the most frequently used approach. However, its key limitation here is the instability of the bonds under physiological conditions, potentially leading to the detachment of ligands [170,171]. Furthermore, current conjugation techniques often neglect the specific positioning of cysteine residues, which are essential for functionalization. For instance, in the case of RVG-29, the ideal conjugation site involves a terminal cysteine, but other cysteine groups within the peptide may interfere with functionalization if not properly accounted for [100–104]. Similarly, ANG has been conjugated through its C-H terminal [94–96,135,137,172,173] or N-H terminal [136,139,174] side. Alternative methods, such as N-hydroxysuccinimide (NHS) ester chemistry, have also been explored for the conjugation of both peptides [149] and mAbs [114]. However, inconsistencies in conjugation strategies and insufficient characterization of the resulting functionalized nanocarriers have led to confusion in the literature, hindering progress in the field.

However, inconsistencies in conjugation strategies and insufficient characterization of the resulting functionalized nanocarriers have led to confusion in the literature, hindering progress in the field.

In conclusion, addressing these knowledge gaps is critical. The selection of appropriate targeting ligands for BBB crossing should also consider the specific characteristics of CNS diseases, as receptor expression varies with pathological conditions. For example, while nAChR is overexpressed in AD [79], LDLR is downregulated [76], making RVG a potentially more suitable ligand in such cases.

3.2.3. Transport based on Temporary disruption of the BBB

A classical approach to disrupting the BBB involves increasing osmotic pressure using mannitol [175]. In this regard the most successful results have been achieved through intra-arterial and intracerebroventricular (ICV) administration [176], although preliminary fluorescence data were also observed for miR-124a-loaded, RVG-functionalized PEI-PEG polyplexes [177].

Recently, potentially more effective approaches to open the BBB have emerged [178]. For example, the combination of focused ultrasound (FUS) with systemically administered microbubbles (MBs) has shown considerable potential to transiently open the BBB, even reaching clinical trials [179,180] (Fig. 3). This opening has been shown to facilitate the delivery of mRNA-loaded lipid NPs (LNPs) to the brain of healthy mice after systemic administration [181].

Similarly, siRNA targeting Smoothed (SMO), loaded into PLGA-based polymer-lipid hybrid NPs showed superior tumor accumulation and effective target protein reduction in a transgenic human medulloblastoma mouse model using FUS/MB technology [182]. In the context of gene therapy for PD, combining FUS with PEI-PEG NPs to deliver glial cell line-derived neurotrophic factor (GDNF)-pDNA to the striatum of PD rats resulted in an 11-fold increase in GDNF expression [183].

From a translational perspective, it is noteworthy that FUS technology has already been implemented in clinical trials for the delivery of drugs such as PTX and Bevacizumab for GBM, with several Phase II and Phase III clinical trials underway [NCT04528680, NCT04446416] [184,185].

Overall, these results highlight the potential of integrating physical methods, i.e. FUS/MB, and nanotechnology. Developing rational strategies for identifying the optimal combination will be crucial for advancing this field.

3.3. Diffusion through the brain parenchyma

Beyond the BBB, drug delivery systems must overcome another biological obstacle: the brain parenchyma [186]. The brain's extracellular matrix (ECM), composed primarily of collagen, proteoglycans, and

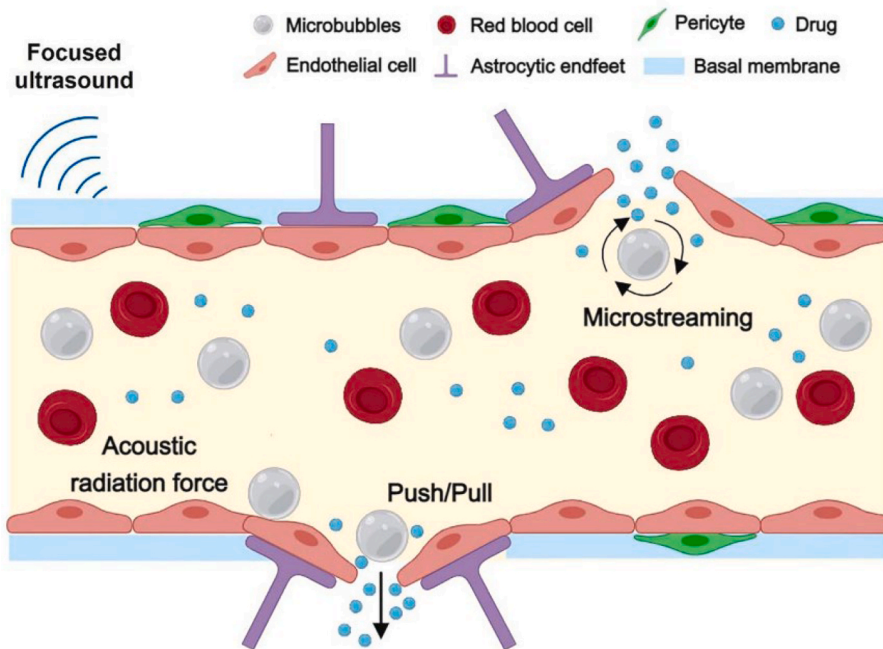


Fig. 3. Scheme of mechanisms involved in FUS/MBs-BBB opening. Three physical mechanisms (acoustic radiation force, push–pull mechanisms, and microstreaming), based on the stable cavitation effect, which might contribute to the opening of BBB [180]. Reproduced with Permission. Copyright® Elsevier (2024).

HA, forms a complex, densely organized barrier that limits the diffusion and migration of large molecules [187]. This structural complexity presents a major challenge for designing nanocarriers capable of widespread distribution across the brain parenchyma, a crucial requirement for effective CNS disease treatment.

In response, microimplants have emerged as a strategy to deliver controlled drug doses directly within the brain parenchyma [188]. A notable development in this area is the Microfluidic Neural Drug Delivery System (MiNDS), which has shown precise modulation of neuronal activity in rat and monkey models through the controlled infusion of artificial cerebrospinal fluid and muscimol. Remarkably, the precision of this controlled infusion was confirmed by injecting ^{64}Cu and assessing its distribution via Positron Emission Tomography (PET), which revealed accurate delivery with no evidence of backflow. This technology holds promise for precise therapeutic interventions in neurological disorders [189].

On the other hand, intrathecal administration enables the direct delivery of drugs into the cerebrospinal fluid (CSF) surrounding the brain and spinal cord. However, this route faces challenges in achieving adequate parenchymal drug uptake from the CSF [190]. To address this, recent efforts have focused on enhancing diffusion across the brain using nanosystems. For instance, oligonucleotide-iron oxide NPs delivered via ICV injection in mice showed, using MRI imaging, extensive brain diffusion [191]. Similarly, ICV administration of fluorescently labeled PEI/siRNA complexes in a mouse model of Parkinson's disease showed neuronal uptake across various brain regions. While these studies highlight the potential of nanosystems to achieve widespread distribution within the brain [192], such evidence is not clear, and their mechanism of diffusion was poorly understood.

To investigate the NP's transport through brain tissue, researchers have examined various local administration strategies. In this context, Hanes and colleagues explored the ability of PEGylated NPs to efficiently diffuse within the brain parenchyma [193]. The NPs were administered into the animal brain by convection-enhanced delivery (CED) a technique that infuses fluid under positive pressure directly into brain tissue [194]. The results showed that PEGylation of PLGA NPs improved NPs dispersion in rat and mouse brain sections, and that this effect was highly dependent on the PEGylation density [193]. This conclusion

explains the behavior of PTX-loaded PLGA-PEG NPs, which were found to diffuse across brain tumor tissue [195]. Similarly, the incorporation of poloxamers into PLGA NPs was reported to facilitate their brain diffusion by reducing interactions with the brain's ECM [196]. In another example, ^{18}F radiolabeled PEG-PLGA NPs of different sizes were evaluated by PET imaging after being administered in the rat striatum via CED. The results showed that smaller NPs (70 nm) gradually spread through the rat brain tissue, whereas larger NPs (150 nm) displayed an irregular distribution [197].

A variety of nanocarriers, including PBAE NPs [198–199], pDNA-polyethyleneimine (PEI) polyplexes [200], liposomes [201] and nano-emulsions (NEs), have been reported to benefit from their PEGylation in terms of enhancing their diffusion in different GBM mice and rat models [202].

The above studies emphasize the importance of non-adhesive surface coatings. However, it has been shown that the NP's composition and particle size are also critical aspects in designing nanosystems capable of traversing the brain's ECM. Alonso and colleagues developed ionizable NEs loaded with mRNA-GFP and stabilized with polysorbate 80 (P80), featuring a particle size of less than 100 nm and an oily core. After intraparenchymal administration, these NEs showed significant diffusion, reaching 3.4 mm from the injection site, as confirmed by GFP protein expression in neurons (Fig. 4) [203]. The combination of P80/DMG-PEG, small particle size, and the deformability provided by the oily core, likely contributed to the enhanced distribution of these nanosystems through the brain parenchyma.

Finally, recent investigations have focused on the capacity of FUS/MB technology to enhance brain diffusion. For example, the combination of FUS and MBs resulted in a 2-fold increase in the transfected area in the case of pDNA-based PEG-PEI NPs after intracranial administration in orthotopic GBM mice [204]. Additionally, cross-sectional analysis of mouse brains treated with Sorafenib NPs combined with FUS/MB technology showcased the enhanced capacity of these NPs to penetrate through the brain parenchyma [205].

4. Delivery of biotherapeutics to the brain

As highlighted in section 3, the use of biological drugs for treating

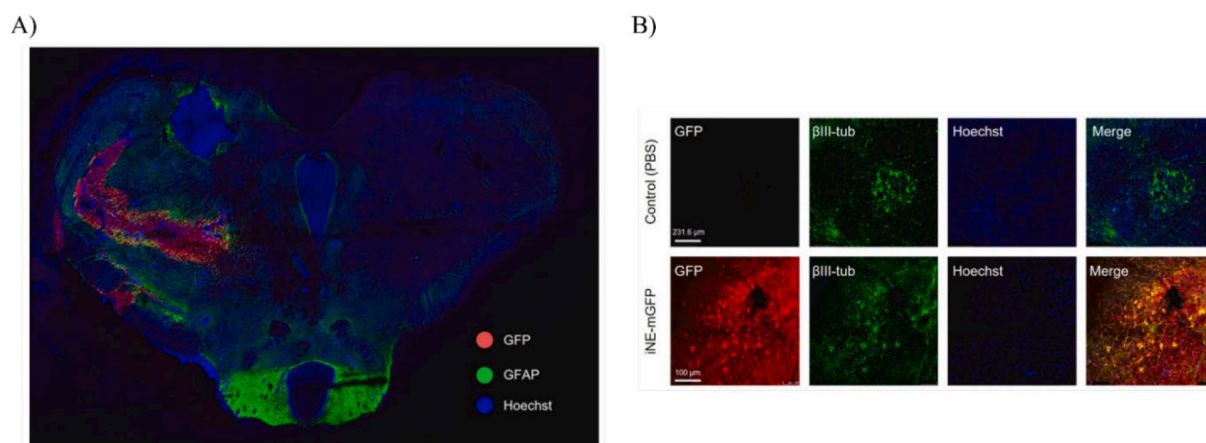


Fig. 4. A) Double immunolabelling in the whole brain for GFP (red) and astrocytes (labeled with GFAP, green) following the intraparenchymal administration of iNE-mGFP. Hoechst was used as nuclei marker. B) Immunolabelling for GFP in neurons (labeled with β III-tubulin) in rats after intraparenchymal administration of either the control (PBS) or iNE-mGFP. Hoechst was used as nuclei marker [203]. Reproduced with Permission. Copyright® Elsevier (2024). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

CNS diseases is highly restricted by barriers that limit their penetration and diffusion within the brain parenchyma [34]. This current section delves into the potential of various nanocarriers in delivering mAbs and RNA-based therapeutics for CNS disease treatment. In line with the focus of this Special Issue, particular emphasis will be given to strategies specifically designed for AD (Fig. 5).

4.1. Nanosystems-Mediated delivery of monoclonal antibodies for CNS diseases

The significant therapeutic potential of mAbs is illustrated by the number of mAb-based treatments marketed or under clinical trials [206]. In this context, CNS diseases, such as AD, are beginning to benefit

from the potential of mAb-based therapies. However, their efficient exploitation in this disease area is still limited due to the difficult access and retention into the brain tissue. Early-stage research in nanomedicine has started to deliver some promising data in terms of overcoming these difficulties.

4.1.1. Delivery of monoclonal antibodies for Alzheimer's disease

mAbs are gaining recognition as promising therapeutic agents for AD, with numerous clinical trials underscoring their potential to address this complex condition. Notably, the FDA recently approved three mAbs: aducanumab (Aduhelm®), lecanemab (Leqembi®), and donanemab (Kisunla™), for the treatment of mild cognitive impairment and early-stage dementia related to AD. These antibodies specifically target A β

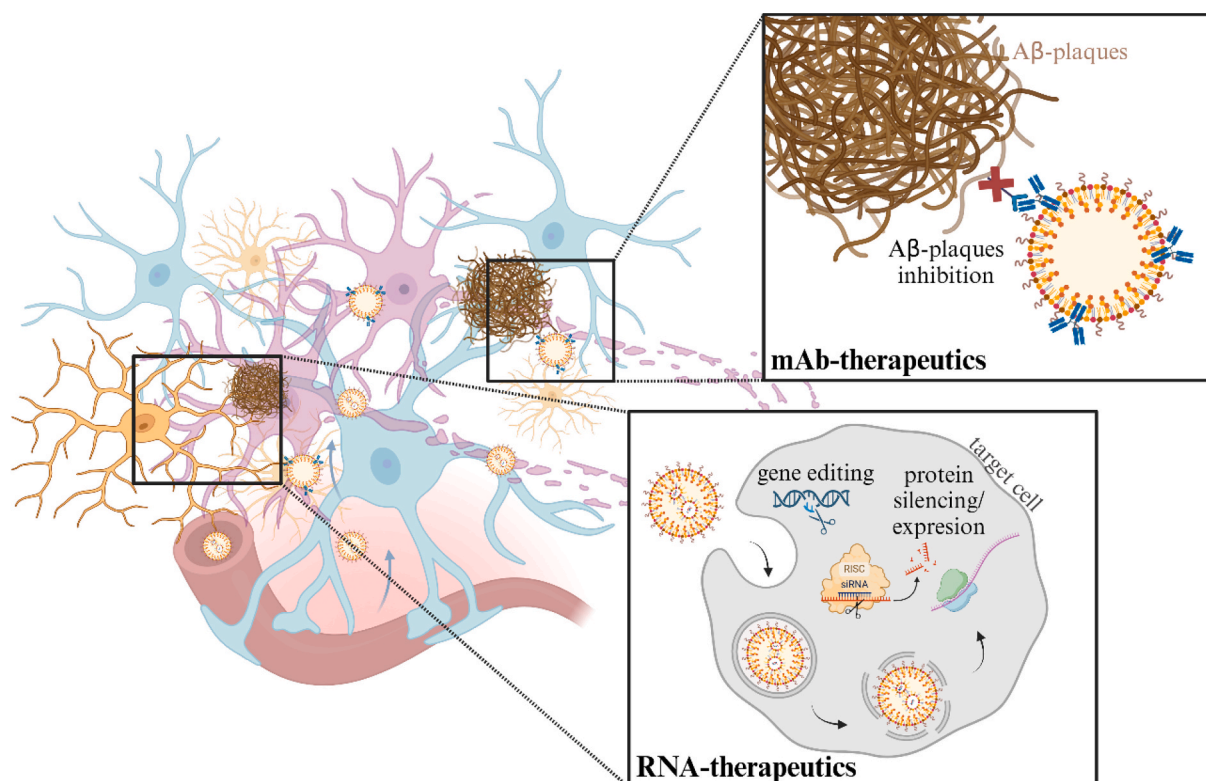


Fig. 5. Schematic representation of the mechanisms of nanosystems encapsulating biomolecules (mAbs and RNA) for AD treatment.

aggregates, a hallmark of AD pathology, with the aim of reducing A β plaque burden in the brain and decelerating cognitive decline [15].

In addition to A β -targeting antibodies, mAbs directed to tau protein, such as ADEL-Y01 (NCT06247345) and JNJ-63733657 (NCT04619420), are also undergoing clinical investigation, representing a broader therapeutic approach to AD by addressing the disease's tau-related neurodegeneration.

Despite these advances, the limited capacity of mAbs to cross the BBB remains an unsolved issue. This barrier likely contributes to the discontinuation of several mAbs in clinical trials, including solanezumab (NCT02008357), bapineuzumab (NCT00575055, NCT00574132), and gantenerumab (NCT05256134), each of which failed to demonstrate meaningful cognitive benefit [207].

This challenge is starting to be addressed through the use nanotechnology [14]. However, research in this area remains limited, with only a handful of studies exploring the synergistic effects of nanotechnology and mAb therapies (Table 2). Notably, mAb-derived fragments and nanobodies targeting A β with the capacity to inhibit aggregation and neurotoxicity of A β peptides, have been encapsulated in nanocarriers [15]. In addition to the poor capacity to permeate the BBB, mAb-derived fragments and nanobodies have a short blood circulation half-life, a limitation that could also be overcome through nanotechnology [208]. For instance, the encapsulation of 3D6-derived anti-A β fragments into glucose-functionalized PEG-PLL polymeric nanomicelles targeting the GLUT1 transporter extended their half-life from approximately 8 min to 1 h in healthy mice. This formulation led to a 42-fold increase in brain delivery in AD mouse models, achieving effective inhibition of A β aggregation with minimal peripheral tissue accumulation [208]. Similar nanomicelles loaded with 6H4-anti-A β derived fragments effectively reduced various toxic A β species in AD mouse models, although comprehensive biodistribution data were not provided [209]. Glutathione-targeted PEGylated liposomes, carrying the nanobody A β -binding (VHH-pa2H), were found to significantly increased brain uptake in transgenic mice, by targeting amyloid depositions [210].

In conclusion, while nanosystem-mediated delivery of mAbs and nanobodies could be promise for improving AD treatment, further research is required, specially from the perspective nanocarrier design, targeting strategies, and rigorous assessment of biodistribution and efficacy.

4.1.2. Delivery of monoclonal antibodies for other CNS diseases

While this review primary focuses on AD, examining brain-targeting strategies in other CNS disorders provides valuable insights. For example, in PD a transferrin-decorated liposomes loaded with fluorescent anti-alpha-synuclein mAb showed a 7-fold fluorescence increase in brain delivery, effectively reducing alpha-synuclein aggregation and decelerating disease progression in mouse models [123].

Brain tumors, particularly GBM, have also been a key target for mAb

delivery. For instance, phospholipid-based nanosystems utilizing the GLUT1 transporter provided substantial brain accumulation of anti-PD-L1, resulting in significant tumor growth inhibition in a non-small cell lung cancer brain metastasis mouse model [211]. Similarly, poly(2-methacryloyloxyethyl phosphorylcholine) (PMPC) NPs encapsulating fluorescent anti-PD-L1 showed a 20-fold improvement in BBB permeability and significant diffusion of the mAb in the tumor area, which was translated into an effective tumor growth suppression in an orthotopic glioma mouse model [212]. Similar results were observed for nimotuzumab in an orthotopic glioma xenograft mouse model [213]. In another study, rituximab-loaded PMPC NPs functionalized with CXCL13, which targets a chemokine receptor on B-cell lymphomas, was efficiently targeted to lymphoma growth areas in a xenograft murine model [214]. A different NP system consisting of (3-Acrylamidophenyl) boronic acid functionalized with GLUT1 was used to co-deliver anti-VEGFR-2 mAb and CPT1C siRNA. This combination treatment resulted in an improved brain tumor accumulation and reduced glioma growth in tumor-bearing nude mice [215].

Despite these advancements in the delivery of mAbs to the brain, challenges remain, particularly in ensuring adequate brain availability and sustained therapeutic efficacy of encapsulated mAbs. Addressing the limited brain penetration of mAbs will require continued exploration, with current evidence suggesting that the integration of nanotechnology with focused ultrasound might be a promising approach, as discussed in Section 3.2.3.

4.2. Delivery of RNA molecules to the brain

Since the approval of NP-based siRNA therapeutics such as Patisiran in 2018, alongside the introduction of various other drugs into the market and advanced clinical trials, this field has garnered substantial attention. mRNA-based therapeutics are now recognized as a highly versatile and scalable option for preventing and managing various diseases due to their ability to produce virtually any desired protein [216]. However, several challenges remain, particularly those associated with RNA delivery, which limit their effective application [217,218].

The field of RNA delivery to the brain is still in its infancy, with most studies relying on intrathecal or intracranial injection [219]. Intrathecal administration has already demonstrated clinical success in treating neurodegenerative diseases [220], exemplified by the FDA-approved drug Nusinersen for spinal muscular atrophy [221,222].

This section highlights recent studies focused on the development of innovative nanodrugs utilizing RNA molecules, with an emphasis on non-invasive delivery methods (Fig. 5).

4.2.1. RNA-based therapy for Alzheimer's disease

As previously mentioned, despite the severity of AD, the current arsenal of FDA-approved drugs is limited, and their efficacy questionable [223]. To unlock the therapeutic potential of siRNA drugs for AD, it is crucial to develop non-invasive, tissue-specific, and efficient delivery technologies (Table 3).

One major target for treating AD has been the β -site amyloid precursor protein (APP)-cleaving enzyme 1 (BACE1). However, despite early promise, several pharmaceutical companies have recently halted clinical trials for small-molecule BACE1 inhibitors due to limited efficacy and safety concerns [224]. This failure associated with small molecules opens the door for advanced therapies, particularly RNA interference (RNAi)-based therapeutics [225].

To date, various nanotechnologies have been explored for BACE1 silencing. For instance, Zheng et al. engineered hybrid PEG-Poly(2-(N,N-dimethylamino)ethyl methacrylate) (PDMAEMA) siRNA nano-complexes that were functionalized with BBB-targeting peptides CGN and QSH for A β binding. Following intravenous administration, this nanosystem achieved significant mRNA knockdown of BACE1, demonstrating a preference for targeting A β plaque-enriched hippocampal regions [108,109]. Additionally, CGN peptide was also used to

Table 2

Summary of some relevant nanosystems with mAb for AD treatment following intravenous administration.

Therapeutic molecule	Target	Nanosystem	BBB targeting molecule	Ref.
3D6-anti-A β derived fragments	A β	PEG-PLL polymeric nanomicelles	Glucose	[208]
6H4-anti-A β derived fragments	A β	PEG-PLL polymeric nanomicelles	Glucose	[209]
VHH-pa2H	A β	Liposomes (EYPC, cholesterol, DSPE-PEG)	Glutathione	[210]

Abbreviations: A β Amyloid beta; DSPE-PEG Distearoylphosphatidylethanolamine-polyethylene glycol; EYPC Egg yolk phosphatidylcholine; PEG-PLL Poly(ethylene glycol)-poly(L-lysine); VHH-pa2H Amyloid beta binding llama single domain antibody fragments.

Table 3

Summary of some relevant siRNA-based nanosystems for the treatment of AD following intravenous injection.

Therapeutic molecule	Target	Nanosystem	BBB targeting molecule	Ref.
siBACE1	BACE1	polymeric nanocomplexes (PEG-PDMAEMA, QSH peptide)	CGN	[108,109]
siBACE1	BACE1	polymeric nanocomplexes (PEG-PDMAEMA, Tet1 peptide)	CGN	[226]
siBACE1	BACE1	polymeric NPs (PEG- <i>b</i> -P(GuF), PEG- <i>b</i> -P(Gu))	Gal	[227]
miRNA-186	BACE1	PMLA/LLL nanoconjugates	D3	[228]
miRNA-124	BACE1, APP + Rutin	DNA nanoconjugates	RVG29	[230]
Cas9 mRNA/sgRNA	APP, TH	GSH-responsive NCs (TESPIC, BTPD, TEOS)	RVG, Glucose	[238]
siPU.1*	PU.1	LNPs (ionizable lipid cKK-E12, cholesterol, phospholipid, PEG-lipid)	–	[232]
miRNA-132**	GATA2 Rb1	PEG-PGA ENCPs	–	[239]

Abbreviations: * Intracisternal Delivery, ** Nose to Brain Delivery, APP Amyloid Precursor Protein, BACE1 Beta-Site Amyloid Precursor Protein Cleaving Enzyme 1, BTPD bis[3-(triethoxysilyl)propyl]-disulfide, ENCP Enveloped nanocomplexes, GATA2 GATA Binding Protein 2, Gal Galactose, GSH Glutathione, LLL Tri-leucin, LNPs Lipid Nanoparticles, NC Nanocapsule, NP Nanoparticle, PEG-PDMAEMA Polyethylene Glycol-Poly[2-(dimethylamino)ethyl methacrylate], PEG-PGA Polyethylene Glycol-Poly(glutamic acid), PEG-*b*-P(Gu) Poly(ethylene glycol)-*block*-poly[(*N*-(3-methacrylamidopropyl) guanidinium, PEG-*b*-P(GuF) Poly(ethylene glycol)-*block*-poly[(*N*-(3-methacrylamidopropyl) guanidinium-co-2,2,3,3-tetrafluoropropyl methacrylate], PLGA Poly(lactic-co-glycolic acid), PMLA Poly(β -L-malic acid), PU.1 Purine-rich Box 1, Rb1 Retinoblastoma 1, TH Tyrosine Hydroxylase, TEOS Tetraethyl orthosilicate, TESPIC *N*-(3-(triethoxysilyl)propyl)-1*H*-imidazole-4-carboxamide.

functionalize siRNA nanocarriers based on PEG-PDMAEMA modified with Tet1 peptide for neuron-specific binding. These nanocomplexes significantly reduced BACE1 mRNA levels and amyloid plaques while restoring cognitive performance in transgenic mice [226]. Similarly, galactose-modified PEG-methacrylate NPs designed for BACE1 targeting achieved 5.8-fold higher brain accumulation in AD mice compared to unmodified NPs, leveraging GLUT1-mediated BBB transport for enhanced delivery [227].

Conversely, miRNA-186 has been identified as a negative regulator of BACE1 expression in neuronal cells and is downregulated in AD. In a study by Israel et al., miRNA-186 was incorporated into poly(β -L-malic acid-trileucine) copolymer conjugates modified with D3 peptide. The delivery of conjugates to neurons in an AD model was confirmed by fluorescence imaging, RNA-seq, and PCR validation [228]. Another mechanistic approach involves miRNA-124, which modulates the expression of both BACE1 and the APP gene [229]. Following intravenous injection, fluorescent nanoconjugates of miRNA-124 functionalized with RVG-29 successfully crossed the BBB in AD mice. This approach, combined with the pharmacological effects of Rutin, significantly reduced the A β burden in the brain [230].

A newly identified target for AD treatment is the transcription factor PU.1, which is predominantly expressed in microglia located near A β plaques. Reducing PU.1 expression has been shown to exert protective effects in both humans and AD models [231]. Researchers have found

that PU.1 siRNA-LNPs could successfully reduce PU.1 levels in microglia-like cells after intracisternal administration. This reduction aligned with a decreased neuroinflammation in CK-p25 mice, which mimics the chronic neuroinflammation observed in AD patients [232].

Another potential target for AD is ApoE4 which is a well-established genetic risk factor for AD [233]. Interestingly, several studies showed the correlation between sleep disturbances and individuals with increased amount of ApoE4 [233–235]. Therefore, targeting ApoE4 in AD may also be beneficial for targeting circadian clock. While gene therapy approaches, such as the adeno-associated virus-mediated delivery of ApoE2 (e.g., LX1001), are currently in clinical trials [236], RNA-based therapeutics specifically targeting ApoE4 remain underexplored. Notably, one study demonstrated that intraventricular administration of ApoE4-targeting siRNA effectively reduced A β plaque burden in AD mice, highlighting its potential efficacy [237]. Further investigation is essential to fully evaluate the feasibility and therapeutic value of targeting ApoE4 with RNA-based strategies.

Gene editing strategies have also been explored in the context of AD. For example, glutathione (GSH)-responsive silica NCs conjugated with glucose and RVG have been designed for dual brain targeting, allowing them to effectively bypass the intact BBB in healthy mice. These NCs, loaded with Cas9 mRNA/sgRNA targeting the APP or TH genes, successfully reduced the expression levels of these genes in a healthy mouse model [238]. While this study demonstrates significant potential, further research is needed to establish whether such reductions can meaningfully contribute to disease control and therapeutic outcomes.

These examples underscore the exciting potential of exploring new therapeutic targets and delivery strategies for AD. Although this review does not cover nose-to-brain RNA delivery, extensively addressed in a recent review [223], notable progress has been made in this non-invasive route. For instance, Alonso and colleagues developed PEG-Polyglutamic acid nanocomplexes loaded with a miRNA-132 mimic, which successfully elevated miRNA-132 levels in the hippocampus of AD mice [239].

Although NP's-based RNA brain delivery has not reached yet clinical trials, tau-targeting antisense oligonucleotides, such as MAPT_{xx}, are currently in Phase 1 clinical trials, though they rely on intrathecal administration [240]. These examples highlight the significant progress being made in the field. However, further research is needed to optimize and expand the applicability of these delivery approaches.

Interestingly, despite the growing amount of LNP research in the field, numerous studies still focus on polymeric NPs in AD research, with various types of like PDMAEMA [241], chosen for its low toxicity and high transfection efficiency, and PMLA, known for being biocompatible and biodegradable [242]. While this section focuses not only on exploring NPs materials but also on novel AD targets, the next section, “RNA-based therapies for other CNS diseases,” will further investigate choice of materials in NP engineering.

4.2.2. RNA-based therapies for other CNS diseases

Although this section highlights RNA-based biotherapeutics for AD, research on GBM (Table 4) and other CNS diseases (Table 5) may also provide valuable insights for advancing AD treatment. These studies demonstrate the versatility of RNA-based technologies and open avenues for cross-disease innovation.

RNA delivery for AD and other CNS diseases often relies on polymeric NPs, with PEI being a common material. However, high-molecular-weight PEI (e.g., 25 kDa) is cytotoxic [243], leading to a shift toward branched low-molecular-weight PEI. For example, siRNA-loaded PEI NPs with 4–12 kDa branched PEI F25-LMW reduced α -synuclein mRNA expression by 65 % and α -synuclein protein levels by ~ 50 % in the striatum after ICV injection, with no observed toxicity in mice [192]. Similarly, iRGD-modified albumin NPs with PEI downregulated STAT3 in GBM, and, combined with ionizing radiation, induced tumor regression and prolonged survival in GBM-bearing mice [244].

Guanidinated polymers have also been used for siRNA delivery

Table 4

Summary of some relevant siRNA-based nanosystems for the treatment of brain cancer following intravenous injection.

Therapeutic molecule	Target	Nanosystem	BBB targeting molecule	Ref.
siSTAT3 + IR	STAT3	SPNPs (HSA, OEG macromer, PEI)	iRGD	[244]
siPLK1	PLK1	NCs (Acrylate guanidine, BAC, PEG)	ANG	[246]
siHDGF	HDGF	PSNPs (PLGA, PEI, DSPE-PEG- H7K(R2)2, lecithin)	H7K(R2)2	[252]
siRNA	Luciferase	PBAE NPs	—	[251]
Cas9/gPLK1 RNP	PLK1	Polymeric NPs (PEG-b-P(GuF), PEG-b-P(Gu))	ANG	[173]
Cas9/sgIGFBP5 RNP	IGFBP5	NCs (Acrylate guanidine, BAC, PEG)	ANG	[247]
siTWF1 + PTX*	TWF1	BRBP1-liposomes (DSPC, cholesterol, DSPE-PEG-BRBP1)	—	[259]
siPKM2 + TMZ	PKM2	MT NCs	ApoE	[99]

Abbreviations: * Brain Metastatic Breast Cancer, ANG Angiopep-2, ApoE Apolipoprotein E, BAC *N,N*-bis(acryloyl) cystamine, BRBP1 A brain metastatic breast carcinoma cell (231-BR)-binding peptide, DSPC 1,2-Distearoyl-*sn*-glycero-3-phosphorylcholine, DSPE-PEG 1, 2-Distearoyl-*sn*-glycero-3-phosphoethanolamine-Poly(ethylene glycol), HDGF Hepatoma-Derived Growth Factor, IGFBP5 Insulin-like Growth Factor Binding Protein 5, HAS Human Serum Albumin, IR Ionizing Radiation, MT Methacrylate-TMZ, NC Nanocapsule, NP Nanoparticle, PEI Polyethyleneimine, PEG Polyethylene glycol, PEG-b-P(Gu) Poly(ethylene glycol)-*block*-poly[(*N*-(3-methacrylamidopropyl) guanidinium, PEG-b-P(GuF) Poly(ethylene glycol)-*block*-poly[(*N*-(3-methacrylamidopropyl) guanidinium-co-2,2,3,3-tetrafluoropropyl methacrylate], PKM2 Pyruvate Kinase M2, PLK1 Polo-Like Kinase 1, PTX Paclitaxel, PSNP pH-sensitive self-assembled nanoparticles, RNP Ribonucleoprotein, STAT3 Signal Transducer and Activator of Transcription 3, SPNP Synthetic Protein Nanoparticle TMZ Temozolomide, TWF1 Twinfilin Actin Binding Protein 1.

[245]. For instance, ANG-guanidinium and fluorine-functionalized NPs delivered Cas9/gRNA RNPs targeting PLK1, extending survival in an orthotopic GBM mouse model [246]. These nanocarriers also enabled IGFBP5 gene editing, achieving BBB penetration, IGFBP5 modification, and prolonged survival in tumor-bearing mice [247]. However, their high positive charge may cause toxicity.

In addition to these positively charged polymers, biodegradable polymers, such as PBAE [248], PLGA [249], and PLA [250], have also been investigated for nucleic acid delivery with reduced toxicity. For example, PBAE-siRNA NPs demonstrated some brain penetration in a patient-derived GBM model, achieving a twofold knockdown of luciferase compared to scrambled RNA control NPs [251]. Additionally, hepatoma-derived growth factor (HDGF) was targeted using siHDGF-loaded PEG-PLGA NPs functionalized with the cell-penetrating peptide H7K(R2)2. In an orthotopic U251 GBM mouse model, this treatment reduced glioma volume and extended survival [252]. Moreover, researchers developed cationic lipid-assisted PEG-PLA NPs loaded with siRNA C3 and found them to be effective in an ischemia/reperfusion mouse model in decreasing C3 expression in microglia [253]. Unfortunately, authors do not disclose the potential deleterious effect of PLA degradation byproducts on the functionality of the polynucleotide.

Chitosan has also been used for gene delivery due to its cationic nature [254–256]. However, the transfection/silencing efficiency results reported for brain diseases has been rather low [257]. An exception could be glutathione-targeted chitosan polyplexes, which enhanced BBB crossing and transfection efficiency in a MS mice model. The authors

Table 5

Summary of some relevant siRNA-based nanosystems for the treatment of different CNS diseases following intravenous injection.

Disease	Therapeutic molecule	Target	Nanosystem	BBB targeting molecule	Ref.
Ischemic Stroke	mRNA encoding IL-10	M2 microglia	LNPs (Ionizable lipid AA3-Dlin, DMG-PEG, DOPE, cholesterol)	Mannose	[267]
I/R Injury	siC3	C3	LNPs (Cationic lipid BHEM-Cholesterol, PEG-PLA)	—	[253]
Multiple Sclerosis	miRNA-219	Plp1, ApoE, Cryab	Ch/TA polyplexes	Glutamine	[258]
Parkinson's disease	α -syn siRNA*	α -syn	PEI NPs	—	[192]

Abbreviations: * Intracerebroventricular Injection, α -syn α -synuclein, ApoE Apolipoprotein E, C3 Complement Component C3, Ch Chitosan, Cryab Crystallin Alpha B, DOPE 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine, DMG-PEG 1,2-dimyristoyl-*rac*-glycero-3-methoxypolyethylene glycol-2000, IL-10 Interleukin-10, I/R Ischemia/reperfusion, LNP Lipid nanoparticle, NP Nanoparticle, PEI – Polyethyleneimine, PEG-PLA Poly(ethylene glycol)-*block*-poly(lactic acid), Plp1 Proteolipid protein 1, TA Tragacanthic acid.

argued that this result is justified by the accumulation of fluorescent miRNA-219a-5p in the brain, likely due to active transport *via* NMDA and AMPA receptors [258].

Based on the recent success of LNPs, researchers have explored their interest in RNA delivery to the brain [260]. Here the use of ionizable lipids, plays a crucial role in RNA encapsulation and intracellular delivery [261]. For example, CRISPR-LNPs targeting PLK1 (sgPLK1-cLNPs) enabled up to ~ 70 % gene editing in aggressive orthotopic GBM models, inducing tumor cell apoptosis, inhibiting tumor growth by 50 %, and improving survival by 30 % [262]. Another pioneering study reported *in vivo* local siRNA therapy in GBM, where hyaluronan –LNPs efficiently delivered siRNA to glioblastoma cells by targeting CD44, a receptor overexpressed in GBM. These LNPs enhanced gene silencing, reduced tumor growth, and improved survival in preclinical models [263].

For less invasive intravenous injection researchers developed MK16 LNPs using a BBB-crossing lipid derived from MK-0752, resulting in around 15.3 % of NPs reaching the brain. In a GBM mouse model, MK16 LNPs loaded with mRNA encoding tumor suppressor protein Pten accumulated in tumors, reduced growth, and extended survival, with 70 % of mice surviving over 120 days [264]. Another study explored peptide-functionalized LNPs for BBB crossing and found that RVG29 LNPs outperformed Ang-2, T7, and mApoe-functionalized nanoparticles in delivering mCherry mRNA into the CNS [265].

Several studies have explored the use of LNPs for stroke therapy, demonstrating their potential for targeted mRNA delivery. VCAM-targeted LNPs were used to deliver IL-10 mRNA to the ischemic brain in a transient middle cerebral artery occlusion (tMCAO) mouse model, resulting in a 62.1 % reduction in infarct volume and 100 % survival [266]. Additionally, mRNA-loaded mannose-decorated LNPs encoding phenotype-switching IL- targeted brain microglia and facilitated IL-10 production upon intravenous administration in a transient MCAO mouse model [267].

Another promising avenue involves overcoming chemotherapy resistance in cancer treatment [268]. Liposomes modified with the BRBP1 peptide were used to deliver PTX and siRNA against twinfilin 1 (TWF1), enhancing PTX sensitivity in brain metastatic breast cancer cells. [259]. Another approach used siRNA against pyruvate kinase M2 (siPKM2) as the core and temozolomide as the shell, combining gene

silencing with direct chemotherapeutic action. This layered design, functionalized with ApoE for BBB targeting, effectively inhibited glioma growth and extended survival in tumor-bearing mice [99].

In conclusion, RNA-based biotherapeutics show considerable promise for the treatment of AD and other CNS diseases, as demonstrated by the diverse applications of RNA delivery in brain cancer, stroke, and chemotherapy resistance. Although many different materials have been tested in various studies, the engineering of the ideal nanovector for RNA delivery has not yet been achieved. The debate between LNPs and polymeric nanoparticles remains open, indicating that many researchers still tend to rely on more well-established materials in the field. Moreover, one of the greatest obstacles to treatment is identifying the right targets for the disease and determining the optimal level of gene silencing, gene editing, or protein enhancement required for a therapeutic effect.

5. Conclusions and future perspectives

Treating brain diseases has become a major focus of modern medicine, with biotherapeutics emerging as key players in CNS therapies. This progress is reflected in the over 80 mAbs and nearly 50 RNA-based brain therapies currently in clinical trials [269]. In the realm of neurodegenerative disorders, to date, there are 164 trials registered in the FDA investigating 127 drugs in the AD pipeline, 43 of which involve biotherapeutics [270]. While mAbs are the most widely studied biotherapeutics (20 trials), other strategies are advancing as well. Gene therapy approaches, such as RNAi with ALN-APP [NCT05231785] and ASO like BIIB080 [NCT05399888], are targeting the inhibition of AD-related protein expression. Additionally, vaccines are also under investigation, with the aim of slowing early-stage AD progression or even preventing it altogether. Notable candidates include ACI-24.060 [NCT05462106] and ALZ-101 [NCT05328115], which are designed to stimulate the brain's immune response against amyloid-beta protein. Therefore, the recent approval of the mAbs donanemab, lecanemab and aducanumab for AD [207], marks a significant advancement, offering renewed hope for effective treatments in this challenging therapeutic area. However, further research is crucial in this field, particularly in exploring the potential of CNS nanomedicine to enhance biotherapeutic delivery.

Nanomedicine has prioritized strategies to improve brain delivery of biotherapeutics through engineered delivery systems, often leveraging surface modification techniques like conjugating mAbs or peptides to NP' surfaces. Despite decades of research, key challenges persist. High molecular weight mAbs are complex to synthesize, difficult to standardize, and face high production costs. Peptides, while promising due to their specificity, face similar challenges, including rapid degradation. These limitations are reflected in the limited clinical translation of functionalized nanosystems, emphasizing the need for research into more stable, cost-effective ligands for active targeting that minimize nonspecific interactions with blood proteins. Furthermore, the wide variability in reported brain accumulation, where even successful studies show values ranging from 2 % to 25 % of the injected dose per gram of tissue (%ID/g) [102,107], underscores the urgent need for standardized and reliable quantification methods. Techniques such as nuclear imaging could provide more accurate and comparable data, enabling better evaluation of targeting efficiency across different studies and ligands.

Emerging strategies that integrate nanosystems with chronotherapy offer additional opportunities to refine drug delivery in alignment with circadian rhythms. This approach could enhance precision and BBB transport. However, no studies to date have targeted the circadian clock in the brain using biopharmaceutical drugs, representing an interesting area for future exploration.

Alternative approaches, such as physically altering the BBB using FUS in combination with MB, represent an interesting strategy. Several studies are in clinical trials, like NCT05469009 investigating its use

alongside aducanumab in AD patients. However, transient BBB openings may allow unintended substances to enter the brain, underscoring the need for further safety evaluations. Additionally, the long-term effects of repeated BBB disruptions remain uncertain. While the combination of FUS/MB with nanomedicine shows promise, most studies are still confined to preclinical models.

In conclusion, advances in biotherapeutics and nanomedicine are transforming the treatment of brain diseases. However, achieving future breakthroughs will require innovative research and the development of novel, integrated approaches to overcome current limitations.

Declaration of competing interest

From FY 2020 to the present, Dr. Robert Langer receives licensing fees (to patents in which he was an inventor on) from, invested in, consults (or was on Scientific Advisory Boards or Boards of Directors) for, lectured (and received a fee), or conducts sponsored research at MIT for which he was not paid for the following entities: https://www.dropbox.com/scl/fo/9hrpxuzs72iwwmoqze7rx/AA_OoThWVjAex7X_Lh_wLRcw?rlkey=llza5qcutubtyx34uqrrfy141&st=zcgqc4qp&dl=0. María José Alonso is founder and shareholder of LiberaBio. The rest of the authors declare no conflict of interest.

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Data availability

No data was used for the research described in the article.

References

- [1] M. Di Luca, D. Nutt, W. Oertel, P. Boyer, J. Jaarsma, F. Destrebecq, G. Esposito, V. Quoidbach, Towards earlier diagnosis and treatment of disorders of the brain, *Bull. World Health Organ.* 96 (2018), 298–298A. <https://doi.org/10.2471/BLT.17.206599>.
- [2] Alzheimer disease, *Nat Rev Dis Primers* 7 (2021) 34. <https://doi.org/10.1038/s41572-021-00275-0>.
- [3] Y. Leng, E.S. Musiek, K. Hu, F.P. Cappuccio, K. Yaffe, Association between circadian rhythms and neurodegenerative diseases, *Lancet Neurol.* 18 (2019) 307–318, [https://doi.org/10.1016/S1474-4422\(18\)30461-7](https://doi.org/10.1016/S1474-4422(18)30461-7).
- [4] K. Fife, A. Videnovic, Circadian and Sleep Dysfunctions in Neurodegenerative Disorders—An Update, *Front. Neurosci.* 14 (2021) 627330, <https://doi.org/10.3389/fnins.2020.627330>.
- [5] A. Videnovic, A.S. Lazar, R.A. Barker, S. Overeem, "The clocks that time us"—circadian rhythms in neurodegenerative disorders, *Nat. Rev. Neurol.* 10 (2014) 683–693, <https://doi.org/10.1038/nrneurol.2014.206>.
- [6] N. Gekakis, D. Staknis, H.B. Nguyen, F.C. Davis, L.D. Wilsbacher, D.P. King, J. S. Takahashi, C.J. Weitz, Role of the CLOCK protein in the mammalian circadian mechanism, *Science* 280 (1998) 1564–1569, <https://doi.org/10.1126/science.280.5369.1564>.

- [7] J.M. Selfridge, T. Gotoh, S. Schiffhauer, J.J. Liu, P.E. Stauffer, A. Li, D.G. S. Capelluto, C.V. Finkelstein, Chronotherapy: intuitive, sound, founded...but not broadly applied, *Drugs* 76 (2016) 1507–1521, <https://doi.org/10.1007/s40265-016-0646-4> FIGURES/3.
- [8] T.G. Dacoba, S. Anthiya, G. Berrecoso, I. Fernández-Mariño, C. Fernández-Varela, J. Crecente-Campo, D. Teijeiro-Osorio, F. Torres Andón, M.J. Alonso, Nanotechnology: a tortoise trail reaching new avenues, *Adv. Funct. Mater.* 31 (2021) 2009860, <https://doi.org/10.1002/adfm.202009860>.
- [9] A.M. López-Estévez, L. Sanjurjo, A. Turrero, I. Arriaga, N.G.A. Abrescia, A. Poveda, J. Jiménez-Barbero, A. Vidal, D. Torres, M.J. Alonso, Nanotechnology-assisted intracellular delivery of antibody as a precision therapy approach for KRAS-driven tumors, *J. Control. Release* 373 (2024) 277–292, <https://doi.org/10.1016/j.jconrel.2024.07.032>.
- [10] A.M. López-Estévez, Y. Zhang, M. Medel, I. Arriaga, L. Sanjurjo, C. Huck-Iriart, N. G.A. Abrescia, M.J. Vicent, D. Ouyang, D. Torres, M.J. Alonso, Engineering hyaluronic acid-based nanoassemblies for monoclonal antibody delivery – design, characterization, and biological insights, *Nano Res.* (2024) 1–15, <https://doi.org/10.1007/s12274-024-6826-8>.
- [11] C.T. Butler, A.M. Rodgers, A.M. Curtis, R.F. Donnelly, Chrono-tailored drug delivery systems: recent advances and future directions, *Drug Deliv. Transl. Res.* 14 (2024) 1756–1775, <https://doi.org/10.1007/s13346-024-01539-4>.
- [12] W. Ruan, X. Yuan, H.K. Eltzschig, Circadian rhythm as a therapeutic target, *Nat. Rev. Drug Discov.* 20 (2021) 287–307, <https://doi.org/10.1038/s41573-020-00109-w>.
- [13] S. Mitragotri, P.A. Burke, R. Langer, Overcoming the challenges in administering biopharmaceuticals: formulation and delivery strategies, *Nat. Rev. Drug Discovery* 14 (2015) 655–672, <https://doi.org/10.1038/nrd4363>.
- [14] M. Durán-Lobato, A.M. López-Estévez, A.S. Cordeiro, T.G. Dacoba, J. Crecente-Campo, D. Torres, M.J. Alonso, Nanotechnologies for the delivery of biologicals: Historical perspective and current landscape, *Adv. Drug Deliv. Rev.* 176 (2021) 113899, <https://doi.org/10.1016/j.addr.2021.113899>.
- [15] A. Barrera-Ocampo, Monoclonal antibodies and aptamers: The future therapeutics for Alzheimer's disease, *Acta Pharm. Sin. B* 14 (2024) 2795–2814, <https://doi.org/10.1016/j.apsb.2024.03.034>.
- [16] FDA approves treatment for adults with Alzheimer's disease | FDA, (n.d.). <https://www.fda.gov/drugs/news-events-human-drugs/fda-approves-treatment-adults-alzheimers-disease> (accessed July 22, 2024).
- [17] M.C.P. Mendonça, A. Kont, M.R. Aburto, J.F. Cryan, C.M. O'Driscoll, Advances in the design of (nano)formulations for delivery of antisense oligonucleotides and small interfering RNA: focus on the central nervous system, *Mol. Pharm.* 18 (2021) 1491–1506, <https://doi.org/10.1021/acs.molpharmaceut.0c01238>.
- [18] V. Di Francesco, A.J. Chua, D. Huang, A. D'Souza, A. Yang, B.S. Bleier, M. M. Amiji, RNA therapies for CNS diseases, *Adv. Drug Deliv. Rev.* 208 (2024) 115283, <https://doi.org/10.1016/j.addr.2024.115283>.
- [19] RNA Therapeutics - IQVIA, (n.d.). <https://www.iqvia.com/blogs/2023/11/rna-therapeutics-rewriting-the-script-of-medical-treatments> (accessed August 13, 2024).
- [20] Overview and Outlook for RNA-Based Therapies, (2024).
- [21] A. Holm, S.N. Hansen, H. Klitgaard, S. Kauppinen, Clinical advances of RNA therapeutics for treatment of neurological and neuromuscular diseases, (2022). <https://doi.org/10.1080/15476286.2022.2066334>.
- [22] A. Curreri, D. Sankholkar, S. Mitragotri, Z. Zhao, RNA therapeutics in the clinic, *Bioeng. Transl. Med.* 8 (2023) e10374, <https://doi.org/10.1002/btm2.10374>.
- [23] H.A. Blair, Tofersen: first approval, *Drugs* 83 (2023) 1039–1043, <https://doi.org/10.1007/s40265-023-01904-6>.
- [24] S. Krol, Challenges in drug delivery to the brain: nature is against us, *J. Control. Release* 164 (2012) 145–155, <https://doi.org/10.1016/j.jconrel.2012.04.044>.
- [25] M. Saeedi, M. Eslamifard, K. Khezri, S.M. Dizaj, Applications of nanotechnology in drug delivery to the central nervous system, *Biomed. Pharmacother.* 111 (2019) 666–675, <https://doi.org/10.1016/j.biopha.2018.12.133>.
- [26] J. Gao, Z. Xia, S. Gunasekar, C. Jiang, J.M. Karp, N. Joshi, Precision drug delivery to the central nervous system using engineered nanoparticles, *Nat. Rev. Mater.* (2024) 1–22, <https://doi.org/10.1038/s41578-024-00695-w>.
- [27] G.C. Terstappen, A.H. Meyer, R.D. Bell, W. Zhang, Strategies for delivering therapeutics across the blood–brain barrier, *Nat. Rev. Drug Discov.* 20 (2021) 362–383, <https://doi.org/10.1038/s41573-021-00139-y>.
- [28] A. Aryani, B. Denecke, Exosomes as a nanodelivery system: a key to the future of nanomedicine? *Mol. Neurobiol.* 53 (2016) 818–834, <https://doi.org/10.1007/s12035-014-9054-5>.
- [29] M.I. Alam, S. Beg, A. Samad, S. Baboota, K. Kohli, J. Ali, A. Ahuja, M. Akbar, Strategy for effective brain drug delivery, *Eur. J. Pharm. Sci.* 40 (2010) 385–403, <https://doi.org/10.1016/j.ejps.2010.05.003>.
- [30] B. Chertok, M.J. Webber, M.D. Succi, R. Langer, Drug Delivery Interfaces in the 21st Century: from science fiction ideas to viable technologies, *Mol. Pharm.* 10 (2013) 3531–3543, <https://doi.org/10.1021/mp4003283>.
- [31] N. Bertrand, P. Grenier, M. Mahmoudi, E.M. Lima, E.A. Appel, F. Dormont, J.-M. Lim, R. Karnik, R. Langer, O.C. Farokhzad, Mechanistic understanding of in vivo protein corona formation on polymeric nanoparticles and impact on pharmacokinetics, *Nat. Commun.* 8 (2017) 777, <https://doi.org/10.1038/s41467-017-00600-w>.
- [32] P. Jain, R. Pawar, R. Pandey, J. Madan, S. Pawar, P. Lakshmi, M. Sudheesh, In-vitro in-vivo correlation (IVIVC) in nanomedicine: Is protein corona the missing link?, (2017). <https://doi.org/10.1016/j.biotechadv.2017.08.003>.
- [33] A.M. López-Estévez, R. Gref, M.J. Alonso, A journey through the history of PEGylated drug delivery nanocarriers, *Drug Deliv. Transl. Res.* (2024), <https://doi.org/10.1007/s13346-024-01608-8>.
- [34] A.M. López-Estévez, P. Lapuhs, L. Pineiro-Alonso, M.J. Alonso, Personalized cancer nanomedicine: overcoming biological barriers for intracellular delivery of biopharmaceuticals, *Adv. Mater.* (2023), <https://doi.org/10.1002/adma.202309355>.
- [35] G. Berrecoso, J. Crecente-Campo, M.J. Alonso, Quantification of the actual composition of polymeric nanocapsules: a quality control analysis, *Drug Deliv. Transl. Res.* 12 (2022) 2865–2874, <https://doi.org/10.1007/s13346-022-01150-5>.
- [36] G. Berrecoso, S.B. Bravo, I. Arriaga, N. Abrescia, J. Crecente-Campo, M.J. Alonso, Controlling the protein corona of polymeric nanocapsules: effect of polymer shell on protein adsorption, *Drug Deliv. Transl. Res.* 14 (2024) 918–933, <https://doi.org/10.1007/s13346-023-01441-5>.
- [37] P. Dash, A.M. Piras, M. Dash, Cell membrane coated nanocarriers - an efficient biomimetic platform for targeted therapy, *J. Control. Release* 327 (2020) 546–570, <https://doi.org/10.1016/j.jconrel.2020.09.012>.
- [38] J.-Y. Wu, Y.-J. Li, J. Wang, X.-B. Hu, S. Huang, S. Luo, D.-X. Xiang, Multifunctional exosome-mimetics for targeted anti-glioblastoma therapy by manipulating protein corona, *J. Nanobiotechnol.* 19 (2021) 405, <https://doi.org/10.1186/s12951-021-01153-3>.
- [39] M. Debayle, E. Balloul, F. Dembele, X. Xu, M. Hanafi, F. Ribot, C. Monzel, M. Coppey, A. Fragola, M. Dahan, T. Pons, N. Lequeux, Zwitterionic polymer ligands: an ideal surface coating to totally suppress protein-nanoparticle corona formation? *Biomaterials* 219 (2019) 119357 <https://doi.org/10.1016/j.biomaterials.2019.119357>.
- [40] K.P. García, K. Zarschler, L. Barbaro, J.A. Barreto, W. O'Malley, L. Spiccia, H. Stephan, B. Graham, Zwitterionic-coated “stealth” nanoparticles for biomedical applications: Recent advances in countering biomolecular corona formation and uptake by the mononuclear phagocyte system, *Small* 10 (2014) 2516–2529, <https://doi.org/10.1002/smll.201303540>.
- [41] K. Wang, B. Zhao, Y. Ao, J. Zhu, C. Zhao, W. Wang, Y. Zou, D. Huang, Y. Zhong, W. Chen, H. Qian, Super-small zwitterionic micelles enable the improvement of blood-brain barrier crossing for efficient orthotopic glioblastoma combinational therapy, *J. Control. Release* 364 (2023) 261–271, <https://doi.org/10.1016/j.jconrel.2023.10.019>.
- [42] R. Liu, J. Yang, L. Liu, Z. Lu, Z. Shi, W. Ji, J. Shen, X. Zhang, An “Amyloid- β cleaner” for the treatment of Alzheimer's disease by normalizing microglial dysfunction, *Adv. Sci.* 7 (2020) 1901555, <https://doi.org/10.1002/advs.201901555>.
- [43] P.S.R. Naidu, N. Gavriel, C.G.G. Gray, C.A. Bartlett, L.M. Toomey, J. A. Kretzmair, D. Patalwala, T. McGonigle, E. Denham, C. Hee, D. Ho, N.L. Taylor, M. Norret, N.M. Smith, S.A. Dunlop, K.S. Iyer, M. Fitzgerald, Elucidating the inability of functionalized nanoparticles to cross the blood–brain barrier and target specific cells in vivo, *ACS Appl. Mater. Interfaces* 11 (2019) 22085–22095, <https://doi.org/10.1021/acsami.9b01356>.
- [44] A. Salvati, A.S. Pitek, M.P. Monopoli, K. Prapainop, F.B. Bombelli, D.R. Hristov, P. M. Kelly, C. Åberg, E. Mahon, K.A. Dawson, Transferrin-functionalized nanoparticles lose their targeting capabilities when a biomolecule corona adsorbs on the surface, *Nat. Nanotechnol.* 8 (2013) 137–143, <https://doi.org/10.1038/nnano.2012.237>.
- [45] W. Kim, N.K. Ly, Y. He, Y. Li, Z. Yuan, Y. Yeo, Protein corona: friend or foe? co-opting serum proteins for nanoparticle delivery, *Adv. Drug Deliv. Rev.* 192 (2023) 114635, <https://doi.org/10.1016/j.addr.2022.114635>.
- [46] A. Salvati, The biomolecular corona of nanomedicines: effects on nanomedicine outcomes and emerging opportunities, *Curr. Opin. Biotechnol.* 87 (2024) 103101, <https://doi.org/10.1016/j.copbio.2024.103101>.
- [47] K. Jiang, Y. Yu, W. Qiu, K. Tian, Z. Guo, J. Qian, H. Lu, C. Zhan, Protein corona on brain targeted nanocarriers: challenges and prospects, *Adv. Drug Deliv. Rev.* 202 (2023) 115114, <https://doi.org/10.1016/j.addr.2023.115114>.
- [48] S. Wohlfart, S. Gelperina, J. Kreuter, Transport of drugs across the blood–brain barrier by nanoparticles, *J. Control. Release* 161 (2012) 264–273, <https://doi.org/10.1016/j.jconrel.2011.08.017>.
- [49] Y. Zhang, W. Xiao, S. He, X. Xia, W. Yang, Z. Yang, H. Hu, Y. Wang, X. Wang, H. Li, Y. Huang, H. Gao, Lipid-mediated protein corona regulation with increased apolipoprotein A-I recruitment for glioma targeting, *J. Control. Release* 368 (2024) 42–51, <https://doi.org/10.1016/j.jconrel.2024.02.020>.
- [50] Z. Zhang, J. Guan, Z. Jiang, Y. Yang, J. Liu, W. Hua, Y. Mao, C. Li, W. Lu, J. Qian, C. Zhan, Brain-targeted drug delivery by manipulating protein corona functions, *Nat. Commun.* 10 (2019) 3561, <https://doi.org/10.1038/s41467-019-11593-z>.
- [51] Z.-A. Zhang, X. Xin, C. Liu, Y. Liu, H.-X. Duan, L. Qi, Y.-Y. Zhang, H. Zhao, L.-Q. Chen, M.-J. Jin, Z.-G. Gao, W. Huang, Novel brain-targeted nanomicelles for anti-glioma therapy mediated by the ApoE-enriched protein corona in vivo, *J. Nanobiotechnol.* 19 (2021) 453, <https://doi.org/10.1186/s12951-021-01097-8>.
- [52] C.-C. Liu, T. Kanekiyo, H. Xu, G. Bu, Apolipoprotein E and Alzheimer disease: risk, mechanisms and therapy, *Nat. Rev. Neurol.* 9 (2013) 106–118, <https://doi.org/10.1038/nrneuro.2012.263>.
- [53] T.R. Daniels, E. Bernabeu, J.A. Rodríguez, S. Patel, M. Kozman, D.A. Chiappetta, E. Holler, J.Y. Ljubimova, G. Helguera, M.L. Penichet, The transferrin receptor and the targeted delivery of therapeutic agents against cancer, *Biochim. Biophys. Acta Gen. Subj.* 1820 (2012) 291–317, <https://doi.org/10.1016/j.bbagen.2011.07.016>.
- [54] T. Huo, Y. Yang, M. Qian, H. Jiang, Y. Du, X. Zhang, Y. Xie, R. Huang, Versatile hollow COF nanospheres via manipulating transferrin corona for precise glioma-targeted drug delivery, *Biomaterials* 260 (2020) 120305, <https://doi.org/10.1016/j.biomaterials.2020.120305>.

- [55] M. Battaglini, N. Feiner, C. Tapeinos, D. De Pasquale, C. Pucci, A. Marino, M. Bartolucci, A. Petretto, L. Albertazzi, G. Ciofani, Combining confocal microscopy, dSTORM, and mass spectroscopy to unveil the evolution of the protein corona associated with nanostructured lipid carriers during blood-brain barrier crossing, *Nanoscale* 14 (2022) 13292–13307, <https://doi.org/10.1039/D2NR00484D>.
- [56] N.J. Abbott, Blood-brain barrier structure and function and the challenges for CNS drug delivery, *J. Inher. Metab. Dis.* 36 (2013) 437–449, <https://doi.org/10.1007/s10545-013-9608-0>.
- [57] P. Henrich-Noack, D. Nikitovic, M. Neagu, A.O. Docea, A.B. Engin, S. Gelperina, M. Shilman, P. Mitsias, G. Tzanakakis, I. Gozes, A. Tsatsakis, The blood-brain barrier and beyond: Nano-based neuropharmacology and the role of extracellular matrix, *Nanomedicine* 17 (2019) 359–379, <https://doi.org/10.1016/j.nano.2019.01.016>.
- [58] C. Saraiva, C. Praça, R. Ferreira, T. Santos, L. Ferreira, L. Bernardino, Nanoparticle-mediated brain drug delivery: Overcoming blood-brain barrier to treat neurodegenerative diseases, *J. Control. Release* 235 (2016) 34–47, <https://doi.org/10.1016/j.jconrel.2016.05.044>.
- [59] E.G. Knox, M.R. Aburto, G. Clarke, J.F. Cryan, C.M. O'Driscoll, The blood-brain barrier in aging and neurodegeneration, *Mol. Psychiatry* 27 (2022) 2659–2673, <https://doi.org/10.1038/s41380-022-01511-z>.
- [60] Z. Huang, L.-W. Wong, Y. Su, X. Huang, N. Wang, H. Chen, C. Yi, Blood-brain barrier integrity in the pathogenesis of Alzheimer's disease, *Front. Neuroendocrinol.* 59 (2020) 100857, <https://doi.org/10.1016/j.ymfe.2020.100857>.
- [61] Y. Pan, J.A. Nicolazzo, Impact of aging, Alzheimer's disease and Parkinson's disease on the blood-brain barrier transport of therapeutics, *Adv. Drug Deliv. Rev.* 135 (2018) 62–74, <https://doi.org/10.1016/j.addr.2018.04.009>.
- [62] B. Obermeier, R. Daneman, R.M. Ransohoff, Development, maintenance and disruption of the blood-brain barrier, *Nat. Med.* 19 (2013) 1584–1596, <https://doi.org/10.1038/nm.3407>.
- [63] M.D. Sweeney, A.P. Sagare, B.V. Zlokovic, Blood-brain barrier breakdown in Alzheimer disease and other neurodegenerative disorders, *Nat. Rev. Neurol.* 14 (2018) 133–150, <https://doi.org/10.1038/nrneurol.2017.188>.
- [64] J.J. Lochhead, J. Yang, P.T. Ronaldson, T.P. Davis, Structure function, and regulation of the blood-brain barrier tight junction in central nervous system disorders, *Front. Physiol.* 11 (2020) 558491, <https://doi.org/10.3389/fphys.2020.00914>.
- [65] S.A. Jagmag, N. Tripathi, S.D. Shukla, S. Maiti, S. Khurana, Evaluation of models of parkinson's disease, *Front. Neurosci.* 9 (2016) 172363, <https://doi.org/10.3389/fnins.2015.00503>.
- [66] A.L. Bartels, A.T.M. Willemsen, R. Kortekaas, B.M. de Jong, R. de Vries, O. de Klerk, J.C.H. van Oostrom, A. Portman, K.L. Leenders, Decreased blood-brain barrier P-glycoprotein function in the progression of Parkinson's disease, PSP and MSA, *J. Neural Transm.* 115 (2008) 1001–1009, <https://doi.org/10.1007/s00702-008-0030-y>.
- [67] J. Niu, H.-H. Tsai, K.K. Hoi, N. Huang, G. Yu, K. Kim, S.E. Baranzini, L. Xiao, J. R. Chan, S.P.J. Fancy, Aberrant oligodendroglial-vascular interactions disrupt the blood-brain barrier, triggering CNS inflammation, *Nat. Neurosci.* 22 (2019) 709–718, <https://doi.org/10.1038/s41593-019-0369-4>.
- [68] S. Zha, H. Liu, H. Li, H. Li, K.L. Wong, A.H. All, Functionalized nanomaterials capable of crossing the blood-brain barrier, *ACS Nano* 18 (2024) 1820–1845, https://doi.org/10.1021/ACS.NANO.3C10674/ASSET/IMAGES/LARGE/NN3C10674_0011.JPEG.
- [69] S.L. Zhang, N.F. Lahens, Z. Yue, D.M. Arnold, P.P. Pakstis, J.E. Schwarz, A. Sehgal, A circadian clock regulates efflux by the blood-brain barrier in mice and human cells, *Nat. Commun.* 12 (2021) 617, <https://doi.org/10.1038/s41467-020-20795-9>.
- [70] C. Copeland, S.E. Stabenfeldt, Leveraging the dynamic blood-brain barrier for central nervous system nanoparticle-based drug delivery applications, *Curr. Opin. Biomed. Eng.* 14 (2020) 1–8, <https://doi.org/10.1016/j.cobme.2020.04.001>.
- [71] R. Dallmann, A. Okyar, F. Lévi, Dosing-time makes the poison: circadian regulation and pharmacotherapy, *Trends Mol. Med.* 22 (2016) 430–445, <https://doi.org/10.1016/j.molmed.2016.03.004>.
- [72] S.L. Zhang, Z. Yue, D.M. Arnold, G. Artiushin, A. Sehgal, A circadian clock in the blood-brain barrier regulates xenobiotic efflux, *Cell* 173 (2018) 130–139.e10, <https://doi.org/10.1016/j.cell.2018.02.017>.
- [73] T. Eckle, K. Hartmann, S. Bonney, S. Reithel, M. Mittelbronn, L.A. Walker, B. D. Lowes, J. Han, C.H. Borchers, P.M. Buttrick, D.J. Kominsky, S.P. Colgan, H. K. Eltzschig, Adora2b-elicited Per2 stabilization promotes a HIF-dependent metabolic switch crucial for myocardial adaptation to ischemia, *Nat. Med.* 18 (2012) 774–782, <https://doi.org/10.1038/nm.2728>.
- [74] S. Helleboid, C. Haug, K. Lamottke, Y. Zhou, J. Wei, S. Daix, L. Cambula, G. Rigou, D.W. Hum, R. Walczak, The identification of naturally occurring neoruscogenin as a bioavailable, potent, and high-affinity agonist of the nuclear receptor ROR α (NR1F1), *SLAS Discovery* 19 (2014) 399–406, <https://doi.org/10.1177/1087057113497095>.
- [75] J. Jang, S. Chung, Y. Choi, H.Y. Lim, Y. Son, S.K. Chun, G.H. Son, K. Kim, Y.-G. Suh, J.-W. Jung, The cryptochrome inhibitor KS15 enhances E-box-mediated transcription by disrupting the feedback action of a circadian transcription-repressor complex, *Life Sci.* 200 (2018) 49–55, <https://doi.org/10.1016/j.lfs.2018.03.022>.
- [76] R.P. Moura, C. Martins, S. Pinto, F. Sousa, B. Sarmiento, Blood-brain barrier receptors and transporters: an insight on their function and how to exploit them through nanotechnology, *Expert Opin. Drug Deliv.* 16 (2019) 271–285, <https://doi.org/10.1080/17425247.2019.1583205>.
- [77] M.J. Mitchell, M.M. Billingsley, R.M. Haley, M.E. Wechsler, N.A. Peppas, R. Langer, Engineering precision nanoparticles for drug delivery, *Nat. Rev. Drug Discov.* 20 (2021) 101–124, <https://doi.org/10.1038/s41573-020-0090-8>.
- [78] Y. Aktaş, K. Andrieux, M.J. Alonso, P. Calvo, R.N. Gürsoy, P. Couvreur, Y. Çapan, Preparation and in vitro evaluation of chitosan nanoparticles containing a caspase inhibitor, *Int. J. Pharm.* 298 (2005) 378–383, <https://doi.org/10.1016/j.ijpharm.2005.03.027>.
- [79] S. Ruan, Y. Zhou, X. Jiang, H. Gao, Rethinking CRITID procedure of brain targeting drug delivery: circulation, blood brain barrier recognition, intracellular transport, diseased cell targeting, internalization, and drug release, *Adv. Sci.* 8 (2021), <https://doi.org/10.1002/advsc.202004025>.
- [80] E.H. Lo, F.M. Faraci, Circadian mechanisms in cardiovascular and cerebrovascular disease, *Circ. Res.* 134 (2024) 615–617, <https://doi.org/10.1161/CIRCRESAHA.124.324662>.
- [81] R.S. Pulido, R.N. Munji, T.C. Chan, C.R. Quirk, G.A. Weiner, B.D. Weger, M. J. Rossi, S. Elmsaouri, M. Malfavon, A. Deng, C.P. Profaci, M. Blanchette, T. Qian, K.L. Foreman, E.V. Shusta, M.R. Gorman, F. Gachon, S. Leutgeb, R. Daneman, Neuronal activity regulates blood-brain barrier efflux transport through endothelial circadian genes, *Neuron* 108 (2020) 937–952.e7, <https://doi.org/10.1016/j.neuron.2020.09.002>.
- [82] W. Pan, A.J. Kastin, Diurnal variation of leptin entry from blood to brain involving partial saturation of the transport system, *Life Sci.* 68 (2001) 2705–2714, [https://doi.org/10.1016/S0024-3205\(01\)01085-2](https://doi.org/10.1016/S0024-3205(01)01085-2).
- [83] S. Ogata, S. Ito, T. Masuda, S. Ohtsuki, Diurnal changes in protein expression at the blood-brain barrier in mice, *Biol. Pharm. Bull.* 45 (2022) b22–00016, <https://doi.org/10.1248/bpb.b22-00016>.
- [84] R. Moreira, C. Nóbrega, L.P. de Almeida, L. Mendonça, Brain-targeted drug delivery - nanovesicles directed to specific brain cells by brain-targeting ligands, *J. Nanobiotechnol.* 22 (2024), <https://doi.org/10.1186/S12951-024-02511-7>.
- [85] L. Wei, X.-Y. Guo, T. Yang, M.-Z. Yu, D.-W. Chen, J.-C. Wang, Brain tumor-targeted therapy by systemic delivery of siRNA with Transferrin receptor-mediated core-shell nanoparticles, *Int. J. Pharm.* 510 (2016) 394–405, <https://doi.org/10.1016/j.ijpharm.2016.06.127>.
- [86] S. Fu, M. Liang, Y. Wang, L. Cui, C. Gao, X. Chu, Q. Liu, Y. Feng, W. Gong, M. Yang, Z. Li, C. Yang, X. Xie, Y. Yang, C. Gao, Dual-modified novel biomimetic nanocarriers improve targeting and therapeutic efficacy in glioma, *ACS Appl. Mater. Interfaces* 11 (2019) 1841–1854, <https://doi.org/10.1021/acsami.8b18664>.
- [87] Z. Liu, X. Gao, T. Kang, M. Jiang, D. Miao, G. Gu, Q. Hu, Q. Song, L. Yao, Y. Tu, H. Chen, X. Jiang, J. Chen, B6 peptide-modified PEG-PLA nanoparticles for enhanced brain delivery of neuroprotective peptide, *Bioconjug. Chem.* 24 (2013) 997–1007, <https://doi.org/10.1021/bc400055h>.
- [88] B. Zhang, Y. Zhao, K. Guo, H. Tian, C. Wang, R. Wang, Y. Chen, X. Chen, H. Zheng, B. Gao, J. Shen, W. Tian, Macromolecular nanoparticles to attenuate both reactive oxygen species and inflammatory damage for treating Alzheimer's disease, *Bioeng. Transl. Med.* 8 (2023), <https://doi.org/10.1002/btm2.10459>.
- [89] Y. Aktaş, M. Yemisci, K. Andrieux, R.N. Gürsoy, M.J. Alonso, E. Fernandez-Megia, R. Novoa-Carballal, E. Quiñoá, R. Riguera, M.F. Sargon, H.H. Çelik, A.S. Demir, A. A. Hincal, T. Dalkara, Y. Çapan, P. Couvreur, Development and brain delivery of chitosan-PEG nanoparticles functionalized with the monoclonal antibody OX26, *Bioconjug. Chem.* 16 (2005) 1503–1511, <https://doi.org/10.1021/bc050217o>.
- [90] M.S. Ashrafzadeh, A. Akbarzadeh, A. Heydarinasab, M. Ardjmand, In vivo glioblastoma therapy using targeted liposomal cisplatin, *Int. J. Nanomed.* 15 (2020) 7035–7049, <https://doi.org/10.2147/IJN.S255902>.
- [91] P. Yue, L. He, S. Qiu, Y. Li, Y. Liao, X. Li, D. Xie, Y. Peng, OX26/CTX-conjugated PEGylated liposome as a dual-targeting gene delivery system for brain glioma, *Mol. Cancer* 13 (2014) 191, <https://doi.org/10.1186/1476-4598-13-191>.
- [92] J. Shen, Z. Zhao, W. Shang, C. Liu, B. Zhang, L. Zhao, H. Cai, Ginsenoside Rg1 nanoparticle penetrating the blood-brain barrier to improve the cerebral function of diabetic rats complicated with cerebral infarction, *Int. J. Nanomed.* 12 (2017) 6477–6486, <https://doi.org/10.2147/IJN.S139602>.
- [93] C. Martins, M. Araújo, A. Malfanti, C. Pacheco, S.J. Smith, B. Ucar, R. Rahman, J.W. Aylott, V. Prát, B. Sarmiento, Stimuli-responsive multifunctional nanomedicine for enhanced glioblastoma chemotherapy augments multistage blood-to-brain trafficking and tumor targeting, *Small* 19 (2023), <https://doi.org/10.1002/sml.202300029>.
- [94] Y. Zhu, Y. Jiang, F. Meng, C. Deng, R. Cheng, J. Zhang, J. Feijen, Z. Zhong, Highly efficacious and specific anti-glioma chemotherapy by tandem nanomicelles co-functionalized with brain tumor-targeting and cell-penetrating peptides, *J. Control. Release* 278 (2018) 1–8, <https://doi.org/10.1016/j.jconrel.2018.03.025>.
- [95] S. Huang, J. Li, L. Han, S. Liu, H. Ma, R. Huang, C. Jiang, Dual targeting effect of Angiopep-2-modified, DNA-loaded nanoparticles for glioma, *Biomaterials* 32 (2011) 6832–6838, <https://doi.org/10.1016/j.biomaterials.2011.05.064>.
- [96] E.-S. Kim, D. Kim, S. Nyberg, A. Poma, D. Cecchin, S.A. Jain, K.-A. Kim, Y.-J. Shin, E.-H. Kim, M. Kim, S.-H. Baek, J.-K. Kim, T.R. Doepfner, A. Ali, J. Redgrave, G. Battaglia, A. Majid, O.-N. Bae, LRP-1 functionalized polymersomes enhance the efficacy of carnosine in experimental stroke, *Sci. Rep.* 10 (2020) 699, <https://doi.org/10.1038/s41598-020-57685-5>.
- [97] X. Tian, S. Nyberg, P.S. Sharp, J. Madsen, N. Daneshpour, S.P. Armes, J. Berwick, M. Azzouz, P. Shaw, N.J. Abbott, G. Battaglia, LRP-1-mediated intracellular antibody delivery to the Central Nervous System, *Sci. Rep.* 5 (2015) 11990, <https://doi.org/10.1038/srep11990>.
- [98] Y. Jiang, J. Zhang, F. Meng, Z. Zhong, Apolipoprotein E peptide-directed chimeric polymersomes mediate an ultrahigh-efficiency targeted protein therapy for

- glioblastoma, *ACS Nano* 12 (2018) 11070–11079, <https://doi.org/10.1021/acsnano.8b05265>.
- [99] Y. Zhang, H. Ma, L. Li, C. Sun, C. Yu, L. Wang, D. Xu, X. Song, R. Yu, Dual-targeted novel temozolomide nanocapsules encapsulating siPKM2 inhibit aerobic glycolysis to sensitize glioblastoma to chemotherapy, *Adv. Mater.* 36 (2024), <https://doi.org/10.1002/adma.202400502>.
- [100] Y. Liu, R. Huang, L. Han, W. Ke, G. Shao, L. Ye, J. Lou, C. Jiang, Brain-targeting gene delivery and cellular internalization mechanisms for modified rabies virus glycoprotein RVG29 nanoparticles, *Biomaterials* 30 (2009) 4195–4202, <https://doi.org/10.1016/j.biomaterials.2009.02.051>.
- [101] L. You, J. Wang, T. Liu, Y. Zhang, X. Han, T. Wang, S. Guo, T. Dong, J. Xu, G. J. Anderson, Q. Liu, Y.-Z. Chang, X. Lou, G. Nie, Targeted brain delivery of rabies virus glycoprotein 29-modified deferoxamine-loaded nanoparticles reverses functional deficits in parkinsonian mice, *ACS Nano* 12 (2018) 4123–4139, <https://doi.org/10.1021/acsnano.7b08172>.
- [102] J.-Y. Kim, W. Il Choi, Y.H. Kim, G. Tae, Brain-targeted delivery of protein using chitosan- and RVG peptide-conjugated, pluronic-based nano-carrier, *Biomaterials* 34 (2013) 1170–1178, <https://doi.org/10.1016/j.biomaterials.2012.09.047>.
- [103] M. Qu, Q. Lin, S. He, L. Wang, Y. Fu, Z. Zhang, L. Zhang, A brain targeting functionalized liposomes of the dopamine derivative N -3,4-bis(pivaloyloxy)-dopamine for treatment of Parkinson's disease, *J. Control. Release* 277 (2018) 173–182, <https://doi.org/10.1016/j.jconrel.2018.03.019>.
- [104] R. Zhou, L. Zhu, Z. Zeng, R. Luo, J. Zhang, R. Guo, L. Zhang, Q. Zhang, W. Bi, Targeted brain delivery of <sc>RVG29-modified rifampicin-loaded</sc> nanoparticles for Alzheimer's disease treatment and diagnosis, *Bioeng. Transl. Med.* 7 (2022), <https://doi.org/10.1002/btm2.10395>.
- [105] H. Gao, J. Qian, S. Cao, Z. Yang, Z. Pang, S. Pan, L. Fan, Z. Xi, X. Jiang, Q. Zhang, Precise glioma targeting of and penetration by aptamer and peptide dual-functionalized nanoparticles, *Biomaterials* 33 (2012) 5115–5123, <https://doi.org/10.1016/j.biomaterials.2012.03.058>.
- [106] C. Zhang, X. Wan, X. Zheng, X. Shao, Q. Liu, Q. Zhang, Y. Qian, Dual-functional nanoparticles targeting amyloid plaques in the brains of Alzheimer's disease mice, *Biomaterials* 35 (2014) 456–465, <https://doi.org/10.1016/j.biomaterials.2013.09.063>.
- [107] Q. Guo, Y. Li, S. Xu, P. Wang, K. Qian, P. Yang, D. Sheng, L. Wang, Y. Cheng, R. Meng, J. Cao, H. Luo, Y. Wei, Q. Zhang, Brain-neuron targeted nanoparticles for peptide synergy therapy at dual-target of Alzheimer's disease, *J. Control. Release* 355 (2023) 604–621, <https://doi.org/10.1016/j.jconrel.2023.01.074>.
- [108] X. Zheng, X. Pang, P. Yang, X. Wan, Y. Wei, Q. Guo, Q. Zhang, X. Jiang, A hybrid siRNA delivery complex for enhanced brain penetration and precise amyloid plaque targeting in Alzheimer's disease mice, *Acta Biomater.* 49 (2017) 388–401, <https://doi.org/10.1016/j.actbio.2016.11.029>.
- [109] Q. Guo, X. Zheng, P. Yang, X. Pang, K. Qian, P. Wang, S. Xu, D. Sheng, L. Wang, J. Cao, W. Lu, Q. Zhang, X. Jiang, Small interfering RNA delivery to the neurons near the amyloid plaques for improved treatment of Alzheimer's disease, *Acta Pharm. Sin.* B 9 (2019) 590–603, <https://doi.org/10.1016/j.apsb.2018.12.010>.
- [110] Y.-C. Kuo, H.-C. Tsai, Rosmarinic acid- and curcumin-loaded polyacrylamide-cardiolipin-poly(lactide-co-glycolide) nanoparticles with conjugated 83-14 monoclonal antibody to protect β -amyloid-insulted neurons, *Mater. Sci. Eng. C* 91 (2018) 445–457, <https://doi.org/10.1016/j.msec.2018.05.062>.
- [111] O.A. Marcos-Contreras, J.S. Brenner, R.Y. Kiseleva, V. Zuluaga-Ramirez, C. F. Greineder, C.H. Villa, E.D. Hood, J.W. Myerson, S. Muro, Y. Persidsky, V. R. Muzykantor, Combining vascular targeting and the local first pass provides 100-fold higher uptake of ICAM-1-targeted vs untargeted nanocarriers in the inflamed brain, *J. Control. Release* 301 (2019) 54–61, <https://doi.org/10.1016/j.jconrel.2019.03.008>.
- [112] M.E. Zamora, S. Omo-Lamai, M.N. Patel, J. Wu, E. Arguiri, V.R. Muzykantor, J. W. Myerson, O.A. Marcos-Contreras, J.S. Brenner, Combination of Physicochemical Tropism and Affinity Moiety Targeting of Lipid Nanoparticles Enhances Organ Targeting, *Nano Lett.* (2024), <https://doi.org/10.1021/acs.nanolett.3c05031>.
- [113] S. Reyes-Esteves, J. Nong, P.M. Glassman, S. Omo-Lamai, S. Ohashi, J. W. Myerson, M.E. Zamora, X. Ma, S.E. Kasner, L. Sansing, V.R. Muzykantor, O. A. Marcos-Contreras, J.S. Brenner, Targeted drug delivery to the brain endothelium dominates over passive delivery via vascular leak in experimental intracerebral hemorrhage, *J. Control. Release* 356 (2023) 185–195, <https://doi.org/10.1016/j.jconrel.2023.02.037>.
- [114] O.A. Marcos-Contreras, C.F. Greineder, R.Y. Kiseleva, H. Parhiz, L.R. Walsh, V. Zuluaga-Ramirez, J.W. Myerson, E.D. Hood, C.H. Villa, I. Tombacz, N. Pardi, A. Seliga, B.L. Mui, Y.K. Tam, P.M. Glassman, V.V. Shuvaev, J. Nong, J.S. Brenner, M. Khoshnejad, T. Madden, D. Weissmann, Y. Persidsky, V.R. Muzykantor, Selective targeting of nanomedicine to inflamed cerebral vasculature to enhance the blood–brain barrier, in: *Proceedings of the National Academy of Sciences* 117, 2020, pp. 3405–3414, <https://doi.org/10.1073/pnas.1912012117>.
- [115] Y. Zhang, P. Guo, Z. Ma, P. Lu, D. Kebebe, Z. Liu, Combination of cell-penetrating peptides with nanomaterials for the potential therapeutics of central nervous system disorders: a review, *J. Nanobiotechnol.* 19 (2021), <https://doi.org/10.1186/s12951-021-01002-3>.
- [116] Y.H. Song, R. De, K.T. Lee, Emerging strategies to fabricate polymeric nanocarriers for enhanced drug delivery across blood-brain barrier: An overview, *Adv. Colloid Interface Sci.* 320 (2023), <https://doi.org/10.1016/j.cis.2023.103008>.
- [117] J.M. Lajoie, E.V. Shusta, Targeting receptor-mediated transport for delivery of biologics across the blood-brain barrier, *Annu. Rev. Pharmacol. Toxicol.* 55 (2015) 613–631, <https://doi.org/10.1146/annurev-pharmtox-010814-124852>.
- [118] A. Duro-Castano, D. Moreira Leite, J. Forth, Y. Deng, D. Matias, C. Noble Jesus, G. Battaglia, Designing peptide nanoparticles for efficient brain delivery, *Adv. Drug Deliv. Rev.* 160 (2020) 52–77, <https://doi.org/10.1016/j.addr.2020.10.001>.
- [119] W.M. Pardridge, Receptor-Mediated Peptide Transport through the Blood-Brain Barrier*, 1986.
- [120] K.B. Johnsen, A. Burkhart, L.B. Thomsen, T.L. Andresen, T. Moos, Targeting the transferrin receptor for brain drug delivery, *Prog. Neurobiol.* 181 (2019) 101665, <https://doi.org/10.1016/j.pneurobio.2019.101665>.
- [121] P. Sonali, R.P. Agrawal, C.V. Singh, S. Rajesh, M.R. Singh, B.L. Vijayakumar, M. S. Pandey, Muthu, Transferrin receptor-targeted vitamin E TPGS micelles for brain cancer therapy: preparation, characterization and brain distribution in rats, *Drug Deliv.* 23 (2016) 1788–1798, <https://doi.org/10.3109/10717544.2015.1094681>.
- [122] R.P. Sonali, N. Singh, G. Singh, M.R. Sharma, B. Vijayakumar, S. Koch, U. Singh, D. Singh, B.L. Dash, M.S. Pandey, Muthu, Transferrin liposomes of docetaxel for brain-targeted cancer applications: formulation and brain theranostics, *Drug Deliv.* 23 (2016) 1261–1271, <https://doi.org/10.3109/10717544.2016.1162878>.
- [123] M. Sela, M. Poley, P. Mora-Raimundo, S. Kagan, A. Avital, M. Kaduri, G. Chen, O. Adir, A. Rozenzweig, Y. Weiss, O. Sade, Y. Leichtmann-Bardog, L. Simchi, S. Aga-Mizrachi, B. Bell, Y. Yeretzi-Peretz, A.Z. Or, A. Choudhary, I. Rosh, D. Cordeiro, S. Cohen-Adiv, Y. Berdichevsky, A. Odeh, J. Shklover, J. Shainsky-Roitman, J.E. Schroeder, D. Hershkovitz, P. Hasson, A. Ashkenazi, S. Stern, T. Laviv, A. Ben-Zvi, A. Avital, U. Ashery, B.M. Maoz, A. Schroeder, Brain-Targeted Liposomes Loaded with Monoclonal Antibodies Reduce Alpha-Synuclein Aggregation and Improve Behavioral Symptoms in Parkinson's Disease, *Adv. Mater.* 35 (2023), <https://doi.org/10.1002/adma.202304654>.
- [124] G. Sharma, A. Modgil, B. Layek, K. Arora, C. Sun, B. Law, J. Singh, Cell penetrating peptide tethered bi-ligand liposomes for delivery to brain in vivo: Biodistribution and transfection, *J. Control. Release* 167 (2013) 1–10, <https://doi.org/10.1016/j.jconrel.2013.01.016>.
- [125] Y. Kuang, S. An, Y. Guo, S. Huang, K. Shao, Y. Liu, J. Li, H. Ma, C. Jiang, T7 peptide-functionalized nanoparticles utilizing RNA interference for glioma dual targeting, *Int. J. Pharm.* 454 (2013) 11–20, <https://doi.org/10.1016/j.ijpharm.2013.07.019>.
- [126] Y. Kuang, X. Jiang, Y. Zhang, Y. Lu, H. Ma, Y. Guo, Y. Zhang, S. An, J. Li, L. Liu, Y. Wu, J. Liang, C. Jiang, Dual Functional Peptide-Driven Nanoparticles for Highly Efficient Glioma-Targeting and Drug Codelivery, *Mol. Pharm.* 13 (2016) 1599–1607, <https://doi.org/10.1021/acs.molpharmaceut.6b00051>.
- [127] Z. Wang, Y. Zhao, Y. Jiang, W. Lv, L. Wu, B. Wang, L. Lv, Q. Xu, H. Xin, Enhanced anti-ischemic stroke of ZL006 by T7-conjugated PEGylated liposomes drug delivery system, *Sci. Rep.* 5 (2015) 12651, <https://doi.org/10.1038/srep12651>.
- [128] T. Zong, L. Mei, H. Gao, W. Cai, P. Zhu, K. Shi, J. Chen, Y. Wang, F. Gao, Q. He, Synergistic Dual-Ligand Doxorubicin Liposomes Improve Targeting and Therapeutic Efficacy of Brain Glioma in Animals, *Mol. Pharm.* 11 (2014) 2346–2357, <https://doi.org/10.1021/mp500057n>.
- [129] Y. Bi, L. Liu, Y. Lu, T. Sun, C. Shen, X. Chen, Q. Chen, S. An, X. He, C. Ruan, Y. Wu, Y. Zhang, Q. Guo, Z. Zheng, Y. Liu, M. Lou, S. Zhao, C. Jiang, T7 Peptide-Functionalized PEG-PLGA Micelles Loaded with Carmustine for Targeting Therapy of Glioma, *ACS Appl. Mater. Interfaces* 8 (2016) 27465–27473, <https://doi.org/10.1021/acsami.6b05572>.
- [130] J. Tang, Q. Wang, Q. Yu, Y. Qiu, L. Mei, D. Wan, X. Wang, M. Li, Q. He, A stabilized retro-inverso peptide ligand of transferrin receptor for enhanced liposome-based hepatocellular carcinoma-targeted drug delivery, *Acta Biomater.* 83 (2019) 379–389, <https://doi.org/10.1016/j.actbio.2018.11.002>.
- [131] M. Yu, D. Su, Y. Yang, L. Qin, C. Hu, R. Liu, Y. Zhou, C. Yang, X. Yang, G. Wang, H. Gao, D-17 Peptide-Modified PEGylated Bilirubin Nanoparticles Loaded with Cediranib and Paclitaxel for Antiangiogenesis and Chemotherapy of Glioma, *ACS Appl. Mater. Interfaces* 11 (2019) 176–186, <https://doi.org/10.1021/acsami.8b16219>.
- [132] H. Zhang, T. Wu, W. Yu, S. Ruan, Q. He, H. Gao, Ligand Size and Conformation Affect the Behavior of Nanoparticles Coated with in Vitro and in Vivo Protein Corona, *ACS Appl. Mater. Interfaces* 10 (2018) 9094–9103, <https://doi.org/10.1021/acsami.7b16096>.
- [133] S. Fan, Y. Zheng, X. Liu, W. Fang, X. Chen, W. Liao, X. Jing, M. Lei, E. Tao, Q. Ma, X. Zhang, R. Guo, J. Liu, Curcumin-loaded PLGA-PEG nanoparticles conjugated with B6 peptide for potential use in Alzheimer's disease, *Drug Deliv.* 25 (2018) 1091–1102, <https://doi.org/10.1080/10717544.2018.1461955>.
- [134] L.L. Israel, A. Galstyan, A. Cox, E.S. Shatalova, T. Sun, M.-H. Rashid, Z. Grodzinski, A. Chiechi, D.-T. Fuchs, R. Patil, M. Koronyo-Hamaoui, K.L. Black, J.Y. Ljubimova, E. Holler, Signature Effects of Vector-Guided Systemic Nano Bioconjugate Delivery Across Blood-Brain Barrier of Normal, Alzheimer's, and Tumor Mouse Models, *ACS Nano* 16 (2022) 11815–11832, <https://doi.org/10.1021/acsnano.1c10034>.
- [135] C. Chen, Z. Duan, Y. Yuan, R. Li, L. Pang, J. Liang, X. Xu, J. Wang, Peptide-22 and Cyclic RGD Functionalized Liposomes for Glioma Targeting Drug Delivery Overcoming BBB and BBB, *ACS Appl. Mater. Interfaces* 9 (2017) 5864–5873, <https://doi.org/10.1021/acsami.6b15831>.
- [136] P. Wu, M. Zhu, Y. Li, Z. Ya, Y. Yang, Y. Yuan, W. Dong, S. Guo, S. Lu, L. Zhang, Y. Zong, M. Wan, Cascade-Amplifying Synergistic Therapy for Intracranial Glioma via Endogenous Reactive Oxygen Species-Triggered “All-in-One” Nanoplateform, *Adv. Funct. Mater.* 31 (2021), <https://doi.org/10.1002/adfm.202105786>.
- [137] Q. Guo, Q. Zhu, T. Miao, J. Tao, X. Ju, Z. Sun, H. Li, G. Xu, H. Chen, L. Han, LRP1-upregulated nanoparticles for efficiently conquering the blood-brain barrier and targetedly suppressing multifocal and infiltrative brain metastases, *J. Control. Release* 303 (2019) 117–129, <https://doi.org/10.1016/j.jconrel.2019.04.031>.

- [138] S.-C. Tang, P. Kumthekar, A.J. Brenner, S. Kesari, D. Piccioni, C.K. Anders, J.A. Carillo, P. Chalasani, P. Kabos, S.L. Puhalla, A. Garcia, K. Tkaczuk, M.S. Ahluwalia, N. Lakhani, N. Ibrahim, CNS tumours 3240 ANG1005, a novel peptide-paclitaxel conjugate crosses the BBB and shows activity in patients with recurrent CNS metastasis from breast cancer, results from a phase II clinical study, Abstract Book of the 41st ESMO Congress (ESMO 2016), 7-11 October 2016, Copenhagen, Denmark 27 (2016) vi103. <https://doi.org/10.1093/annonc/mdw367.2>.
- [139] G. Han, K. Bai, X. Yang, C. Sun, Y. Ji, J. Zhou, H. Zhang, Y. Ding, "Drug-Carrier" Synergy Therapy for Amyloid- β Clearance and Inhibition of Tau Phosphorylation via Biomimetic Lipid Nanocomposite Assembly, *Adv. Sci.* 9 (2022), <https://doi.org/10.1002/advs.202106072>.
- [140] X. Tian, D.M. Leite, E. Scarpa, S. Nyberg, G. Fullstone, J. Forth, D. Matias, A. Apriceno, A. Poma, A. Duro-Castano, M. Vuyyuru, L. Harker-Kirschneck, A. Šarić, Z. Zhang, P. Xiang, B. Fang, Y. Tian, L. Luo, On the shuttling across the blood-brain barrier via tubule formation: Mechanism and cargo avidity bias, 2020. <http://www.science.org>.
- [141] J.T. Guptill, S.M. Raja, F. Boakye-Agyeman, R. Noveck, S. Ramey, T.M. Tu, D. T. Laskowitz, Phase 1 Randomized, Double-Blind, Placebo-Controlled Study to Determine the Safety, Tolerability, and Pharmacokinetics of a Single Escalating Dose and Repeated Doses of CN-105 in Healthy Adult Subjects, *J. Clin. Pharmacol.* 57 (2017) 770–776, <https://doi.org/10.1002/jcph.853>.
- [142] M.L. James, J. Troy, N. Nowacki, J. Komisarow, C.B. Swisher, K. Tucker, K. Hatton, M.A. Babi, B.B. Worrall, C. Andrews, D. Woo, P.G. Kranz, C. Lascola, M. Maughan, D.T. Laskowitz, CN-105 in Participants with Acute Supratentorial Intracerebral Hemorrhage (CATCH) Trial, *Neurocrit. Care* 36 (2022) 216–225, <https://doi.org/10.1007/s12028-021-01287-0>.
- [143] Z. Zheng, J. Zhang, J. Jiang, Y. He, W. Zhang, X. Mo, X. Kang, Q. Xu, B. Wang, Y. Huang, Remodeling tumor immune microenvironment (TIME) for glioma therapy using multi-targeting liposomal codelivery, *J. Immunother. Cancer* 8 (2020) e000207, <https://doi.org/10.1136/jitc-2019-000207>.
- [144] C. Fu, Y. Xiang, X. Li, A. Fu, Targeted transport of nanocarriers into brain for theranosis with rabies virus glycoprotein-derived peptide, *Mater. Sci. Eng. C* 87 (2018) 155–166, <https://doi.org/10.1016/j.msec.2017.12.029>.
- [145] R. Huey, S. Hawthorne, P. McCarron, The potential use of rabies virus glycoprotein-derived peptides to facilitate drug delivery into the central nervous system: a mini review, *J. Drug Target.* 25 (2017) 379–385, <https://doi.org/10.1080/1061186X.2016.1223676>.
- [146] P. Jiang, Y. Xiao, X. Hu, C. Wang, H. Gao, H. Huang, J. Lv, Z. Qi, Z. Wang, RVG29 Peptide-Modified Exosomes Loaded with Mir-133b Mediate the RhoA-ROCK Pathway to Improve Motor and Neurological Symptoms in Parkinson's Disease, *ACS Biomater. Sci. Eng.* 10 (2024) 3069–3085, <https://doi.org/10.1021/acsbomaterials.3c01622>.
- [147] L. Alvarez-Erviti, Y. Seow, H. Yin, C. Betts, S. Lakhali, M.J.A. Wood, Delivery of siRNA to the mouse brain by systemic injection of targeted exosomes, *Nat. Biotechnol.* 29 (2011) 341–345, <https://doi.org/10.1038/nbt.1807>.
- [148] Y. Yu, W. Li, L. Mao, W. Peng, D. Long, D. Li, R. Zhou, X. Dang, Genetically engineered exosomes display RVG peptide and selectively enrich a neprilysin variant: a potential formulation for the treatment of Alzheimer's disease, *J. Drug Target.* 29 (2021) 1128–1138, <https://doi.org/10.1080/1061186X.2021.1929257>.
- [149] G. Cui, H. Guo, H. Li, Y. Zhai, Z. Gong, J. Wu, J. Liu, Y. Dong, S. Hou, J. Liu, RVG-modified exosomes derived from mesenchymal stem cells rescue memory deficits by regulating inflammatory responses in a mouse model of Alzheimer's disease, *Immun. Ageing* 16 (2019) 10, <https://doi.org/10.1186/s12979-019-0150-2>.
- [150] P. Kumar, H. Wu, J.L. McBride, K.-E. Jung, M. Hee Kim, B.L. Davidson, S. Kyung Lee, P. Shankar, N. Manjunath, Transvascular delivery of small interfering RNA to the central nervous system, *Nature* 448 (2007) 39–43, <https://doi.org/10.1038/nature05901>.
- [151] Y. Gao, Z.-Y. Wang, J. Zhang, Y. Zhang, H. Huo, T. Wang, T. Jiang, S. Wang, RVG-Peptide-Linked Trimethylated Chitosan for Delivery of siRNA to the Brain, *Biomacromolecules* 15 (2014) 1010–1018, <https://doi.org/10.1021/bm401906p>.
- [152] J. Li, L. Feng, L. Fan, Y. Zha, L. Guo, Q. Zhang, J. Chen, Z. Pang, Y. Wang, X. Jiang, V.C. Yang, L. Wen, Targeting the brain with PEG-PLGA nanoparticles modified with phage-displayed peptides, *Biomaterials* 32 (2011) 4943–4950, <https://doi.org/10.1016/j.biomaterials.2011.03.031>.
- [153] J. Li, C. Zhang, J. Li, L. Fan, X. Jiang, J. Chen, Z. Pang, Q. Zhang, Brain Delivery of NAP with PEG-PLGA Nanoparticles Modified with Phage Display Peptides, *Pharm. Res.* 30 (2013) 1813–1823, <https://doi.org/10.1007/s11095-013-1025-4>.
- [154] J. Gu, C. Yan, S. Yin, H. Wu, C. Liu, A. Xue, X. Lei, N. Zhang, F. Geng, Erythrocyte membrane-coated nanocarriers modified by TGN for Alzheimer's disease, *J. Control. Release* 366 (2024) 448–459, <https://doi.org/10.1016/j.jconrel.2023.12.030>.
- [155] H. Gao, Z. Yang, S. Zhang, Z. Pang, Q. Liu, X. Jiang, Study and evaluation of mechanisms of dual targeting drug delivery system with tumor microenvironment assays compared with normal assays, *Acta Biomater.* 10 (2014) 858–867, <https://doi.org/10.1016/j.actbio.2013.11.003>.
- [156] J. Yang, Q. Zhang, Y. Liu, X. Zhang, W. Shan, S. Ye, X. Zhou, Y. Ge, X. Wang, L. Ren, Nanoparticle-Based Co-Delivery of siRNA and Paclitaxel for Dual-Targeting of Glioblastoma, *Nanomedicine* 15 (2020) 1391–1409, <https://doi.org/10.2217/nnm-2020-0066>.
- [157] Q. Guo, S. Xu, P. Yang, P. Wang, S. Lu, D. Sheng, K. Qian, J. Cao, W. Lu, Q. Zhang, A dual-ligand fusion peptide improves the brain-neuron targeting of nanocarriers in Alzheimer's disease mice, *J. Control. Release* 320 (2020) 347–362, <https://doi.org/10.1016/j.jconrel.2020.01.039>.
- [158] R. Fan, D. Chuan, H. Hou, H. Chen, B. Han, X. Zhang, L. Zhou, A. Tong, J. Xu, G. Guo, Development of a hybrid nanocarrier-recognizing tumor vasculature and penetrating the BBB for glioblastoma multi-targeting therapy, *Nanoscale* 11 (2019) 11285–11304, <https://doi.org/10.1039/C9NR01320B>.
- [159] W.A. Jefferies, M.R. Brandon, S.V. Hunt, A.F. Williams, K.C. Gatter, D.Y. Mason, Transferrin receptor on endothelium of brain capillaries, *Nature* 312 (1984) 162–163, <https://doi.org/10.1038/312162a0>.
- [160] S.J. Barker, M.B. Thayer, C. Kim, D. Tatarakis, M.J. Simon, R. Dial, L. Nilewski, R. C. Wells, Y. Zhou, M. Afetian, P. Akkapeddi, A. Chappell, K.S. Chew, J. Chow, A. Clemens, C.B. Discenza, J.C. Dugas, C. Dwyer, T. Earr, C. Ha, Y.S. Ho, D. Huynh, E.I. Lozano, S. Jayaraman, W. Kwan, C. Mahon, M. Pizzo, Y. Robles-Colmenares, E. Roche, L. Sanders, A. Stergioulis, R. Tong, H. Tran, Y. Zuchero, A. A. Estrada, K. Gadkar, C.M.M. Koth, P.E. Sanchez, R.G. Thorne, R.J. Watts, T. Sandmann, L.A. Kane, F. Rigo, M.S. Dennis, J.W. Lewcock, S.L. DeVos, Targeting the transferrin receptor to transport antisense oligonucleotides across the mammalian blood-brain barrier, *Sci. Transl. Med.* 16 (2024), <https://doi.org/10.1126/scitranslmed.adi2245>.
- [161] M.S. Kariolis, R.C. Wells, J.A. Getz, W. Kwan, C.S. Mahon, R. Tong, D.J. Kim, A. Srivastava, C. Bedard, K.R. Henne, T. Giese, V.A. Assimon, X. Chen, Y. Zhang, H. Solano, K. Jenkins, P.E. Sanchez, L. Kane, T. Miyamoto, K.S. Chew, M. E. Pizzo, N. Liang, M.E.K. Calvert, S.L. DeVos, S. Baskaran, S. Hall, Z.K. Sweeney, R.G. Thorne, R.J. Watts, M.S. Dennis, A.P. Silverman, Y.J.Y. Zuchero, Brain delivery of therapeutic proteins using an Fc fragment blood-brain barrier transport vehicle in mice and monkeys, *Sci. Transl. Med.* 12 (2020), <https://doi.org/10.1126/scitranslmed.aay1359>.
- [162] P. Harmatz, R. Giugliani, A.M. Martins, T. Hamazaki, T. Kubo, R. Kira, K. Minami, T. Ikeda, H. Moriuchi, S. Kawashima, N. Takasao, S. So, H. Sonoda, T. Hirato, K. Tanizawa, M. Schmidt, Y. Sato, α -L-iduronidase fused with humanized anti-human transferrin receptor antibody (Iepunafus alfa) for mucopolysaccharidosis type I: A phase 1/2 trial, *Mol. Ther.* 32 (2024) 609–618, <https://doi.org/10.1016/j.jymthe.2024.01.009>.
- [163] I.T. Papademetriou, C. Garnacho, E.H. Schuchman, S. Muro, In vivo performance of polymer nanocarriers dually-targeted to epitopes of the same or different receptors, *Biomaterials* 34 (2013) 3459–3466, <https://doi.org/10.1016/j.biomaterials.2013.01.069>.
- [164] K.B. Johnsen, M. Bak, F. Melander, M.S. Thomsen, A. Burkhart, P.J. Kempen, T. L. Andresen, T. Moos, Modulating the antibody density changes the uptake and transport at the blood-brain barrier of both transferrin receptor-targeted gold nanoparticles and liposomal cargo, *J. Control. Release* 295 (2019) 237–249, <https://doi.org/10.1016/j.jconrel.2019.01.005>.
- [165] F. Zheng, Y. Pang, L. Li, Y. Pang, J. Zhang, X. Wang, G. Raes, Applications of nanobodies in brain diseases, *Front. Immunol.* 13 (2022), <https://doi.org/10.3389/fimmu.2022.978513>.
- [166] Y. Wouters, T. Jaspers, B. De Strooper, M. Dewilde, Identification and in vivo characterization of a brain-penetrating nanobody, *Fluids Barriers CNS* 17 (2020) 62, <https://doi.org/10.1186/s12987-020-00226-z>.
- [167] F. Man, P.J. Gawn, R.T.M. de Rosales, Nuclear imaging of liposomal drug delivery systems: A critical review of radiolabelling methods and applications in nanomedicine, *Adv. Drug Deliv. Rev.* 143 (2019) 134–160, <https://doi.org/10.1016/j.addr.2019.05.012>.
- [168] M. Liu, A. Apriceno, M. Sipin, E. Scarpa, L. Rodriguez-Arco, A. Poma, G. Marchello, G. Battaglia, S. Angioletti-Uberti, Combinatorial entropy behaviour leads to range selective binding in ligand-receptor interactions, *Nat. Commun.* 11 (2020), <https://doi.org/10.1038/s41467-020-18603-5>.
- [169] R.L. Manthe, M. Loeck, T. Bhowmick, M. Solomon, S. Muro, Intertwined mechanisms define transport of anti-ICAM nanocarriers across the endothelium and brain delivery of a therapeutic enzyme, *J. Control. Release* 324 (2020) 181–193, <https://doi.org/10.1016/j.jconrel.2020.05.009>.
- [170] D. Schauenburg, T. Weil, Not So Bioorthogonal Chemistry, *J. Am. Chem. Soc.* 147 (2025) 8049–8062, <https://doi.org/10.1021/jacs.4c15986>.
- [171] M. Lahnsteiner, A. Kastner, J. Mayr, A. Roller, B.K. Keppler, C.R. Kowol, Improving the Stability of Maleimide–Thiol Conjugation for Drug Targeting, *Chemistry – A European Journal* 26 (2020) 15867–15870, <https://doi.org/10.1002/chem.202003951>.
- [172] P. Song, N. Song, L. Li, M. Wu, Z. Lu, X. Zhao, Angiopep-2-Modified Carboxymethyl Chitosan-Based pH/Reduction Dual-Stimuli-Responsive Nanogels for Enhanced Targeting Glioblastoma, *Biomacromolecules* 22 (2021) 2921–2934, <https://doi.org/10.1021/acs.biomac.1c00314>.
- [173] W. Ruan, M. Jiao, S. Xu, M. Ismail, X. Xie, Y. An, H. Guo, R. Qian, B. Shi, M. Zheng, Brain-targeted CRISPR/Cas9 nanomedicine for effective glioblastoma therapy, *J. Control. Release* 351 (2022) 739–751, <https://doi.org/10.1016/j.jconrel.2022.09.046>.
- [174] S. Palma-Florez, A. López-Canosa, F. Morales-Zavala, O. Castaño, M.J. Kogan, J. Samitier, A. Lagunas, M. Mir, BBB-on-a-chip with integrated micro-TEER for permeability evaluation of multi-functionalized gold nanorods against Alzheimer's disease, *J. Nanobiotechnology* 21 (2023), <https://doi.org/10.1186/s12951-023-01798-2>.
- [175] D. Paolino, D. Cosco, R. Molinaro, C. Celia, M. Fresta, Supramolecular devices to improve the treatment of brain diseases, *Drug Discov. Today* 16 (2011) 311–324, <https://doi.org/10.1016/j.drudis.2011.02.006>.
- [176] C.P. Foley, D.G. Rubin, A. Santillan, D. Sondhi, J.P. Dyke, Y. Pierre Gobin, R. G. Crystal, D.J. Ballon, Intra-arterial delivery of AAV vectors to the mouse brain after mannitol mediated blood brain barrier disruption, *J. Control. Release* 196 (2014) 71–78, <https://doi.org/10.1016/j.jconrel.2014.09.018>.
- [177] D.W. Hwang, S. Son, J. Jang, H. Youn, S. Lee, D. Lee, Y.-S. Lee, J.M. Jeong, W. J. Kim, D.S. Lee, A brain-targeted rabies virus glycoprotein-disulfide linked PEI

- nanocarrier for delivery of neurogenic microRNA, *Biomaterials* 32 (2011) 4968–4975, <https://doi.org/10.1016/j.biomaterials.2011.03.047>.
- [178] H.J. Liu, P. Xu, Strategies to overcome/penetrate the BBB for systemic nanoparticle delivery to the brain/brain tumor, *Adv. Drug Deliv. Rev.* 191 (2022) 114619, <https://doi.org/10.1016/j.addr.2022.114619>.
- [179] S.M. Chowdhury, L. Abou-Elkacem, T. Lee, J. Dahl, A.M. Lutz, Ultrasound and microbubble mediated therapeutic deliveries: Underlying mechanisms and future outlook, *J. Control. Release* 326 (2020) 75–90, <https://doi.org/10.1016/j.jconrel.2020.06.008>.
- [180] P. Zhao, T. Wu, Y. Tian, J. You, X. Cui, Recent advances of focused ultrasound induced blood-brain barrier opening for clinical applications of neurodegenerative diseases, *Adv. Drug Deliv. Rev.* 209 (2024) 115323, <https://doi.org/10.1016/j.addr.2024.115323>.
- [181] K. Ogawa, N. Kato, M. Yoshida, T. Hiu, T. Matsuo, S. Mizukami, D. Omata, R. Suzuki, K. Maruyama, H. Mukai, S. Kawakami, Focused ultrasound/microbubbles-assisted BBB opening enhances LNP-mediated mRNA delivery to brain, *J. Control. Release* 348 (2022) 34–41, <https://doi.org/10.1016/j.jconrel.2022.05.042>.
- [182] Y. Guo, H. Lee, Z. Fang, A. Velalopoulou, J. Kim, M. Ben Thomas, J. Liu, R. G. Abramowitz, Y. Kim, A.F. Coskun, D.P. Krummel, S. Sengupta, T.J. MacDonald, C. Arvanitis, Single-cell analysis reveals effective siRNA delivery in brain tumors with microbubble-enhanced ultrasound and cationic nanoparticles, *Sci Adv* 7 (2021), <https://doi.org/10.1126/sciadv.abf7390>.
- [183] B.P. Mead, N. Kim, G.W. Miller, D. Hodges, P. Mastorakos, A.L. Klivanov, J. W. Mandell, J. Hirsh, J.S. Suk, J. Hanes, R.J. Price, Novel Focused Ultrasound Gene Therapy Approach Noninvasively Restores Dopaminergic Neuron Function in a Rat Parkinson's Disease Model, *Nano Lett.* 17 (2017) 3533–3542, <https://doi.org/10.1021/acs.nanolett.7b00616>.
- [184] A.M. Sonabend, A. Gould, C. Amidei, R. Ward, K.A. Schmidt, D.Y. Zhang, C. Gomez, J.F. Bebawy, B.P. Liu, G. Bouchoux, C. Desseaux, I.B. Helenowski, R. V. Lukas, K. Dixit, P. Kumthekar, V.A. Arrieta, M.S. Lesniak, A. Carpentier, H. Zhang, M. Muzzio, M. Canney, R. Stupp, Repeated blood–brain barrier opening with an implantable ultrasound device for delivery of albumin-bound paclitaxel in patients with recurrent glioblastoma: a phase 1 trial, *Lancet Oncol.* 24 (2023) 509–522, [https://doi.org/10.1016/S1470-2045\(23\)00112-2](https://doi.org/10.1016/S1470-2045(23)00112-2).
- [185] K.-C. Wei, CTNI-63 A PHASE IIA CLINICAL TRIAL FOR COMBINATION OF NAVIGATION-GUIDE FOCUSED ULTRASOUND AND BEVACIZUMAB FOR PATIENTS WITH RECURRENT GLIOBLASTOMA, *Neuro Oncol.* 25 (2023) v91–v, <https://doi.org/10.1093/neuonc/oad179.0345>.
- [186] R.G. Thorne, C. Nicholson, In vivo diffusion analysis with quantum dots and dextrans predicts the width of brain extracellular space, *PNAS* 103 (2006) 5567–5572, <https://doi.org/10.1073/PNAS.0509425103/ASSET/B2151F6F-3058-4A73-8775-CF9A856FD3E9/ASSETS/GRAPHIC/ZPQ0140617840007.JPG>.
- [187] D.R. Zimmermann, M.T. Dours-Zimmermann, Extracellular matrix of the central nervous system: From neglect to challenge, *Histochem. Cell Biol.* 130 (2008) 635–653, <https://doi.org/10.1007/s00418-008-0485-9>.
- [188] R.J. Tamargo, R. Langer, H. Brem, Interstitial Drug Delivery to the Central Nervous System Using Controlled Release Polymers: Chemotherapy for Brain Tumors, in: *Methods in Neurosciences*, Academic Press, 1994, pp. 135–149, <https://doi.org/10.1016/B978-0-12-185291-7.50014-X>.
- [189] C. Dagdeviren, K.B. Ramadi, P. Joe, K. Spencer, H.N. Schwerdt, H. Shimazu, S. Delcasso, K. Amemori, C. Nunez-Lopez, A.M. Graybiel, M.J. Cima, R. Langer, Miniaturized neural system for chronic, local intracerebral drug delivery, *Sci. Transl. Med.* 10 (2018) 1–10, <https://doi.org/10.1126/scitranslmed.aan2742>.
- [190] M.J. Fowler, J.D. Cotter, B.E. Knight, E.M. Seveck-Muraca, D.I. Sandberg, R. W. Sirianni, Intrathecal drug delivery in the era of nanomedicine, *Adv. Drug Deliv. Rev.* 165–166 (2020) 77–95, <https://doi.org/10.1016/j.addr.2020.02.006>.
- [191] C.H. Liu, J.Q. Ren, J. Yang, C. Liu, J.B. Mandeville, B.R. Rosen, P.G. Bhide, Y. Yanagawa, P.K. Liu, DNA-Based MRI Probes for Specific Detection of Chronic Exposure to Amphetamine in Living Brains, *J. Neurosci.* 29 (2009) 10663–10670, <https://doi.org/10.1523/JNEUROSCI.2167-09.2009>.
- [192] C. Helmschrodt, S. Höbel, S. Schöninger, A. Bauer, J. Bonicelli, M. Gringmuth, S. A. Fietz, A. Aigner, A. Richter, F. Richter, Polyethylenimine Nanoparticle-Mediated siRNA Delivery to Reduce α -Synuclein Expression in a Model of Parkinson's Disease, *Mol. Ther. Nucleic Acids* 9 (2017) 57–68, <https://doi.org/10.1016/j.omtn.2017.08.013>.
- [193] E.A. Nance, G.F. Woodworth, K.A. Sailor, T. Shih, Q. Xu, G. Swaminathan, D. Xiang, C. Eberhart, J. Hanes, A Dense Poly(Ethylene Glycol) Coating Improves Penetration of Large Polymeric Nanoparticles Within Brain Tissue, *Sci. Transl. Med.* 4 (2012), <https://doi.org/10.1126/scitranslmed.3003594>.
- [194] W. Zhan, C.-H. Wang, Convection enhanced delivery of chemotherapeutic drugs into brain tumour, *J. Control. Release* 271 (2018) 74–87, <https://doi.org/10.1016/j.jconrel.2017.12.020>.
- [195] E. Nance, C. Zhang, T.-Y. Shih, Q. Xu, B.S. Schuster, J. Hanes, Brain-Penetrating Nanoparticles Improve Paclitaxel Efficacy in Malignant Glioma Following Local Administration, *ACS Nano* 8 (2014) 10655–10664, <https://doi.org/10.1021/nn504210g>.
- [196] K. Negron, G. Kwak, H. Wang, H. Li, Y.-T. Huang, S.-W. Chen, B. Tyler, C. G. Eberhart, J. Hanes, J.S. Suk, A Highly Translatable Dual-arm Local Delivery Strategy To Achieve Widespread Therapeutic Coverage in Healthy and Tumor-bearing Brain Tissues, *Small* 19 (2023), <https://doi.org/10.1002/smll.202207278>.
- [197] R.W. Sirianni, M.-Q. Zheng, T.R. Patel, T. Shafbauer, J. Zhou, W.M. Saltzman, R. E. Carson, Y. Huang, Radiolabeling of Poly(lactic-co-glycolic acid) (PLGA) Nanoparticles with Biotinylated F-18 Prosthetic Groups and Imaging of Their Delivery to the Brain with Positron Emission Tomography, *Bioconjug. Chem.* 25 (2014) 2157–2165, <https://doi.org/10.1021/bc500315j>.
- [198] A. Mangraviti, S.Y. Tzeng, K.L. Kozielski, Y. Wang, Y. Jin, D. Gullotti, M. Pedone, N. Buaron, A. Liu, D.R. Wilson, S.K. Hansen, F.J. Rodriguez, G.-D. Gao, F. DiMeco, H. Brem, A. Olivi, B. Tyler, J.J. Green, Polymeric nanoparticles for nonviral gene therapy extend brain tumor survival in vivo, *ACS Nano* 9 (2015) 1236–1249, <https://doi.org/10.1021/nn504905q>.
- [199] K. Negron, C. Zhu, S.-W. Chen, S. Shahab, D. Rao, E.H. Raabe, C.G. Eberhart, J. Hanes, J.S. Suk, Non-adhesive and highly stable biodegradable nanoparticles that provide widespread and safe transgene expression in orthotopic brain tumors, *Drug Deliv. Transl. Res.* 10 (2020) 572–581, <https://doi.org/10.1007/s13346-020-00759-8>.
- [200] P. Mastorakos, C. Zhang, S. Berry, Y. Oh, S. Lee, C.G. Eberhart, G.F. Woodworth, J.S. Suk, J. Hanes, Highly PEGylated DNA Nanoparticles Provide Uniform and Widespread Gene Transfer in the Brain, *Adv. Healthc. Mater.* 4 (2015) 1023–1033, <https://doi.org/10.1002/adhm.201400800>.
- [201] A.J. Pickering, N.G. Lamson, M.H. Marand, W. Hwang, J.P. Straehla, P. T. Hammond, Layer-by-Layer Polymer Functionalization Improves Nanoparticle Penetration and Glioblastoma Targeting in the Brain, *ACS Nano* 17 (2023) 24154–24169, <https://doi.org/10.1021/acsnano.3c09273>.
- [202] D. Séhédic, I. Chourpa, C. Tétaud, A. Griveau, C. Loussouarn, S. Avril, C. Legendre, N. Lepereur, D. Wion, F. Hindré, F. Davodeau, E. Garcion, Locoregional Confinement and Major Clinical Benefit of 188 Re-Loaded CXCR4-Targeted Nanocarriers in an Orthotopic Human to Mouse Model of Glioblastoma, *Theranostics* 7 (2017) 4517–4536, <https://doi.org/10.7150/thno.19403>.
- [203] M.L. Borrajo, A. Quijano, P. Lapuhs, A.I. Rodriguez-Perez, S. Anthiya, J. L. Labandeira-Garcia, R. Valenzuela, M.J. Alonso, Ionizable nanoemulsions for RNA delivery into the central nervous system – importance of diffusivity, *J. Control. Release* 372 (2024) 295–303, <https://doi.org/10.1016/j.jconrel.2024.06.051>.
- [204] C.T. Curley, B.P. Mead, K. Negron, N. Kim, W.J. Garrison, G.W. Miller, K. M. Kingsmore, E.A. Thim, J. Song, J.M. Munson, A.L. Klivanov, J.S. Suk, J. Hanes, R.J. Price, Augmentation of brain tumor interstitial flow via focused ultrasound promotes brain-penetrating nanoparticle dispersion and transfection, *Sci. Adv.* 6 (2020), <https://doi.org/10.1126/sciadv.aay1344>.
- [205] D. Dahis, D.M. Azagury, F. Obeid, M.Z. Dion, A.M. Cryer, M.A. Riquelme, P. Dosta, A.W. Abraham, M. Gavish, N. Artzi, Y. Shamay, H. Azhari, Focused Ultrasound Enhances Brain Delivery of Sorafenib Nanoparticles, *Adv. NanoBiomed Res.* 3 (2023) 2200142, <https://doi.org/10.1002/anbr.202200142>.
- [206] AntibodySociety, Antibody therapeutics approved or in regulatory review in the EU or US, (n.d.), <https://www.antibodysociety.org/resources/approved-antibodies/>.
- [207] L.X. Yi, E.K. Tan, Z.D. Zhou, Passive immunotherapy for Alzheimer's disease: challenges & future directions, *J. Transl. Med.* 22 (2024) 430, <https://doi.org/10.1186/s12967-024-05248-x>.
- [208] J. Xie, D. Gonzalez-Carter, T.A. Tockary, N. Nakamura, Y. Xue, M. Nakakido, H. Akiba, A. Dirisala, X. Liu, K. Toh, T. Yang, Z. Wang, S. Fukushima, J. Li, S. Quader, K. Tsumoto, T. Yokota, Y. Anraku, K. Kataoka, Dual-sensitive nanomicelles enhancing systemic delivery of therapeutically active antibodies specifically into the brain, *ACS Nano* 14 (2020) 6729–6742, <https://doi.org/10.1021/acsnano.9b09991>.
- [209] A. Amano, N. Sanjo, W. Araki, Y. Anraku, M. Nakakido, E. Matsubara, T. Tomiyama, T. Nagata, K. Tsumoto, K. Kataoka, T. Yokota, Peripheral administration of nanomicelle-encapsulated anti-A β oligomer fragment antibody reduces various toxic A β species in the brain, *J. Nanobiotechnol.* 21 (2023) 36, <https://doi.org/10.1186/s12951-023-01772-y>.
- [210] M. Rotman, M.M. Welling, A. Bunschoten, M.E. de Backer, J. Rip, R.J.A. Nabuurs, P.J. Gaillard, M.A. van Buchem, S.M. van der Maarel, L. van der Weerd, Enhanced glutathione PEGylated liposomal brain delivery of an anti-amyloid single domain antibody fragment in a mouse model for Alzheimer's disease, *J. Control. Release* 203 (2015) 40–50, <https://doi.org/10.1016/j.jconrel.2015.02.012>.
- [211] S. Li, C. Meng, Q. Hao, L. Dai, J. Shi, J. Xu, X. Zhou, S. Zhao, J. Yang, H. Kang, Y. Hou, M. Zhang, X. Ma, M. Tan, L. W. Wu, A Multistage-responsive antibody-delivery strategy to improve immunotherapy for NSCLC brain metastasis by ultrasensitive releasing and tumor-anchoring, *Adv. Funct. Mater.* 34 (2024) 2312595, <https://doi.org/10.1002/adfm.202312595>.
- [212] H. Wang, Y. Chao, H. Zhao, X. Zhou, F. Zhang, Z. Zhang, Z. Li, J. Pan, J. Wang, Q. Chen, Z. Liu, Smart nanomedicine to enable crossing blood–brain barrier delivery of checkpoint blockade antibody for immunotherapy of glioma, *ACS Nano* 16 (2022) 664–674, <https://doi.org/10.1021/acsnano.1c08120>.
- [213] L. Han, C. Liu, H. Qi, J. Zhou, J. Wen, D. Wu, D. Xu, M. Qin, J. Ren, Q. Wang, L. Long, Y. Liu, I. Chen, X. Yuan, Y. Lu, C. Kang, Systemic delivery of monoclonal antibodies to the central nervous system for brain tumor therapy, *Adv. Mater.* 31 (2019), <https://doi.org/10.1002/adma.201805697>.
- [214] J. Wen, D. Wu, M. Qin, C. Liu, L. Wang, D. Xu, H.V. Vinters, Y. Liu, E. Kranz, X. Guan, G. Sun, X. Sun, Y. Lee, O. Martinez-Maza, D. Widney, Y. Lu, I.S.Y. Chen, M. Kamata, Sustained delivery and molecular targeting of a therapeutic monoclonal antibody to metastases in the central nervous system of mice, *Nat. Biomed. Eng.* 3 (2019) 706–716, <https://doi.org/10.1038/s41551-019-0434-z>.
- [215] Y. Zhang, Y. Ren, H. Xu, L. Li, F. Qian, L. Wang, A. Quan, H. Ma, H. Liu, R. Yu, Cascade-responsive 2-DG nanocapsules encapsulate aV-siCPT1C conjugates to inhibit glioblastoma through multiple inhibition of energy metabolism, *ACS Appl. Mater. Interf.* 15 (2023) 10356–10370, <https://doi.org/10.1021/acsami.2c19285>.

- [216] Y. Zhu, L. Zhu, X. Wang, H. Jin, RNA-based therapeutics: an overview and prospectus, *Cell Death Dis.* 13 (2022) 644, <https://doi.org/10.1038/s41419-022-05075-2>.
- [217] T.R. Damase, R. Sukhovshin, C. Boada, F. Taraballi, R.I. Pettigrew, J.P. Cooke, The limitless future of RNA therapeutics, *Front. Bioeng. Biotechnol.* 9 (2021), <https://doi.org/10.3389/fbioe.2021.628137>.
- [218] M. Imran Sajid, F. Sultan Sheikh, F. Anis, N. Nasim, R.K. Sumbria, S.M. Nauli, R. Kumar Tiwari, siRNA drug delivery across the blood-brain barrier in Alzheimer's disease, *Adv. Drug Deliv. Rev.* 199 (2023) 114968, <https://doi.org/10.1016/j.addr.2023.114968>.
- [219] X. Dong, Current Strategies for Brain Drug Delivery, *Theranostics* 8 (2018) 1481–1493, <https://doi.org/10.7150/thno.21254>.
- [220] T.M. Miller, A. Pestronk, W. David, J. Rothstein, E. Simpson, S.H. Appel, P. L. Andres, K. Mahoney, P. Allred, K. Alexander, L.W. Ostrow, D. Schoenfeld, E. A. Macklin, D.A. Norris, G. Manousakis, M. Crisp, R. Smith, C.F. Bennett, K. M. Bishop, M.E. Cudkowicz, An antisense oligonucleotide against SOD1 delivered intrathecally for patients with SOD1 familial amyotrophic lateral sclerosis: a phase 1, randomised, first-in-man study, *Lancet Neurol.* 12 (2013) 435–442, [https://doi.org/10.1016/S1474-4422\(13\)70061-9](https://doi.org/10.1016/S1474-4422(13)70061-9).
- [221] M. Haché, K.J. Swoboda, N. Sethna, A. Farrow-Gillespie, A. Khandji, S. Xia, K. M. Bishop, Intrathecal injections in children with spinal muscular atrophy: nusinersen clinical trial experience, *J. Child Neurol.* 31 (2016) 899–906, https://doi.org/10.1177/0883073815627882/ASSET/IMAGES/LARGE/10.1177_0883073815627882-FIG1.JPEG.
- [222] E. Mercuri, B.T. Darras, C. Chiriboga, J.W. Day, C. Campbell, A.M. Connolly, S. T. Iannaccone, J. Kirschner, N.L. Kuntz, K. Saito, P.B. Shieh, M. Tulinius, E. S. Mazzone, J. Montes, K.M. Bishop, Q. Yang, R. Foster, S. Gheuens, C.F. Bennett, W. Farwell, E. Schneider, D.C. De Vivo, R.S. Finkel, Nusinersen versus Sham Control in Later-Onset Spinal Muscular Atrophy, *N. Engl. J. Med.* 378 (2018) 625–635, <https://doi.org/10.1056/NEJMoa1710504>.
- [223] M.L. Borrajo, M.J. Alonso, Using nanotechnology to deliver biomolecules from nose to brain — peptides, proteins, monoclonal antibodies and RNA, *Drug Deliv. Transl. Res.* 12 (2022) 862–880, <https://doi.org/10.1007/s13346-021-01086-2>.
- [224] S.A. Doggrell, Lessons that can be learnt from the failure of verubecestat in Alzheimer's disease, *Expert Opin. Pharmacother.* 20 (2019) 2095–2099, <https://doi.org/10.1080/14656566.2019.1654998>.
- [225] M. Hrabanova, J. Pejchal, T. Kucera, D. Jun, M. Schmidt, O. Soukup, Is It the Twilight of BACE1 Inhibitors? *Curr. Neuropharmacol.* 19 (2020) 61–77, <https://doi.org/10.2174/1570159X18666200503023323>.
- [226] P. Wang, X. Zheng, Q. Guo, P. Yang, X. Pang, K. Qian, W. Lu, Q. Zhang, X. Jiang, Systemic delivery of BACE1 siRNA through neuron-targeted nanocomplexes for treatment of Alzheimer's disease, *J. Control. Release* 279 (2018) 220–233, <https://doi.org/10.1016/j.jconrel.2018.04.034>.
- [227] Y. Zhou, F. Zhu, Y. Liu, M. Zheng, Y. Wang, D. Zhang, Y. Anraku, Y. Zou, J. Li, H. Wu, X. Pang, W. Tao, O. Shimoni, A.I. Bush, X. Xue, B. Shi, Blood-brain barrier-penetrating siRNA nanomedicine for Alzheimer's disease therapy, *Sci. Adv.* 6 (2020), <https://doi.org/10.1126/sciadv.abc7031>.
- [228] L.L. Israel, T. Sun, O. Braubach, A. Cox, E.S. Shatalova, H.-M. Rashid, A. Galstyan, Z. Grodzinski, X.Y. Song, O. Chepurina, V.A. Ljubimov, A. Chiechi, S. Sharma, C. Phebus, Y. Wang, J.Y. Ljubimova, K.L. Black, E. Holler, β -Amyloid targeting nanodrug for neuron-specific delivery of nucleic acids in Alzheimer's disease mouse models, *J. Control. Release* 361 (2023) 636–658, <https://doi.org/10.1016/j.jconrel.2023.08.001>.
- [229] S.S. Hébert, K. Horré, L. Nicolai, B. Bergmans, A.S. Papadopolou, A. Delacourte, B. De Strooper, MicroRNA regulation of Alzheimer's Amyloid precursor protein expression, *Neurobiol. Dis.* 33 (2009) 422–428, <https://doi.org/10.1016/j.nbd.2008.11.009>.
- [230] Q. Ouyang, K. Liu, Q. Zhu, H. Deng, Y. Le, W. Ouyang, X. Yan, W. Zhou, J. Tong, Brain-penetration and neuron-targeting DNA nanoflowers co-delivering miR-124 and rutin for synergistic therapy of alzheimer's disease, *Small* 18 (2022), <https://doi.org/10.1002/sml.202107534>.
- [231] A.A. Pimenova, M. Herbinet, I. Gupta, S.I. Machlovi, K.R. Bowles, E. Marcora, A. M. Goate, Alzheimer's-associated PU.1 expression levels regulate microglial inflammatory response, *Neurobiol Dis* 148 (2021) 105217, <https://doi.org/10.1016/j.nbd.2020.105217>.
- [232] W.T. Ralvenius, J.L. Andresen, M.M. Huston, J. Penney, J.M. Bonner, O.S. Fenton, R. Langer, L. Tsai, Nanoparticle-Mediated Delivery of Anti-PU.1 siRNA via Localized Intracisternal Administration Reduces Neuroinflammation, *Adv. Mater.* 36 (2024), <https://doi.org/10.1002/adma.202309225>.
- [233] A.-C. Raulin, S.V. Doss, Z.A. Trottier, T.C. Ikezu, G. Bu, C.-C. Liu, ApoE in Alzheimer's disease: pathophysiology and therapeutic strategies, *Mol. Neurodegener.* 17 (2022) 72, <https://doi.org/10.1186/s13024-022-00574-4>.
- [234] L.L. Drogos, S.J. Gill, A. V Tyndall, J.K. Raneri, J.S. Parboosingh, A. Naef, K.D. Guild, G. Eskes, P.J. Hanly, M.J. Poulin, MEDICAL HYPOTHESIS (2016).
- [235] J. Blackman, S. Love, L. Sinclair, R. Cain, E. Coulthard, APOE ϵ 4 Alzheimer's disease neuropathology and sleep disturbance, in individuals with and without dementia, *Alzheimers Res. Ther.* 14 (2022) 47, <https://doi.org/10.1186/s13195-022-00992-y>.
- [236] T. Williams, D.R. Borchelt, P. Chakrabarty, Therapeutic approaches targeting Apolipoprotein E function in Alzheimer's disease, *Mol. Neurodegener.* 15 (2020) 8, <https://doi.org/10.1186/s13024-020-0358-9>.
- [237] C.M. Ferguson, S. Hildebrand, B.M.D.C. Godinho, J. Buchwald, D. Echeverria, A. Coles, A. Grigorenko, L. Vangjeli, J. Sousa, N. McHugh, M. Hassler, F. Santarelli, M.T. Heneka, E. Rogae, A. Khvorova, Silencing ApoE with divalent-siRNAs improves amyloid burden and activates immune response pathways in Alzheimer's disease, *Alzheimer's Dement.* 20 (2024) 2632–2652, <https://doi.org/10.1002/alz.13703>.
- [238] Y. Wang, X. Wang, R. Xie, J.C. Burger, Y. Tong, S. Gong, Overcoming the Blood-Brain Barrier for Gene Therapy via Systemic Administration of GSH-Responsive Silica Nanocapsules, *Adv. Mater.* 35 (2023), <https://doi.org/10.1002/adma.202208018>.
- [239] E. Samaridou, H. Walgrave, E. Salta, D.M. Álvarez, V. Castro-López, M. Loza, M. J. Alonso, Nose-to-brain delivery of enveloped RNA - cell permeating peptide nanocomplexes for the treatment of neurodegenerative diseases, *Biomaterials* 230 (2020) 119657, <https://doi.org/10.1016/j.biomaterials.2019.119657>.
- [240] C.J. Mummery, A. Börjesson-Hanson, D.J. Blackburn, E.G.B. Vijverberg, P.P. De Deyn, S. Ducharme, M. Jonsson, A. Schneider, J.O. Rinne, A.C. Ludolph, R. Bodenschatz, H. Kordasiewicz, E.E. Swayze, B. Fitzsimmons, L. Mignon, K. M. Moore, C. Yun, T. Baumann, D. Li, D.A. Norris, R. Crean, D.L. Graham, E. Huang, E. Ratti, C.F. Bennett, C. Junge, R.M. Lane, Tau-targeting antisense oligonucleotide MAPTRx in mild Alzheimer's disease: a phase 1b, randomized, placebo-controlled trial, *Nat. Med.* 29 (2023) 1437–1447, <https://doi.org/10.1038/s41591-023-02326-3>.
- [241] S. Lin, F. Du, Y. Wang, S. Ji, D. Liang, L. Yu, Z. Li, An acid-labile block copolymer of PDMAEMA and PEG as potential carrier for intelligent gene delivery systems, *Biomacromolecules* 9 (2008) 109–115, <https://doi.org/10.1021/bm7008747>.
- [242] X. Huang, L. Xu, H. Qian, X. Wang, Z. Tao, Polymalic acid for translational nanomedicine, *J. Nanobiotechnol.* 20 (2022), <https://doi.org/10.1186/s12951-022-01497-4>.
- [243] W.T. Godbey, K.K. Wu, A.G. Mikos, Poly(ethylenimine)-mediated gene delivery affects endothelial cell function and viability (2001).
- [244] J.V. Gregory, P. Kadiyala, R. Doherty, M. Cadena, S. Habel, E. Ruoslahti, P. R. Lowenstein, M.G. Castro, J. Lahann, Systemic brain tumor delivery of synthetic protein nanoparticles for glioblastoma therapy, *Nat. Commun.* 11 (2020) 5687, <https://doi.org/10.1038/s41467-020-19225-7>.
- [245] I. Tabujew, C. Freidel, B. Krieg, M. Helm, K. Koynov, K. Müllen, K. Peneva, The guanidinium group as a key part of water-soluble polymer carriers for siRNA complexation and protection against degradation, *Macromol. Rapid Commun.* 35 (2014) 1191–1197, <https://doi.org/10.1002/marc.201400120>.
- [246] Y. Zou, X. Sun, Y. Wang, C. Yan, Y. Liu, J. Li, D. Zhang, M. Zheng, R.S. Chung, B. Shi, Single siRNA Nanocapsules for Effective siRNA Brain Delivery and Glioblastoma Treatment, *Adv. Mater.* 32 (2020), <https://doi.org/10.1002/adma.202000416>.
- [247] W. Lin, R. Niu, S.-M. Park, Y. Zou, S.S. Kim, X. Xia, S. Xing, Q. Yang, X. Sun, Z. Yuan, D. Zhang, H.J. Kwon, S. Park, C. Il Kim, H. Koo, Y. Liu, H. Wu, M. Zheng, H. Yoo, B. Shi, J.B. Park, J. Yin, IGFBP5 is an ROR1 ligand promoting glioblastoma invasion via ROR1/HER2-CREB signaling axis, *Nat. Commun.* 14 (2023) 1578, <https://doi.org/10.1038/s41467-023-37306-1>.
- [248] J.J. Green, R. Langer, D.G. Anderson, A combinatorial polymer library approach yields insight into nonviral gene delivery, *Acc. Chem. Res.* 41 (2008) 749–759, <https://doi.org/10.1021/ar7002336>.
- [249] S.Y. Tzeng, J.J. Green, Subtle changes to polymer structure and degradation mechanism enable highly effective nanoparticles for siRNA and DNA delivery to human brain cancer, *Adv. Healthc. Mater.* 2 (2013) 468–480, <https://doi.org/10.1002/adhm.201200257>.
- [250] B. Tyler, D. Gullotti, A. Mangraviti, T. Utsuki, H. Brem, Polylactic acid (PLA) controlled delivery carriers for biomedical applications, *Adv Drug Deliv Rev* 107 (2016) 163–175, <https://doi.org/10.1016/j.addr.2016.06.018>.
- [251] J. Karlsson, Y. Rui, K.L. Kozielski, A.L. Placone, O. Choi, S.Y. Tzeng, J. Kim, J. J. Keyes, M.I. Bogorad, K. Gabrielson, H. Guerrero-Cazares, A. Quinones-Hinojosa, P.C. Searson, J.J. Green, Engineered nanoparticles for systemic siRNA delivery to malignant brain tumours, *Nanoscale* 11 (2019) 20045–20057, <https://doi.org/10.1039/C9NR04795F>.
- [252] M. Zhou, N. Jiang, J. Fan, S. Fu, H. Luo, P. Su, M. Zhang, H. Shi, J. Zeng, Y. Huang, Y. Li, H. Shen, A. Zhang, R. Li, H7K(R)2-modified pH-sensitive self-assembled nanoparticles delivering small interfering RNA targeting hepatoma-derived growth factor for malignant glioma treatment, *J. Control. Release* 310 (2019) 24–35, <https://doi.org/10.1016/j.jconrel.2019.08.010>.
- [253] Y. Wang, S.-Y. Li, S. Shen, J. Wang, Protecting neurons from cerebral ischemia/reperfusion injury via nanoparticle-mediated delivery of an siRNA to inhibit microglial neurotoxicity, *Biomaterials* 161 (2018) 95–105, <https://doi.org/10.1016/j.biomaterials.2018.01.039>.
- [254] M. de la Fuente, B. Seijo, M.J. Alonso, Bioadhesive hyaluronan-chitosan nanoparticles can transport genes across the ocular mucosa and transfect ocular tissue, *Gene Ther.* 15 (2008) 668–676, <https://doi.org/10.1038/gt.2008.16>.
- [255] A.E. De Salamanca, Y. Diebold, M. Calonge, C. García-Vazquez, S. Callejo, A. Vila, M.J. Alonso, Chitosan nanoparticles as a potential drug delivery system for the ocular surface: Toxicity, uptake mechanism and in vivo tolerance, *Invest. Ophthalmol. Vis. Sci.* 47 (2006) 1416–1425, <https://doi.org/10.1167/iovs.05-0495>.
- [256] X. Wang, J. Yao, J.P. Zhou, Y. Lu, W. Wang, Synthesis and evaluation of chitosan-graft-polyethylenimine as a gene vector, *Pharmazie* 65 (2010) 572–579, <https://doi.org/10.1691/ph.2010.9422>.
- [257] F. Bordin, L. Chronopoulou, C. Palocci, F. Bomboi, A. Di Martino, N. Cifani, B. Pompili, F. Ascenzioni, S. Sennato, Chitosan-DNA complexes: effect of molecular parameters on the efficiency of delivery, *Colloids Surf A Physicochem Eng Asp* 460 (2014) 184–190, <https://doi.org/10.1016/j.colsurfa.2013.12.022>.
- [258] N. Shamaeizadeh, J. Varshosaz, M. Miran, M. Aliomrani, Glutathione targeted tragacanth acid-chitosan as a non-viral vector for brain delivery of miRNA-219a-5P: An in vitro/in vivo study, *Int. J. Biol. Macromol.* 200 (2022) 543–556, <https://doi.org/10.1016/j.ijbiomac.2022.01.100>.

- [259] J. Du, Y. Shao, Y. Hu, Y. Chen, J. Cang, X. Chen, W. Pei, F. Miao, Y. Shen, M. Muddassir, Y. Zhang, J. Zhang, G. Teng, Multifunctional liposomes enable active targeting and twifilin 1 silencing to reverse paclitaxel resistance in brain metastatic breast cancer, *ACS Appl Mater Interfaces* 13 (2021) 23396–23409, <https://doi.org/10.1021/acsami.1c02822>.
- [260] Y. Eygeris, M. Gupta, J. Kim, G. Sahay, Chemistry of Lipid Nanoparticles for RNA Delivery, *Acc. Chem. Res.* 55 (2022) 2–12, <https://doi.org/10.1021/acs.accounts.1c00544>.
- [261] X. Hou, T. Zaks, R. Langer, Y. Dong, Lipid nanoparticles for mRNA delivery, *Nat. Rev. Mater.* 6 (2021) 1078–1094, <https://doi.org/10.1038/s41578-021-00358-0>.
- [262] D. Rosenblum, A. Gutkin, R. Kedmi, S. Ramishetti, N. Veiga, A.M. Jacobi, M.S. Schubert, D. Friedmann-Morvinski, Z.R. Cohen, M.A. Behlke, J. Lieberman, D. Peer, CRISPR-Cas9 genome editing using targeted lipid nanoparticles for cancer therapy, 2020. <https://www.science.org>.
- [263] Z.R. Cohen, S. Ramishetti, N. Peshes-Yaloz, M. Goldsmith, A. Wohl, Z. Zibly, D. Peer, Localized RNAi therapeutics of chemoresistant grade IV glioma using hyaluronan-grafted lipid-based nanoparticles, *ACS Nano* 9 (2015) 1581–1591, <https://doi.org/10.1021/nn506248s>.
- [264] C. Wang, Y. Xue, T. Markovic, H. Li, S. Wang, Y. Zhong, S. Du, Y. Zhang, X. Hou, Y. Yu, Z. Liu, M. Tian, D.D. Kang, L. Wang, K. Guo, D. Cao, J. Yan, B. Deng, D. W. McComb, R.E. Parsons, A.M. Minier-Toribio, L.M. Holt, J. Pan, A. Hashemi, B. H. Kopell, A.W. Charney, E.J. Nestler, P.C. Peng, Y. Dong, Blood–brain-barrier-crossing lipid nanoparticles for mRNA delivery to the central nervous system, *Nat. Mater.* (2025), <https://doi.org/10.1038/s41563-024-02114-5>.
- [265] E.L. Han, S. Tang, D. Kim, A.M. Murray, K.L. Swingle, A.G. Hamilton, K. Mrksich, M.S. Padilla, R. Palanki, J.J. Li, M.J. Mitchell, Peptide-Functionalized Lipid Nanoparticles for Targeted Systemic mRNA Delivery to the Brain, *Nano Lett.* (2024), <https://doi.org/10.1021/acs.nanolett.4c05186>.
- [266] J. Nong, P.M. Glassman, V.V. Shuvaev, S. Reyes-Estevés, H.C. Descamps, R. Y. Kiseleva, T.E. Papp, M.G. Alameh, Y.K. Tam, B.L. Mui, S. Omo-Lamai, M. E. Zamora, T. Shuvaeva, E. Argüiri, X. Gong, T.V. Brysgel, A.W. Tan, A. G. Woolfork, A. Weljie, C.A. Thaiss, J.W. Myerson, D. Weissman, S.E. Kasner, H. Parhiz, V.R. Muzykantov, J.S. Brenner, O.A. Marcos-Contreras, Targeting lipid nanoparticles to the blood-brain barrier to ameliorate acute ischemic stroke, *Mol. Ther.* 32 (2024) 1344–1358, <https://doi.org/10.1016/j.ymthe.2024.03.004>.
- [267] M. Gao, Y. Li, W. Ho, C. Chen, Q. Chen, F. Li, M. Tang, Q. Fan, J. Wan, W. Yu, X. Xu, P. Li, X.-Q. Zhang, Targeted mRNA nanoparticles ameliorate blood–brain barrier disruption postischemic stroke by modulating microglia polarization, *ACS Nano* 18 (2024) 3260–3275, <https://doi.org/10.1021/acs.nano.3c09817>.
- [268] C. Holohan, S. Van Schaeybroeck, D.B. Longley, P.G. Johnston, Cancer drug resistance: an evolving paradigm, *Nat. Rev. Cancer* 13 (2013) 714–726, <https://doi.org/10.1038/nrc3599>.
- [269] Search for: cns OR Brain Cancer or Alzheimer, Other terms: monoclonal antibody OR RNA therapeutics OR mRNA OR siRNA OR ASO OR aptamers OR miRNA, Not yet recruiting, Recruiting, Active, not recruiting studies, Phase: Early Phase 1, 1, 2, 3, 4, Interventional studies | List Results | ClinicalTrials.gov, (n.d.). <https://clinicaltrials.gov/search?cond=cns%20OR%20Brain%20Cancer%20or%20Alzheimer&aggFilters=phase:0%201%202%203%204,status:rec%20act%20not,studyType:int&limit=100&term=monoclonal%20antibody%20OR%20RNA%20therapeutics%20OR%20mRNA%20OR%20siRNA%20OR%20ASO%20OR%20aptamers%20OR%20miRNA%20> (accessed November 20, 2024).
- [270] J. Cummings, Y. Zhou, G. Lee, K. Zhong, J. Fonseca, F. Cheng, Alzheimer's disease drug development pipeline: 2024, *Alzheimer's Dement. Transl. Res. Clin. Intervent.* 10 (2024) e12465, <https://doi.org/10.1002/trc2.12465>.