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Targeting Specific Cells in the Brain with Nanomedicines for CNS Therapies

Fan Zhang^{#a,b}, Yi-An Lin^{#a}, Sujatha Kannan^c, and Rangaramanujam M. Kannan^{a,d,*}

^aCenter for Nanomedicine at the Wilmer Eye Institute, Department of Ophthalmology, Johns Hopkins School of Medicine, Baltimore, MD 21231, USA

^bDepartment of Material Science and Engineering, Johns Hopkins University, Baltimore, MD 21218, USA

^cDepartment of Anesthesiology and Critical Care Medicine, The Johns Hopkins Medical Institutions, MD, 21287 USA

^dDepartment of Chemical & Biomolecular Engineering, Johns Hopkins University, Baltimore, MD 21218, USA

These authors contributed equally to this work.

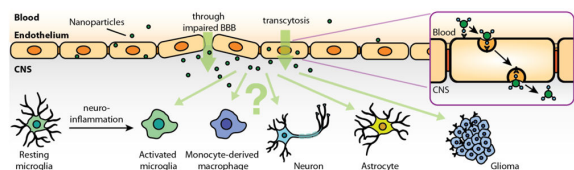
Abstract

Treatment of Central Nervous System (CNS) disorders still remains a major clinical challenge. The Blood-brain-barrier (BBB), known as the major hindrance, greatly limits therapeutics penetration into the brain. Moreover, even though some therapeutics can cross BBB based on their intrinsic properties or *via* the use of proper nanoscale delivery vehicles, their therapeutic efficacy is still often limited without the specific uptake of drugs by the cancer or disease-associated cells. As more studies have started to elucidate the pathological roles of major cells in the CNS (for example, microglia, neurons, and astrocytes) for different disorders, nanomedicines that can enable targeting of specific cells in these diseases may provide great potential to boost efficacy. In this review, we aim to briefly cover the pathological roles of endothelial cells, microglia, tumor-associated microglia/macrophage, neurons, astrocytes, and glioma in CNS disorders and to highlight the recent advances in nanomedicines that can target specific disease-associated cells. Furthermore, we summarized some strategies employed in nanomedicine to achieve specific cell targeting or to enhance the drug neuroprotective effects in the CNS. The specific targeting at the cellular level by nanotherapy can be a more precise and effective means not only to enhance the drug availability but also to reduce side effects.

Graphical abstract

*correspondence to krangar1@jhmi.edu.

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Keywords

Nanomedicine; CNS disorders; targeted therapy; BBB; microglia; neuron

1. Introduction

Achieving effective therapy for many diseases in the central nervous system (CNS) remains a great challenge. To date, clinical treatments for CNS diseases provide only limited improvement in outcomes and are often accompanied by severe side effects. For instance, patients diagnosed with glioblastoma only have a median survival period of 14 months following surgical resection, radiation, or concomitant chemotherapy [1]. The difficulty in achieving improved outcome for CNS diseases stems from the inability to deliver therapeutically relevant doses of the therapeutic to diseased cells or regions. The Blood-Brain Barrier (BBB) poses as the main obstacle that prevents most systematically administered drugs from entering the CNS.

Nanomedicine offers great potential for improving the therapeutic efficacy or diagnosis efficiency in the clinical settings for many CNS disorders. The use of nanoparticles as drug delivery vehicles is not only widely reported in pre-clinical studies but is now also being implemented in clinical applications. While the entry of drugs to the brain is highly restricted, drug-carrying nanoparticles can significantly improve the CNS pharmacokinetics and biodistribution relative to free drugs [2]. Recent discoveries have shed more light on the CNS disease pathology at the cellular or even molecular level, and have greatly impacted the design of nanomedicine to target specific cell populations in the CNS. This review will describe the pathological roles of major CNS cells (such as neurons, microglia/macrophage, astrocytes, endothelial cells, and brain tumor cells) in CNS disorders. We will then focus on recent advances of nanomedicine design that have been implemented to target specific cell populations in the CNS.

2. Cells of Interest for Designing Nanomedicine for CNS Delivery and Their Functions in Pathology

2.1 Endothelium at BBB in Normal Physiology

The BBB is composed of endothelial cells adjoined with tight junctions which act as physical barriers and constrain the passage of most molecules through the barrier *to* transcellular trafficking. More than 98% of small molecular drugs and almost 100% of large-molecules (>500 Da) are simply excluded from CNS because they cannot cross the BBB [3]. It not only functions as a protective obstruction that blocks possible neurotoxic substances from CNS but also mediates several important functions, such as i) selectively transporting

molecules that provides essential nutrients to the brain, ii) effluxing waste products, and iii) regulating homeostasis in the brain [4].

Small molecules are known to pass through endothelial cells by passive diffusion (for lipophilic molecules) or by active transport (as in the case of required nutrients into the brain, such as glucose, amino acids, nucleosides, or nucleobases) [4]. Small lipophilic molecules (<400 Da) exhibit certain abilities to pass the BBB by passive diffusion through the endothelial cells [5]. Unfortunately, many therapeutic molecules do not belong to this category. Most hydrophobic substances over 400 Da fail to enter the brain through this mechanism, often due to the efflux system regulated by ATP-binding cassette (ABC) transporters [5, 6]. These transporters are protective mediator that actively exclude possible neurotoxic substances from entering the CNS and are expressed on the endothelial cells. For instance, P-glycoprotein, an ATP-dependent transport protein localized in the blood luminal membrane, deters the entry of potentially toxic substances by pumping them back to the blood. While many small molecular drugs are substrates of ABC transporters, they possess fairly limited capabilities of entering the brain.

Large molecules, peptides or proteins, are incapable of passive diffusion across the BBB. Thus, their preferred mode of transport is through transcytosis, given that endothelial cells at the BBB exhibit much lower activity of internalization compared with other endothelia [4]. This mechanism involves either adsorptive-mediated (non-specific) or receptor-mediated (specific) transcytosis to allow macromolecules, captured in vesicles, to be internalized by endothelial cells and eventually exocytosed into the brain [4]. Adsorptive transcytosis involves endothelial internalization induced by non-specific binding caused by the interaction between cell surface and excess positive charges on the macromolecules. Some cationic proteins or peptides, such as cationic albumin, undergo this type of pathway to cross the endothelial cells [7]. On the other hand, internalization of substances can also occur by receptor-mediated transcytosis when ligands interact with their respective endothelial membrane receptors, such as transferrin receptor (TfR), melanotransferrin receptor (MTfR), lactoferrin (LfR), or LDL-receptor-related proteins [8-11]. Researchers that have designed nanomedicines in an attempt to increase drug delivery to the brain have heavily considered these two pathways, as discussed later.

2.2 BBB Dysfunction and Pathological Roles of Glia, Neurons in CNS disorders

2.2.1 BBB Dysfunction—Dysfunction of BBB endothelial cells is an important feature in many CNS pathologies [12]. This dysfunction is often associated with neuroinflammation which leads to alternation of permeability of the BBB. Under these conditions, the tight junctions between endothelial cells are disrupted, which enables additional substances to cross BBB or permits circulating macrophages to infiltrate into the CNS (Figures 1 and 2). This permeability change can be observed in neuroinflammation initiated by bacterial lipopolysaccharides (LPS), or in disease pathologies for stroke, traumatic brain injury, MS, and brain tumors [4, 13]. Such dysfunction can also affect the efflux system in endothelial cells. In some diseases such as AD or PD, the BBB permeability is altered due to decreased level of P-glycoprotein expression, which lowers the efficiency of efflux pumps in transporting neurotoxic substances back to the blood [14-18].

2.2.2 Microglia in Neurodegenerative Diseases—The CNS possesses a distinctive immune system since circulating immune cells cannot access to the CNS in physiological conditions. Microglial cells serve as the major resident immune cells in the CNS where they constantly monitor their surroundings and act as the mediator in innate immunity for early control of infections [19, 20]. They serve as key therapeutic targets because their functions are closely associated with the pathologies of many CNS disorders, including ischemic stroke, traumatic brain injury, spinal cord injury, AD, Parkinson’s disease (PD), amyotrophic lateral sclerosis (ALS), multiple sclerosis (MS), and most types of brain tumors [19-25]. Under normal conditions, quiescent microglial cells constantly survey their surroundings, providing a defense mechanism for the brain. Upon sensing pathogen invasion or tissue damage, they rapidly switch to the activated phenotype that secretes cytokines to initiate secondary inflammatory responses. (Figure 1). This phenotype change of microglia, characterized by a morphological change from ramified form to amoeboid form, is often induced by the recognition of pathogen-associated moieties by toll-like receptors (TLRs), a family of receptors that plays a role in both innate and adaptive immunity [19, 20]. When recognizing stimuli by such receptors, microglia can produce cytokines to stimulate either pro-inflammatory or anti-inflammatory responses. It should be noted that the termed “activation” could indicate a range of activated states for microglia, broadly divided into two main phenotypes, M1 and M2, as indicated in other reviews [26, 27]. Activated microglia in the M1 state, or also called “classically activated”, promote the production of inflammatory cytokines, such as TNF- α , IL-1 β , or IL-6 [19, 20]. On the other hand, M2 microglia contain phenotypes which suppress inflammation by cytokines TGF- β or IL-10 (M2a phenotype), or assist tissue remodeling and matrix deposition (M2c phenotype) [26]. The dynamic between M1 and M2 phenotypes of microglia enables the response to early stages of infection and is responsible for the clearance of cellular debris. However, in many pathological conditions, pro-inflammatory effects can persistently dominate without effective anti-inflammatory responses. For example, M1 microglia may release cytotoxic nitric oxide (NO), reactive oxygen species (ROS), or excessive cytokines (such as TNF- α) that cause neurotoxicity and lead to the injury/death of neurons, oligodendrocytes, and/or damage extracellular matrix [27].

Dysfunction of microglial responses has been observed in CNS diseases associated with both acute and chronic neuroinflammation. In acute inflammation, such as ischemic stroke, spinal cord injury, or traumatic brain injury, microglia activation is triggered by mechanisms that cope with damage-associated molecular pattern molecules (DAMPs) released from injured cells or cellular necrosis processes [28, 29]. Though this is a crucial step for tissue repair, a failure of follow-up anti-inflammation responses in these instances can result into more damages. As stated before, this activation (dominated by M1 phenotype) could lead to over-production of cytotoxic NO and ROS, which exacerbate the cellular death, or cytokines (for example, IL-1, IL-6, and TNF- α) that amplify inflammation response [30]. Chronic inflammation, in contrast to acute inflammation, is a self-propagating inflammatory response initiated from certain stimuli. This process generally starts from an initial microglia activation, which further develops into increased inflammation and glial activation when M2 responses occur and fail to exert their neuroprotective roles [26]. M1 microglia participates in the chronic inflammation of many neurodegenerative diseases, including AD, PD, ALS,

or MS [25, 31-33]. In these instances, microglia respond to stimuli, such as aggregation of A β in AD and α -synuclein in PD, motor neuronal death in ALS, or signals from infiltrating lymphocytes in MS; and further produce more NO, ROS, proinflammatory cytokines (such as TNF- α , IL-1 β , IL-6) that eventually cause progressive injury to other neurons/astrocytes without appropriate neuroprotective effects from M2 phenotypes [25-27, 34-37]. Since this cascade is often accompanied with BBB impairment in pathology, circulating macrophages can possibly infiltrate into the CNS and function similarly to microglial cells to amplify the inflammatory responses.

The production of proinflammatory cytokines and neurotoxic substances from microglia/infiltrating macrophages are linked to several pathways, including, but not limited to, the activation of NOX2 complex (an NADPH oxidase), mitogen-activated protein kinase (MAPK), and sphingosine kinase 1 (Sphk1) as detailed elsewhere [38-40]. In general, these pathways can be stimulated by variety of disease proteins (A β precursor protein, α -synuclein, myelin), LPS, cytokines (TNF- α , IL-1 β , IL-4, IL-13), or environmental toxins. Since these pathways account for the production of neurotoxic molecules, inhibition of these pathways in microglia can possibly promote neuroprotective effects. For example, inhibition of NOX2 or p38 MAPK by treating dexamethasone or minocycline could attenuate the activation of microglia, as well as the production level of NO and IL-1 β [39, 41, 42]. Dimethylsphingosine, an inhibitor of Sphk1, reportedly suppresses the expression of proinflammatory cytokines including TNF- α , IL-1 β , and the production of NO of microglial cells [40]. But to optimize the therapeutic effects, these potent drugs require specific, effective delivery to microglia/infiltrating macrophages.

2.2.3 Tumor-Associated Microglia/Macrophages in Tumor Microenvironments

—Tumor-associated microglia and infiltrating macrophages (TAMs) pose as important mediators in CNS cancer (Figure 2). Various studies regarding the synergic effects between TAMs and gliomas that allow them to co-exist have been reviewed previously [21, 43]. TAMs can be recruited by gliomas by chemokines, cytokines, or matrix metalloproteinases (MMPs)[21]. Although TAMs may polarize to M1 phenotype to give rise to pro-inflammatory effects (for initiating the clearance of potential hazards in CNS), their inflammatory functions are often suppressed in glioma pathology [23, 43-46]. Gliomas produce anti-inflammatory cytokines such as IL-10, IL-4, IL-6, and TGF- β [21], where tumor cells are able to establish an immunosuppressive microenvironment after recruiting TAMs. Under such condition, TAMs are instead polarized to the M2 phenotype that reduces classic neuroinflammation, and allows further tumor progression [21, 43]. In addition, TAMs themselves can also secrete cytokines (TGF- β) and MMPs (MMP-2, MMP-9, and MT1-MMP) that promote tumor growth and invasion [47-50]. These findings suggest TAMs can serve as a crucial target, as their pathological functions are strongly associated with tumor cells. Delivering therapeutics to alter the functions of TAMs or using TAMs as delivery vehicles could potentially provide an effective approach for the treatment of brain tumors.

Microglia/macrophages, under such circumstance, are polarized to M2 phenotypes and produce cytokines (TGF- β) or MMPs that are associated with tumor progression.

2.2.4 Astrocytes—Astrocyte is another major glial cells in the brain which perform a majority of functions related to maintenance of extracellular microenvironment and cell-cell communications in the CNS. They participate in the metabolism of amino acids, nutrients, and ions in the brain, communication with neurons and BBB endothelium, as well as the modulation of excitatory synaptic transmission [51, 52]. Abnormalities and dysfunctions of astrocytes are also linked with pathologies of CNS diseases, including stroke, neuroinfection, epilepsy, AD, PD and ALS [53, 54]. Changes of astrocyte functions in response to potent perturbations or injury, termed “astrogliosis”, is characterized by the increase in number, size, motility of astrocytes, and upregulation of pro- or anti-inflammatory cytokines. In addition, increased level of glial fibrillary acidic protein (GFAP) expression by reactive astrocytes is a common pathological observation of astrogliosis in various CNS diseases. However, the pathological functions of this upregulation of GFAP remain unclear [51]. Activated astrocytes are often found in malignant regions in neurodegenerative diseases, producing ROS and NO when neuroinflammation occurs [24]. In spite of the limited understanding of the pathological mechanisms of astrocytes, medicine that targets these activated astrocytes could potentially provide neuroprotective effects within local malignant tissues.

2.2.5 Neurons—Neuronal cells are the major cells that process and transmit information in the brain. They are also considered an important therapeutic target because they are often impaired in the pathologies of many CNS diseases. Ischemia related CNS diseases, such as stroke, spinal cord injury and traumatic brain injury, excitotoxicity often leads to subsequent neuronal death [55-58]. This process involves the neuronal up-regulation of excitatory neurotransmitter glutamate as well as glutamate receptors (such as NMDA and AMPA receptors) on neurons [58]. This cascade not only causes neuron damages, but also initiates microglia activation, which often exacerbates the injury as discussed previously [59]. On the other hand, neuronal cells can also undergo degeneration or apoptosis in chronic neuroinflammation. When neuroinflammation persists, neurons can possibly initiate apoptosis upon sensing cytokines (TNF- α) from activated microglia, due to ROS and NO from both activated microglia and astrocyte, or via interacting with neurotoxic substances (such as A β) [60]. These pathological roles of neurons in CNS diseases suggest that the effective delivery of therapeutics to neurons may alleviate the damage.

2.2.6 Brain Tumor Cells—Brain tumors are highly heterogeneous and present with a variety of phenotypes. Gliomas are the most common invasive tumors in the CNS and are derived from glial cells. For the treatment of CNS cancers, tumor cells could be the therapeutic targets for delivering chemotherapeutics. Chemotherapeutic agents such as carmustine, lomustine, and temozolomide (all approved by the FDA for the treatment of brain tumors) often damage the DNA and trigger the death of tumor cells. However, these agents normally do not carry targeting properties, and therefore do not distinguish between malignant cells and healthy cells, further causing severe side effects. As a result, surface receptors that over-expressed on glioma cells could serve as molecules of interest for active targeting when designing nanomedicine, which will be discussed in later context.

3.1 Targeting endothelial cells in the CNS

3.1.1 Targeting Endothelial Cells for Disease Diagnosis—Diagnostic tools aiming to target specific cell population are often engineered according to the pathology of diseases. In neuroinflammatory disorders (such as stroke, cerebral ischemia, and AD), endothelial activation plays a critical role at the initial stage in recruiting immune cells to the site of inflammation [61]. Such activation is marked by over-expression of some adhesion molecules—such as vascular cell adhesion molecule-1 (VCAM-1)—on the membrane of endothelial cells [62] (Table 1). In some reported work, VCAM-1 was chosen as the targeting molecule for the early detection of vascular damages. Grafting VCAM-1 targeting peptides to ultrasmall superparamagnetic iron oxide allowed the designed nanoparticles being used for the detection of cerebrovascular cell activation in multiple models (such as stroke and ischemia), serving as a valuable strategy for non-invasive and quantitative evaluation for neuroinflammation in pre-clinical models [63-65]. However, VCAM-1 expression varies at different stages after injury in different models. One should note that successful targeting of activated endothelial cells is highly associated with the time course of disease progression.

3.1.2 Targeting Endothelial Cells for Increasing the Brain Uptake—Targeting endothelial cells in the CNS could be used as a means to increase the efficiency of drug across BBB. Various strategies with this feature have been applied in the treatments of diverse CNS disorders such as AD [68-70], primary or secondary malignant glioma [72, 73, 76-80, 84], stroke [74], and cerebral ischemia [83]. These applications are summarized according to their platforms, targeting ligands, and models of evaluation in Table 1. Currently, this strategy has led to profound results in large animal models such in primates [81] and are being evaluated in clinical trials (NCT01480583) [76]. In these instances, ligands that were involved in the specific interactions with endothelial transporters were often incorporated into the therapeutic agents/cargos for the initiation of receptor-mediated transcytosis at BBB endothelium (Figure 3). Potential receptors of interest in recent studies include the choline transporter [85], the transferrin receptor (TfR) [68, 72-74], integrin $\alpha_v\beta_3$ (the RGD receptor) [71], the insulin receptor [81, 82], and the low-density lipoprotein receptor-related protein-1 (LRP1) [75, 76, 84].

To bring this to light, surface modification of large nanoparticles (>700 nm) with TfR targeting peptides could enhance the particle accumulation in the brain by the specific interactions with TfR on the endothelial cells as applied in a stroke model [74]. However, the successful delivery of TfR targeting devices to the CNS requires careful control of the binding affinity between the targeting device and TfR. Recent studies have shown that reducing TfR affinity is crucial for successful transcytosis and increased brain exposure of therapeutics. Therapeutic antibody with bivalent binding showed higher binding affinity with TfR, but resulted in incomplete transcellular trafficking and less brain exposure due to the higher binding affinity. Reducing the binding affinity to TfR by 5-8 fold, by using vehicles with monovalent binding, greatly increased the efficacy of this therapeutic antibody by more than 50-fold compared with the parent antibody [68-70].

Low-density lipoprotein receptor-related protein-1(LRP1) is another successful example that has been extensively considered as the targeting receptor on endothelial cells.

Nanomedicines that target LRP1 have been shown to enhance the brain accumulation of chemotherapeutic agents, siRNA, and proteins in glioma models [76-80, 84, 86]. In these studies, angiopeps-2 (An2), known as the LRP1 targeting peptide, was incorporated into the nanodevices or drugs to enhance the therapeutic efficacy. It has been shown in *in vivo* human xenograft models that incorporating paclitaxel with An2 significantly improved the brain uptake of paclitaxel by 5-fold [77]. Moreover, an An2-conjugate of paclitaxel (ANG1005) has reached clinical trials and demonstrated encouraging preliminary efficacy in Phase I/II for the treatment of primary and secondary brain tumors (NCT01480583) [76].

Dual targeting may be an effective strategy to enhance the specific cellular uptake of therapeutics after the nanodevices pass the BBB. In addition to incorporating ligands that bind to endothelial transporter receptors, adding a targeting ligand that interacts with another cell in the CNS can potentially increase the accumulation of nanomedicine in targeted cell population after the entry to the CNS. For example, the combination of a folate receptor (tumor cells) and a TfR (BBB endothelial cell)[72], or a TAT receptor (tumor cells) and a TfR (BBB endothelial cells) [73], both demonstrated the increased brain tumor uptake of therapeutics *in vitro* and extended survival compared to single targeting therapy. Still, it should be noted that this strategy would only be effective when both targeting ligands could maintain their respective targeting activity or provide synergic effects. A thorough understanding of the mechanism of how two targeting ligands synergically affect each other when integrated into one single system will provide more insight.

In addition to targeting ligands or antibodies, surfactants such as polysorbate 80 can also be chosen as surface moieties to enhance the interactions of nanoparticles with BBB. Coating polysorbate 80 to the surface of nanoparticles has been shown to enhance the efficiency for nanoparticles to interact with BBB through the following mechanisms: (1) binding with apolipoprotein E in the blood and the follow-up endocytosis by endothelial cells, and (2) suppressing the function of P-glycoprotein (P-gp), consequently reducing the efflux of nanoparticles from brain parenchyma [67]. Recent studies have successfully applied this concept both in therapeutics and diagnostics [66, 67]. These polysorbate 80 coated nanoparticles could significantly increase the brain uptake of doxorubicin at 1 hour post administration (nanoparticle with doxorubicin: undetectable amount, polysorbate 80 coated nanoparticle with doxorubicin: 2 $\mu\text{g/g}$) [87]. Another study has employed clinical relevant MRI imaging technologies and further demonstrated higher accumulation levels of gadolinium (5.34% injected dose at 2.5 hours post injection) when formulated with polysorbate 80 compared with free gadolinium (0.009% injected dose), which accordingly resulted into a 1.5-fold increase in signals [66]. However, as polysorbate 80 was passively adsorbed to the surface of nanoparticles in these studies, the *in vivo* stability of these formulations should be tested before moving polysorbate 80 based therapies into clinical studies.

3.2 Targeting microglia/macrophages and TAMs in the CNS

As stated in previous paragraphs, neuroinflammation, a hallmark of many neurodegenerative diseases and CNS cancer, is mediated by microglia and macrophages in the CNS. They are considered as the main candidates to function as “Trojan horses” in targeted drug delivery,

because of their inherent phagocytic nature and disease homing properties in pathology [88]. Many nanoparticle platforms have been designed to target microglia/macrophage so that they can bring the therapeutics to the diseased regions for the treatments of neurodegenerative disorders (such as AD/PD) or glioblastoma [89-102]. Based on the size, surface chemistry (cationic/anionic/neutral, hydrophilic/lipophilic) and chemical structures, the inherent properties of nanoparticles can accordingly impact the targeting ability and pharmacokinetics. The following paragraph will depict different strategies related to microglia/macrophage targeting by nanomedicine for therapeutic purposes and diagnosis.

3.2.1 Mechanism of Nanoparticles Uptake by Microglia/Macrophage—

Understanding the mechanistic internalization of nanoparticles by microglia/macrophage can provide great insights towards designing effective platforms with improved targeting efficiency. *In vitro* studies have suggested that clathrin-mediated endocytosis serves as the major pathway for microglia to uptake quantum dots and polymeric nanoparticles, regardless of the activation status of microglia [89, 92, 95]. These results echoed well with the findings from another study on how nanoparticle geometry affects their uptake by microglia [103]. Since clathrin-mediated endocytosis involves the binding of clathrin receptor with the surface of nanoparticles, the geometries with larger surface area to volume ratio can have higher uptake efficiency. This was confirmed by Maysinger *et al.*, where they discovered that gold nanoparticles with “urchin-mimicking” geometry (10 times larger surface area than the rod and sphere nanoparticle) were taken up more readily (detectable under confocal microscopy) by microglial cells when compared with spherical and rod-like gold nanoparticles (undetectable under confocal microscopy) [103]. In addition to clathrin mediated endocytosis, mannose receptors and macrophage scavenger receptors have also been implicated in playing a role in nanoparticle uptake in non-activated microglia [89].

Several studies have demonstrated that activated microglia have a greater ability to internalize nanoparticles than non-activated microglia [90, 92, 95, 101, 104]. However, future studies focused on determining the differences in uptake mechanisms between activated and non-activated microglia are necessary to address this crucial knowledge gap. Specifically, under *in vitro* condition, LPS-activated microglial cells displayed a 1.7-fold higher uptake of poly-ε-caprolactone and polyethylene glycol (PLC) and 1.4-fold higher uptake of QD665 than non-activated microglial cells (Figure 4 A,B) [92]. In a rodent model of glioblastoma, activated TAMs accounted for most of the internalization of G4 hydroxyl-terminated PAMAM dendrimers (~4 nm) in the brain tumor microenvironments after systematic administration, as revealed from the strong co-localization of TAMs with fluorescence-labeled nanoparticles that were uniformly distributed in the tumor mass (Figure 4C–F) [101]. The enhanced nanoparticle uptake by activated microglia could be demonstrated by comparing the nanoparticle accumulation in the contralateral hemisphere of tumor bearing rats or in healthy rats, in which there was no apparent internalization of the dendrimers by non-activated microglial cells.

Along with the nanoparticles surface geometry, nanoparticle surface functionalities also affect the amount and rate of nanoparticle uptake by microglia. For instance, Veglianese *et al.* tested the uptake of poly(methyl methacrylate) nanoparticles (PMMA-NPs) with different surface charges by activated microglia; in this study, the surface properties of PMMA-NP

varied between positively charged (PMMA-pos NPs, ζ -potentia: 21.5 mV), negatively charged (PMMA-NPs, ζ -potentia: -18.0 mV), and PEGylated (PMMA-PEG NPs, ζ -potentia: -2.52 mV, the size of nanoparticles did not vary significantly). After LPS-activated microglial cells were incubated with nanoparticles for 3 hours, it was found that the internalization of PMMA-pos was significantly higher (4.83 ± 5.3 mean integrated density \pm SD) than that of PMMA-NPs (3.46 ± 4.53 mean integrated density \pm SD). It was also found that the PEGylation reduced the nanoparticle uptake by 1.5 fold and delayed the rate of NPs uptake for 45 hours compare to non-PEGylated nanoparticles [95].

3.2.2 strategies of Manipulating Microglia/Macrophages—Recently, it was demonstrated that the manipulation of microglia/macrophages could provide beneficial effects in attenuating inflammation and reducing subsequent tissue damages [90-94, 104]. Most cases utilized two pathways: (I) the modulation of microglia/macrophage behavior, such as the activation status and the release of inflammatory signals, and (II) the control of microglia/macrophage population through microglia depletion or the prevention of infiltrating monocytes/macrophages (Table 2).

(I) Modulation of microglial activation and control of cytokine release: Microglia/macrophage modulation has been well-documented in *in vitro* studies (see Table 2). Successful modulation of LPS-induced cytokine release from microglial cells was achieved through the inhibition of p38 MAPK pathway with the treatment of celastrol-encapsulated hydroxyl terminated generation 4 PAMAM dendrimers [111]. Similarly, anti-inflammatory effects were also shown through the use of minocycline-encapsulated polymeric micelles [112]. Methylprednisolone (MP)-loaded dendrimer nanoparticles have shown to affect the microglial cell viability as a function of released MP [93]. Nanoparticles with the ability to target inflammation-associated subcellular organelles such as lipid droplets in microglial cells were even realized in *in vitro* studies [113].

Nevertheless, successful application of these strategies in specific disease animal models is highly dependent with the type and status of inflammation, suggesting that effective treatments may differ from disease to disease. In general, the successful resolution of inflammation requires considering the stage of inflammation, physiochemical properties, toxicity of delivery vehicles and the mechanism of therapeutics. As examples in Table 2 have shown, treating inflammation at different stages of diseases may result in differing outcomes. In a recent study, Papa *et al.* locally injected minocycline encapsulated polymeric nanoparticles in a spinal cord injury mice model, and discovered that there were decreased levels of pro-inflammatory cytokines and reduced microglial activation *in vivo* [92]. However, treatments with anti-inflammatory agents may not always result in a reduction of pro-inflammatory cytokines, especially during later stages of inflammation. In another study of spinal cord injury model, Saxena *et al.* systemically injected liposomal formulation of macrophage migration inhibitory factor at 48 hours post injury, when inflammation is considered well established. In this case, the treatment in turn resulted in a significant increase of pro-inflammatory cytokines transcripts (such as CCL2, IL1- β , iNOS), and a simultaneous increase of anti-inflammatory cytokines transcript (such as Arginase-1 and TGF- β) that are believed to assist wound healing [91].

Properties of nanocarriers also affect the results of microglial/macrophage modulation. Sizes of nanoparticles determine the amount and the retention period of nanoparticles that stay in microglia/macrophages. Nanoparticles with larger sizes provide longer systemic circulation, providing additional temporal opportunities for microglia/macrophage uptake. Yet, their size needs to be ‘appropriate’ to achieve sufficient brain extravasation at the injured site before the nanoparticles are finally internalized by microglia/macrophages. In cases of spinal cord injury, the optimal size for systemic therapy was found to be around 200 nm, at which the treatment elongated the therapeutic window up to 90 hours [91, 92]. In a rabbit model of cerebral palsy, the systemic injection of single dose of PAMAM dendrimer-*N*-acetylcysteine conjugates (with ~4 nm diameter in size) at postnatal day 1 resulted in a long intracellular retention of this therapy in activated microglia and consequently elongated the therapeutic window of this disease [104]. It should be noted that the vehicles themselves may affect the activities of microglia/macrophage as well. For instance, dendritic polyglycerol sulfate (dPGS), as a polyglycerol scaffold, has been proved to be able to inhibit microglia activation in chronic inflammation [114]. Iron oxide nanoparticles could reportedly inhibit the production of IL1- β when microglial cells were activated with LPS *in vitro* [106]. Single wall carbon nanohorns were also shown to inhibit microglial activation, leading to reduction proliferation, delay mitotic entry, and increased apoptosis of activated mice microglial cells [92]. Hence, the intrinsic properties of the vehicle should also be taken into consideration when designing new formulations of anti-inflammatory drugs.

(II) Control of microglia/macrophage population: Regulation of microglia/macrophage population in CNS can modulate the pathological environments mediated by microglia/macrophage, although the effects can be either beneficial [117, 118] or detrimental [119, 120] according to disease models. Depletion of microglia/macrophage and inhibition of infiltrating monocytes/macrophages are the most commonly employed strategies to regulate microglia/macrophage population. Cytotoxic drugs, such as clodronate, glucocorticoids, doxorubicin, and methotrexate were usually applied for microglia/macrophage depletion therapies, inducing the reduction of local microglia or global macrophage population [88]. For example, clodronate encapsulated in liposome was shown to successfully eliminate circulating macrophages but not the resident microglia, with concomitant reduction in clinical signs of experimental autoimmune encephalomyelitis (EAE) [96, 97]. In another case, locally delivered methylprednisolone encapsulated into PLGA nanoparticles dramatically reduced the number of macrophages/reactive microglia [94]. The other type of microglia/macrophage depletion approach is through photothermal therapy, which utilizes photoabsorbers such as organic dyes to generate localized hyperthermia upon illumination by visible-NIR light [121]. Based on this concept, Baek *et al.* synthesized gold nanoshell which can convert near-infrared light (NIR) to heat. Microphage can efficiently take up these nanoshells and infiltrate into the *in vitro* glioma sphere. NIR laser irradiation of spheroids incorporating NS loaded macrophages resulted in complete glioma growth inhibition in an irradiance dependent manner [99, 100]. However, microglia/macrophage depletion therapies are inherently aggressive, and can potentially cause significant side effects such as global immune suppression. Therefore, this approach is more valuable in life-threatening diseases such as cancer.

On the other hand, increasing the immune cell population is needed in some diseases such as glioma. The tumor microenvironment is known to be immunosuppressive; therefore, delivering immunostimulatory agents to TAMs can provide beneficial outcomes. Badie *et al.* showed that carbon nanotubes could target TAMs in glioma through intratumoral administration [108]. Based on this finding, they formulated CNT-cPG therapy and successfully exerted its anti-tumor activity by promoting the influx of inflammatory cells such as macrophages, nature killer cells, CD8 and CD4 cells [109]. Their recent study has even shown that, through the use of iron oxide nanoparticles, they could control the migration of targeted microglia *in vitro* under an external magnetic field and could further increase the infiltration of inflammatory macrophage to the brain tumor *in vivo* [110].

3.2.3 Imaging microglia/macrophage activity—Nanotechnology can also be employed to monitor microglia or macrophage activities (examples are listed in Table 2). Caspase-1 is a key participant in inflammation, yet there is currently no available assay for assessing its activity. Surface-modified quantum dots as nanosensors were developed for caspase-1 ratiometric measurements, and this platform has been applied in *in vivo* studies [115]. In another study, phosphorus dendrimers was used as a spectral nano-sensor for macrophage polarization and fate tracking in spinal cord injury [116]. Since these studies are still in early stages, the successful translation into clinics will need further optimization on instrumentation to improve the detection sensitivities.

3.3 Targeting neuronal cells

3.3.1 anoparticles intrinsically target neurons—Neuron-specific delivery is challenging because neurons have a non-phagocytic nature relative to glial cells, and are highly outnumbered by the glial cell population in the brain. In some studies, neuron-specific targeting was achieved by taking advantage of the vehicle's intrinsic ability to interact with neurons (Table 3) [122, 123]. For example, apolipoprotein E (ApoE) is a dominant lipoprotein in the brain produced by astrocytes. It delivers essential lipids to neurons via binding of the cell membrane. MacVicar *et al.* utilized the nature of lipid nanoparticles (LPNs) to deliver siRNA to the neuron cells through the absorption of ApoE to LPNs and the subsequent endocytosis into neurons mediated by ApoE receptor [122]. Batrakova *et al.* used the property of exosome as a mechanism for intercellular communication and delivered exosomes based therapeutics to CNS neurons in a mouse model of PD [123]. In this study, the exosomes (~100-200 nm) were administrated intranasally and mainly accumulated in neurons and microglia (Figure 5). This platform was then used to deliver catalase (a potent drug that could deactivate free radicals) to yield neuroprotective effects in the PD model.

3.3.2 rategies of actively targeting neurons—To enhance the neuronal targeting, active targeting strategies, utilizing ligands such as Tet1 [85, 124, 125], dopamine [126] and rabies virus glycoprotein peptide (RVG and RDP peptide) [126-128] are being pursued (Table 3). Active targeting requires the specific expression of the target molecule on the cell of interest. GT1b gangliosides, sphingophospholipids are such molecules highly expressed on the neuronal cell types. Therefore, Tet1, a 12-amino acid peptide that binds to these molecules, was used in many studies to modify the nanoparticles surface to increase

targeting efficiency [85, 124, 125]. Another type of active targeting is well adapted for CNS entry. A 29-amino-acid sequence of the RVG was identified to be critical for the entry and transport of virus in the CNS, [129] and therefore it was incorporated on the surface of delivery vehicles or directly used to deliver therapy for CNS diseases. Examples of RVG based delivery include: transvascular delivery of small interfering RNA to the CNS neurons through RVG peptide. This delivery efficiently silenced the specific gene and afforded robust protection against fatal viral encephalitis in mice [127]. Further studies have demonstrated that systemic delivery of siRNA using RVG peptide modified exosomes result in gene knockdown in neurons, microglia, oligodendrocytes [128].

However, modifying the surface of nanoparticles with excessive targeting ligands could reduce selectivity, due to enhanced binding to both target and non-target cells and organs [130]. Therefore, the balance between enhanced targeting efficiency and selectivity would be critical. Pun *et al.* developed an approach to precisely control over ligands density, orientation of display, and architectural display in the final polymeric construct. They found that the optimized Tet1 ligands density was around 3-5% (mol% by feed) in their *in vivo* model to provide neuron specific selectivity [85].

3.3.3 Targeting neurons based on pathology of disease—Neuron-specific targeting can also be achieved by taking advantage of the disease pathology. A recent example, reported by Kannan and coworkers, has indicated that hydroxyl-terminated generation-4 PAMAM dendrimers (size ~ 4 nm) could cross the impaired BBB and only be localized in injured neurons in the superficial dentate granule cell layer without accumulation in un-injured neurons via systematic administration in a canine model of hypothermic circulatory arrest induced brain injury, where neurons in the hippocampus and cerebellum region underwent apoptosis due to glutamate excitotoxicity [134]. This study further demonstrated excitotoxicity associated neuronal uptake of dendrimer can be utilized for neurological and neurobehavioral benefits by delivering dendrimer based therapy to the injured neuron [134].

3.3.4 Targeting neurons for stimulation—Neuron-targeting nanoparticles can also be used as tools to stimulate neurons. Gold nanoparticles modified with neuron-targeting ligands have been demonstrated to transduce millisecond pulses of light into heat, which changes neuron membrane capacitance, depolarizing the cell and eliciting action potentials [132]. Magnetic nanoparticles delivered to the brain can dissipate heat, triggering widespread and reversible firing in specific neurons [131]. These studies extended the use of neuron-targeting nanoparticles from therapeutics application to non-invasive methods for the study of neuronal activities or brain circuits.

3.3 Targeting Astrocytes

Although the contribution of astrocytes in pathological conditions was discussed earlier, drug delivery systems designed to specifically target these cells is still limited. Several viral vectors have been described to inherently target astrocytes *in vivo* (Table 4). Lentiviral vectors (LV) were found to have pseudotype-dependent neuron and glia tropism. Based on such vectors, it is possible to target astrocytes *in vivo* through modifying LV pseudotype,

integrating cell-specific promoters, and using miRNA post-transcriptional regulatory elements [135-137]. However, more studies are needed to take the heterogeneity of astrocytes into consideration, especially when astrocytes are in pathological conditions. Another promising type of viral vectors, adeno-associated virus (AAV) vectors, has already been studied in clinical trials for the treatment of PD. Recently AAV vector-based therapy was found to target astrocytic CN/NFAT signaling in a mouse model of AD, resulting in reduced glial activation, lower amyloid levels, and subsequent improvement of cognitive and synaptic functions [138]. Nevertheless, there are concerns regarding the safety, tolerance, and immunogenicity of viral vector-based therapies, which has limited their translational potential.

Instead of utilizing viral vectors as therapeutic vehicles, synthetic nanoparticles such as iron oxide nanoparticles, silver nanoparticles, and some polymeric nanoparticles can be potential candidates for astrocyte specific targeting, even though many related studies remain in the *in vitro* stage [139, 140]. A few *in vivo* studies have shown that the internalization of nanoparticle by astrocyte was always accompanied by a more dominant nanoparticle uptake by other CNS cell types such as microglia [104, 142] or neurons [141]. In a study where PAMAM dendrimer was used for the treatment of ischemia-induced neonatal white matter injury, systemically administrated dendrimers were first found in astrocytes and oligodendrocytes at the initial stage of the injury. However, at later stage of neuroinflammation, dendrimer uptake switched from astrocytes into activated microglia, indicating that pathological environments in the brain might play a major role in the uptake of nanoparticles by different cells [90].

3.4 Targeting brain cancer cells

Brain cancer can be classified into different types according to the tissue of origin. Current studies have focused on developing therapies for glioblastoma, the most common and aggressive type of brain cancer which derives from astrocytes. Delivering drugs specifically to the tumor mass is considered as an effective means to increase the specific uptake by cancer cells. To achieve this, both passive and active strategies have been employed.

A study that utilized non-targeting polyglutamate-paclitaxel (CT2103, Xyotax) has reached clinical trials for the treatments of brain cancer, where it was tested in combination with temozolomide and radiotherapy [143, 144]. As no targeting ligand was used in this case, passive diffusion is major pathway for drug accumulation in the brain tumor.

In terms of active targeting strategies, a recent study has shown that CD44 receptor is highly expressed in GBM cell lines and primary glioma cells [145]. Grafting lipid-based nanoparticles (LNPs) with hyaluronan (HA), which specifically target CD44 receptor significantly increase the binding of LNPs to these GBM cell lines and primary glioma cells. When HA-grated LNPs were complexed with therapeutic siRNA, this increase in binding efficiency could translate into an effective glioma cell death (90%) *in vitro*, and 60% survival at day 95 post-tumor inoculation (the median survival of Mock-treated mice was 33 days) after 4 systemic doses treatment *in vivo*.

In many cases, both endothelial cells at the blood-tumor brain barrier (BBTB) and the glioma cells overexpress similar types of receptors (such as LPR1), ligands that actively target the BBTB often possess intrinsic targeting to glioma cells as well [70, 72, 73]. This included ANG1005 (now in clinical trial), a paclitaxel-angiopep conjugate where angiopep was a peptide designed to target LPR1 expressed on both BBTB and glioma cells [76-80]. However, it is worth noting that even when targeting ligands were incorporated, the total amount of injected dose that is delivered to the brain was only ~1% or less [144].

Recently, some other studies suggested a new strategy for the treatment of glioma by using polymer nanoparticle-delivered gene therapy [146, 147]. Although the polymer was not conjugated with any targeting ligand, but the possibility to recombine the building blocks of nanoparticle made it possible to select a combination with highest targeting and transfection efficiency.

4. Translation of CNS cell-targeting nanomedicine to the clinics

In the previous sections, we have discussed several novel strategies that have been used in recent years to enhance the cell-specific targeting of nanomedicine for different CNS diseases. It should be noted that most of these studies are still in pre-clinical stage. Drugs for CNS disorders are less than one-half as likely to achieve marketing approval than other drugs, and it also takes more than a year longer for the development [November/December Tufts CSDD Impact Report]. In addition to the widely-acknowledged barriers such as the BBB [148] and the extracellular space of brain parenchyma (both known for preventing nanoparticles from reaching the targeting site), [149] there are a few additional factors that need to be considered during the translation of cell-targeting nanomedicine.

In most studies, targeting effects are tightly associated with disease pathologies in the tested models. Therefore, it is crucial to select a clinically relevant disease model for the development and evaluation of nanomedicine for translational purposes. For instance, to develop a therapy that targets the endothelial cells for the treatment of glioblastoma, an orthotopic brain tumor model is a better representative of the tumor microenvironment than a subcutaneous tumor model. To develop therapies that are based on active target mechanisms, it will be essential to use cell lines that possess similar expression to the target cells in the patients as opposed to those do not carry similar patterns.

Secondly, many studies have reported that less than 1% of total injected dose eventually reach in the brain by intravenous administration [150]. Though it could be of interest to enhance the overall brain uptake of therapeutics by using other convective administration routes (such as intracerebral or intraventricular injection), even less than 1% of total systematic-injected dose in the targeted cells could provide significant effects if a majority of the drug accumulation is contributed by the uptake in targeted cells [104]. Therefore, maintaining a high targeting efficiency to the key cells can also greatly enhance the therapeutic efficacy.

Lastly, the toxicity of nanomedicine should be addressed. It is undoubtedly crucial to select a drug-delivering vehicle that is intrinsically safe at the therapeutic relevant dose for the

clinical translation. Many drug delivery platforms were discovered to cause CNS toxicity. For example, intra-nasally administrated generation 5 *cationic* PAMAM dendrimers can modulate gene expression of brain-delivered neurotrophic pathway at a dose of 15 µg per mouse [151]. This dendrimer can also cause neurotoxicity by inducing autophagic flux and upregulation of ROS in the glioma cell lines in a concentration dependent manner (concentration ranging from 0 to 100 µg/mL) [152]. Similarly, many non-biodegradable vehicles such as carbon nanotubes and gold NPs can also cause immunogenicity in the brain: a single Intracortical dose (500 ng/mouse) of multi-walled carbon nanotubes was found to cause glial activation in the mouse brain at 30 days post injection [153]; intranasal administration of rod shape gold nanoparticles (5 µL at a concentration of 10⁹ particle/mL) could induce biphasic activation of microglia at one day and a week post administration [103] The toxic properties of drug delivery vehicles need to be cautiously scrutinized in clinically relevant animal models before being translated into clinical study stage.

5. Conclusions

Nanomedicine offers significant opportunities to enhance drug delivery across the BBB, and for targeting specific cells. These advancements have shown various possibilities for effective treatments of many CNS disorders. The improved understanding of the pathologies of CNS disorders, from the cellular and molecular levels in recent years, has been embraced when it comes to design smart nanomaterials. Developing nanotherapies that could target the pathological cells in the CNS has attracted significant recent interest, and has led to promising results in animal models. In this review, we have systematically introduced the functions of several CNS cell types: endothelial cells, microglia/macrophages, TAMs, neurons, astrocytes, brain tumor cells, and their pathological roles in CNS disorders. We then connected the recent studies utilizing nanomedicine with targeting properties in accordance with the pathologies in these cells, and the key strategies being considered for engineering an appropriate delivery platform. This provides the general guidelines for designing of cell-specific targeting nanotherapies for CNS disorders.

Abbreviations

ABC	ATP-binding cassette
AD	Alzheimer's disease
ALS	amyotrophic lateral sclerosis
BBB	blood brain barrier
BBTB	blood brain tumor barrier
CCL	chemokine ligand
CNS	central nervous system
dPGS	dendritic polyglycerol sulfate
GFAP	glial fibrillary acidic protein

IL	interleukin
LFR	lactoferrin
LPS	lipopolysaccharides
MMP	matrix metalloproteinase
MS	multiple sclerosis
MTFR	melanotransferrin receptor
NO	nitro oxide
PAMAM	polyamidoamine
PD	Parkinson's disease
ROS	reactive oxygen species
TAM	tumor-associated microglia/macrophage
TfR	transferrin receptor
TNF	tumor necrosis factor
VCAM	vascular adhesion molecule

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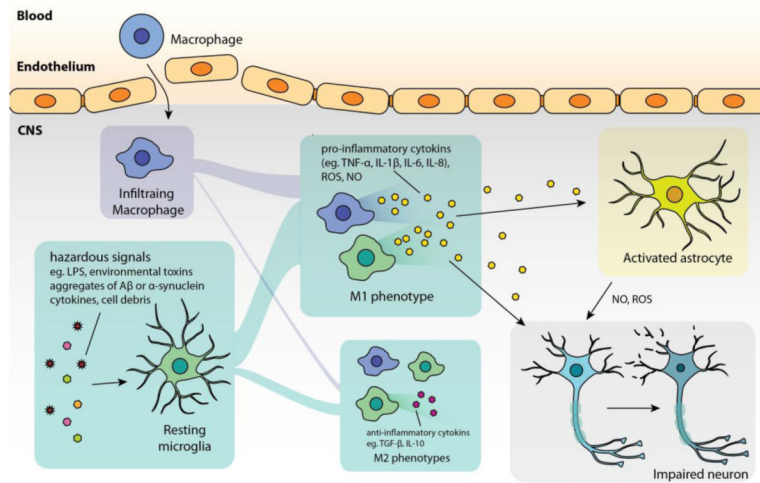


Figure 1. Common features in neuroinflammation

In general, microglial cells are initially activated by hazardous signals, such as damage-associated molecular pattern molecules and cell debris from damaged cells in acute inflammation, aggregates of A β in Alzheimer's disease, α -synuclein aggregates from abnormal dopaminergic neurons in Parkinson's disease, SOD1 aggregates from diseased neuron in amyotrophic lateral sclerosis. In such pathologies, microglia (together with infiltrating macrophages) tend to polarize to M1 phenotype that amplifies neuroinflammation by producing pro-inflammatory cytokines (TNF- α , IL-1 β , IL-6) as well as ROS and NO which lead to neuronal injury. Some pro-inflammatory cytokines (such as TNF- α , IL-1 β) can also activate astrocytes to induce over-production of ROS and NO that exacerbate the injury. The impaired neurons are then susceptible to undergo apoptosis or necrosis, which, in turn, release molecules that further activate microglia.

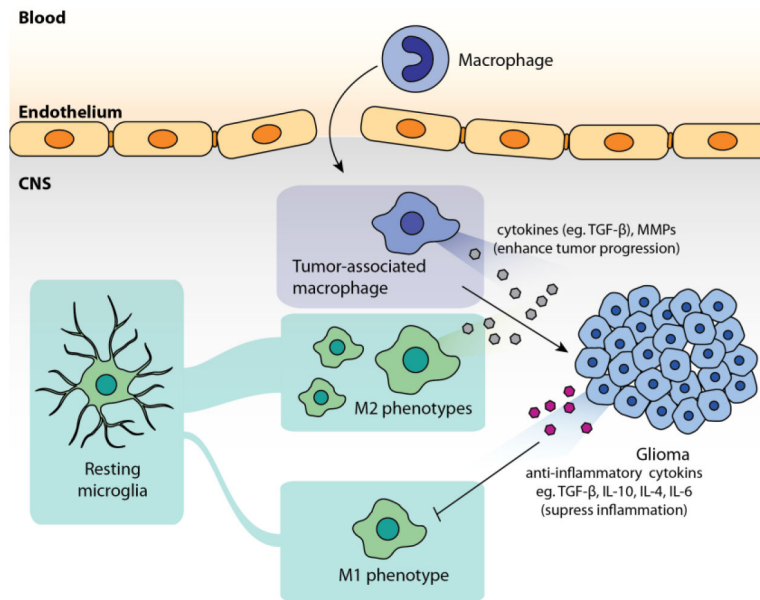


Figure 2. Role of tumor-associated microglia/macrophages and tumor cells in tumor microenvironment

Glioma cells generally create an environment that suppresses classic inflammation (or, the functions of M1 phenotypes of microglia/infiltrating macrophage) by cytokines such as TGF-β, IL-10, IL-4, IL-6.

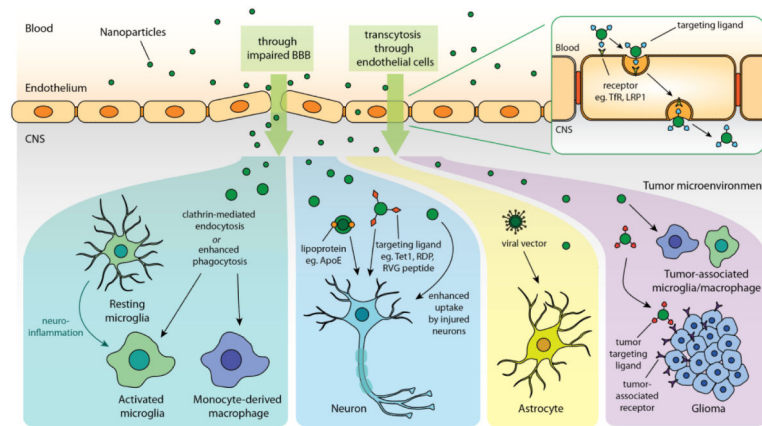


Figure 3. Schematic representation of the strategies for targeted drug delivery to different cell phenotypes in CNS

First, nanomedicine can either enter CNS through impaired BBB or cross BBB endothelium via receptor-mediated transcytosis when the drug/cargo circulates systemically. Nanomedicine can potentially be uptake by activated microglia, infiltrating macrophage, or tumor-associated microglia/macrophage through clathrin-mediated endocytosis or phagocytosis. The drug/cargo can also possess cell-targeting properties when a targeting moiety is incorporated. Vesicles with lipoprotein ApoE or nanoparticles with ligands (eg. Tet1, RDP, RVG peptide) can be particularly uptake by neurons. Viral vector homing to astrocyte could be an effective cargo to deliver therapeutics. Nanoparticles functionalized with tumor targeting ligand can also be introduced to target glioma with enhanced accumulation.

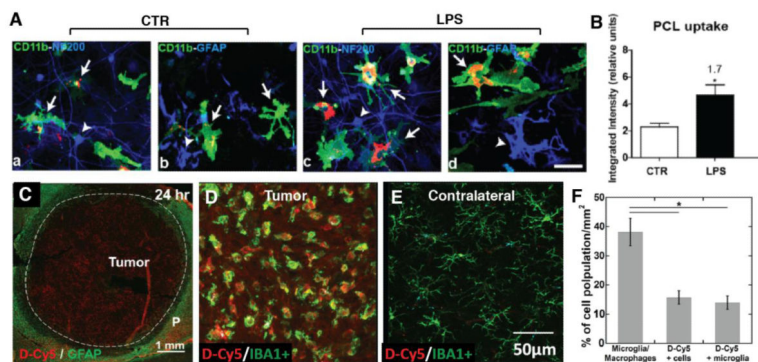


Figure 4. Specific uptake of nanoparticles by microglial cells and tumor-associated microglia/macrophage

(A) Representative confocal image of PCL nanoparticles uptake by control (non-activated) microglia LPS stimulated (activated) microglia *in vitro*, (B) Semi-quantification based on integrated fluorescence intensity demonstrated higher 1.7-fold higher uptake of particles by LPS stimulated microglia than control microglia. Reprinted (adapted) with permission from Ref [92]. Copyright (2015) American Chemical Society. (C–F) Distribution of fluorescently labeled generation 4 hydroxyl terminated PAMAM dendrimers (D-Cy5) in a rat brain bearing 9L glioblastoma. D-Cy5 was administered systemically at day 9 after tumor inoculation. Confocal images of tumor region suggested that D-Cy5 (~4 nm in size) uniformly distributed across the 6 mm tumor after 24 hr post administration. (C), and was co-localized with Iba1+ TAM in tumor but not in the 'resting' microglia in the contralateral hemisphere of the same rat brain after 4 hr of D-Cy5 administration (D and E). Imaging analysis showed almost all D-Cy5+ cells were Iba1+ cells, indicating the high efficiency of TAMs taking up dendrimers (F) [101]. Green: Iba1+ microglia/macrophages; red: D-Cy5; blue: DAPI; bar: 100 μm. Reprinted from Biomaterials, Vol 52, F Zhang, *et al.*, Uniform brain tumor distribution and tumor associated macrophage targeting of systemically administered dendrimers, 507-516, Copyright (2015), with permission from Elsevier.

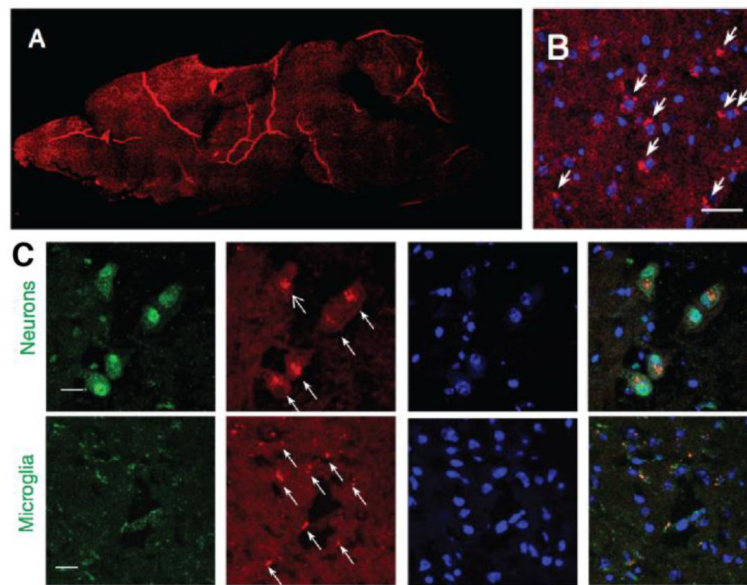


Figure 5. Exosomes as vehicles for CNS drug delivery that targets neurons and microglia (A–B) Distribution of fluorescence labeled exosomes (DIL-exosomes) in a mouse brain at 4 hr after the dose was given. The exosomes were administered intranasally to mice with 6-hydroxydopamine-induced neuroinflammation. Red: DIL-exosomes; blue: DAPI; bar: 40 μ m (C) Exosomes were localized mostly in neurons and microglia in this mouse model of neuroinflammation according to confocal microscopy [123]. Green: neuron/CD11b+ microglia; red: DIL-exosomes; blue: DAPI; bar: 10 μ m. Reprinted from *Journal of Controlled Release*, Vol 207, MJ Haney, *et al.*, Exosomes as drug delivery vehicles for Parkinson's disease therapy, 18-30, Copyright (2015), with permission from Elsevier.

Table 1

A summary of endothelial cell-targeting platforms reported in recent studies.

Targeted cells	Platform	Targeting ligand	Route of administration	Delivered therapeutics?	Model of evaluation/Application	Effects on targeted cells
Activated endothelial cells	Iron oxide microparticles [63]	Vascular cell adhesion molecule-1 binding peptide	Intravenous	No	Mouse model of cerebral ischemia	Imaging endothelial cell activation
Activated endothelial cells	Iron oxide microparticles [64]	Vascular cell adhesion molecule-1 binding peptide	Intravenous	No	Mouse model of acute brain inflammation, vascular dementia, Alzheimer's disease (AD) and multiple sclerosis	Imaging endothelial cell activation
Activated endothelial cells	Iron oxide microparticles [65]	Vascular cell adhesion molecule-1 binding peptide	Intravenous	No	Mouse model of Stroke	Imaging endothelial cell activation
Endothelial cells	Poly(butylcyanoacrylate) nanoparticles (PBCN) [66]	Polysorbate 80	Intravenous	No	Healthy mouse, mouse model of AD	Enhance BBB interaction
Endothelial cells	Poly(butylcyanoacrylate) nanoparticles (PBCN) [67]	Polysorbate 80	Intravenous	Puerarin	Rat model of ischemia reperfusion	Enhance BBB interaction
Endothelial cells	Monovalent molecular shuttle (modified anti-TTR monoclonal antibody) [68-70]	Anti-TTR monoclonal antibody	Intravenous	Antibody	Mouse model of Alzheimer disease	Enhance BBB penetration
Endothelial cells (BBTB)	Liposome [71]	Cyclic Arg-Gly-Asp (RGD) peptide	Intravenous	No	Mouse model of C6 glioma	Enhance BBB penetration
Endothelial cells (BBTB)	Liposome [72]	Folate and transferrin (TF)	Intravenous	Doxorubicin	Rat model of C6 glioma	Enhance BBB penetration
Endothelial cells (BBTB)	Liposome [73]	Cell penetration peptide TAT and transferrin (TF)	Intravenous	Doxorubicin	Mouse model of glioma	Enhance BBB penetration
Endothelial cells	Chitosan nanoparticle [74]	Transferrin (TF)	Intravenous	Peptide	Mouse model of stroke	Enhance BBB penetration
Endothelial cells	Angiopep2-neurotensin conjugates [75]	Angiopep-2	Intravenous	Neurotensin	Rat model of pain	Enhance BBB penetration

Targeted cells	Platform	Targeting ligand	Route of administration	Delivered therapeutics?	Model of evaluation/Application	Effects on targeted cells
Endothelial cells (BBTB)	Paclitaxel–Angiopep-2 peptide drug conjugate (GRN1005) [76]	Angiopep-2	Intravenous	Paclitaxel	Glioma patients (Phase I)	Enhance BBB penetration
Endothelial cells (BBTB)	Paclitaxel–Angiopep-2 peptide drug conjugate (GRN1005) [77]	Angiopep-2	Intravenous	Paclitaxel	Mouse human glioma cell xerograph model	Enhance BBB penetration
Endothelial cells (BBTB)	Paclitaxel–Angiopep-2 peptide drug conjugate (GRN1005) [78]	Angiopep-2	Intravenous	Paclitaxel	Mouse orthotopic glioma model	Enhance BBB penetration
Endothelial cells (BBTB)	Paclitaxel–Angiopep-2 peptide drug conjugate (GRN1005) [79]	Angiopep-2	Intravenous	Paclitaxel	Mouse model of brain metastases of breast cancer	Enhance BBB penetration
Endothelial cells (BBTB)	Doxorubicin–Angiopep-2 peptide drug conjugate (ANG1007 and ANG1009) [80]	Angiopep-2	Intravenous	Doxorubicin	Mouse model of glioma	Enhance BBB penetration
Endothelial cells	Fused protein [81]	Monoclonal antibody (mAb) against human insulin receptor (HIRMAb)	Intravenous	Iduronate 2-sulfatase (IDS)	Rhesus monkey	Enhance BBB penetration
Endothelial cells	Gold nanoparticles [82]	Insulin	Intravenous	No	Healthy mouse	Enhance BBB penetration
Endothelial cells (BBTB)	Trail [83]	Choline derivate	Intravenous	Trail	Mouse model of glioma	Enhance BBB penetration
Endothelial cells (BBB)	PEG-dendrigrift poly-l-lysine (DGL)/DNA nanoparticles [83]	Dermorphin	Intravenous	DNA	Cerebral ischemia reperfusion injury	Enhance BBB penetration

For each platform, targeting ligand; delivered therapeutics; model of evaluation/application and effects on targeted cells are described.

Table 2

A summary of microglia/macrophage-targeting platforms reported in recent studies.

Targeted cells	Platform	Route of administration	Delivered therapeutics?	Model of evaluation/Application	Effects on targeted cells
Microglia	Quantum dots (QD) [89]	Intracranial	No	Mixed primary glia culture, healthy mouse	N/A
Microglia	Gold Nanoparticle [103]	Intranasal	No	Mixed primary glia culture, healthy mouse	N/A
Microglia, Astrocytes, Oligodendrocytes	Hydroxyl terminated G4 PAMAM Dendrimer [90]	Intravenous	N-acetyl cysteine (NAC)	Mouse model of ischemia-induced neonatal white matter injury	Control of microglial/macrophage activation and inflammatory cytokine release
Microglia, Astrocytes	Hydroxyl terminated G4 PAMAM Dendrimer [104]	Intravenous	N-acetyl cysteine (NAC)	Rabbit model of Cerebral Palsy	Control of microglial/macrophage activation and inflammatory cytokine release
Microglia	Liposome [91]	Intravenous	Inhibitor of macrophage migration inhibitory factor (MIF)/Chicago sky blue	Rat model of Spinal Cord injury	Control of microglial/macrophage activation and inflammatory cytokine release
Microglia	Poly-ε-caprolactone (PCL) nanoparticles [92]	Intraspinal	Minocycline	Mouse model of Spinal Cord injury	Control of microglial/macrophage activation and inflammatory cytokine release
Microglia, Astrocytes, Oligodendrocytes	Carboxymethylchitosan/polyamidoamine (CMChit/PAMAM) dendrimer [93]	-	Methylprednisolone	Mixed primary glia culture	N/A
Microglia	PLGA nanoparticles [94]	Intraspinal	Methylprednisolone	Rat model of Spinal Cord injury	Reduce activated microglia/macrophage population
Microglia	poly(methyl methacrylate) (PMMA) nanoparticles [95]	Intraspinal	No	Mouse model Spinal Cord injury	N/A
Microglia	Carbon Nanotube (CNT) [105]	-	Dexamethasone	in vitro microglia cell culture	Control of microglial/macrophage activation and inflammatory cytokine release
Microglia	Iron oxide nanoparticle [106]	-	No	in vitro microglia cell culture	Control of microglial/macrophage activation and inflammatory cytokine release

Targeted cells	Platform	Route of administration	Delivered therapeutics?	Model of evaluation/Application	Effects on targeted cells
Circulating macrophage	Mannose modified liposome [96, 97]	Intravenous	Dichloromethylene diphosphonate (Cl ₂ MDP)	Rat model of Experimental Allergic Encephalomyelitis (EAE)	Control infiltrating macrophages
M2 Macrophage	Nanozyme [98]	Intravenous	Catalase	Mouse model of Parkinson's disease	Attenuates oxidative stress
Tumor associated macrophage	Gold nanoshell [100, 107]	-	Photothermal therapy	in vitro microglia cell culture in vitro glioma spheroids culture	Control of microglia/macrophage population
Tumor associated macrophage	Hydroxyl terminated G4 PAMAM Dendrimer [101]	Intravenous	No	Rat model of Glioblastoma (GBM)	N/A
Tumor associated macrophage	Carbon Nanotube (CNT) [108, 109]	Intratumoral	CpG oligodeoxynucleotides	Mouse model of GBM	Increase infiltration of anti-tumor inflammatory cells
Microglia	Iron oxide nanoparticle [110]	-	CpG oligodeoxynucleotides	in vitro microglia cell culture	Control microglia migration
Microglia	G4 PAMAM Dendrimer hydroxyl terminated (G4-OH) and amine terminated (G4-NH ₂) [111]	-	Celastrol	in vitro microglia cell culture	Control of microglial/macrophage activation and inflammatory cytokine release
Microglia	Carboxymethyl dextran-block-poly(ethylene glycol) [112]	-	Minocycline	in vitro microglia cell culture	Control of microglial/macrophage activation and inflammatory cytokine release
Microglia	Multivalent niacin nanocoujugates [113]	-	No	in vitro microglia cell culture	Target lipid droplet in microglia
Microglia	Dendritic polyglycerol sulfate (dPGS) [114]	-	No	in vitro microglia cell culture and ex vivo hippocampus slice culture	Control of microglial/macrophage activation and inflammatory cytokine release
Microglia	Quantum Dots (QD) [115]	-	No	in vitro microglia cell culture	Study inflammation in molecular level
Macrophage	Phosphorus Dendrimer [116]	-	No	in vitro macrophage cell culture	Imaging macrophage polarization

For each platform, delivered therapeutics; model of evaluation/application and effects on targeted cells are described.

Table 3

A summary of neuron-targeting platforms reported in recent studies.

Targeted cells	Platform	Targeting ligand	Route of administration	Delivered therapeutics?	Model of evaluation/Application	Effects on targeted cells
Neuron	Magnetic nanoparticles [131]	N/A	Intracranial	No	Healthy mouse	Neuron stimulation
Neuron	Gold nanoparticles [132]	Ts1 neurotoxin based synthetic molecule	-	No	ex vivo hippocampus slice	Neuron stimulation
Neuron	Cationic block copolymer [124]	Tet-1	-	Plasmid DNA	in vitro neuronal cell culture	Transfection
Neural progenitor cells	PEGylated PEI nanoparticles [125]	Tet-1	Intraventricular	Plasmid DNA	Healthy mouse	Transfection
Neuron	HPMA-co-oligolysine copolymer nanoparticles [85]	Tet-1	-	Plasmid DNA	in vitro neuronal cell culture	Transfection
Neuron	RVG-siRNA conjugate [127]	RVG	Intravenous	siRNA	Mouse model of encephalitis	Silence pathological gene
Neuron	Endosome [128]	RVG	Intravenous	siRNA	Healthy mouse	RNA and protein knockdown
Neuron	Gold nanoparticles [126]	RDG	Intravenous	No	Healthy mouse	N/A
Neuron	Organically modified silica nanoparticles [133]	N/A	-	No	Healthy larvae	N/A
Injured neuron	Hydroxyl terminated G4 PAMAM Dendrimer [134]	N/A	Intravenous, Intraventricular	N-acetyl cysteine (NAC); Valproic acid (VPA)	Canine model of hypothermic circulatory arrest (HCA)	Neuroprotection
Neuron	Silica nanoparticles [126]	Dopamine	-	No	in vitro neuronal cell culture	N/A
Neurons, Microglia, Endothelial cells	Exosome [123]	N/A	Intranasal	No	Mouse model of Parkinson's disease	N/A
Neuron	Lipid nanoparticles [122]	N/A	Intracortical, Intraventricular	siRNA	Healthy rat	N/A

For each platform, targeting ligand; delivered therapeutics; model of evaluation/application and effects on targeted cells are described.

Table 4

A summary of astrocytes-targeting platforms reported in recent studies.

Targeted cells	Platform	Route of administration	Delivered therapeutics?	Model of evaluation/Application	Effects on targeted cells
Astrocytes	Lentiviral Vector [135]	Intracranial	Gene	Healthy mouse	Transfection
Astrocytes	Lentiviral Vector [136]	Intracranial	Gene	Healthy rat	Transfection
Astrocytes	Adeno-Associated Virus (AAV) [138]	Intracranial	Astrocyte-specific Gfa2 promoter	Mouse Model of Alzheimer's Disease	Reduced glia activation
Astrocytes	Iron oxide, silver nanoparticles [139]	-	No	in vitro	N/A
Microglia, Astrocytes, Oligodendrocytes	Carboxymethylchitosan/polyamidoamine (CMCh/PAMAM) dendrimer [140]	-	No	in vitro	N/A
Astrocytes, neurons	PLGA nanoparticles [141]	Intraspinal	Neurotrophic factor (GDNF)	Spinal cord injury	N/A
Astrocytes, microglia	Hydroxyl terminated G4 PAMAM Dendrimer [104]	Intravenous	NAC	CP	Reduced glia activation
Astrocytes, Oligodendrocytes	Hydroxyl terminated G4 PAMAM Dendrimer [90]	Intravenous	NAC	Mouse model of Ischemia-induced neonatal white matter injury	N/A

For each platform; delivered therapeutics; model of evaluation/application and effects on targeted cells are described.