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Nanosystems for gene therapy targeting brain damage caused by viral infections

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ABSTRACT

Several human pathogens can cause long-lasting neurological damage. Despite the increasing clinical knowledge about these conditions, most still lack efficient therapeutic interventions. Gene therapy (GT) approaches comprise strategies to modify or adjust the expression or function of a gene, thus providing therapy for human diseases. Since recombinant nucleic acids used in GT have physicochemical limitations and can fail to reach the desired tissue, viral and non-viral vectors are applied to mediate gene delivery. Although viral vectors are associated to high levels of transfection, non-viral vectors are safer and have been further explored. Different types of nanosystems consisting of lipids, polymeric and inorganic materials are applied as non-viral vectors. In this review, we discuss potential targets for GT intervention in order to prevent neurological damage associated to infectious diseases as well as the role of nanosized non-viral vectors as agents to help the selective delivery of these gene-modifying molecules. Application of non-viral vectors for delivery of GT effectors comprise a promising alternative to treat brain inflammation induced by viral infections.

1. Introduction

Several human pathogens can cause long-lasting symptoms and devastating effects on life quality of patients [1,2]. Viral infections can often lead to neurological symptoms, and while some pathogens can invade the Central Nervous System (CNS) directly triggering inflammation, others affect normal brain function by inducing severe systemic immune responses. In most cases, the factors that lead to increased susceptibility of individuals to develop neurological complications following viral infections remain unknown. In addition, although clinical knowledge about these diseases has improved in recent years, efficient therapeutic interventions are still lacking [3]. Therefore, gene therapy can emerge as a strategy to target neuroinflammation and brain damage caused by infectious diseases.

Gene therapy (GT) approaches comprise strategies to modify or adjust the expression or function of a gene, thus providing therapy for human diseases. GT agents comprise recombinant nucleic acids (DNA or RNA) which can adjust, repair, replace, add or remove a given gene sequence [4]. Plasmid DNAs, anti-sense oligonucleotides, small interfering RNA (siRNA)-lipid complexes, live viruses, and genetically engineered cells are examples of effectors used in GT [5]. A wide range of GT agents and delivery techniques have been designed and tested as treatment for many disorders [6] including cardiovascular, muscular, metabolic, hematological, ophthalmological and neurological diseases [7–9]. Viral encephalitis are especially interesting for GT application due to the smaller timeframe between disease onset and diagnosis, compared to chronic neurodegenerative diseases. Therefore, GT strategies can be employed at early stages, before irreversible tissue damage is established.

Since recombinant nucleic acids used in GT have physicochemical limitations and can fail to reach the desired tissue, viral and non-viral vectors are applied to mediate gene delivery. Although viral vectors are associated to high levels of transfection, non-viral vectors are safer and have been further explored. Different types of nanosystems consisting of lipids and polymeric materials are applied as non-viral vectors. In this review, we will discuss potential targets for GT intervention in order to prevent brain damage associated with infectious diseases as well as the

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role of organic and inorganic non-viral vectors as agents to help the selective delivery of these gene-modifying molecules. Therefore, the aim of this work is to highlight the application of non-viral vectors for delivery of GT effectors, as an alternative to treat brain inflammation induced by viral infections.

2. Methods

Searches were performed in PubMed, Google Scholar and Science Direct. Search terms were divided into three groups, as follows: group 1 ("gene therapy", "transfection", "siRNA", "miRNA", "gene silencing", "CRISPR-Cas9", "neuroinflammation"); group 2 ("viral infection", "viral neuroinflammation", "brain", "glial cells", "WHO", "neurological disorders", "encephalitis", "treatment", "malaria", "Zika", "Herpes Simplex Virus", "Human Immunodeficiency Virus", "West Nile Virus", "SARS-CoV-2"); group 3 ("viral vectors", "non-viral vectors", "liposomes", "cationic lipids", "helper lipids", "endocytosis", "lipoplexes", "inorganic nanoparticles", "gold nanoparticles", "quantum dots nanoparticles", "graphene quantum dots", "carbon quantum dots", "polymer nanoparticle", "dendrimers", "branched polymers", "polyplexes", "cationic polymers", "sponge effect", "polymeric micelles" and "lipopolyplexes"). Searches were performed using different combinations of words from groups 1, 2 and 3; 1 and 3 or 2 and 3. Only articles published after 2010 were considered, but relevant publications cited by these articles were also added to the study.

3. Potential targets for gene therapy in brain inflammation caused by viral infections

As infectious diseases are emerging or re-emerging due to urbanization and climate changes, the population is susceptible to viral epidemics and pandemics [10]. Neurotropic viruses invade the CNS and have great affinity for neural cells, having reemerged as public health threats. Other pathogens, which do not show specific tropism for neural tissue, can still induce immune responses that affect brain homeostasis and blood-brain barrier (BBB) integrity, leading to altered CNS function [11,12], cerebral edema, encephalitis and myelitis [13]. Table 1 shows a list of pathogens most frequently associated to brain damage and some of the potential targets for intervention by GT. For some of these conditions, prototype GT agents have been developed and tested at some level. The main findings concerning the development of GT agents to treat viral-induced brain inflammation are illustrated in Fig. 1.

4. Approaches for gene delivery in encephalitis: non-viral vectors

Although GT strategies are promising alternatives to treat several diseases, there are some challenges that need to be overcome when designing a new gene therapy system [36]. Since nucleic acids are large and negatively charged, they do not freely cross the cell membrane. When delivered as free molecules, they are susceptible to cellular endonucleases and renal clearance. Therefore, a delivery vector should be applied to allow effective cellular internalization and transfection of nucleic acids [37–39]. Accordingly, both viral and non-viral vectors have been effectively employed in gene therapy [40]. Table 2 summarizes studies that have applied vectors for gene therapy delivery for treatment of viral encephalitis. Adenoviral, adeno-associated viral and lentiviral vectors are some examples of viral vectors applied in these cases [41]. Viral systems present high transfection potential and constant expression of therapeutic genes [40].

Even though viral vectors have been the most frequently used vehicles for GT delivery, they present disadvantages such as the high cost, the risk of provoking undesired immune response and the limited size of the genetic material that can be incorporated [48,49]. In addition, product quality protocols and the mean shelf-life of products involving the use of viral vectors are unknown in most cases [50]. Therefore, the application of non-viral transfection for GT strategies rises as a promising, cheaper and safer approach.

Non-viral transfection approaches include physical methods such as electroporation, biolistic, microinjection, laser, heat exposure and ultrasound, which can injure the cell during the transfection process [51,52]. Chemical methods are alternative non-viral approaches of transfection and frequently involve the use of compounds such as calcium phosphate, DEAE-dextran, cationic lipids, and cationic polymers [52–54]. Cationic lipids and polymers can be part of nanosystem structures since they directly interact with the negative charge from nucleic acid.

Biomaterials have been employed to structure non-viral vectors, mediating a more controlled gene delivery than viral vectors, which

Table 1
Viral infections and encephalic manifestations. The main viruses frequently associated to brain damage are listed, as well as the frequency of these symptoms and the most widely used approaches to prevent or treat these conditions.

Pathogens	Neurological manifestations	Frequency of neurological manifestations	Available treatments	References
West-Nile	WNND, Meningitis, encephalitis, acute flaccid paralysis and coma	1 in every 150 infections	Limited to supportive care	FULTON et al., 2020 [14]; SANTINI et al., 2022 [15]; YAKASS; FRANCO; QUAYE, 2020 [16]; ZIDOVEC-LEPEJ et al., 2021 [17]
Zika in newborns	CZS, brain calcifications, hydrocephaly, Microcephaly seizures and developmental delay	6–12% of infected pregnancies will result in CZS, 60% of normocephalic babies presented seizures, 2.3% presented microcephaly	No available treatments	BARBEITO-ANDRÉS et al., 2020 [18]; BENAZZATO; RUSSO; BELTRÃO-, 2022 [19]; CAMPOS COELHO; CROVELLA, 2017 [20]; SOUZA et al., 2019 [21]; TAKAHASHI; UI-TEI, 2020 [22]
Zika in adults	Encephalitis, meningitis, meningoencephalitis, myelitis, memory impairments, cognitive declines, sensory polyneuropathy and Guillain-Barré syndrome	6 of 41 patients with Zika infection had neurological manifestation	No approved drug treatments or vaccines	ARTAL; ARAUJO, 2020 [23]; BIDO-MEDINA et al., 2018 [24]; SOUZA et al., 2019 [21]
Herpes simplex virus	Herpes simplex encephalitis (HSE)	HSE in children 6% and 13% in adults	Acyclovir, antivirals and anticonvulsants	BARTOLINI et al., 2019 [25]; DA SILVA et al., 2016 [26]; LIU et al., 2019a [27]; MCGRATH et al., 1997 [28]; SELLNER; TRINKA, 2012 [29]; SOLBRIG et al., 2006 [30]
HIV	Stages of HIV-Associated Neurocognitive Disorder (HAND): asymptomatic neurocognitive impairment (ANI), mild neurocognitive disorder (MND) and HIV-associated dementia (HAD)	23.24 per 1000 people	Lacks effective medical treatment	HOLROYD et al., 2020 [31]; HWANG et al., 2007 [32]; SACKTOR, 2018 [33]; TSAI et al., 2017 [34]; WINSTON; SPUDICH, 2020 [35]

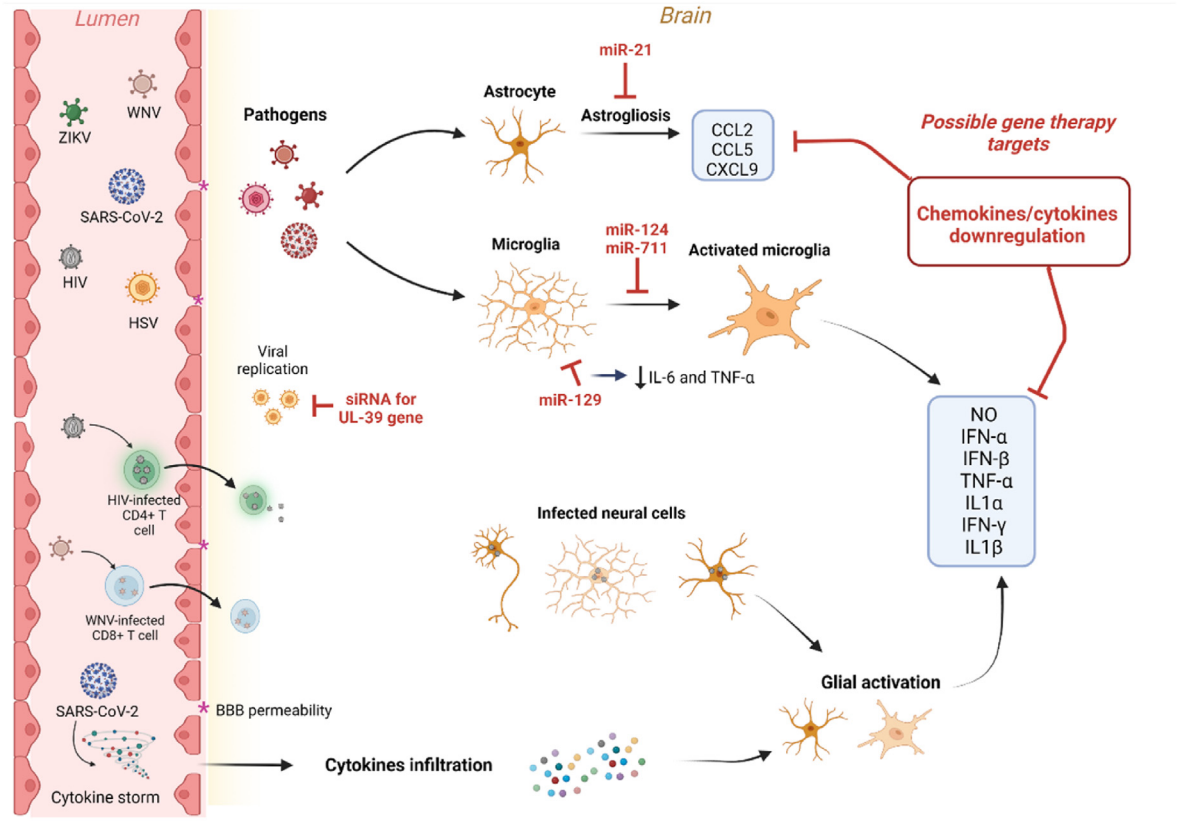

Fig. 1. Potential sites of GT intervention in brain inflammatory conditions associated to infectious diseases. Human pathogens as West Nile Virus (WNV), Zika virus (ZIKV) and Herpes-simplex virus (HSV) are known to directly infect neuronal cells, stimulating the release of chemokines and cytokines from glial cells, also causing BBB disruption. Although the mechanisms involved in WNV and human immunodeficiency virus (HIV) neuroinvasion are not completely elucidated, it is possible that both viruses cross the BBB inside T cells, causing neural cell infection and glial activation. Mechanisms of SARS-CoV-2 brain damage are also not fully elucidated yet, but studies have shown that the virus can directly infect glial cells, while others have shown that the cytokine storm could be involved in brain damage. Glial activation allows the release of cytokines and chemokines that could be targeted by iRNA. For example, miR-129 was shown to reduce IL-6 and TNF-α expression in the brain. miR-21 mediates anti-inflammatory effects, controlling astrogliosis, while downregulation of miR-124 and miR-711 coincides with the resting state from microglia. Besides, gene therapy can also be applied to reduce viral replication, such as the siRNA that targets UL-39 gene. Created with BioRender.com.

Table 2
Studies that have already applied gene therapy for viral encephalitis.

Virus	Gene target	Vector	Reference
HSV-1	siRNA against bifunctional polynucleotide phosphatase/kinase (PNKP)	Lipofectamine 2000	Yue et al. (2013) [42]
HSV-1	siRNA against ribonucleotide reductase enzyme (gene UL-39)	Rhabdovirus glycoprotein (RVG-9R)	Da Silva et al. (2016) [26]
JEV	shRNA against non-structural viral enzymes (NS3 and NS4A)	Lipofectamine 2000	Yuan et al. (2016) [43]
HIV	siRNA against Nef protein	Carbosilane dendrimer	Serramía et al. (2015) [44]
HIV	miRNA-34a for downregulation of negative regulator of NFκB signaling	Lipofectamine™ RNAiMAX	Periyasamy et al. (2016) [45]
HIV	Beclin1 siRNA	PEI nanoparticles	Rodriguez et al. (2021) [46]
ZIKV	siRNA targeting small extracellular vesicles	Rabies virus glycoprotein derived peptide (RVG)	Zhang et al. (2022) [47]

usually show limited tissue tropism and random delivery [55]. Hence, stimuli-responsive biomaterials have been applied to constitute multi-functional nanosystems [56]. In this review, nanotechnology from non-viral vectors such as lipid and polymeric nanosystems are highlighted along with their application in GT as promising strategies to treat viral encephalopathies. Fig. 2 shows some non-viral vectors forming complexes, highlighting their different components and exemplifies how poly(β-amino ester) (PBAE) could condensate nucleic acids to form polyplexes.

The prefix "nano" represents a scale reduction of 10^{-9} fold, in which 1 nm (nm) is one billionth of a meter, also equivalent to 10 Å. Nanotechnology refers to the ability to manipulate or control matter, by physical or chemical means, in order to obtain materials on a nanometer scale, with functional properties (i.e., optical, magnetic or electrical) different from the macroscopic bulk materials [57]. Particular properties and functions of the substances can be changed or enhanced as their size is scaled down, enabling the development of new applications for materials in diverse fields [58]. Specifically in nanomedicine, nanodevices can be used as efficient delivery systems owing to their advantages over ordinary conventional dosage forms. Nanoparticles can interact with biological systems with high specificity. Due to their smaller size, these nanoparticles show increased tissue penetration, effectively delivering drugs or biologically active substances into cells. Nanostructures reportedly aid in protecting substances from degradation, causing fewer plasma fluctuations and, consequently, reducing adverse effects associated with its use [59,60]. Also, by altering the surface properties of nanomaterials with the addition of ligands, it is possible to target tissues in a more specific and controlled way [61].

Nanotechnology can help to design safe and efficient non-viral gene delivery systems to overcome deficiencies normally associated with

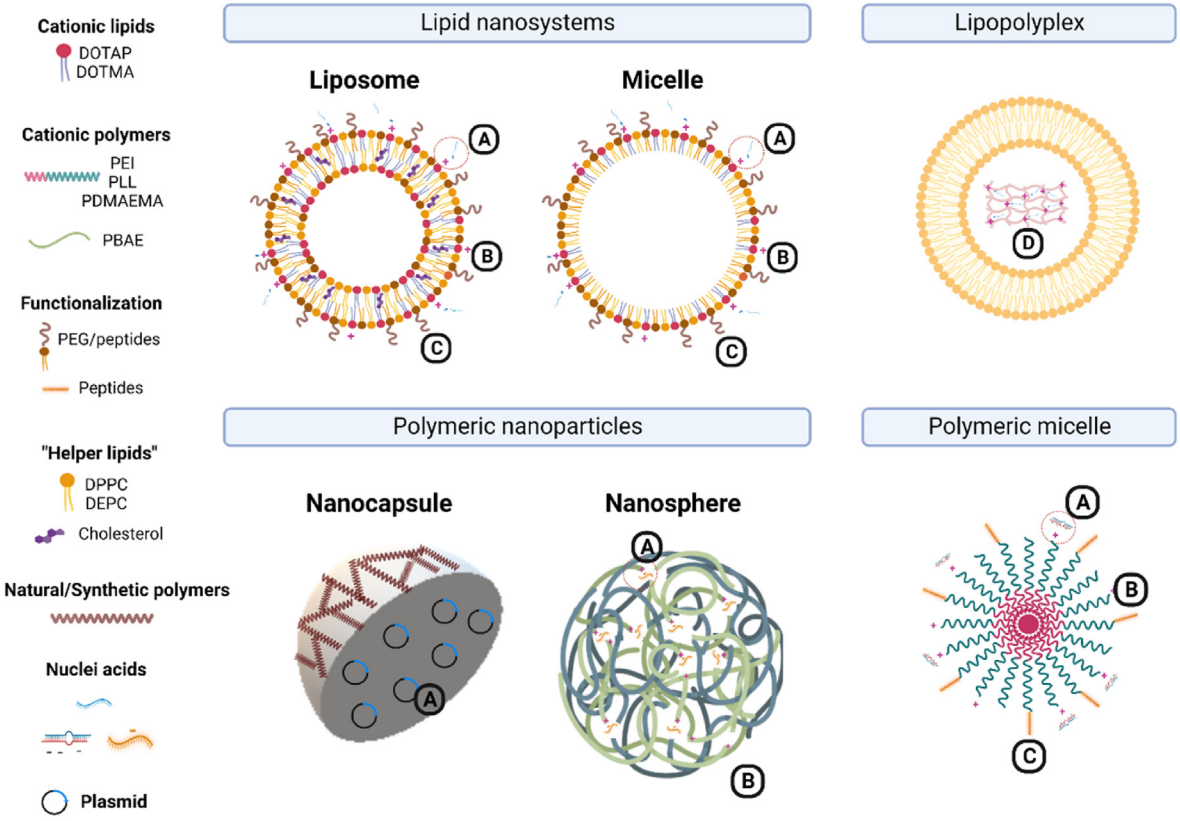

Fig. 2. – Examples of lipid and polymeric nanosystems, lipoplexes and polymeric micelles with their respective structures to form complexes with nuclei acid or plasmids. Physical-chemical interactions are highlighted due to their advantages: (A) charge interactions between nucleic acid and cationic lipids or polymers, allowing gene incorporation; (B) cationic charges that could lead to sponge effect and improve interaction with glial cell membranes; (C) functionalization of lipids or polymers with polyethylene glycol (PEG) and peptides which prevent opsonization and facilitate endocytosis, respectively; (D) polycation interaction with nucleic acids in lipopolyplexes; (E) plasmid incorporation inside of polymeric nanocapsules. Phospholipids: 1,2-Dioleoyl-3-trimethylammonium-propane (DOTAP), 1,2-di-O-octadecenyl-3-trimethylammonium propane (DOTMA), dipalmitoylphosphatidylcholine (DPPC), 1,2-Dierucoyl-sn-glycero-3-PC (DEPC), polyethyleneglycol (PEG), polyethylenimines (PEI), poly(L-lysine) (PLL), poly(2-*N*-(dimethylaminoethyl) methacrylate) (PDMAEMA) and poly(β-amino ester) (PBAE). Created with BioRender.com.

genetic drugs. Nanodevices can reduce genetic drug degradation and, consequently, enhance its stability by blocking the access of nucleases [62]. Genetic drugs formulated as nanoparticles can avoid the renal filtration and present extended blood half-life and biodistribution [63]. Nucleic acids carry highly negative charges and their electrostatic repulsion with cell membrane results in poor cellular uptake. Vectors can cover up the negative charges, promoting its absorption. Also, by active targeting, nanocarriers can ensure a preferential accumulation of genetic drugs in the brain [62,63]. Nanocarriers are generally classified as organic and inorganic nanomaterials, depending on their chemical nature. Fig. 3 exemplifies how the chemical nature from these nanosystems can facilitate their interaction with the targeted glial cells. Fig. 3 also highlights the main advantages of each system and how they can provide better interactions with the cell which improve transfection rates.

4.1. Lipid nanosystems

Lipid nanosystems have been widely studied for delivery of small drug molecules and have been proposed as a relevant alternative for the development of non-viral carriers for GT agents [64]. Lipid-based nanosystems include solid lipid nanoparticles (SLNs), liposomes, nanoemulsions, nanosuspensions and micelles. Liposomes and micelles are examples of amphiphilic nanocarriers systems.

Phospholipids are amphiphilic molecules composed of phosphatidylcholine head groups and two hydrophobic hydrocarbon chains tails that, in aqueous environment, assemble to form different structures such as bilayers and vesicles [65]. Liposomes are self-assembled, closed spherical vesicles composed of an aqueous core surrounded by one or more phospholipid bilayers. Conventional liposomes are synthesized from natural or synthetic phospholipids with or without cholesterol, which is added to improve liposomes fluidity and stability, altering the bilayer rigidity [66].

Depending on their size and number of bilayers, liposomes can be classified in unilamellar vesicles (ULV) and multilamellar liposomes (MLV). ULV are enclosed by one lipid bilayer and according to their size can be divided into three categories: small unilamellar vesicles (SUV, <100 nm), large unilamellar vesicles (LUV,>100 nm) and giant unilamellar vesicles (GUV,>1 μm). Multilamellar liposomes consist of more than one lipid bilayer, usually with diameters of hundreds of nanometers. Size and membrane lamellarity will influence liposomal encapsulation efficiency and circulation time [67].

Due to their structure, liposomes can simultaneously entrap substances of different polarities: hydrophilic molecules can be encapsulated into the core of the vesicles while hydrophobic active substances are incorporated into the lipid bilayer. As delivery systems, lipid nanosystems have the advantage of being highly biocompatible, non-toxic and biodegradable [60]. Lipid bilayers of liposomes, analogous to the cell membrane, protect substances from enzymatic degradation and immunologic/chemical inactivation, allowing their sustained release [68,69]. Various ligands, as polymers (polyethylene glycol - PEG) and polysaccharides (dextran, mannose), targeting molecules or aptamers, can be attached to the surface of liposomes, in order to extend blood circulation time and improve targeting efficiency [70]. PEG-modified liposomes are denoted as “stealth liposomes” and are used as a strategy to hinder

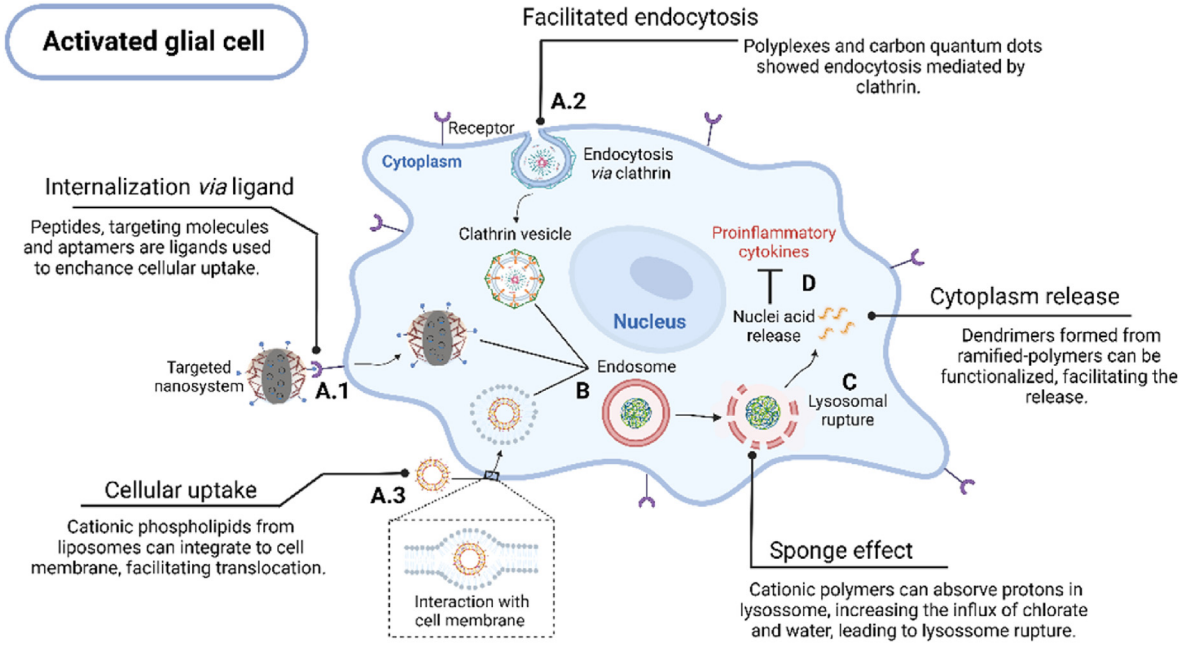

Fig. 3. Different nanosystems have different forms of interacting with glial cells. Internalization *via* ligand (A.1) initiates with the functionalization of nanosystem surface. Polymeric nanoparticles and carbon quantum dots can present facilitated endocytosis due to clathrin-mediated uptake mechanism (A.2). Cellular uptake can also be mediated by the interaction between cationic phospholipids from liposomes or micelles and cell membrane (A.3). After the uptake, nanosystems can be targeted by lysosomes disrupting their structure and leading to nucleic acid release. In the case of cationic polymers, such as PEI, the polymeric nanoparticles suffer the sponge effect where PEI can absorb protons leading to influx of chlorate and water, resulting in the lysossomal rupture and the easiest gene release (C). This last step could be improved using dendrimers due to their ramified structure with end terminal that can be easily functionalized (D). Created with BioRender.com.

opsonization by the reticuloendothelial system, resulting in an increased half-life and consequent better target accumulation than conventional liposomes [66].

The overall surface charge of the liposomal system is determined by its phospholipid composition. Most lipid carriers employed for GT delivery are formed by hydrophilic cationic head groups that contain amino groups and hydrophobic tails [52]. Cationic lipids such as 1,2-Dioleoyl-3-trimethylammonium-propane (DOTAP), cetyltrimethylammonium bromide (CTAB) and 1,2-di-O-octadecenyl-3-trimethylammonium propane (DOTMA) have already been used for nucleic acid delivery. The net positive charge of these lipids facilitates interaction with cell surface [71]. Also, lipid formulations present high target affinity and are able to incorporate nucleic acids ten times larger than viral vectors [72]. When lipids in nanosystems interact with nucleic acids due to charge differences, a complex known as lipoplex is formed. Once the lipoplex reaches the cell surface, it is internalized by endocytosis and enzymatically digested in the phagosome allowing cytosolic distribution of the delivered nucleic acid [73]. Lipid complexes have already been used to reduce the severity of herpes simplex encephalitis infected animal models. HSV-1-infected mice were treated with siRNA to silence matrix metalloproteinase-9 (MMP-9) and control cerebrovascular complications derived from HSE [74]. The lipid-DOPE complex presents interaction with murine brain cells without *in vivo* toxicity. Therefore, the intracerebral injection of siRNA-lipid complex targeting murine MMP-9 was able to reduce levels of this protein and also of pro-inflammatory cytokines.

Addition of neutral lipids as "helper lipids" into the nanosystem increases transfection efficiency, facilitates the lipid self-assembly and the level of hydration from the lipid film [75]. The presence of helper lipids as dipalmitoylphosphatidylcholine (DPPC), cholesterol, dioleoylphosphatidylethanolamine (DOPE) and 1,2-Dioleoyl-sn-glycero-3--phosphocholine (DOPC) in the lipoplex increases nanocarrier stability in the endosome due to the conformation change at acidic pH, allowing the intracellular bilayer destabilization [76,77]. These lipids can promote a phase change of lipoplex from lamellar to non-lamellar structure, which can improve the cationic lipid mediated transfection efficiency by allowing efficient dissociation of the gene from the lipoplex followed by the release into the cytosol [78]. In a recent study, Dhaliwal and colleagues [79] developed mRNA-containing lipoplexes obtained with DPPC, DOTAP and cholesterol for intranasal administration, in order to increase transfection rates in the brain. In this work, the composition of phospholipids formed a monodisperse population of liposomes capable of efficiently delivering mRNA to the murine cortex and midbrain.

Nanosystems containing PEG are also more stable formulations, since PEG shields the lipoplex surface charge and prevents the binding of opsonin to the surface of the lipoplexes [80]. After escaping opsonization, lipoplexes must be endocytosed by the target cells in order to deliver the carried nucleic acid. For this, the surface from these complexes can be functionalized with cell penetrating peptides (CPPs) that facilitate the cellular uptake. Rodrigues et al. [81] produced liposomes containing PEG and CPP, obtaining vesicles with optimized surface and high BBB penetrability, allowing a more efficient delivery of GT agents to the brain. DOTAP, DOPE, cholesterol and 1,2-Distearoyl-sn-glycero-3-phosphorylethanolamine DSPE-PEG were the lipids used to compose liposomes. The use of CPP increased lipoplex penetration in primary astrocytes and neurons, leading to an improvement in transfection. Du et al. [82] observed that dipalmitoyl phosphatidylserine (DPPS) was a key component in the formulation of the liposomes to enhance their phagocytosis by microglia. Once microglia were transfected, these cells were used as a transport vector to deliver paclitaxel for the treatment of glioma. The use of ligands could also increase phagocytosis by microglia as shown by Bhattacherjee et al. [83]. In this work, authors found that intracerebroventricular injection of liposomes containing CD33 ligands could modulate microglial cell function in mice. Likewise, CPPs can be used to functionalize the surface of liposomes.

Liposomes have already been employed in GT designed to treat glioma [84,85] and neurological disorders such as Alzheimer, Parkinson, stroke, traumatic brain injury and epilepsy [80,86–89]. Regarding neurological infections, there are only a few studies that incorporate nucleic acids into liposomes specifically for viral neurological disorders. Despite this, there are studies that already applied cationic-lipid

transfection reagents as Lipofectamine™ to deliver iRNA to treat neuroinflammation following Japanese encephalitis virus and HIV-infection [43,45].

A recent work from Sánchez-arribas et al. [90] showed that lipid-type nanovectors containing gemini cationic lipids (GCLs) showed higher transfection rates of plasmids DNA (pDNA) encoding IL-12 *in vitro*, when compared to formulations containing Lipofectamine™ 2000. GCLs are surfactants constituted by two hydrophobic chains and two hydrophilic cationic heads linked by a spacer, and comprise interesting alternatives for non-viral vector systems for gene delivery [91,92]. Based on this last work, GCLs could be applied in gene therapy in other disease models such as viral infections. Therefore, there is still a field to develop more lipid nanoparticles to obtain high levels of transfection with more interesting cost-benefit ratios when compared to commercial reagents.

Regarding disadvantages of cationic liposomes, some toxicity has been associated with the presence of cationic lipids in nanoparticles, including cytoplasmic vacuolization, cell shrinkage and protein denaturation. In addition, cationic lipids can aggregate following intravenous administration. This results in vector disintegration and release of genetic material. Although lipoplexes are less efficient than viral vectors when administered intravenously, lipid vectors still have greater safety for *in vivo* application [93,94].

4.2. Polymeric nanoparticles

Polymeric nanoparticles (PNPs) typically represent homogeneous spherical structures produced by a polymerization reaction of many monomer units that, under certain conditions, can be organized and self-assemble with a nanometric size (10–100 nm) [95]. Depending on the preparation method, the therapeutically active compound can be dissolved, attached, encapsulated or entrapped to the matrix of the nanoparticle, defining nanocapsules and nanospheres [96]. Nanocapsules are systems with a vesicular structure, in which the retained active substances are reserved in an aqueous or nonaqueous liquid core cavity and enclosed by the solidified polymeric shell. Nanospheres can be described as colloidal particles in which therapeutic compounds may be trapped within the sphere matrix by physical dispersion or adsorbed at the mass surface [97].

Both natural and synthetic polymers can be used in the synthesis of polymeric nanoparticles [69]. Natural polymers include chitosan, alginate, collagen and gelatin. They are biocompatible and biodegradable. However, batch-to-batch variability, broad molecular weight and microbiological contamination may limit its use [96,98]. Among natural polymers, chitosan (poy-Dglucosamine) has a great potential in biomedical field due to its biocompatibility, low toxicity and easy preparation [99].

Poly (lactide-co-glycolide) (PLGA), poly (butylcyano-acrylate) (PBCA), poly (glycolic acid) (PGA) and poly (lactic acid) (PLA) are the most common synthetic polymers used in polymeric nanoparticles carriers. They are also biodegradable and biocompatible. Unlike natural polymers, however, synthetic polymers present controlled and reproducible chemical composition and lower immunogenicity [98,100].

In recent years, PNPs are extensively proposed as biomaterials in therapeutic applications. Polymeric nanoparticles are comparatively more stable than liposomes, contributing to their longer blood circulation time and better stability both in plasma and during storage [101]. PNPs are also less complex to prepare, with easy size distribution control and high loading capacity [96]. Although less permeable through BBB, the pharmacokinetics and targeting efficiency of polymeric nanoparticles can be enhanced by surface functionalization with suitable substances or molecules [61]. The great synthetic versatility and broad structures variety of polymers allows the adjustment of physicochemical properties (size, hydrophobicity, surface charge) and drug release parameters of polymeric nanoparticles obtained [98].

Besides cationic lipids, cationic polymers have also emerged as promising candidates for non-viral gene delivery systems because they are easier to synthesize and because they possess flexible properties [102]. Concerning these properties, nanocarriers containing cationic polymers are easy to produce, present robustness, can be responsive to certain stimuli and can be designed to balance higher transfection efficiency with low cytotoxicity [103–105]. The polymer can interact with nucleic acid negative charges, which allows large genes to be packed into small structured complexes called polyplexes [106]. Naturally occurring polymers such as chitosan, pullulan, dextran and hyaluronic acid show low grades of toxicity and have been already applied for gene delivery [107]. As for cationic synthetic polymers, polyethylenimines (PEI), poly(2-*N*-(dimethylaminoethyl) methacrylate) (PDMAEMA), and poly(-L-lysine) (PLL) are some examples [108].

As an example of nanocarrier with natural polymers, Gu and colleagues [109] developed chitosan nanoparticles to deliver siRNA across the BBB and inhibit HIV replication in astrocytes. In this work, siRNAs targeting spliceosome associated factor 3 (SART3) and human cyclin T1 (hCycT1) genes reduced the viral transcription *in vitro.* Chitosan enables the interaction between the polymer amino groups and siRNA, associated with minimal immunogenicity and the ability to open the cellular tight junctions. Also, the chitosan nanoparticles were conjugated with monoclonal antibodies to induce rapid receptor mediated endocytosis and reduce the nonspecific cellular uptake.

Illustrating the use of synthetic polymers in nanosystem, stands PEI and its application in GT. Rodriguez et al. [110] developed PEI nanoparticles containing siRNA to control inflammatory responses in HIV-infected human microglial cells. Authors showed that siRNA against Beclin1, a protein that regulates autophagic activity which is essential for HIV-1 replication and brain inflammation, led to a significant decrease in the production of chemokines such as IL-6, regulated upon activation normal T cell expressed and secreted (RANTES) and monocyte chemoattractant protein 1 (MCP-1). In this work, intranasally administered nanoparticles were found in different glial cells in mice, after 24 h.

Concerning the cell internalization of polyplexes, it depends on the polymer class and architecture [111]. For example, PEI polyplexes show caveolin and clathrin-mediated endocytosis [112,113]. After endocytosis, cationic polymers as PEI also facilitate “proton sponge” effect which allows the influx of protons and water into the endosome, causing the polyplexes to swell and release their contents into the cytoplasm [114]. Because of this effect, the most frequently used cationic polymers for GT targeting the brain are PEI and PLL [62].

Ryu et al. [115] demonstrated that branched PEI is a promising non-viral vector to deliver CRISPR/Cas9 systems to Neuro2a cells. Moreover, PEI-containing polyplexes allow a more efficient gene transfection than DOTAP lipoplexes since polyplexes are internalized by cells through a caveolae-dependent pathway. Since caveolae-dependent internalization is independent from the lysosome, the GT agent is protected from enzymatic degradation. Rodriguez et al. [46] also complexed genes with PEI to control HIV brain replication and microglial neuroinflammation in mice. Incorporation of PEI to nanosystems improved the biodistribution in the brain via intranasal delivery. Although PEI presents high effectiveness in transfection, it interacts with serum proteins *in vivo* which could cause red blood cells (RBCs) aggregation, cell lysis and thrombosis [116]. To improve polymer biocompatibility, PEI can be conjugated to water soluble molecules such as sugars, amino acids, hydroxyl, and PEG, which prevent opsonization. Following this, a previous work from Morris and Labhasetwar [117] conjugated PEI with PEG and arginine and analyzed whether RBCs compatibility was improved. It was found that combined use of PEI:arginine:PEG in the proportion of 1:5:50 provided the right balance in cytocompatibility and BBB permeability with effective gene delivery into the hippocampus of mice. Similarly, Joshi et al. [118] conjugated PEI, arginine and PEG, observing favorable results in gene delivery for murine and human astrocytes. Polyplexes with higher amounts of arginine showed hemocompatibility with human RBCs. Cytotoxicity assays demonstrated that polyplexes were also biocompatible to human astrocytes even after 48 h of exposure and gene expression was sustained for more than a week. Moreover, the treatment

of mice via intravenous tail-vein showed that polyplexes could also cross the BBB with higher transfection after 24 h. Finally, the use of arginine in polymers allowed a more hydrolysable polyplex structure with more permeability to neuronal cells [119].

4.3. Dendrimers

Dendrimers are highly branched, monodisperse, three-dimensional polymeric macromolecules, formed by the cross linking of repetitive monomers subunits around a central core [120]. They are nanometric structures radially symmetric, ranging from 1 to 15 nm [121, 122]. The basic components of dendrimers are the initiator central core or nucleus, the repetitive concentric layers starting from the core (branching units) called dendrons and the terminal groups on the surface. Dendrimers are classified based on their molecular weight: the number of ramifications points in one dendron corresponds to the number of generations [123]. As the functional groups attached to the core increase, the three-dimensional (3D) configuration of the dendrimers is altered to a globular-shaped symmetrical structure, leading to solubility and reactivity changes of the terminal groups [101].

Compared to liposomes, dendrimers are more stable, highly permeable, easy to modify with functional ligands and can covalently bind drugs [123]. Compared to linear polymeric nanoparticles, dendrimers have specific advantages such as uniform size distribution and chemical homogeneity, ability to associate with a large number of active compounds and high stability [123–125]. Its surface can be functionalized with additional polymers or ligands for targeting release [126].

Bioactive compounds can be encapsulated or entrapped in the internal cavities or flexible spaces within dendrimers and surface functional groups can interact with guest molecules through electrostatic and/or hydrophobic interactions or through covalent attachment [69]. So, substances entrapment and release can be controlled by modifying both dendrimer surfaces and generations [127].

Dendrimers features such as spatial arrangement, terminal groups on the surface, generation number and size are controlled by the synthesis and are crucial for optimum gene delivery [123]. The controlled synthetic mechanisms led to the development of different classes of dendrimers which find potential applications in diagnostics and carrier systems for drugs, proteins and genes [121]. Polyamidoamine (PAMAM), Poly (propylene imine) (PPI), and Poly-L-lysine (PLL) are dendrimers commonly applied as delivery systems. For CNS regenerative medicine, polyester-based dendrimers have been proposed [125].

In addition to polymer end terminal conjugation, the polymer structure is a crucial parameter since the 3D structure with multiple terminal groups allows more reactions for conjugation with nucleic acids [27, 128]. Branched polymers such as PLL and PDMAEMA present superior transfection efficiency than linear polymers [128]. The use of controlled polymerization reaction techniques allows to obtain more tailored polymers with defined chain length, composition, and architecture [129]. As an example, the hydrolysis of poly(2-oxazoline)s (POx) produced PEI with linear architecture, while branched PEI can be synthesized via the polymerization of aziridine [130]. The side reactions of polymers allow them to grow from linear structure to perfectly branched, and fractal-like dendrimers which are represented by a core with internal layers and terminal ends [131]. Since the functionalization of the terminal ends from dendrimers can overcome rapid clearance, cytotoxicity, and low transfection efficiency, these highly branched structures are considered promising for gene delivery.

Concerning their application for GT delivery, dendrimers display high density of charges, interacting with DNA, siRNA or miRNA and forming dendriplexes which have already been applied to reach the brain. For instance, PAMAM is another polymer that can produce BBB-permeable dendrimers, which can selectively target astrocytes and microglia in animal models of neuroinflammation [132]. To this extent, a previous work from Zarebkohan et al. [133] showed that PAMAM-dendrimers functionalized with serine-arginine-leucine were able to reach the brain of rats after intravenous injection. The modified dendriplexes provided a good transfection efficacy with low toxicity *in vitro*. Besides, the functionalization with peptides allowed the cellular internalization of nanoparticles via caveolin endocytosis due to the interaction between peptides and lipoprotein receptor-related protein (LRP) expressed in BBB. In a more recent work, Sharma et al. [132] observed that PAMAM-dendrimers could be found in cortex, hippocampus and periventricular regions of the brains of rabbits after intravenous administration of the nanosystem. Also, authors observed that microglia and macrophages with pro-inflammatory phenotype were targeted by dendrimers. Since there is evidence that these nanoparticles are safe and present transfection capacity, PAMAM dendrimers have been commercialized as PolyFect Transfection Reagent [123].

Another example of synthetic polymer applied in branched model is PBAE which has properties inherent to tertiary amines and esters, such as pH responsiveness and biodegradability [134]. In low pH buffer, branched PBAE presents more protonation of the tertiary amines which results in a better condensation from DNA [135]. As an example, Liu et al. [136] developed compact/spherical nanoparticles by condensing PBAE with DNA to improve transfection to astrocytes. Results showed that moderately branched PBAE showed higher transfection efficiency than molecules with linear structure due to the amounts of disulfide bonds introduced into the polymers backbone, facilitating PBAE degradation and DNA release.

Cao et al. [137] developed a new linear-branched PBAE for GT, offering another approach for neurological diseases and disorders by controlling the differentiation from neural stem cells into neurons. To obtain PBAE, four different monomers were used and the copolymerization of amines and diacrylates through Michael reaction allowed proper DNA condensation. Also, esters present in nanoplexes can be hydrolyzed and easily release DNA with lower cytotoxicity. As expected, the efficient transfection resulted in silencing of SOX9 gene in neural stem cells, increasing their differentiation into neurons.

4.4. Lipopolyplexes

Acknowledging polymeric and lipid based nanocomplexes advantages, lipopolyplexes (LPPs) were developed to deliver nucleic acids. Polyplexes are systems formed by the compression of nucleic acids with the aid of polycationic polymer, while lipoplexes are composed of polyanionic nucleic acids with cationic lipids. LPPs are ternary nanoparticles prepared by the combination of both structures [138]. These nanoparticles comprise phospholipids and present strongly reduced surface charges, enhancing transfection, decreasing cytotoxicity and high colloidal stability [139]. PLL, PEI, spermidine, spermine and protamine sulfate are commonly used polycation in LPPs, while DOPE, DOTMA, DOTAP, *N*-(2-hydroxyethyl)-*N*,*N*-dimethyl-2, 3-bis(tetradecyloxy-1-propanaminiumbromide) (DMRIE) are some examples of cationic lipids that can be applied [80]. Thus, LPPs are versatile nanosystems formed by polycationic molecules which can form complexes with the carried nucleic acid, improving permeability and brain retention as already seen in gene therapy for glioblastoma [140].

Nanotechnology-enabled carriers are sophisticated tools to overcome several challenges in delivering genes to the central nervous system and the limitations of conventional pharmaceutical formulations, such as premature gene release, active pharmaceutical ingredient (API) efflux and low brain bioavailability and stability. Such nanocarriers are nanosized vehicles with the capacity of encapsulating or complexing with nucleic acids, APIs and other therapeutic molecules, providing protection, increasing circulation time and both temporal and site-controlled release of their cargo. The surface characteristics of nanosystems, such as charge, shape, ligand properties and density, can be crucial to the success of nano-based gene therapy targeting the central nervous system.

Therapeutic approaches as patisiran and givosiran comprise lipid-based nanosystems for GT and demonstrate that non-viral vectors are the future of effective transfection. As the stability from lipid

nanosystems can be achieved by their composition, lipoplexes are viable to reach astrocytes and microglia. Concerning polymeric nanoparticles, their 3D structure with functional end groups improve interaction not only with nucleic acid, but also their *in vivo* responses, overcoming both cytotoxicity and low transfection rates. These complexes can be designed according to their target by modifying their surface structure with polymers or ligands. The stimuli-responsive characteristics of nanoparticles prolong their blood circulation time, improve the interaction with the target cell and cellular lysosomes, enabling the release of nucleic acid in the cytosol. These materials are promising alternatives for treatment of neuroinflammation, especially since most neurological disorders still lack effective treatment.

4.5. Polymeric micelles

Polymeric micelles comprise nano-sized core/shell carriers surrounded by block copolymers with interfacial and amphiphilic properties. They are often considered aggregation colloids as amphiphilic block copolymers can self-assemble into a vesicle micellar structure when in solution [141]. They are known for their nanometric size, possibility of enhancing both APIs solubility, permeability across biological barriers and relatively simple production/scale up [142,143].

The versatility of polymer chemistry enables a great range of micelle arrangement and structures with novel properties and applications. In general, the self-assemble of hydrophobic and hydrophilic copolymers in solution forms micelles as a way to decrease free energy, since the hydrophobic segments form vesicles to minimize the contact with the aqueous phase [144]. The choice of the core-forming segment is significantly important to obtain crucial characteristics of polymeric micelles such as stability, API encapsulation ability, and drug release profiles. For instance, poly(propylene oxide), hydrophobic poly(L-amino acid)s, poly(ester)s, copolymers of lactic acid and glycolic acids, and poly(-caprolactone) (PCL) constitute the most studied hydrophobic core blocks [145]. Besides, the chemical structure and molecular weight of hydrophilic segments, which comprise the outer shell of polymeric micelles, dictates their *in vivo* behavior and their capacity to interact with proteins, receptors and other biological structures [146]. PEG is the most used hydrophilic block in polymeric micelles. It is an FDA-approved nontoxic polymer with important physicochemical properties, such as high water solubility, high flexibility, and large exclusion volume, providing steric stabilization for polymeric micelles [147].

When it comes to brain-targeted gene therapy, polymeric micelles are unique carriers due to their specific properties such as nano-sized structure, both charge-switching and stimuli-responsive therapeutic release and flexible film morphology [148]. Polymeric micelles can improve BBB permeability through the interaction of copolymers brush with cell membranes, which causes membrane fluidification, inhibit P-grycoprotein and other efflux transporters, as well as enhances insulin receptor-mediated transport [149]. Additionally, cationic polymers can be selected to compose the outer branch of polymeric micelles, so that negatively charged therapeutic nucleic acids can form polyelectrolytes complexes, comprising micelleplexes. Micelleplexes feature promising properties, such as the possibility for a dual therapy with both drugs and nucleic acids, overcoming multidrug resistance, biochemical and physiological barriers which still limit the RNA-based therapeutics [150,151].

Zhang et al. [152] developed micelleplexes based on block copolymers poly(ethylene glycol-b-lactide-b-arginine) to deliver GT systems to the human cerebrospinal fluid (CSF). Interestingly, the micelleplexes were found, for the first time, to have significantly better stability and high anti-miRNA activity in CSF than in human plasma. The micelleplexes showed the capacity to separate from the cationic peptides *in vivo* associated with an enhanced miRNA silencing efficiency and no toxicity *in vitro*. Such results suggest that micelleplexes may show advantages for targeting CNS disorders *in vivo* [152].

Gwak et al. [153] have developed polymeric micelles constituted by [poly(lactide-co-glycolide)-graft-polyethylenimine] and demonstrated their ability to efficiently transfect siRNA in various neural cell lines and primary chick forebrain neurons *in vitro*, as well as in the normal rat spinal cord. Later, authors also reported the delivery of siRNA [poly(lactide-co-glycolide)-graft-polyethylenimine] polymeric micelles targeting RhoA to the injured spinal cord. This treatment induced RhoA knockdown in chick forebrain neurons for up to 4 weeks post-injection, reducing astrogliosis and cavitation, and increasing axonal regeneration [154].

Huo et al. [155] produced pegylated micelleplexes decorated with rabies glycoprotein (RVG). Their results showed that micelleplexes presented a spherical and monodispersed morphology with low cytotoxicity, good serum stability and high gene silencing efficiency *in vitro*. Besides, the surface modification with RVG produced higher cellular uptake efficiency. Additionally, *in vivo* biodistribution studies showed that RVG-modified micelles can cross BBB and achieve the CNS.

Despite great advances in both polymer science and in the application of polymeric micelles for poorly soluble small molecule drug delivery, their application for nucleic acid delivery to the CNS is still limited. Thus, more studies are required before these nano systems can reach clinical trials, and therefore, they are often neglected for further consideration. However, polymeric micelles and micelleplexes show interesting properties that surely can contribute to the successful application of RNA-based therapeutics for CNS targeting into the clinic. The most highlighting features include self-assembly capacity, thermodynamic and steric stability, effective condensation and protection of nucleic acids, ability to overcome biological barriers, increasing cell interaction and gene transfection and avoiding escape mechanisms [151].

4.6. Inorganic nanoparticles

Inorganic nanoparticles have gained interest in biomedical application as a result of their optical, electrical and magnetic properties [156]. Inorganic materials may be formulated as nanoparticles delivery systems with clinically approved properties [157]. Inorganic nanoparticles (IN) such as iron oxide, gold nanoparticles (AuNPs), quantum dots (QDs) and carbon nanoparticles have already been applied into brain gene delivery [112,158]. Inorganic structures can provide robust nanocarriers mostly made of noble metals. Those structures can be applied to both photothermal therapy and photoacoustic imaging. Considering the brain as a target, iron oxide nanoparticles can be used to diagnose purposes in diseases such as Alzheimer's, Parkinson's, and amyotrophic lateral sclerosis. IN could be also used for treatment of viral-induced brain inflammatory conditions. Similar to the previous nanosystems, IN can be modified to be internalized by glial cells to deliver nucleic acids.

In contrast to organic materials, IN can provide real-time tracking of the nucleic acids in the brain to guide and track the treatment [62]. Furthermore, IN as AuNPs can easily penetrate BBB to enhance gene delivery. For neurodegenerative diseases, the AuNPs can present many advantages such as low cytotoxicity, optical properties suitable for detection/imaging, high surface area for drug loading and the ability to cross the BBB [159]. As an example, KIM et al. [160] used IN to treat neuroinflammation induced by amyloid beta fibers. In addition, AuNPs induced downregulation of pro-inflammatory cytokines [161]. AALINKEEL et al. [162] demonstrated that AuNPs were neuroprotective for microglial cells in HAND pathology. These neuroprotective properties suggest that AuNPs comprise a suitable strategy for GT targeting brain neuroinflammation, allowing a synergistic anti-inflammatory response from both material and nucleic acids.

QDs, in turn, can also be developed to cover a broad optical range of fluorescence ranging from ultraviolet to near infrared, allowing their biomedical application [156]. QDs are usually mineral semiconductor nanocrystals with a diameter of 1–10 nm that can emit light after adequate excitation [163]. Similar to some AuNPs, the surface of QDs can be easily functionalized with siRNAs, aptamers, antibodies or peptides [164]. QDs present many physical-chemical advantages that can justify their use into GT such as tunable size, shape, simultaneous excitation of

different-sized QDs by a single light source, brightness and broad-spectrum windows [165]. Conventional heavy metal-based QDs can induce toxicity, and may not be approved for clinical applications [166]. Still, they can be tailored according to a specific treatment. Therefore, graphene quantum dots (GQDs) and carbon quantum dots (CQDs) have a favorable adequacy for biocompatibility and low toxicity for biomedical applications [166,167].

The GQDs are the product of chemical oxidation of carbonaceous materials that can be considered extremely small derivatives of graphene oxide which may contain oxygenated functional groups [168]. The GQDs have been considered a key element in biomedical and neuroscience fields due to their ability to cross BBB [169]. In addition, GQDs have been applied in the management of NDDs, since they present anti-amyloid activity [170]. As for GT application, graphene-bound biomacromolecules can improve GQDs bioactivity, but they need additional functionalization to interact electrostatically or covalently with genes. A recent work from AHN and SONG [171] synthesized GQDs for the transfection of mRNAs and plasmid deoxyribonucleic acids (pDNAs). In this work, GQDs were functionalized with PEI and citric acid as precursors to give positive charges and then to form complexes with mRNA and pDNAs. As result, transfection from GQDs complexes presented better performance than Lipofectamine which is considered as a "gold standard".

In addition to GQDs, CQDs are considered an excellent substitute to replace QDs for biological applications [172]. CQDs are a category of carbon nanostructures with high resistance to photobleaching and can be fine-tuned by size control [173]. Regarding gene delivery, the CQDs present low toxicity, strong fluorescence emission, broad excitation spectra and show superior capacity to condense with genes [174]. CQDs can be easily associated with cationic polymers and liposomes to suppress the positive charge from nucleic acids which facilitates transfection [175]. As an example, PARK et al. [176] developed a QD platform that can be applied into cellular labeling, targeting and gene delivery. In this work, transfection efficiency between QDs encapsulated with Lipofectamine and QDs conjugated with PEI was compared. Results showed that PEI-complex had higher transfection efficiency and brightness than Lipofectamine complexes, since PEI formed a hydrophobic inside pocket where many QDs can equilibrate which allowed reversible interactions with oxygen indicating phosphorescence dyes for ratiometric photoluminescence.

Although IN are considered more stable than organic materials, IN have also disadvantages as they might not be degraded or eliminated [177]. Considering the brain as the GT target, IN shows toxicity, poor drug release profile and non-biodegradability [178]. In some cases, IN presented more severe neurotoxic effects, leading to seizures episodes and astrogliosis due to their limited excretion [158]. Afterwards, for biomedical applications, IN physical-chemical aspects as solubility, stability and toxicity must be considered [156]. These aspects can be reached by proper surface modifications through chemical reactions as free thiols, aldehydes, ketones, amines, carboxylates, hydroxyls, and azides [179]. In addition, biocompatible polymers such as PEG can also be used to modify IN surface to reduce uptake from RES organs and achieve faster clearance. The chemical core can be adjusted by the addition of other metal ions to enhance chemical stability against pH-dependent degradation [180]. Even with all modifications, inorganic nanoparticles are not superior to other non-vectors systems for treatment of brain neuroinflammation.

5. Limitations and future directions

The population worldwide is susceptible to infections by emerging and reemerging viral agents. Several viruses can directly or indirectly impact normal brain function, which can cause significant impact on life quality of patients. These neurological manifestations are frequent, and some may endure for several weeks or months. In most cases, the factors that lead to increased susceptibility of individuals to develop neurological complications following viral infections remain unknown. Gene therapy (GT) comprises promising strategies to treat these conditions, since they can modify or adjust the expression or function of a gene in a specific tissue or cell type. Nevertheless, few studies are translated in clinical applications, especially in the neurological field [63,181]. The widespread use of GT is limited due to instability, limited specificity and unfavorable biodistribution with poor cellular uptake of genetic drugs [62,181].

Non-viral vectors have the potential to overcome the drawbacks of genetic drugs and viral vectors due to their biocompatibility, easy and flexible synthesis, tunable surface properties and safety profile [62,182,183]. However, they must face many systemic obstacles from delivery to the target tissue and further into their specific cellular site of action. Whereas viruses have evolved to efficiently access the genome of mammalian cells, most synthetic vectors are unable to effectively transport their cargoes across the multiple existing biological barriers [184]. These extracellular and intracellular barriers primarily include systemic factors, the BBB for CNS diseases, nonspecific cellular uptake, endosomal escape and nuclear uptake [62,185]. Factors such as nanoparticle toxicity and costs of production must also be considered.

The delivery of nonviral gene vehicles almost invariably involves endocytosis [186]. Following endocytosis, the vacuoles with non-viral vectors become accessible to early endosomes. The early endosomes are fusing with late endosomes and further with lysosomes, where there are many hydrolytic enzymes, which can rapidly degrade a broad range of non-viral vectors and their attached genetic drugs [62]. Therefore, endosome trapping has been identified to be one of the rate-limiting steps for non-viral vector-based gene therapy, since they must be able to escape from the endosome into the cell cytoplasm to avoid degradation [62,186].

Considering specifically the CNS delivery of non-viral vectors, the primary condition is to cross the BBB. Due to the selectivity of the BBB, brain-targeted nanotechnologies struggle to achieve significant distribution, since BBB prevents most non-viral vectors from entering CNS [187]. It has been shown that usually less than 1% of a nanoparticle-formulated drug dose is found in the brain after systemic injection [188]. To achieve therapeutic concentrations in the brain via intravenous injection, nanomedicines must be administered at very high doses [182]. Repeated administration combined with the intricate brain organization that makes targeted treatment difficult, can lead to an immune or cytotoxic response, increasing the risk of toxicity and side effects. Off-target accumulation is still a relevant obstacle to the use of nanomedicines, making it essential to increase their brain perfusion and neuronal targeting capacity, while avoiding the fast renal and reticuloendothelial clearances [62,181]. To enhance the rate of entry of non-viral vectors into specific cells and prevent accumulation in other tissues, many delivery systems incorporate ligands, including proteins, peptides, glycosaminoglycans, which can specifically bind to receptors on targeted cells to trigger endocytosis and vesicular trafficking processes [63]. Transferrin receptor, low-density lipoprotein receptor (LRP) and insulin receptor (IR) and their ligands are some of the surface receptors with potential to be used for brain-targeted delivery [181].

In conclusion, increasing evidence suggests that the use of nanosystems carrying gene modifying agents comprise new avenues for treatment of brain diseases. This is especially important concerning viral-induced brain inflammation, which may require glia-targeted therapies. As shown throughout this review, nanocarries can be used as efficient delivery systems with increased specificity for certain biological systems and tissue penetration. They also provide a more controlled delivery of biologically active substances and increase their stability. The use of nanosystems for GT delivery comprises an overall interesting strategy to overcome the limitations and adverse effects of anti-inflammatory and antiviral drugs. Also, the use of these materials for treatment or neuroinflammatory conditions is promising and studies testing the *in vivo* effectiveness of these agents should be pursued.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

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

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References

[1] P. Tavčar, et al., Neurotropic viruses, astrocytes, and COVID-19, Front. Cell. Neurosci. 15 (2021) 1–14.
[2] A. Zumla, D.S.C. Hui, Emerging and reemerging infectious diseases: global overview, Infect. Dis. Clin. 33 (2019) xiii–xix.
[3] S. Willems, D. Zaienne, D. Merk, Targeting nuclear receptors in neurodegeneration and neuroinflammation, J. Med. Chem. 64 (2021) 9592–9638.
[4] S. Ingusci, G. Verlengia, M. Soukupova, S. Zucchini, M. Simonato, Gene therapy tools for brain diseases, Front. Pharmacol. 10 (2019) 1–19.
[5] C.C. Ma, Z.L. Wang, T. Xu, Z.Y. He, Y.Q. Wei, The approved gene therapy drugs worldwide: from 1998 to 2019, Biotechnol. Adv. 40 (2020), 107502.
[6] N.A. Alhakamy, D.T. Curiel, C.J. Berkland, The era of gene therapy: from preclinical development to clinical application, Drug Discov. Today 26 (2021) 1602–1619.
[7] C. Li, R.J. Samulski, Engineering adeno-associated virus vectors for gene therapy, Nat. Rev. Genet. 21 (2020) 255–272.
[8] S.A. Pena, et al., Gene therapy for neurological disorders: challenges and recent advancements, J. Drug Target. 28 (2020) 111–128.
[9] X. Dong, Current strategies for brain drug delivery, Theranostics 8 (2018).
[10] U. Meyding-Lamadé, E. Craemer, P. Schnitzler, Emerging and re-emerging viruses affecting the nervous system, Neurol Res Pract 1 (2019) 1–9.
[11] Z. Chen, G. Li, Immune response and blood–brain barrier dysfunction during viral neuroinvasion, Innate Immun. 27 (2021) 109–117.
[12] M. Ludlow, et al., Neurotropic virus infections as the cause of immediate and delayed neuropathology, Acta Neuropathol. 131 (2016) 159–184.
[13] M. Ren, et al., Functionalized nanoparticles in prevention and targeted therapy of viral diseases with neurotropism properties, special insight on COVID-19, Front. Microbiol. 12 (2021).
[14] C.D.M. Fulton, D.W.C. Beasley, D.A. Bente, K.T. Dineley, Long-term, West Nile virus-induced neurological changes: a comparison of patients and rodent models, Brain Behav Immun Health 7 (2020), 100105.
[15] M. Santini, et al., Severe West nile virus neuroinvasive disease : clinical characteristics, short- and long-term outcomes, Pathogens 11 (2022).
[16] M.B. Yakass, D. Franco, O. Quaye, Suppressors of cytokine signaling and protein inhibitors of activated signal transducer and activator of transcriptions as therapeutic targets in flavivirus infections, J. Interferon Cytokine Res. 40 (2020) 1–18.
[17] S. Zidovec-Lepej, et al., Antiviral cytokine response in neuroinvasive and non-neuroinvasive west nile virus infection, Viruses 13 (2021) 1–12.
[18] J. Barbeito-Andrés, et al., Congenital Zika syndrome is associated with maternal protein malnutrition, Sci. Adv. 6 (2020).
[19] C. Benazzato, F.B. Russo, P.C.B. Beltrão, An update on preclinical pregnancy models of Zika virus infection for drug and vaccine discovery, Expet Opin. Drug Discov. 17 (2022) 19–26.
[20] A.V. Campos Coelho, S. Crovella, Microcephaly prevalence in infants born to zika virus-infectedwomen: a systematic review and meta-analysis, Int. J. Mol. Sci. 18 (2017) 1–10.
[21] I.N.O. Souza, F.G.Q. Barros-Aragão, P.S. Frost, C.P. Figueiredo, J.R. Clarke, Late neurological consequences of zika virus infection: risk factors and pharmaceutical approaches, Pharmaceuticals 12 (2019) 1–21.
[22] T. Takahashi, K. Ui-Tei, Mutual regulation of RNA silencing and the IFN response as an antiviral defense system in mammalian cells, Int. J. Mol. Sci. 21 (2020).
[23] F.J.C. Artal, A.Q.C. Araujo, Neurological complications in adults with Zika and chikungunya virus infection, Lancet Neurol. 19 (2020) 799–801.
[24] R. Bido-medina, et al., Impact of Zika Virus on adult human brain structure and functional organization, Ann Clin Transl Neurol (2018) 752–762, https://doi.org/10.1002/acn3.575.
[25] L. Bartolini, et al., Viral triggers and inflammatory mechanisms in pediatric epilepsy, Mol. Neurobiol. 56 (2019) 1897–1907.
[26] A.S. da Silva, J.v. Raposo, T.C. Pereira, M.A. Pinto, V.S. de Paula, Effects of RNA interference therapy against herpes simplex virus type 1 encephalitis, Antivir. Ther. 21 (2016) 225–235.
[27] H. Liu, K. Qiu, Q. He, Q. Lei, W. Lu, Mechanisms of blood-brain barrier disruption in herpes simplex encephalitis, J. Neuroimmune Pharmacol. 14 (2019) 157–172.
[28] N. McGrath, N.E. Anderson, M.C. Croxson, K.F. Powell, Herpes simplex encephalitis treated with acyclovir: diagnosis and long term outcome, J. Neurol. Neurosurg. Psychiatry 63 (1997) 321–326.
[29] J. Sellner, E. Trinka, Seizures and epilepsy in herpes simplex virus encephalitis: current concepts and future directions of pathogenesis and management, J. Neurol. 259 (2012) 2019–2030.
[30] M.v. Solbrig, R. Adrian, D.Y. Chang, G.C. Perng, Viral risk factor for seizures: pathobiology of dynorphin in herpes simplex viral (HSV-1) seizures in an animal model, Neurobiol. Dis. 23 (2006) 612–620.
[31] K.B. Holroyd, A. Vishnevetsky, M. Srinivasan, D. Saylor, Neurologic complications of acute HIV infection, Curr. Treat. Options Infect. Dis. 12 (2020) 227–242.
[32] S. Hwang, et al., Updated research nosology for HIV-associated neurocognitive disorders, Neurology 69 (2007) 1789–1799.
[33] N. Sacktor, Changing clinical phenotypes of HIV-associated neurocognitive disorders, J. Neurovirol. 24 (2018) 141–145.
[34] Y.T. Tsai, Y.C. Chen, C.Y. Hsieh, W.C. Ko, N.Y. Ko, Incidence of neurological disorders among HIV-infected individuals with universal health care in taiwan from 2000 to 2010, J. Acquir. Immune Defic. Syndr. 75 (1988) 509–516, 2017.
[35] A. Winston, S. Spudich, Cognitive disorders in people living with HIV, Lancet HIV 7 (2020) e504–e513.
[36] R. Goswami, et al., Gene therapy leaves a vicious cycle, Front. Oncol. 9 (2019) 1–25.
[37] A.G. Bader, P. Lammers, The therapeutic potential of microRNAs, Innovat. Pharmaceut. Technol. (2012) 52–55.
[38] H. Ling, M. Fabbri, G.A. Calin, MicroRNAs and other non-coding RNAs as targets for anticancer drug development, Nat. Rev. Drug Discov. 12 (2013) 847–865.
[39] Z. Wu, T. Li, Nanoparticle-mediated cytoplasmic delivery of messenger RNA vaccines: challenges and future perspectives, Pharm. Res. (N. Y.) 38 (2021) 473–478.
[40] J.T. Bulcha, Y. Wang, H. Ma, P.W.L. Tai, G. Gao, Viral vector platforms within the gene therapy landscape, Signal Transduct. Targeted Ther. 6 (2021).
[41] O.W. Merten, B. Gaillet, Viral vectors for gene therapy and gene modification approaches, Biochem. Eng. J. 108 (2016) 98–115.
[42] L. Yue, et al., PNKP knockdown by RNA interference inhibits herpes simplex virus-1 replication in astrocytes, Virol. Sin. 28 (2013) 345–351.
[43] L. Yuan, et al., The NS3 and NS4A genes as the targets of RNA interference inhibit replication of Japanese encephalitis virus in vitro and in vivo, Gene 594 (2016) 183–189.
[44] M.J. Serramía, et al., Vivo delivery of siRNA to the brain by carbosilane dendrimer, J. Contr. Release 200 (2015) 60–70.
[45] P. Periyasamy, A. Thangaraj, V.S. Bendi, S. Buch, HIV-1 Tat-mediated microglial inflammation involves a novel miRNA-34a-NLRC5-NFκB signaling axis Palsamy, Physiol. Behav. 176 (2016) 139–148.
[46] M. Rodriguez, et al., Targeting beclin1 as an adjunctive therapy against hiv using mannosylated polyethylenimine nanoparticles, Pharmaceutics 13 (2021) 1–17.
[47] R. Zhang, et al., sEVsRVG selectively delivers antiviral siRNA to fetus brain, inhibits ZIKV infection and mitigates ZIKV-induced microcephaly in mouse model, Mol. Ther. 30 (2022) 2078–2091.
[48] D. Ibraheem, A. Elaissari, H. Fessi, Gene therapy and DNA delivery systems, Int. J. Pharm. 459 (2014) 70–83.
[49] Z. Zhao, A.C. Anselmo, S. Mitragotri, Viral vector-based gene therapies in the clinic, Bioeng Transl Med 7 (2022) 1–20.
[50] A. Srivastava, K.M.G. Mallela, N. Deorkar, G. Brophy, Manufacturing challenges and rational formulation development for AAV viral vectors, J Pharm Sci 110 (2021) 2609–2624.
[51] X. Du, et al., Advanced physical techniques for gene delivery based on membrane perforation, Drug Deliv. 25 (2018) 1516–1525.
[52] N. Sayed, et al., Gene therapy: comprehensive overview and therapeutic applications, Life Sci. 294 (2022), 120375.
[53] A. Fus-kujawa, et al., An Overview of Methods and Tools for Transfection of Eukaryotic Cells in Vitro vol. 9, 2021, pp. 1–15.
[54] L. Salimzadeh, M. Jaberipour, A. Hosseini, A. Ghaderi, Non-Viral Transfection Methods Optimized for Gene Delivery to a Lung Cancer Cell Line vol. 5, 2013.
[55] Y. Wang, et al., Is viral vector gene delivery more effective using biomaterials? Adv Healthc Mater 10 (2021) 1–25.
[56] S.W.L. Lee, et al., MicroRNA delivery through nanoparticles, J. Contr. Release 313 (2019) 80–95.
[57] S. Bayda, M. Adeel, T. Tuccinardi, M. Cordani, F. Rizzolio, The history of nanoscience and nanotechnology: from chemical-physical applications to nanomedicine, Molecules 25 (2020), https://doi.org/10.3390/molecules25010112. Preprint at.
[58] R. Poupot, D. Bergozza, S. Fruchon, Nanoparticle-based strategies to treat neuro-inflammation, Materials 11 (2018).
[59] B. Begines, et al., Polymeric nanoparticles for drug delivery: recent developments and future prospects, Nanomaterials 10 1 (2020), https://doi.org/10.3390/nano10071403. –41 Preprint at.
[60] J.K. Patra, et al., Nano based drug delivery systems: recent developments and future prospects 10 technology 1007 nanotechnology 03 chemical sciences 0306 physical chemistry (incl. Structural) 03 chemical sciences 0303 macromolecular and materials chemistry 11 medical and He, J. Nanobiotechnol. 16 (2018) 1–33.
[61] R. Kumar, K.R. Aadil, S. Ranjan, V.B. Kumar, Advances in nanotechnology and nanomaterials based strategies for neural tissue engineering, J. Drug Deliv. Sci. Technol. 57 (2020), 101617.

[62] Y. Li, L. Liu, W. Ji, H. Peng, R. Zhao, Strategies and materials of SMART vectors: overcoming the barriers for brain gene therapy, Nano Today 35 (2020), 101006.
[63] S. Kimura, H. Harashima, Current status and challenges associated with CNS-targeted gene delivery across the BBB, Pharmaceutics 12 (2020) 1–33.
[64] P.R. Cullis, M.J. Hope, Lipid nanoparticle systems for enabling gene therapies, Mol. Ther. 25 (2017) 1467–1475.
[65] K.S. Ahmed, et al., Liposome: composition, characterisation, preparation, and recent innovation in clinical applications, J. Drug Target. 27 742 (2019), https://doi.org/10.1080/1061186X.2018.1527337. –761 Preprint at.
[66] D. Luo, et al., Doxorubicin encapsulated in stealth liposomes conferred with light-triggered drug release, Biomaterials 75 (2016) 193–202.
[67] D.E. Large, R.G. Abdelmessih, E.A. Fink, D.T. Auguste, Liposome composition in drug delivery design, synthesis, characterization, and clinical application, Adv. Drug Deliv. Rev. 176 (2021), https://doi.org/10.1016/j.addr.2021.113851. Preprint at.
[68] G. Bozzuto, A. Molinari, Liposomes as nanomedical devices, Int. J. Nanomed. 10 975 (2015), https://doi.org/10.2147/IJN.S68861. –999 Preprint at.
[69] M. Chakravarty, A. Vora, Nanotechnology-based antiviral therapeutics, Drug Deliv Transl Res 11 (2021) 748–787.
[70] Y.J. Kang, E.G. Cutler, H. Cho, Therapeutic nanoplatforms and delivery strategies for neurological disorders, Nano Converg 5 (2018).
[71] B. Ozpolat, A.K. Sood, G. Lopez-Berestein, Liposomal siRNA nanocarriers for cancer therapy, Adv. Drug Deliv. Rev. 66 (2014) 110–116, https://doi.org/10.1016/j.addr.2013.12.008. Preprint at.
[72] R.D. Jayant, et al., Current status of non-viral gene therapy for CNS disorders, Expet Opin. Drug Deliv. 13 (2016) 1433–1445, https://doi.org/10.1080/17425247.2016.1188802. Preprint at.
[73] H. Daraee, A. Etemadi, M. Kouhi, S. Alimirzalu, A. Akbarzadeh, Application of liposomes in medicine and drug delivery, Artif. Cell Nanomed. Biotechnol. 44 (2016) 381–391.
[74] Y. Zhou, Z.N. Lu, Y.J. Guo, Y.W. Mei, Favorable effects of MMP-9 knockdown in murine herpes simplex encephalitis using small interfering RNA, Neurol. Res. 32 (2010) 801–809.
[75] S. Even-chen, R. Cohen, Y. Barenholz, Factors affecting DNA binding and stability of association to cationic liposomes, Chem. Phys. Lipids 165 (2012) 414–423.
[76] X. Cheng, R.J. Lee, The role of helper lipids in lipid nanoparticles (LNPs) designed for oligonucleotide delivery, Adv. Drug Deliv. Rev. 99 (2016) 129–137.
[77] A. Lechanteur, et al., Cationic liposomes carrying siRNA: impact of lipid composition on physicochemical properties, cytotoxicity and endosomal escape, Nanomaterials 8 (2018) 270.
[78] L. Wasungu, D. Hoekstra, Cationic lipids, lipoplexes and intracellular delivery of genes, J. Contr. Release 116 (2006) 255–264.
[79] H.K. Dhaliwal, Y. Fan, J. Kim, M.M. Amiji, Intranasal delivery and transfection of mRNA therapeutics in the brain using cationic liposomes, Mol. Pharm. 17 (2020) 1996–2005.
[80] W. Chen, H. Li, Z. Liu, W. Yuan, Lipopolyplex for therapeutic gene delivery and its application for the treatment of Parkinson's disease, Front. Aging Neurosci. 8 (2016) 1–10.
[81] B. dos Santos Rodrigues, S. Lakkadwala, T. Kanekiyo, J. Singh, Dual-modified liposome for targeted and enhanced gene delivery into mice brain, J. Pharmacol. Exp. Therapeut. 374 (2020) 354–365.
[82] Y. Du, et al., Engineered Microglia Potentiate the Action of Drugs against Glioma through Extracellular Vesicles and Tunneling Nanotubes vols. 1–14, 2021, 2002200.
[83] A. Bhattacherjee, et al., Increasing phagocytosis of micoglia by targeting CD33 with liposomes displaying glycan ligands, J. Contr. Release 338 (2021) 680–693.
[84] Z. Chen, et al., Targeted delivery of CRISPR/Cas9-Mediated cancer gene therapy via liposome-templated hydrogel nanoparticles, Adv. Funct. Mater. 27 (2017).
[85] Y. Hu, et al., Core-shell lipoplexes inducing active macropinocytosis promote intranasal delivery of c-Myc siRNA for treatment of glioblastoma, Acta Biomater. 138 (2022) 478–490.
[86] S. Arora, J. Singh, Vitro and in vivo optimization of liposomal nanoparticles based brain targeted vgf gene therapy, Int. J. Pharm. 608 (2021), 121095.
[87] H. Bender, et al., PrP C knockdown by liposome-siRNA-peptide complexes (LSPCs) prolongs survival and normal behavior of prion-infected mice immunotolerant to treatment, PLoS One (2019) 1–25.
[88] E.G. Pedachenko, V. v Biloshytsky, S.A.M. Sky, N.Y.A. Gridina, T.Y.U. Kvitnitskaya-ryzhova, The effect of APOE3 gene therapy on structural and functional manifestations of secondary hippocampal damages in experimental traumatic brain injury, PROBLEMS OF NEUROSURGERY NAMED AFTER N.N. BURDENKO (2015) 17–27, https://doi.org/10.17116/engneiro201579217-27.
[89] H.P. Vemana, A. Saraswat, S. Bhutkar, K. Patel, V. Vikas, A Novel Gene Therapy for Neurodegenerative Lafora Disease via EPM2A -loaded DLinDMA Lipoplexes vol. 16, 2021, pp. 1081–1095.
[90] N. Sánchez-Arribas, et al., Gemini cationic lipid-type nanovectors suitable for the transfection of therapeutic plasmid dna encoding for pro-inflammatory cytokine interleukin-12, Pharmaceutics 13 (2021).
[91] E. Junquera, E. Aicart, Recent progress in gene therapy to deliver nucleic acids with multivalent cationic vectors, Adv. Colloid Interface Sci. 233 (2016) 161–175.
[92] M. Martínez-Negro, et al., Multidisciplinary approach to the transfection of plasmid DNA by a nonviral nanocarrier based on a gemini−bolaamphiphilic hybrid lipid, ACS Omega 3 (2018) 208–217.
[93] I. Posadas, Nanoparticles for brain-specific drug and genetic material delivery , imaging and diagnosis 11 (2016) 833–849.
[94] H. Lv, S. Zhang, B. Wang, S. Cui, J. Yan, Toxicity of cationic lipids and cationic polymers in gene delivery, J. Contr. Release 114 (2006) 100–109, https://doi.org/10.1016/j.jconrel.2006.04.014. Preprint at.
[95] Y. Herdiana, N. Wathoni, S. Shamsuddin, M. Muchtaridi, Scale-up polymeric-based nanoparticles drug delivery systems: development and challenges, Open 7 (2022), https://doi.org/10.1016/j.onano.2022.100048. Preprint at.
[96] S. Sur, et al., Recent developments in functionalized polymer nanoparticles for efficient drug delivery system, Nano-Structures and Nano-Objects 20 (2019), https://doi.org/10.1016/j.nanoso.2019.100397. Preprint at.
[97] K.M. El-Say, H.S. El-Sawy, Polymeric nanoparticles: promising platform for drug delivery, Int. J. Pharm. 528 (2017) 675–691, https://doi.org/10.1016/j.ijpharm.2017.06.052. Preprint at.
[98] N. Kamaly, B. Yameen, J. Wu, O.C. Farokhzad, Degradable controlled-release polymers and polymeric nanoparticles: mechanisms of controlling drug release, Chem. Rev. 116 (2016) 2602–2663, https://doi.org/10.1021/acs.chemrev.5b00346. Preprint at.
[99] E.K. Goharshadi, K. Goharshadi, M. Moghayedi, The use of nanotechnology in the fight against viruses: a critical review, Coord. Chem. Rev. 464 (2022), https://doi.org/10.1016/j.ccr.2022.214559. Preprint at.
[100] C.I.C. Crucho, M.T. Barros, Polymeric nanoparticles: a study on the preparation variables and characterization methods, Mater. Sci. Eng. C 80 (2017) 771–784, https://doi.org/10.1016/j.msec.2017.06.004. Preprint at.
[101] S. Shah, et al., Nanomedicine based approaches for combating viral infections, J. Contr. Release 338 (2021) 80–104.
[102] R. Rai, S. Alwani, I. Badea, Polymeric nanoparticles in gene therapy: new avenues of design and optimization for delivery applications, Polymers 11 (2019).
[103] S. Deka, A. Sharma, P. Kumar, Cationic polymers and their self-assembly for antibacterial applications, Curr. Top. Med. Chem. 15 (2015) 1179–1195.
[104] Z. Liu, Z. Zhang, C. Zhou, Y. Jiao, Hydrophobic modifications of cationic polymers for gene delivery, Prog. Polym. Sci. 35 (2010) 1144–1162.
[105] S.K. Samal, et al., Cationic polymers and their therapeutic potential, Chem. Soc. Rev. 41 (2012) 7147–7194.
[106] Y.K. Sung, S.W. Kim, Recent advances in the development of gene delivery systems, Biomater. Res. 23 (2019) 1–7.
[107] T.J. Thomas, H.A. Tajmir-Riahi, C.K.S. Pillai, Biodegradable polymers for gene delivery, Molecules 24 (2019).
[108] C.K. Chen, et al., Biodegradable polymers for gene-delivery applications, Int. J. Nanomed. 15 (2020) 2131–2150.
[109] J. Gu, K. Al-Bayati, E.A. Ho, Development of antibody-modified chitosan nanoparticles for the targeted delivery of siRNA across the blood-brain barrier as a strategy for inhibiting HIV replication in astrocytes, Drug Deliv Transl Res 7 (2017) 497–506.
[110] M. Rodriguez, et al., Intranasal drug delivery of small interfering RNA targeting Beclin1 encapsulated with polyethylenimine (PEI) in mouse brain to achieve HIV attenuation, Sci. Rep. 7 (2017) 1–10.
[111] K.M. Fichter, N.P. Ingle, P.M. Mclendon, T.M. Reineke, Polymeric nucleic acid vehicles exploit active interorganelle trafficking mechanisms, ACS Nano 7 (2013) 347–364.
[112] A. Li, et al., The gene transfection and endocytic uptake pathways mediated by PEGylated PEI-entrapped gold nanoparticles, Arab. J. Chem. 13 (2020) 2558–2567.
[113] M.J. Reilly, J.D. Larsen, M.O. Sullivan, Polyplexes Traffic through Caveolae to the Golgi and Endoplasmic Reticulum en Route to the Nucleus, 2012.
[114] A. Parodi, et al., Enabling cytoplasmic delivery and organelle targeting by surface modification of nanocarriers, Nanomedicine 10 (2015), https://doi.org/10.2217/nnm.15.39, 1923–1940 Preprint at.
[115] N. Ryu, et al., Effective PEI-mediated delivery of CRISPR-Cas9 complex for targeted gene therapy, Nanomedicine 14 (2018) 2095–2102.
[116] L.L. Israel, et al., Acute in vivo toxicity mitigation of PEI-coated maghemite nanoparticles using controlled oxidation and surface modifications toward siRNA delivery, ACS Appl. Mater. Interfaces 7 (2015) 15240–15255.
[117] V.B. Morris, V. Labhasetwar, Arginine-rich polyplexes for gene delivery to neuronal cells, Biomaterials 60 (2015) 151–160.
[118] C.R. Joshi, et al., Reaching for the stars in the brain : polymer-mediated gene delivery to human astrocytes, Mol. Ther. Nucleic Acids 12 (2018) 645–657.
[119] B.J. Zern, H. Chu, A.O. Osunkoya, J. Gao, Y. Wang, A biocompatible arginine-based polycation blaine, Adv. Funct. Mater. 21 (2013) 434–440.
[120] M. Saeedi, M. Eslamifar, K. Khezri, S.M. Dizaj, Applications of nanotechnology in drug delivery to the central nervous system, Biomed. Pharmacother. 111 (2019) 666–675.
[121] A. Aurelia Chis, et al., Applications and limitations of dendrimers in biomedicine, Molecules 25 (2020), https://doi.org/10.3390/molecules25173982. Preprint at.
[122] P. Mittal, et al., Dendrimers: a new race of pharmaceutical nanocarriers, BioMed Res. Int. 2021 (2021).
[123] A.P. Dias, et al., Dendrimers in the context of nanomedicine, Int. J. Pharm. 573 (2020), 118814.
[124] A. Mehrabian, et al., Nanocarriers call the last shot in the treatment of brain cancers, Technol. Cancer Res. Treat. 21 (2022), https://doi.org/10.1177/15330338221080974. Preprint at.
[125] V. Patel, P. Patel, J.v. Patel, P.M. Patel, Dendrimer as a versatile platform for biomedical application: a review, J. Indian Chem. Soc. 99 (2022), https://doi.org/10.1016/j.jics.2022.100516. Preprint at.
[126] M.J. Mitchell, et al., Engineering precision nanoparticles for drug delivery, Nat. Rev. Drug Discov. 20 (2021) 101–124.
[127] A.S. Chauhan, Dendrimers for drug delivery, Molecules 23 (2018).

[128] M. Zeng, D. Zhou, S. Ng, J.O.K. Ahern, F. Alshehri, Highly branched poly(5-amino-1-pentanol- co -1,4- butanediol diacrylate) for high performance gene transfection, Polymers 9 (2017).

[129] A.C. Rinkenauer, S. Schubert, A. Traeger, U.S. Schubert, The influence of polymer architecture on in vitro pDNA transfection, J. Mater. Chem. B 3 (2015) 7477–7493.

[130] E. Haladjova, S. Rangelov, C. Tsvetanov, Thermoresponsive Polyoxazolines as Vectors for Transfection of Nucleic Acids. *Polymers (Basel)*, 2020.

[131] S.E. Seo, C.J. Hawker, The beauty of branching in polymer science, Macromolecules 53 (2020) 3257–3261.

[132] A. Sharma, J.E. Porter, E. Smith, R. Sharma, Effect of mannose targeting of hydroxyl PAMAM dendrimers on cellular and organ biodistribution in a neonatal brain injury model, J. Contr. Release 283 (2018) 175–189.

[133] A. Zarebkohan, et al., European Journal of Pharmaceutical Sciences Synthesis and characterization of a PAMAM dendrimer nanocarrier functionalized by SRL peptide for targeted gene delivery to the brain, Eur. J. Pharmaceut. Sci. 78 (2015) 19–30.

[134] Y. Liu, Y. Li, D. Keskin, L. Shi, Poly (β-Amino esters): synthesis, formulations, and their biomedical applications, Adv Healthc Mater (2019) 1–24, https://doi.org/10.1002/adhm.201801359.

[135] Y. Wang, et al., Effects of branching strategy on the gene transfection of highly branched poly(β-amino ester)s, Chin. J. Polym. Sci. 38 (2020) 830–839.

[136] S. Liu, et al., Highly branched poly(β-amino ester) delivery of minicircle DNA for transfection of neurodegenerative, Nat. Commun. (2019), https://doi.org/10.1038/s41467-019-11190-0.

[137] Y. Cao, et al., Linear-branched Poly (β-amino Esters)/DNA Nano- Polyplexes for Effective Gene Transfection and Neural Stem Cell Differentiation Linear-Branched Poly (β -amino Esters)/DNA Nano-Polyplexes for Effective Gene Transfection and Neural Stem Cell Different, Biomedical Materials, 2022.

[138] P. Shende, N. Ture, R.S. Gaud, F. Trotta, Lipid- and polymer-based plexes as therapeutic carriers for bioactive molecules, Int. J. Pharm. 558 (2019) 250, https://doi.org/10.1016/j.ijpharm.2018.12.085. –260 Preprint at.

[139] A. Ewe, et al., Liposome-polyethylenimine complexes (DPPC-PEI lipopolyplexes) for therapeutic siRNA delivery in vivo, Nanomedicine 13 (2017) 209–218.

[140] R. Spellerberg, et al., Selective sodium iodide symporter (NIS) gene therapy of glioblastoma mediated by EGFR-targeted lipopolyplexes, Mol. Ther. 23 (2021) 432–446.

[141] M. Ghezzi, et al., Polymeric micelles in drug delivery: an insight of the techniques for their characterization and assessment in biorelevant conditions, J. Contr. Release 332 (2021) 312–336.

[142] B.L. Banik, P. Fattahi, J.L. Brown, Polymeric nanoparticles: the future of nanomedicine, Nanomedicine and Nanobiotechnology 8 (2016) 271–299.

[143] K. Miyata, R.J. Christie, K. Kataoka, Polymeric micelles for nano-scale drug delivery, React. Funct. Polym. 71 (2011) 227–234.

[144] M.G. Carstens, C.J. Rijcken, C.F.v. Nostrum, W.E. Hennink, Pharmaceutical Micelles: Combining Longevity, Stability, and Stimuli Sensitivity, Multifunctional pharmaceutical nanocarriers, 2008, https://doi.org/10.1201/9781420075496-36.

[145] A. Waris, et al., Applications of various types of nanomaterials for the treatment of neurological disorders, Nanomaterials 12 (2022).

[146] W. Xu, P. Ling, T. Zhang, Polymeric micelles, a promising drug delivery system to enhance bioavailability of poorly water-soluble drugs, J Drug Deliv 1–15 (2013), 2013.

[147] D. Hwang, J.D. Ramsey, A.v. Kabanova, Polymeric micelles for the delivery of poorly soluble drugs: from nanoformulation to clinical approval, Adv. Drug Deliv. Rev. 156 (2020) 80–118.

[148] J. Kaur, et al., Advances in designing of polymeric micelles for biomedical application in brain related diseases, Chem. Biol. Interact. 361 (2022), 109960.

[149] M.A.S. Abourehab, O.A.A. Ahmed, G.F. Balata, W.H. Almalki, Self-assembled biodegradable polymeric micelles to improve dapoxetine delivery across the blood–brain barrier, Int. J. Nanomed. 13 (2018) 3679–3687.

[150] C. Melim, I. Jarak, F. Veiga, A. Figueiras, The potential of micelleplexes as a therapeutic strategy for osteosarcoma disease, 3 Biotech 10 (2020) 1–15.

[151] P. Pereira, et al., Smart micelleplexes as a new therapeutic approach for RNA delivery, Expet Opin. Drug Deliv. 14 (2017) 353–371.

[152] Y. Zhang, J.S. Buhrman, Y. Liu, J.E. Rayahin, R.A. Gemeinhart, Reducible micelleplexes are stable systems for anti-miRNA delivery in cerebrospinal fluid, Mol. Pharm. 13 (2016) 1791–1799.

[153] S.J. Gwak, et al., Cationic, amphiphilic copolymer micelles as nucleic acid carriers for enhanced transfection in rat spinal cord, Acta Biomater. 35 (2016) 98–108.

[154] S.-J. Gwak, et al., RhoA knockdown by cationic amphiphilic copolymer/siRhoA polyplexes enhances axonal regeneration in rat spinal cord injury model, Biomaterials 121 (2017) 155–166.

[155] H. Huo, et al., Polyion complex micelles composed of pegylated polyasparthydrazide derivatives for siRNA delivery to the brain, J. Colloid Interface Sci. 447 (2015) 8–15.

[156] A.M. Wagner, J.M. Knipe, G. Orive, N.A. Peppas, Quantum dots in biomedical applications, Acta Biomater. 94 (2019) 44–63.

[157] A.C. Anselmo, S. Mitragotri, A Review of Clinical Translation of Inorganic Nanoparticles vol. 17, 2015.

[158] H. Zu, D. Gao, Non-viral vectors in gene therapy: recent development, challenges, and prospects, AAPS J. 23 (2021), https://doi.org/10.1208/s12248-021-00608-7. Preprint at.

[159] N. Mishra, S. Ashique, A. Garg, V. Kumar, K. Dua, Role of siRNA-based nanocarriers for the treatment of neurodegenerative diseases, Drug Discov. Today 27 (2022) 1431–1440.

[160] M.J. Kim, S.U. Rehman, F.U. Amin, M.O. Kim, Enhanced neuroprotection of anthocyanin-loaded PEG-gold nanoparticles against A β 1-42 -induced neuroinflammation and neurodegeneration via the NF- K B/JNK/GSK3 β signaling pathway, Nanomedicine 13 (2017) 2533–2544.

[161] P. César, et al., Gold Nanoparticles Application to the Treatment of Brain Dysfunctions Related to Metabolic Diseases : Evidence from Experimental Studies, 2022.

[162] R. Aalinkeel, et al., Galectin-1 reduces neuroinflammation via modulation of nitric oxide-arginase signaling in HIV-1 transfected microglia, a Gold Nanoparticle-Galectin-1 " Nanoplex " a Possible Neurotherapeutic ? (2017) 133–151, https://doi.org/10.1007/s11481-016-9723-4.

[163] X. Fu, et al., Positive Effects of Taxol Pretreatment on Morphology , Distribution and Ultrastructure of Mitochondria and Lipid Droplets in Vitrification of in Vitro Matured Porcine Oocytes vol. 115, 2009, pp. 158–168.

[164] A. Bonoiu, et al., MMP-9 gene silencing by a quantum dot – siRNA nanoplex delivery to maintain the integrity of the blood brain barrier, Brain Res. 1282 (2009) 142–155.

[165] Z. He, et al., Advances in quantum dot-mediated siRNA delivery, Chin. Chem. Lett. 28 (2017) 1851–1856.

[166] J. Seo, et al., Recent advances in quantum dots for biomedical applications, J Pharm Investig 48 (2018) 209–214.

[167] A. Alaghmandfard, et al., Materials Science & Engineering C Recent advances in the modification of carbon-based quantum dots for biomedical applications, Mater. Sci. Eng. C 120 (2021), 111756.

[168] S.M. Mousavi, S.A. Hashemi, M.Y. Kalashgrani, N. Omidifar, W. Chiang, Bioactive Graphene Quantum Dots Based Polymer Composite for Biomedical Applications, 2022.

[169] G. Perini, V. Palmieri, G. Ciasca, M. de Spirito, M. Papi, Unravelling the Potential of Graphene Quantum Dots in Biomedicine and Neuroscience, 2020.

[170] T.K. Henna, V.R. Raphey, R. Sankar, V.K.A. Shirin, H.v. Gangadharappa, Carbon nanostructures : the drug and the delivery system for brain disorders, Int. J. Pharm. 587 (2020), 119701.

[171] M. Ahn, J. Song, Facile Synthesis of N-Doped Graphene Quantum Dots as Novel Transfection Agents for mRNA and pDNA, 2021, pp. 1–11.

[172] S. Iravani, R.S. Varma, Green synthesis , biomedical and biotechnological applications of carbon and graphene quantum dots . A review, Environ. Chem. Lett. 18 (2020) 703–727.

[173] P. Devi, S. Saini, K. Kim, The advanced role of carbon quantum dots in nanomedical applications, Biosens. Bioelectron. 141 (2019), 111158.

[174] M.J. Molaei, Carbon Quantum Dots and Their Biomedical and Therapeutic Applications : a Review, 2019, pp. 6460–6481, https://doi.org/10.1039/c8ra08088g.

[175] V.G. Reshma, P.v. Mohanan, Quantum dots : Applications and safety consequences 205 (2019) 287–298.

[176] J. Park, et al., Quantum Dots in an Amphiphilic Polyethyleneimine Derivative Platform for Cellular Labeling , Targeting , Gene Delivery , and Ratiometric Oxygen Sensing, 2015, pp. 6511–6521.

[177] C. Saraiva, et al., Nanoparticle-mediated brain drug delivery: overcoming blood-brain barrier to treat neurodegenerative diseases, J. Contr. Release 235 (2016) 34–47, https://doi.org/10.1016/j.jconrel.2016.05.044. Preprint at.

[178] A.M. Shehata, M.A. Shaker, Recent expansions of novel strategies towards the drug targeting into the brain (2019) 5895–5909.

[179] T. Komsthöft, G. Bovone, S. Bernhard, M.W. Tibbitt, Polymer functionalization of inorganic nanoparticles for biomedical applications, Curr Opin Chem Eng 37 (2022), 100849.

[180] S.J. Soenen, W.J. Parak, J. Rejman, B. Manshian, (Intra)Cellular stability of inorganic nanoparticles: effects on cytotoxicity, particle functionality and biomedical applications, Chem. Rev. 115 (2015) 2109–2135.

[181] M. Luo, et al., Delivering the promise of gene therapy with nanomedicines in treating central nervous system diseases, Adv. Sci. 9 (2022), https://doi.org/10.1002/advs.202201740. Preprint at.

[182] D.L. Puhl, A.R. D'Amato, R.J. Gilbert, Challenges of gene delivery to the central nervous system and the growing use of biomaterial vectors, Brain Res. Bull. (2019) 150 216–230, https://doi.org/10.1016/j.brainresbull.2019.05.024. Preprint at.

[183] M. Sainz-ramos, et al., How far are non-viral vectors to come of age and reach clinical translation in gene therapy? Int. J. Mol. Sci. 22 (2021) https://doi.org/10.3390/ijms22147545. Preprint at.

[184] H. Yin, et al., Non-viral vectors for gene-based therapy, Nat. Rev. Genet. 15 (2014) 541–555, https://doi.org/10.1038/nrg3763. Preprint at.

[185] C. Zylberberg, K. Gaskill, S. Pasley, S. Matosevic, Engineering liposomal nanoparticles for targeted gene therapy, Gene Ther. 24 (2017) 441–452, https://doi.org/10.1038/gt.2017.41. Preprint at.

[186] Y. Zhang, A. Satterlee, L. Huang, In vivo gene delivery by nonviral vectors: overcoming hurdles, Mol. Ther. 20 (2012) 1298–1304, https://doi.org/10.1038/mt.2012.79. Preprint at.

[187] J. Kreuter, Drug delivery to the central nervous system by polymeric nanoparticles: what do we know? Adv. Drug Deliv. Rev. 71 (2014) 2–14, https://doi.org/10.1016/j.addr.2013.08.008. Preprint at.

[188] A. Aigner, D. Kögel, Nanoparticle/siRNA-based therapy strategies in glioma: which nanoparticles, which siRNAs? Nanomedicine 13 (2018) 89–103, https://doi.org/10.2217/nnm-2017-0230. Preprint at.