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Passive immunotherapies for central nervous system disorders – current delivery challenges and new approaches.

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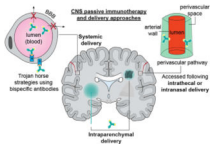
Abstract

Passive immunotherapy, i.e., the administration of exogenous antibodies that recognize a specific target antigen, has gained significant momentum as a potential treatment strategy for several central nervous system (CNS) disorders, including Alzheimer's disease, Parkinson's disease, Huntington's disease, and brain cancer, among others. Advances in antibody engineering to create therapeutic antibody fragments or antibody conjugates have introduced new strategies that may also be applied to treat CNS disorders. However, drug delivery to the CNS for antibodies and other macromolecules has thus far proven challenging, due in large part to the blood-brain barrier and blood-cerebrospinal fluid barriers that greatly restrict transport of peripherally administered molecules from the systemic circulation into the CNS. Here, we summarize the various passive immunotherapy approaches under study for the treatment of CNS disorders, with a primary focus on disease-specific and target site-specific challenges to drug delivery and new, cutting edge methods.

Graphical Abstract



INTRODUCTION

Antibodies are a class of serum glycoproteins called immunoglobulins (Igs) that facilitate the adaptive humoral immune response in vertebrates [1]. Immunoglobulin G (IgG; ~150 kDa)

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is the most abundant serum isotype and consists of a crystallizable fragment (Fc; ~ 50 kDa) that binds to Fc receptors and elicits immune effector functions [1] and two antigen-binding fragments (Fab; ~ 50 kDa each), both of which contain a variable region capable of recognizing a highly specific target antigen (Figure.1). The administration of antibodies to target disease-specific antigens, also referred to as 'passive immunotherapy', has steadily gained momentum since César Milstein and Georges Köhler's seminal discovery demonstrating the production of monoclonal antibodies using hybridomas [2]. Indeed, antibody-based therapeutics have emerged as one of the fastest growing class of drugs [3] due to their high target specificity and capacity to be customized [4]. Therapeutic antibodies can be full-length antibodies (e.g., IgG), which have a long half-life due to Fc binding to the Brambell receptor/ neonatal Fc receptor (FcRn) [5] and elicit effector functions by interacting with Fcγ receptors [6–9], or antibody fragments such as Fab or single domain antibodies (sdAbs) which are useful when long half-lives and effector functions are not needed [10]. Additionally, antibody fragments are smaller and may penetrate physiological barriers better, as well as recognize more inaccessible antigen epitopes [10]. The ability to engineer antibody fusion proteins, bispecific antibodies, and antibody-drug conjugates has further expanded the use of therapeutic antibodies [4,10,11].

The massive burden placed on the healthcare system due to the increasing incidence of central nervous system (CNS) disorders and the paucity of disease-modifying drugs for these disorders underscores the need for better therapies [12]. Antibodies have many promising applications in the treatment CNS disorders; they may elicit disease-modifying effects for neurodegenerative diseases by interfering with the aggregation of abnormal proteins and aiding their clearance, or they may have cytotoxic effects on tumor cells and be used in the treatment of brain cancers. However, therapeutic antibodies are large proteins, making their delivery to the CNS difficult due to the restrictive properties of the blood-brain barrier (BBB) [13] and blood-cerebrospinal fluid barriers (BCSFBs) [14,15]. In this review, we discuss the application of antibody-based therapeutics for the treatment of several CNS disorders in the context of disease-specific pathology as well as strategies for their successful delivery to the brain and spinal cord.

BRAIN CANCER

There are several types of cancers that occur within the CNS and they may be classified based on their site of origin (primary or metastatic), the cell type they are derived from (e.g., astrocyte, neuron, meningeal cell, etc.), their level of malignancy, and the CNS region they affect. Primary and metastatic brain tumors may have adverse effects due to several reasons: increased mass causing a rise in intracranial pressure [16], physical encroachment on normal brain areas, and necrosis in tumors which may cause inflammation and cognitive decline due to neuronal cell death. Brain metastases from peripheral cancers are the most common type of intracranial tumors and typically arise from non-small cell lung cancer, breast cancer, or melanoma [17]. Brain metastases are associated with a poor (8%) 2-year survival rate [18] and a median survival time of 4–12 months [19], with few treatment options thus far [17]. Primary brain cancers originate from abnormal cells within the brain. The most common type of primary brain cancer is the glioma, which as the name suggests originates from glial cells. Gliomas cause the second highest level of morbidity in individuals under 15 and the fourth

highest level of morbidity in individuals between 35 and 54 [20] and account for over 60% of primary neoplasms [21]. The prognosis for the most malignant form referred to as glioblastoma multiforme (GBM) continues to be poor, with most patients dying within a year of the initial diagnosis [20]. The median survival time for GBM patients following diagnosis is 14.6 months and the 5-year survival rate is 9.8 % [22–24]. Gliomas and brain metastases are typically diagnosed by neuroimaging in patients who present with symptoms such as chronic headaches, onset of seizures, nausea and vomiting, neurological deficits, and signs of increased intracranial pressure [18,25]. Despite advances in new cancer therapeutics, the typical standard of care consists of surgical resection (when possible) followed by a combination of radio and chemotherapy with temozolomide (TMZ), which has limited benefit [23].

Passive immunotherapies for brain cancers.

Passive immunotherapies have emerged as a promising class of therapeutics for the treatment of brain cancers and can overcome several challenges specific to this pathology. First, there is significant heterogeneity in brain tumors observed across individuals, between different cells within a tumor, as well as at different stages of tumor growth [26,27]. This heterogeneity underlies the need for therapies that are combinatorial and can be tailored to a specific antigen profile at different stages of brain cancer in a patient and across patients. Passive immunotherapy lends itself well to this purpose since antibody-based therapeutics have the ability to be highly selective in recognizing tumor-specific or relevant anti-tumor immunomodulatory antigens that can be targeted to either directly inhibit tumor growth or selectively target a cytotoxic payload of chemo or radiotherapy to kill tumor cells. Another challenge in the treatment of brain cancers is their aggressive growth. For example, gliomas often cannot be fully surgically resected due to their infiltrative and diffuse spread [28]. Surgical resection is also far more challenging in the case of many pediatric glioma patients since the tumors are often in non-hemispheric regions such as the brainstem [29]. Additionally, it is often challenging to strike a balance between the efficacy, pharmacokinetic characteristics, and safety profile for small molecule therapeutics, putting them at a disadvantage compared to highly specific and potent antibody-based therapies [30]. Overall, passive immunotherapies have many potential advantages for the treatment of brain cancers. To facilitate our discussion of antibodies investigated as potential therapies for brain cancer, we will describe them in the context of five categories based on their targets and modes of action: (i) anti-angiogenic antibodies, (ii) checkpoint inhibitors, (iii) lymphocyte target, (iv) antibody drug conjugates, and (v) metastatic brain tumor target.

Anti-angiogenic antibodies: The strategy to use anti-angiogenic agents as anti-cancer therapies was founded based on the correlation between pathological angiogenesis and tumorigenesis, first established by Judah Folkman over 40 years ago [31,32]; it is summarized schematically in Figure 2. Folkman's findings spurred the eventual isolation of the pro-angiogenic vascular endothelial growth factor (VEGF) [33–35]. $VEGF_{165}$ or VEGFA is the most physiologically relevant isoform and may get cleaved by plasmin or matrix metalloproteinase-9 (MMP-9) to release bioactive fragments that promote angiogenesis. In January 1997, Genentech filed an Investigational New Drug application and initiated clinical trials for bevacizumab (commercial name – Avastin) – a humanized monoclonal

recombinant antibody that binds to all VEGFA isoforms and their bioactive fragments with high affinity and specificity, inhibiting their interaction with VEGFRs, and thus suppressing VEGF signaling [30]. The US Food and Drug Administration (FDA) approved the use of bevacizumab first for the treatment of colorectal cancer in 2004 and later expanded the range of approved oncology indications to include the treatment of lung, breast, brain, cervical, and ovarian cancer over the next decade in keeping with new clinical trial data. Since GBM is associated with significant necrosis and high VEGF mRNA expression within clusters of necrotic tumor cells [36], it was hoped that 'anti-angiogenesis' therapies might offer a powerful treatment strategy for gliomas, which demonstrate the highest degree of angiogenesis of all human neoplasms [37,38].

Initial Phase 2 clinical studies investigating systemically administered bevacizumab monotherapy or combinatorial therapies for recurrent glioblastoma demonstrated a reduced radiological contrast enhancement and increase in progression free survival (PFS) with bevacizumab [39–43]. The FDA subsequently provided accelerated approval for systemically administered bevacizumab as a monotherapy to treat patients with recurrent GBMs that had progressed following initial treatment with chemotherapy and radiation [44]. However, the benefit of systemically administered bevacizumab for the treatment of recurrent glioblastoma as a monotherapy or in combination with radiotherapies and chemotherapies remains controversial [39]. Results from initial phase 2 clinical studies [39–43] must be interpreted with care and have several caveats such as small sample sizes, insufficient controls, instances of poor correlation between radiological contrast enhancement and anti-tumor effects, and no significant indication of increased overall survival [39–43]. The more recent bevacizumab and lomustine for recurrent GBM (BELOB) clinical trial was a randomized controlled multicenter phase 2 study that included three treatment arms receiving bevacizumab monotherapy, lomustine monotherapy, or bevacizumab in combination with lomustine. By including a treatment group that did not receive bevacizumab the BELOB trial provided the first objective phase 2 clinical assessment of bevacizumab monotherapy versus chemotherapy alone or in combination with bevacizumab [39,45]. The primary outcome of overall survival at 9 months was lowest in the group receiving bevacizumab alone and did not justify further clinical study for systemically administered bevacizumab monotherapy for recurrent GBM. Additionally, initial clinical investigation in a randomized controlled trial also demonstrated that systemically administered bevacizumab provided no benefit for newly diagnosed glioblastoma [46]. The poor clinical outcomes of systemically administered bevacizumab for GBM may be attributed in some part to insufficient delivery to the brain target site. The elevated production of VEGF by tumor cells [47] and the occurrence of VEGF/VEGFRs on both luminal and abluminal sides of tumor vasculature underscores the importance of successful delivery of anti-angiogenic therapies to the brain tumor and migrating tumor cells by overcoming or circumventing the blood-tumor barrier (BTB) and BBB [48] Furthermore, many of the adverse side-effects of bevacizumab treatment, e.g., hypertension, fatigue, headache, hemorrhage, and thromboembolic events [49], may in fact be a consequence of off-target anti-angiogenic effects at non-tumor sites [50]. Thus, drug delivery strategies that minimize exposure to non-tumor sites will prove beneficial.

Checkpoint inhibitors: To ensure specific targeting of abnormal or pathogenic entities versus normal host tissue, the immune system relies on the recognition of molecular checkpoints to make go/no-go decisions. Cancer cells have the ability to modulate these molecular checkpoints and thus escape attack from the immune system. Therefore, checkpoint inhibitors that may be antibodies or small molecules have emerged as a promising strategy to prevent checkpoint modulation by cancer cells and thus improve anti-tumor immune responses.

For example, although cancer cells often express antigens that can be recognized by T cells of the host immune system, they often escape T cell mediated elimination. This is because the appropriate priming and accomplishment of T cell effector functions requires not only the engagement of the T cell receptor (TCR) by antigen peptides presented on the major histocompatibility complex (MHC) of antigen presenting cells (APCs) and tumor cells, but also activation of additional co-stimulatory signals and suppression of inhibitory signals (immune check points) expressed by APCs and tumor cells (Figure. 3) [52]. TCR engagement without the support of co-stimulatory signals results in a suppressed T cell immune responsive state referred to as ‘anergy’ [52]. Both co-stimulatory and inhibitory signals that influence T cell response occur in peripheral lymphoid organs as well as in the tumor microenvironment. Augmenting co-stimulatory signals and blocking inhibitory signals to increase anti-tumor T cell activity has thus emerged as a viable strategy for cancer therapy [52].

Checkpoint inhibitor immunotherapies for cancer currently include six FDA approved IgG antibodies that target CTLA-4 (ipilimumab), PD-1 (pembrolizumab, nivolumab), or PD-L1 (atezolimumab, avelumab, and durvalumab). Ipilimumab (commercial name – Yervoy; Bristol-Myers Squibb; approved in 2011) was the first checkpoint inhibitor immunotherapy approved by the FDA for the treatment of melanoma. Pembrolizumab (commercial name – Keytruda; Merck), nivolumab (commercial name – Opdivo; Bristol-Myers Squibb) were approved by the FDA in 2014 for advanced melanoma. Atezolimumab (commercial name – Tecentriq; Genentech) was approved by the FDA in 2016 for urothelial carcinoma and metastatic lung cancer. Avelumab (commercial name – Bavencio; Merck, Pfizer, & Eli Lilly) was approved in 2017 for urothelial carcinoma and metastatic merkel cell carcinoma). Durvalumab (commercial name – Imfinzi; Medimmune/Astrazeneca) was approved in 2017 for advanced bladder cancer and in 2018 for advanced non-small cell lung cancer.

The CD-28 receptor on T cells and its B7–1/B7–2 ligands expressed by APCs constitute an important co-stimulatory pathway that can increase anti-tumor T cell activity. Conversely, CTLA-4 (cytotoxic T lymphocyte antigen-4)[56], an inducible CD-28 homologue expressed by T cells, binds to B7–1/B7–2 ligands with a higher affinity than CD-28 [57] and initiates an inhibitory response that can suppress anti-tumor T cell activity [52]. CTLA-4 expression has been shown to be upregulated on anti-tumor T cells and in particular on an immunosuppressive T cell population called regulatory T cells or Tregs. Thus, blocking CTLA-4 with antibodies such as ipilimumab offers a potentially promising strategy to allow immune recognition of cancer cells (Figure. 4). Initial clinical investigation in a small cohort of glioblastoma patients testing ipilimumab in combination with bevacizumab showed that

the combination was well tolerated and was associated with positive radiographic responses over a 3 month period [58], possibly warranting further clinical examination.

The expression of a receptor called PD-1 (programmed cell death-1) on activated T cells [59] and its ligand PD-L1 (programmed cell death ligand-1) [60] on APCs constitutes an important inhibitory pathway that under normal physiological conditions plays an important role in preventing autoimmunity. However, the high expression of PD-L1 on several tumor cell types results in the PD-1/PD-L1 inhibitory pathway preventing an appropriate anti-tumor T cell response [61]. PD-L1 is highly expressed by GBM tumor cells, in particular at the tumor periphery, resulting in the formation of a "molecular shield" between the tumor boundary and host anti-tumor T cells [62] and is a promising target for passive immunotherapy (Figure. 4). Nivolumab and pembrolizumab are anti-PD-1 monoclonal antibodies that are currently being clinically investigated for the treatment of primary and metastatic brain cancers as monotherapies and in combination with radiation therapies, chemotherapies, or other immunotherapies [54,62–64]. It remains to be seen whether CNS access of antibodies acting as checkpoint inhibitors is needed and, if so, whether such access is sufficient to alter the course of primary as well as metastatic brain cancers. As checkpoint inhibitors have been expected to primarily act in the periphery on T cells, it has been suggested that CNS access may not be needed for effects [17]; however, the observation that brain metastases continue to occur with systemic application of these newer therapies and that extracranial responses are generally superior to intracranial responses suggests that CNS delivery may in fact be needed for more robust responses [17]. Indeed, clinical trials are under way in which checkpoint inhibitor immunotherapies are administered both systemically and intrathecally (e.g., nivolumab; [65]).

Lymphocyte target: Lymphocytes are not typically present in the central compartment (cerebrospinal fluid (CSF) and CNS tissue) in large numbers except in disease conditions (e.g., multiple sclerosis); however, T cells commonly perform a CNS immune surveillance function even in healthy individuals [66,67]. Although lymphocyte numbers in the CSF are very low under normal physiological conditions, recirculating lymphocytes have been shown to migrate into the CSF at levels similar to those observed in subcutaneous lymph [68]. Primary CNS lymphoma (PCNSL) is a rare form of non-Hodgkin lymphoma that occurs in the brain, leptomeninges, or eyes [69,70]. Median survival of PCNSL patients is 13 months with a 5 year survival rate less than 5 % [71]. Immunodeficiency is a major risk factor for PCNSL, with a high rate of incidence observed in patients infected with the human immunodeficiency virus (HIV) or organ transplant recipients [70,72,73]. PCNSL is thought to typically involve the malignant transformation of B cells within the brain microenvironment, although the precise biological details are still lacking [69]. Malignant lymphocytes from the periphery extravasate at the level of arterioles and venules to first enter and spread along enlarged perivascular spaces, and eventually move into the CNS parenchyma as the outer boundary of the perivascular space is compromised [74]. Once malignant lymphocytes enter the CNS they are not easily eradicated since the CNS is a relatively immune-privileged site. The adhesion molecule CD44 and its ligands likely play an important role in the extravasation of malignant lymphocytes; high CD44 expression is observed within PCNSL lesions in the white matter [74]. Due to their diffuse progression, surgical resection is not a

useful strategy for CNS lymphomas. High dose methotrexate (HD-MTX) chemotherapy for newly diagnosed PCNSL and whole brain radiotherapy (WBRT) for recurring PCNSL are the current standard of care [75]. Poor penetration of methotrexate through the BBB due to the presence of efflux transporters (methotrexate is a substrate for many such transporters, including p-glycoprotein and breast cancer resistance protein [76]) and toxicity associated with high doses of methotrexate pose additional challenges for this treatment strategy [77]. Passive immunotherapy approaches targeting abnormal lymphocytes in PCNSLs are currently being explored (Figure. 5). For example, systemic administration of rituximab, a chimeric murine monoclonal antibody that recognizes the B cell specific cell surface antigen CD20 [78], has been reported to elicit radiographic responses in 4 out of 12 patients in a small clinical study, and these may be synergistic when delivered in combination with chemotherapy [79]. Osmotic disruption of the BBB in combination with intra-arterial methotrexate has also been demonstrated to improve patient outcomes by improving chemotherapeutic delivery to the CNS [71,80].

Antibody drug conjugates: Antibody drug conjugates (ADCs) are targeted antibodies linked to anti-tumor cytotoxic moieties and have been successfully used in the treatment of peripheral solid tumors [81]. ADCs may allow tumor specific targeting of radio and chemotherapies, while reducing off-target side effects. However, the benefit of ADCs may be lost over chronic application if the expression of the targeted tumor antigen gets downregulated [25]. Most passive immunotherapies with naked (unconjugated) antibodies for brain cancers have thus far demonstrated limited success in improving overall survival (e.g., bevacizumab in GBM). There are two possible reasons for these disappointing outcomes – (i) unconjugated mAbs are not eliciting sufficient pharmacological efficacy at their target site, possibly due to downregulation of target antigens or other tumor compensatory mechanisms, and (ii) antibodies are not being delivered effectively to the target sites due to challenges posed by CNS barriers such as the BBB and the BCSFBs. Using ADCs as a therapy for brain cancers is a potential way to navigate the first pharmacological challenge since they provide an additional benefit of delivering an effective cytotoxic payload (Figure. 6). Several radioimmuno-conjugates are being investigated in clinical trials for the treatment of brain cancer. For example, ^{188}Re-nimotuzumab, a beta-emitting radioisotope of rhenium linked to an anti-epithelial growth factor receptor (EGFR) antibody is being investigated for the treatment of gliomas overexpressing EGFR [82]. ^{211}At-ch81C6, an alpha-emitting radioisotope of astatine linked to an anti-tenascin antibody and ^{131}I-BC2/BC4, a beta and gamma emitting radioisotope of iodine linked to an anti-tenascin antibody are being investigated for the treatment of GBM [83]. Tenascin C is an extracellular matrix protein whose expression is controlled by Notch signaling; in GBM tumor cells, aberrant notch signaling results in over-expression of Tenascin C resulting in increased tumor cell migration which aids the invasiveness of GBM tumors [84,85]. Bacterial toxins conjugated to proteins such as transferrin and interleukin-13 are being investigated in the treatment of high-grade gliomas. A similar strategy with bacterial toxins conjugated to targeted antibodies might also serve as a related promising strategy. These toxins include molecules such as the diphtheria toxin and the *Pseudomonas aeruginosa* exotoxin A, among others [81]. ABT-414 (Abbvie) – anti-EGFR antibody conjugated to the cytotoxin monomethyl auristatin F (MMAF) – an anti-mitotic agent that inhibits cell division – is currently under clinical evaluation for newly

diagnosed GBM with EGFR amplification [86]. AMG-595 (Amgen), an anti-EGFR antibody conjugated to the cytotoxin maytansinoid emtansine (DM1), is currently under clinical evaluation for newly diagnosed GBM with EGFR amplification; DM1 binds to the ends of microtubules and thereby destabilizes the cytoskeleton of tumor cells [87].

Metastatic brain tumor target: Primary tumors in the periphery can metastasize to the brain [17,88]. Metastatic brain cancers are as much as ten times more common than primary brain cancers, with brain metastases from lung (~50%), breast (~15–25%) and melanoma (~5–20%) being the most common [17,88,89]. Brain cancer metastases are often non-angiogenic tumors, i.e., the metastatic cancer cells co-opt the existing brain vasculature, which may make anti-angiogenic therapies less effective in treating these tumors [90]. Systemic treatment with trastuzumab, an anti-human epidermal growth factor receptor 2 (HER2) antibody (commercial name – Herceptin; Genentech/Roche) has been used to effectively treat extracranial breast cancer that overexpresses HER2 [91] (Figure. 7). However, systemic trastuzumab treatment also has a significant correlation to increased incidence of brain metastasis [92] and this correlation is most likely the consequence of trastuzumab not being effectively delivered to the metastatic brain tumors across the BTB and BBB [93]. Intrathecal administration of trastuzumab in breast cancer patients with leptomeningeal carcinomatosis has shown some promise warranting further investigation in a larger study [94,95]. Similar considerations hold true of passive immunotherapies for the treatment of other types of brain metastases as such as non-small cell lung cancer (treatment – nivolumab; anti-PD1 antibody; commercial name – Opdivo; Bristol-Myers Squibb) [96], and melanoma (treatment – ipilimumab; anti-CTLA-4 antibody; commercial name – Yervoy; Bristol-Myers Squibb) [97,98].

Current strategies and challenges in delivering passive immunotherapies to brain tumors.

Delivering passive immunotherapies to treat brain cancers is difficult. Both systemic and central delivery approaches used clinically face unique challenges in the treatment of brain cancers, emphasizing the need for new approaches and strategies for tumor drug delivery. Targeting passive immunotherapies to brain tumors via systemic delivery suffers the inherent drawback of having a large fraction of the administered dose being potentially lost to the rest of the body and is heavily dependent on the capacity of antibodies to not only cross the BTB but also areas of normal BBB that tumor cells may be hidden behind. Hydrophilic macromolecules like antibodies are thought to cross the walls of peripheral microvessels typically via passive movement across fenestrations and interendothelial clefts or via active receptor-mediated transcytosis [99]. In order of increasing permeability, brain tumor microvasculature may include non-fenestrated, continuous capillaries, which closely resemble those observed in normal brain tissue, fenestrated continuous capillaries, and fenestrated capillaries with interendothelial gaps as large as 1 μm [100,101]; importantly, BTB permeability in animal models of brain cancer has been shown to exhibit marked heterogeneity ranging from minimal to marked permeability that is not easily predictable [102]. Passive movement of large biologics like antibodies may only occur appreciably across capillaries with open fenestrations, large interendothelial gaps, or via transcytosis [103,104]. To harness the potential of receptor mediated transcytosis across the walls of tumor microvessels, bispecific antibodies that recognize both a transcytosis receptor at the BTB

and an anti-tumor antigen within the brain tumor may be used [105]. However, transcytosis receptors at the BTB may also be expressed elsewhere within the body, which increases the possibility of off-target side effects [106,107]. Therapeutic antibodies designed to exploit receptor-mediated transcytosis at the BTB for transport into the tumor may also face the challenge of having to compete with the endogenous ligand of the receptor [100]. Typically, microvessel permeability within the tumor core is high and drops sharply at the tumor margins [108]. However, cancer cells may reside in the tumor periphery and remain protected by the BBB, facilitating the possibility of tumor spread or recurrence. Overall, the permeability of microvessels within brain tumors and surrounding brain varies considerably depending on the type of tumor and the location of the microvessels [100], making systemic delivery of passive immunotherapies to brain tumors a complex task. Strategies such as transiently disrupting the BTB to enhance systemic drug delivery to brain tumors by systemic infusion of hyperosmolar mannitol appear to have some benefit [71,80]. However, permeability of the BBB in normal brain tissue may be relatively more affected than the BTB by systemic osmotic approaches [109], resulting in neurotoxic sequelae in healthy tissue. Distribution of antibody-based therapeutics within solid tumors has often been found to be heterogeneous and sites of antibody accumulation often do not correlate with sites of high antigen expression [110]. This phenomenon of problematic and uneven distribution of systemically administered antibody-based therapeutics within tumors has been attributed to the high interstitial pressure that builds within tumors due to the increased angiogenesis and vascular hydraulic conductivity in tumors [111], although other factors may also be at play. The more or less uniformly high interstitial pressure within tumors and sharp drop in pressure at the tumor periphery may result in systemically administered macromolecules like antibody-based therapeutics to accumulate close to blood vessels (points of entry) and the tumor periphery, with little delivery occurring to the rest of the tumor [112,113].

Strategies involving the direct administration of antitumor drugs into the CNS have emerged to overcome some of the challenges faced by systemic delivery. Methods such as convection-enhanced delivery (CED) [114] or injection/infusion of drugs directly into cavities following surgical tumor resection, can deliver passive immunotherapies directly to the brain while bypassing the BTB, the BBB, and the BCSFBs. However, in addition to being highly invasive, such strategies are likely to be practically restricted to local drug delivery due to the transport limitations associated with the brain extracellular spaces where long range distribution is limited by diffusion (*224, 225*); diffusive transport in brain extracellular spaces is size-dependent and will be particularly limited for large macromolecules like antibodies (Wolak 2015). While this transport limitation may be desirable to ‘target’ drugs to a small area of a brain tumor, cancer cells within the tumor periphery may still be beyond reach. Direct injection or infusion (including the aforementioned CED) into brain tumors (intratumoral, intracystic, and intralesional) or surrounding tissue has been utilized clinically to deliver a variety of antibody therapeutics [65]. Examples of passive immunotherapies administered via CED to treat brain cancers include: ^{131}I-chTNT-1/B (commercial name – Cotara; Peregrine Pharmaceuticals/Avid bioservices) – an ADC consisting of an iodine radioisotope conjugated to an anti-DNA-histone H1 complex monoclonal antibody [115]; ^{123}I- or ^{131}I-labeled 81C6 (commercial name – Neurodiab; Bradmer Pharmaceuticals) – an ADC consisting of an iodine radioisotope conjugated to an anti-tenascin monoclonal antibody [116];

^{131}I-8H9 (commercial name – Burtomab; Y-mAbs Therapeutics) – an ADC consisting of an iodine radioisotope conjugated to a murine anti-human B7-H3 monoclonal antibody [117,118]; D2C7-IT – an ADC consisting of a *Pseudomonas* exotoxin (PE38KDEL) conjugated to a single chain variable fragment of an anti-EGFRwt/EGFRvIII monoclonal antibody [119]; and Me1–14 F(ab')$_2$ – a F(ab')$_2$ antibody fragment of the anti-proteoglycan chondroitin sulfate-associated protein murine monoclonal antibody Me1–14) [120]. Clinical trials infusing antibodies into a surgically created resection cavity have also been conducted with ADCs (e.g., ^{123}I- or ^{131}I-labeled 81C6 [121]).

Other methods of delivery that circumvent the BTB, BBB, and BCSFBs are intrathecal and intracerebroventricular (ICV) administration into CSF; these routes may provide more global delivery of antibody-based therapeutics within the CNS due to their capacity to access low-resistance pathways such as perivascular spaces surrounding leptomeningeal and cerebral blood vessels that potentially allow rapid distribution throughout the brain and exchange between the interstitial fluid and CSF [122]. Numerous clinical trials have been or are currently being conducted for treatment of CNS cancer using CSF-administered antibodies. Intrathecal/ICV rituximab - an anti-CD20 monoclonal antibody (commercial name – MabThera/Rituxan; Genentech/Roche) -has been administered to treat CNS lymphoma [123]. Intrathecal/ICV ^{131}I-3F8 - a radiolabeled anti-GD2 ganglioside monoclonal antibody - has been used to treat primary and metastatic leptomeningeal or brain tumors, including a trial for medulloblastoma [118]. Intrathecal/ICV administration of two anti-HER2 antibodies has been investigated for the treatment of leptomeningeal metastases associated with HER2+ breast cancer - trastuzumab (commercial name – Herceptin; Genentech/Roche) monotherapy or in combination with pertuzumab (commercial name – Perjeta; Genentech/Roche) [123,124]. Intrathecal/ICV ^{131}I-8H9 has been given Breakthrough Therapy Designation by the FDA for the treatment of neuroblastoma [118]. ^{123}I- or ^{131}I-labeled 81C6, Me1–14 F(ab')$_2$, and LMB-7 (or B3(Fv)-PE38, a single-chain variable fragment of the murine B3 anti-Lewis Y-related carbohydrate monoclonal antibody conjugated to the a portion of the *Pseudomonas* exotoxin PE38) [125] have also been administered into the CSF for primary or metastatic brain cancer and leptomeningeal cancer. Finally, other non-conventional routes of administration (e.g., intranasal delivery) are also being actively investigated to target therapies to brain tumors [126]. The intranasal route for drug delivery is thought to achieve some degree of CNS targeting by accessing pathways associated with the olfactory and trigeminal nerve systems in the nasal mucosae that allow brain entry at the level of the olfactory bulbs and brainstem, respectively [127]. Intranasal delivery in particular may prove to be relevant for the treatment of brainstem gliomas, which are not particularly amenable to surgical resection or invasive drug delivery methods [128].

ALZHEIMER'S DISEASE

Alzheimer's disease (AD) and related dementia are estimated to affect more than 47 million patients worldwide [129], with more than 5.7 million patients in the United States as of 2018 [130]. These numbers are likely to double by 2050, partly due to the rise of a more susceptible ageing demographic (Alzheimer's association, [130]. The clinical definition of AD has evolved over the last three decades from a cognitive syndrome [131] to a multi-faceted gamut of pathological changes that gradually lead to cognitive impairment over decades [132]. Among

AD patients, cognitive impairment often manifests as one or more progressively declining core domains (memory, executive function, language, visuospatial perception, and intellect) [133,134]. AD can be difficult to diagnose since symptoms for AD may overlap with a variety of other neurological conditions, including (but not limited to) vascular dementia, dementia with Lewy bodies, frontotemporal dementia and cardiovascular disease [133]. AD pathophysiology is typically characterized by simultaneous accumulation of two abnormal proteins and their aggregates – beta-amyloid and hyperphosphorylated tau [134].

Passive immunotherapies for Alzheimer's disease.

Beta-amyloid and hyperphosphorylated tau occur in several different forms and stages of aggregation during the progression of AD pathology providing a wide range of targets for therapies. For example, amyloid β-peptide (Aβ) occurs as a heterogeneous mixture of monomeric peptides from the sequential cleavage of amyloid precursor protein (APP) by several different enzymes. Aβ peptides are typically in the range of 38–43 amino acids although other isoforms are also generated [135,136]. APP cleavage by α-secretase or β-secretase generates amino-terminal fragments and carboxy-terminal fragments; the amino-terminal fragments are called secreted APP (sAPP) α or β respectively and the carboxy-terminal fragments (CTFs) are called CTF83 and CTF99 respectively [136]. γ-secretase cleavage of CTF83 and CTF99 results in the generation of p3 and Aβ peptides respectively and the amino-terminal APP intracellular domain (AICD) [136]. In the amyloidogenic pathway APP is primarily cleaved by β-secretase (beta-site APP-cleaving enzyme 1, or BACE1, in the brain) and γ-secretase resulting in the production of pathogenic Aβ isoforms [136–138]. In the non-amyloidogenic pathway, observed in healthy individuals, APP is primarily cleaved by α-secretase and γ-secretase [136]. α-secretase cleavage is thought to prevent Aβ formation since the α-secretase cleavage site occurs within the Aβ sequence [136]. In AD pathology the less amyloidogenic Aβ40 is the predominant species present around the cerebral vasculature while the more amyloidogenic Aβ42 is the earliest and most abundant isoform within the parenchyma [132,139]. N-terminally truncated forms of Aβ40/42 may also form very harmful pyroglutamate Aβ isomers (pGlu-Aβ(3–40/42)) following cyclization of the N-terminal glutamate residue [140]. Increased production or the lack of efficient clearance of Aβ spurs CNS buildup and aggregation as multiple Aβ units fuse together to form toxic, soluble oligomers [141,142]. These soluble oligomers further act as seeds for aggregation of insoluble, fibrillar species of beta-amyloid [143,144] leading to accumulation within the brain parenchyma as well as abnormal deposition around the smooth muscle layer of cerebral arteries, referred to as cerebral amyloid angiopathy (CAA) [132]; both of these processes (and others) ultimately are responsible for the neurodegeneration observed in AD [132,135]. While the focus typically has been on targeting insoluble fibrillar oligomers or 'plaques', recent evidence suggests that soluble oligomers may drive the levels of other Aβ aggregates; so therapeutic strategies engaging oligomers might be a promising approach moving forward [135,145]. Likewise, abnormal hyperphosphorylated tau protein aggregates to form several different types of pathologic conformations such as paired helical filaments (PHFs), pre-neurofibrillary tangles (pre-NFTs) and eventually neurofibrillary tangles (NFTs) that disrupt the neuronal cytoskeleton and lead to cell death [146]. Passive immunotherapy lends itself well to the task of targeting these different Aβ and hyperphosphorylated tau antigen profiles over the course of AD progression and has therefore emerged as a promising course of treatment

[147,148]. The fact that there are currently no disease-modifying therapies approved for AD [149] further emphasizes the need to investigate AD therapies.

In this review we will discuss antibodies as potential therapies for AD (Figure. 8) in the context of four broad categories based on their antigen/target: (i) anti-beta amyloid antibodies, (ii) anti-tau antibodies, (iii) anti-BACE 1antibodies, (iv) anti-apolipoprotein E (APOE) antibodies, and (v) anti-inflammatory antibodies.

Anti-beta amyloid antibodies: Aβ was the first antigen target investigated for potential AD passive immunotherapies based on two independent studies that showed a reduction in Aβ levels in the brain via different mechanisms following chronic systemic administration of two different monoclonal anti-Aβ antibodies (3D6 and m266) [150,151]. It was proposed that 3D6 demonstrated reduced plaque burden by engaging various forms of Aβ within the brain parenchyma while also mediating Aβ clearance via cell-mediated immune mechanisms (Fc-receptor-mediated phagocytosis) [150]. In contrast, m266 was believed to primarily act by sequestering Aβ in the peripheral compartment, shifting the equilibrium between the CNS and the peripheral Aβ pools towards a greater accumulation in the periphery [151]. In 2006, humanized versions of these ‘first-generation’ antibodies were eventually tested for clinical efficacy in phase 2 studies under the labels bapineuzumab (3D6; Janssen/Pfizer) and solanezumab (m266; Eli Lilly), respectively [152]. Bapineuzumab clinical trials for AD were discontinued in 2013 for their inability to meet clinical endpoints [153]. Systemic administration of solanezumab – a humanized monoclonal antibody that recognizes soluble Aβ – also failed to show significant improvement in primary cognitive outcomes in two phase 3 clinical trials (EXPEDITION 1 and EXPEDITION 2) in patients diagnosed with mild-to-moderate AD [154]. Disappointing outcomes of the bapineuzumab and solanezumab clinical trials may potentially be attributed to many possible factors: (i) initiation of treatment too late in the disease process [155]; (ii) the possibility that targeting Aβ alone may be insufficient to alter disease progression in some cases [155]; and (iii) insufficient central delivery of systemically applied antibodies to the appropriate target sites [156]. Further investigation into the possibility of therapeutic effects at an earlier stage of AD was spurred by secondary analysis of the EXPEDITION 1 and 2 trial data which showed that solanezumab treatment resulted in lesser cognitive and functional decline than placebo among trial participants diagnosed with mild AD [157]. However, investigation of systemic solanezumab passive immunotherapy in a third Phase 3 trial specifically for mild AD (EXPEDITION 3; 400 mg solanezumab or placebo administered intravenously every 4 weeks for 76 weeks) also recently failed to show any significant effect on cognitive outcomes [158]. Higher doses of solanezumab are currently being investigated in prodromal populations at risk for AD in two major clinical studies: (i) the Dominantly Inherited Alzheimer Network (DIAN) clinical trial investigating solanezumab as a preventative treatment in individuals at risk for early onset AD due to a dominantly inherited genetic mutation [159] and (ii) the A4 trial investigating solanezumab as a preventative treatment in older individuals at risk for AD due to amyloid plaque build-up but who do not yet show any cognitive impairment [160]. The results of these later trials may ultimately better inform on solanezumab efficacy and its limitations.

Other anti-Aβ passive immunotherapies for which (i) clinical investigation has been discontinued due to failure to meet clinical endpoints and/or (ii) clinical study outcomes have not been fully reported include: ponezumab (Rinat Neuroscience/Pfizer), an anti-Aβ monoclonal antibody that specifically binds to the Aβ40 fragment that accumulates in the walls of blood vessels as part of the CAA process; GSK933776 (GlaxoSmithKline), an anti-Aβ monoclonal antibody that binds with higher affinity to Aβ monomers and has a modified Fc region that reduces effector-mediated functions to minimize the risk of side effects such cerebral edema or microhaemorrhages that are detected as amyloid-related imaging abnormalities (ARIA) [161]; AAB-003 (Pfizer/Janssen), a modified version of bapineuzumab that has a modified Fc region that reduces effector-mediated functions to minimize the risk of ARIA [162]; SAR228810 (Sanofi), an anti-Aβ monoclonal antibody that binds with higher affinity to Aβ protofibrils than Aβ oligomers or monomers and has reduced effector-mediated function to minimize the risk of ARIA; and MEDI1814 (AstraZeneca and Eli Lilly), an anti-Aβ monoclonal antibody that binds with high affinity to Aβ42.

Numerous anti-Aβ passive immunotherapy trials are still under clinical investigation [65](clinicaltrials.gov), e.g., BAN2401 (Biogen/Eisai), gantenerumab (Hoffman-La Roche), crenezumab (Genentech/Hoffman-La Roche), and KHK6640 (Kyowa Hakko Kirin), anti-Aβ monoclonal antibodies that bind with higher affinity to more aggregated insoluble conformations of Aβ such as protofibrils and/or fibrils compared to soluble Aβ monomers and/or oligomers; aducanumab (Biogen), an anti-Aβ monoclonal antibody that binds with higher affinity to soluble oligomeric as well as insoluble fibrillar Aβ aggregates compared to monomeric Aβ; LY3002813 (Eli Lilly), an anti-Aβ monoclonal antibody that recognizes the pyroglutamate Aβ monomer Aβp3–42; and intravenous immunoglobulin (IVIg; Octapharm), an immunoglobulin serum fraction obtained from healthy donors, used to supplement/replace the immunoglobulin fraction in AD patients.

Anti-tau antibodies: Similar to anti-amyloid therapeutic interventions, anti-tau antibodies targeting hyperphosphorylated toxic tau conformations are being investigated as potential passive immunotherapies for AD [163,164]. Since antibodies are obviously most efficacious when targeted against extracellular antigens, immunotherapy approaches aimed at the typically intracellular tau aggregates [165] initially appeared to face many challenges. However, emerging evidence has suggested that secreted extracellular tau species may initiate the spread of pathology and act as seeds for further tau aggregation [166–168], providing a clear rationale for tau immunotherapy approaches.

Anti-tau passive immunotherapy trials that are still under clinical investigation [65] include: RO7105705 (Hoffman-La Roche), an anti-tau antibody that specifically recognizes a phosphorylated serine residue (Tau/pS409) present in intracellular pre-NFTs as well as extracellular neuropil threads and mature NFTs; LY3303560 (Eli Lilly), an anti-tau antibody that specifically binds to the N-terminus of tau aggregates over monomers; ABBV-8E12 (AbbVie), an anti-tau antibody that has high affinity for all forms of extracellular aggregated tau; and BIIB092 (Bristol-Myers Squibb and Biogen), an anti-tau antibody that has high affinity for tau residues 15–24 and specificity for extracellular secreted forms of tau, as well as tau aggregates. Gene therapy strategies to target intracellular tau are also being investigated; these include the use of anti-tau intracellular antibodies or ‘intrabodies’ [169].

Intrabodies are antibody fragments (e.g., single chain variable fragments) that can recognize specific antigens such as tau and are expressed intracellularly using viral gene therapy approaches to transduce desired cell populations [169–171].

Anti-BACE1 antibodies: Another strategy to reduce the production of Aβ is to inhibit BACE1, one of the enzymes that cleaves APP to produce Aβ. Anti-BACE1 antibodies are being investigated to inhibit APP cleavage by either sterically blocking the BACE1 active site or by blocking the allosteric site that regulates enzyme activity. Preclinical testing has shown that this strategy holds promise [172,173] and clinical investigation will likely follow.

Anti-ApoE antibodies: Apolipoprotein E (ApoE) is the primary carrier of lipids and cholesterol within the brain [174] and the ε4 isoform of ApoE has been identified as one of the strongest genetic risk factors for late-onset AD [175,176]. Recent studies have highlighted the potential of anti-ApoE antibodies as passive immunotherapy candidates in AD [177,178] and clinical investigation is likely to follow.

Anti-inflammatory antibodies: Several preclinical studies have shown that systemic inflammatory stimuli in the periphery can trigger an adverse central immune response, which subsequently leads to neurotoxicity [179,180]. Indeed, AD patients with elevated levels of the pro-inflammatory cytokine TNF-α have a typically faster cognitive decline [181]. Etanercept (Amgen and Pfizer) is a fusion protein consisting of human IgG1 Fc portion linked to a dimeric ligand-binding region of tumor necrosis factor alpha cell surface receptor (p75 TNF-α) [182] that was under clinical investigation as a potential passive immunotherapy for AD. However, the inability to meet clinical endpoints has currently halted further investigation of this strategy.

Current strategies and challenges in delivering passive immunotherapies for Alzheimer's disease.

Pathological changes in AD initiate as localized protein aggregation but spread globally throughout the course of disease progression [186,187]. Hence, whole brain delivery of antibodies will eventually become crucial to obtain widespread CNS effects and acceptable clinical efficacy. Currently, most AD passive immunotherapy clinical trials utilize the systemic route of administration. Unfortunately, it is likely that systemically administered exogenous antibodies do not cross the BBB or BCSFBs to an appreciable extent and often remain restricted to the endothelial compartment where they cannot engage target antigens [156]. High doses of systemic exogenous antibodies, often administered in order to attempt overcoming poor delivery to the CNS, have been linked to adverse events such as vasogenic edema and microhemorrhages (ARIA-E or ARIA-H respectively) [188]. Strategies to enhance delivery of systemically administered exogenous antibodies to the CNS such as transient disruption of the BBB with focused ultrasound (e.g., BAM-10 [189]), or shuttling antibodies across CNS barriers using bispecific antibodies (e.g., anti-BACE-1/TfR [190–192]) are also being tested.

Strategies exploring the administration of passive immunotherapies for AD directly into the central compartment have also received increasing interest. For example, preclinical studies

have shown that ICV administration of anti-amyloid antibodies results in widespread brain delivery and reduces parenchymal plaque burden [193–196]. ICV administration of passive immunotherapies for AD also outperforms systemic delivery approaches, both in efficacy and safety (reduced incidence of ARIAs) [197]. Perispinal administration has received renewed attention as a potential means to deliver drugs to the intracranial venous system, which is potentially in communication with the CSF [198]. Perispinal injection involves injecting the drug between the spinous processes of the lower dorsal vertebrae, outside the spinal canal, and posterior to the ligamentum flavum with the expectation that the drug is rapidly absorbed by local vertebral venous vasculature and eventually drains into the external vertebral venous plexus (EVVP) [198]. Vertebral veins are valveless and are in communication with intracranial veins allowing drug in the EVVP to access the intracranial venous system, and eventually the CSF [198] potentially via communication between the intracranial venous system, arachnoid villi [199], dural lymphatics [200,201], and other extracellular pathways [202,203]. Perispinal administration of etanercept has showed rapid anti-inflammatory response in some studies [204–207]; however the outcomes of this route are somewhat controversial [208]. Intranasal delivery is also emerging as a promising non-invasive central delivery approach to target passive immunotherapies to the CNS; indeed, delivery of antibodies [209–211], as well as antibody fragments [212], have been reported to reduce pathology in rodent models of AD. However, the detailed CNS distribution, mechanisms responsible for transport from the nasal epithelia to the CNS, and strategies to optimize CNS delivery of intranasally applied antibodies have only recently been explored [213]. Further work is clearly needed to better define alternative delivery approaches for targeting antibodies to the CNS.

PARKINSON'S DISEASE

Parkinson's disease (PD) affects nearly 10 million individuals worldwide and nearly 1 million individuals in the United States alone and, like other neurodegenerative disorders, PD poses a significant financial burden due to large healthcare costs and lost earning potential associated with those afflicted and their caregivers (e.g., it may be estimated that PD accounts for over $20 billion in direct and indirect costs in the U.S. today with PD prevalence / costs expected to rise dramatically by 2040) [214,215]. Bradykinesia, postural instability, rigidity, and tremor are the major clinical symptoms observed in Parkinsonian disorders [216]. Accumulation of an abnormal form of the presynaptic neuronal protein alpha synuclein within neuronal perikarya as Lewy bodies is a hallmark of idiopathic PD [216]. As with AD, there are currently no disease-modifying therapeutics for the treatment of PD; strategies that can target different alpha synuclein aggregation profiles and other pathological targets observed with PD progression will be crucial for success. Passive immunotherapies are well suited to this challenge and are therefore currently being investigated for the treatment of PD [217].

Passive immunotherapies for Parkinson's disease.

In this section, we will limit the discussion to antibodies as potential therapies for PD (Figure. 9) in the context of three broad categories based on their antigen/target: (i) anti-alpha synuclein antibodies, (ii) fusion proteins, and (iii) anti-LAG3 antibodies.

Anti-alpha synuclein antibodies: A study by Masliah and coworkers showing a reduction in alpha-synuclein pathology in the CNS was the first preclinical study to investigate passive immunotherapy targeting alpha-synuclein for PD treatment [218]. Several other preclinical studies followed to investigate the efficacy of anti-alpha synuclein antibodies in PD therapy [219]. These antibodies demonstrated varied specificity for epitopes and conformations of alpha synuclein and included the C-terminus [220,221], N-terminus [222], or central region of alpha synuclein [222], as well as alpha synuclein protofibrils [223]. Clinical investigation of passive immunotherapies for PD has been fairly limited thus far. A monoclonal anti-alpha synuclein antibody PRX002 (Prothena Corp.) has been shown to be safe in humans but its efficacy remains to be demonstrated in a clinical setting [224].

Fusion proteins: Glial-derived neurotrophic factor (GDNF) has been shown to promote neuronal cell survival and has long been thought to be a promising potential therapy for PD. However, GDNF cannot appreciably cross the barriers of the CNS following systemic administration; several different strategies have been tried in the hope of successful central GDNF therapy over the years (e.g., intraventricular or intraparenchymal GDNF infusions) but these have so far met with challenges [225]. This initially spurred efforts to engineer an immunoglobulin fusion protein that might utilize a putative BBB transcytosis system (e.g., the transferrin receptor or the human insulin receptor) to shuttle GDNF from the blood circulation into the brain parenchyma [226]; however, despite initially positive pre-clinical findings, systemic delivery of a GDNF-human insulin receptor antibody fusion protein ultimately did not show behavioral or anatomical efficacy in a macaque PD model and, further, produced metaplastic and neoplastic pancreatic lesions in rhesus monkeys that caution against use of such a systemically applied growth factor-insulin receptor antibody conjugate for future clinical trials [227].

Anti-LAG3 antibodies: Although Lewy bodies and other alpha synuclein aggregates typically occur intracellularly, a secreted form of abnormal alpha synuclein has also been reported to contribute to the spread of pathology to other brain regions in a prion-like manner [228–230]. This transfer of abnormal alpha synuclein between neurons was recently reported to involve the lymphocyte-activation gene 3 (LAG3) transmembrane protein [231]. LAG3 is a transmembrane protein that structurally resembles the T cell co-receptor CD4, which binds MHC class II molecules and is expressed by neurons in the cortex and cerebellum, as well as cells/cellular processes in developing white matter and the choroid plexus [232]. Although the physiological function of LAG3 remains largely unknown, it has been demonstrated that LAG3 binds to abnormal alpha synuclein preformed fibrils (PFFs) but not monomers and facilitates the entry of pathologic alpha synuclein PFFs into neurons via clathrin-mediated endocytosis [231]; based on these findings an anti-LAG3 passive immunotherapy approach to inhibit the spread of pathologic alpha synuclein within the CNS may be promising and warrants future investigation.

Current strategies and challenges in delivering passive immunotherapies for Parkinson's disease.

Among the innovative approaches that have been considered to enable anti-alpha synuclein antibodies to access and engage intracellular aggregates, is the use of intracellular antibodies

(i.e., intrabodies) [233,234]. Expression of antigen specific intrabodies within the CNS requires that brain cells be transfected with anti-alpha synuclein scFv cDNA containing plasmids or viruses [171]. However, delivering plasmids and viral vectors to brain tissue remains challenging due to their highly limited capacity to cross CNS barriers following systemic delivery [235] and their limited spread away from the site of administration following central delivery approaches such as direct intraparenchymal or intrathecal administration [235–237]. Delivering viral vectors to the CNS also may pose safety concerns with certain vector types [238]. Another unique challenge for intrabodies to engage their target is the instability of antibody disulfide bonds in the reducing environment of the cell cytoplasm [217]. Endogenous immunoglobulin disulfide bonds are formed under highly controlled redox potential conditions within the endoplasmic reticulum; these conditions favor the formation and stability of disulfide bonds [239]. Endogenous immunoglobulins remain protected from the reducing environment of the cell cytoplasm by vesicles until they are secreted [240] into a physiological fluid (e.g., blood or CSF), which has a redox potential that can sustain disulfide bond stability [241]; intrabodies may fail to fully access this complex intracellular protein trafficking pathway.

In general, the challenges faced for delivering passive therapies to the CNS for the treatment of PD faces some of the same challenges as those for other neurodegenerative disorders. Systemically administered exogenous therapeutic antibodies may not appreciably cross CNS barriers to engage their pathologic target [156], while most central routes of delivery are limited by their invasiveness and, at least so far, a suspected inability to provide global drug delivery [236,237].

HUNTINGTON'S DISEASE

Huntington's disease (HD) is a hereditary neurodegenerative disease marked by progressive cognitive, behavioral, and motor decline. HD prevalence worldwide is around 3 per 100,000 people, ranging from a high in Europe, North America, and Australia (~6 per 100,000) to a low in Asia (<1 per 100,000) [242]. Late-stage HD brains reveal severe atrophy of the cortex and striatum [243–245]. Pathology in HD is caused by an expanded trinucleotide repeat pattern CAG (>36–40 repeats) encoding an abnormally long string of the amino acid glutamine (polyQ tract) in exon 1 of the huntingtin gene (HTT) thereby producing a misfolded mutant huntingtin protein (mHTT) [246]. While the precise function of the Huntingtin protein remains unknown, it has been hypothesized that Huntingtin is a membrane-associated protein that is involved in vesicular trafficking [247]. Approved treatment options for HD are currently quite limited and only address the symptoms of the disease (i.e., symptomatic, not disease-modifying therapies). Among the currently approved therapies are small molecule therapeutics that suppress involuntary movement and anti-psychotic drugs. Potential new HD therapies under preclinical and clinical investigation include macromolecules with a variety of targets implicated in HD pathology.

Passive immunotherapies for Huntington's disease.

Although there are currently no approved antibody-based therapeutics for HD, there are a number of different antibodies in the preclinical pipeline that target mHTT as well as other

proteins involved in neuronal cell survival and neuroinflammation. In this section, we will discuss antibodies as potential therapies for HD (Figure. 10) in the context of three broad categories based on their antigen/target: (i) anti-mHTT antibodies, (ii) anti-inflammatory antibodies, and (iii) BDNF mimetics.

Anti-mHTT antibodies: Antibody-based therapeutics targeting both the intracellular and extracellular forms of mHTT are potential strategies for HD intervention that are still in the preclinical stage of investigation [248]. Targeting intracellular and/or membrane bound mHTT has the potential to slow down and/or prevent cell death [249], while targeting extracellular secreted mHTT has the potential to slow down and/or prevent cell-to-cell transmission and spread of pathology [250]. Indeed, an anti-mHTT monoclonal antibody developed by AFFiRis that binds to extracellular mHTT has been shown to reduce levels of the abnormal Huntingtin protein in plasma and organs in the YAC128 mouse model of Huntington's disease after intraperitoneal administration [251].

Anti-inflammatory antibodies: Immune dysfunction has emerged as an early hallmark in HD pathology; indeed, proinflammatory signals have been shown to exacerbate HD progression in humans [248,252]. One such proinflammatory signal is the semaphorin 4D (SEMA4D) protein, which is expressed by infiltrating immune cells while its receptor is expressed by neurons, endothelial cells, and oligodendrocytes [253]. Expressions of both SEMA4D and its CNS receptor plexin-B1 have been shown to be upregulated in HD, suggesting a possible correlation between the SEMA4D proinflammatory signal and HD pathology [254]. Importantly, preclinical studies have shown that anti-SEMA4D antibodies dampen neuroinflammation and can rescue the disease phenotype in a transgenic mouse model of HD [255]. Vaccinex is currently investigating the efficacy of anti-SEMA4D monoclonal antibody (VX15/2503) for the treatment of HD in clinical trials and received a fast-track designation from the FDA in 2016 for the development of this therapy.

BDNF mimetics: Given that HD causes cortical and striatal atrophy, another therapeutic target for HD is an important signaling pathway for neuronal survival activated by brain-derived neurotrophic factor (BDNF). Pfizer has identified two mouse monoclonal antibodies, known as 38B8 and 29D7, that act as BDNF mimetics and activate the Tropomyosin receptor kinase B (TrkB) signaling pathway leading to cell survival. These two antibodies have been shown to have some neuroprotective effects in rat primary striatal neurons *in vitro* [256], although *in vivo* efficacy has to our knowledge not yet been established.

Conventional anti-HTT antibodies may be used to target extracellular mHTT and prevent its cell-to-cell transmission. Additionally, antibodies that mimic BDNF may be used to activate the TrkB signaling pathway to promote neuronal survival, while anti-SEM4D antibodies may be used to interrupt the SEM4D/plexinB1 pro-inflammatory signaling pathway. Adapted from: [248,250,251]. Abbreviations: BDNF – brain derived neurotrophic factor; SEM4D - semaphorin 4D; TrkB – Tropomyosin receptor kinase B.

Current strategies and challenges in delivering passive immunotherapies for Huntington's disease.

Intrabodies are typically used to target intracellular mHTT pathology; however, this strategy faces the same challenges posed by gene therapy delivery and safety as in other CNS disease contexts. Typically intrabodies are smaller antibody fragments such as scFvs, in order to simplify protein expression [248]. Intrabodies for HD passive immunotherapy are currently under preclinical investigation. While intrabodies targeting the abnormally expanded polyQ tract unfortunately caused rapid cell death and worsened mHTT aggregation in preclinical studies [259], intrabodies targeting other mHTT domains have demonstrated a reduction in aggregates [248,260].

As with most other CNS disorders, passive immunotherapies for HD have typically been administered via the systemic route and face the challenge of inadequate access to the brain parenchyma (i.e., the site of target engagement) due to the presence of the CNS barriers [156]. Central routes of delivery, while invasive, may be promising for delivery directly to the most vulnerable brain regions such as the striatum and cerebral cortex. Non-invasive routes of central delivery such as intranasal administration also hold some promise. For example, intranasal application of a small molecule BDNF mimetic was found reduce motor dysfunction and pathology by acting via the TrkB signaling pathway in a mouse model of HD [261]; these studies may be extended to investigate the efficacy of HD passive immunotherapies in the near future.

SUMMARY OF SYSTEMIC ADMINISTRATION STRATEGIES TO DELIVER PASSIVE IMMUNOTHERAPIES FOR CNS DISORDERS

Systemic administration (i.e., delivery of drugs via the blood circulation) of passive immunotherapies to investigate potential treatments for CNS disorders has historically been the primary focus of both industry and academic studies for several reasons. First, most biologics, such as antibody-based therapeutics are susceptible to protease degradation and permeate poorly across physiological barriers (e.g., the gastrointestinal mucosa) due to their large size and charge [262,263]. Second, the pharmacokinetics of the most typical parenteral routes of administration (i.e., intravenous, intramuscular, or subcutaneous) are relatively simpler and better understood than that for other routes of administration such as oral or intranasal where an often complex initial absorption step must be accounted for. Third, the brain is a highly vascularized organ with capillary density as high as several thousand mm/mm^3 (total capillary length per tissue volume) [264]. The typical distance between capillaries and neurons within the brain ranges between –25 μm [264]. However, a major hurdle to systemic drug delivery to the CNS is the existence of the BBB and BCSFBs [265–267]. While it has been reported that a small fraction of endogenous IgG circulating in the blood may access the CNS [268,269] via sites where the BBB is absent (e.g., the circumventricular organs) [202,203] the capacity of these pathways to allow entry of exogenous systemically administered antibodies into the CNS at therapeutically relevant levels is limited [156]. Indeed only 0.009% of IVIg has been detected in the brain and a large portion of this fraction has been observed to be sequestered within the endothelial compartment of cerebral microvessels, i.e., it is unable to access the brain parenchyma to engage with target

antigens [156]. The difficulty in being able to distinguish between the systemically administered exogenous antibody fraction sequestered within the cerebral endothelial cells versus the antibody fraction that truly gains access to the brain parenchyma has resulted in an overall poor quantitative estimation of antibody CNS levels following systemic delivery [156,270–272]. Fourth, it is often assumed that the BBB is compromised under pathological conditions and that its ability to restrict systemically administered drugs from entering the brain is altered under such conditions. However, the degree of BBB disruption varies greatly depending on the stage of disease progression and may be heterogeneous in different brain regions [273]; indeed, careful study of BBB permeability to systemically applied human IgG in several common mouse models of AD (mutant PS2-APP, tau and APOE lines) and amyotrophic lateral sclerosis (mutant superoxide dismutase 1(SOD1) line) revealed no change in IgG levels in cortex, cerebellum, or spinal cord [274].

The poor outcomes of clinical trials investigating systemically administered passive immunotherapies for CNS disorders and a better understanding of the limited levels of exogenous IgG capable of passively reaching the CNS has spurred new efforts to enhance transport across or around the CNS barriers. For example, one such strategy has involved the transient disruption of the BBB via methods such as MRI-guided focused ultrasound with microbubbles [275,276] or systemic infusion of hyperosmolar solutions (e.g., hyperosmolar mannitol) [277–279]. However, it has long been appreciated that disruption of the BBB poses a risk since it non-specifically allows entry of not just drugs but other serum macromolecules into the CNS [280]. A more specific approach being investigated to enhance the CNS delivery of antibodies across the BBB is the application of methods (sometimes referred to as ‘Trojan Horse’ strategies) that utilize endogenous receptor-mediated vesicular transport systems (primarily clathrin-coated vesicles [281]) to shuttle their ligands (nutrients, metabolites, proteins etc.) from the luminal to the abluminal surface of the brain endothelium, i.e., from the blood to the brain [282]. Endogenous receptors involved in putative receptor-mediated transport (RMT) at the BBB include the transferrin receptor, insulin receptor, and low-density lipoprotein-receptor related protein, among others [282,283]. Although RMT in brain endothelium is relatively downregulated compared to endothelium in other parts of the body, it may be crucial for macromolecule transport across the BBB [281]. Such BBB-crossing strategies typically involve a drug consisting of an antibody, antibody-fusion protein, or antibody-decorated nanoparticle with two main components, the first of which targets one of the aforementioned RMT pathways in brain endothelial cells while the second consists of a therapeutic payload (e.g., lysosomal enzyme) or disease-modifying Fab portion directed against CNS pathology (e.g., amyloid beta or alpha-synuclein) [281,282,284,285]. However, ‘molecular hitchhiking’ of therapeutic molecules across the BBB by harnessing endogenous transport mechanisms [285] may sometimes come at a price, e.g., the expression of transcytosis receptor targets (e.g., transferrin receptor) in other regions of the body poses the risk of off-target side effects, depending on the nature of the antibody [106,107,286]. Additionally, it has been suggested that antibodies directed against BBB RMT systems should ideally demonstrate low affinity binding in order to allow the antibody to be successfully released following transit across endothelial cells of the BBB; such low affinity interactions will often require larger systemic doses to be administered in order to achieve therapeutically relevant levels within the brain parenchyma [107,190]. Lastly, exogenous

antibodies targeted to an endogenous RMT system at the BBB may compete with the endogenous ligand of the receptor in some cases, a situation that may cause complications over time depending on the nature of the receptor system being targeted [100]. Identification of new RMT pathways at the BBB is under investigation [191] in order find delivery mechanisms with a larger transport capacity and fewer off-target side effects. An important caveat to such approaches is that any antibody or antibody conjugate that first crosses the brain endothelium must then navigate endothelial-, pericyte-, and astrocyte-associated basement membranes in crossing the perivascular compartment (which may consist of fused basement membranes or a potential pericapillary space), before finally moving beyond astrocytic endfeet to reach the brain extracellular spaces and then diffuse to target neurons [122,287,288]. These steps may pose a particular barrier to larger macromolecule therapeutics like antibodies, which may not always easily escape the perivascular spaces to enter the parenchyma [122,213]; however, further studies are needed to better understand the distribution of endothelial cell-crossing therapeutics between the PVS and brain ECS.

SUMMARY OF CENTRAL ADMINISTRATION STRATEGIES TO DELIVER PASSIVE IMMUNOTHERAPIES FOR CNS DISORDERS

Central administration strategies that bypass the BBB and BCSFBs entirely are emerging as a necessary tool to facilitate the delivery of large biologics like therapeutic antibodies to the CNS. One approach to bypass the BBB is direct injection or infusion of substances into the brain parenchyma; this may be particularly suitable when narrow, focal delivery to specific brain regions is desired. Transport within the brain extracellular spaces is particularly limited to short range distribution by diffusion [236,237], a size-dependent process that may be slow and inefficient over longer distances for large macromolecules like antibodies [289]. This transport limitation may be desirable to ‘target’ drugs to a small area of the brain, e.g., a brain tumor (although invading cancer cells migrating away from tumors to other brain sites may still be beyond reach). Treatment of whole brain disorders that may require chronic drug administration paradigms (e.g., Alzheimer’s disease, neuropathic lysosomal storage disorders, Parkinson’s disease, and Huntington’s disease, to name a few) with direct parenchymal injections will likely be neither practical nor feasible due to the number of injection sites required for therapeutic delivery.

One well-described drug delivery method for direct brain injection is CED [114]. It was originally thought that this technique could accomplish delivery to a larger brain volume than simple injections due to a resulting pressure gradient imposed between the catheter tip and the tissue interstitium that might force an infusate to flow through the extracellular space. However, it is now better appreciated that parenchymally-infused substances are much more likely to distribute via faster bulk flow along low-resistance pathways in the brain, e.g., cerebral perivascular spaces and white matter tracts [122,290–296], than along narrow gray matter extracellular spaces that exhibit high hydraulic resistance to flow of any kind [236]; indeed, these low-resistance pathways associated with the perivascular spaces and white matter are now more commonly credited for the large area of tracer distribution following CED [295,297]. Important considerations for CED include optimal injection volumes and rates that ideally achieve the desired target volume of distribution. In keeping with these

considerations, the FDA approved the ‘iPlan Flow’ software to help target therapies more accurately to specific brain regions and without significantly losing the drug to the CSF via white matter tracts or the pial/ependymal surfaces [298]. Improved cannula designs [299,300] that may help prevent backflow are also under active investigation. Recent work includes the investigation of multifunctional microfabricated devices (e.g., a miniaturized neural drug delivery system, or MiNDS) with smaller catheters that can more precisely deliver smaller volumes at lower flow rates (e.g., an order of magnitude lower than typical CED) and with rapid on/off dosing; such devices have recently been tested for feasibility and functionality in both rodents and non-human primates [301]. Though the above strategies are invasive, and the transport of large molecules may still be limited with some of them, intraparenchymal delivery methods nonetheless continue to hold promise for certain types of targeted therapies. To date there are no approved macromolecule therapeutics delivered directly into the brain parenchyma; a recent FDA approval (the Cleveland Multiport Catheter CED device) [302] may open the way for further studies (this particular device has so far been utilized in clinical trials administering topotecan, a small molecule chemotherapeutic, intratumorally; [65]).

Another central delivery approach to bypass the BBB and BCSFBs involves administration directly into the CSF within and surrounding the brain and spinal cord. Possible routes of administration include lumbar intrathecal (into the lower/caudal spinal CSF space), cisternal intrathecal (into the cisterna magna, near the brainstem, the reason this route is rarely used clinically), or ICV into the lateral, third, or fourth ventricles in the brain’s interior) [303]. A recent study reviewed the safety and usage of ICV devices in patients and suggested that it is a reasonable long-term drug delivery strategy [304]. CSF-administration shows some promise for larger macromolecules, though clinically almost all are still in trials [124]. It is important to note that many clinical trials listing ‘intrathecal’ as the route of delivery actually administer the therapeutic agent into the CSF by intrathecal injection/lumbar puncture or by administration into the ventricles using a device such as the Ommaya reservoir or the Rickham device (subcutaneous implanted reservoirs with a catheter placed into a ventricle or surgically created resection cavity that can be accessed through the skin); indeed, some clinical trials require an implanted ventricular access device for eligibility. Intraventricular delivery is not technically equivalent to intrathecal, (with obvious differences in location and application) so caution should be exercised when assuming the actual route of delivery based on use of the term ‘intrathecal’; the implications of an entirely different site of delivery may be important for drug distribution between the cranial and spinal CSF compartments and critical for understanding the pharmacokinetics. There are currently three biologics approved in the United States by the FDA for CSF-administration: (i) ziconotide, a 2.6 kDa peptide delivered via lumbar intrathecal infusion approved in 2004 for chronic pain [305,306], (ii) nusinersen, a 7.5 kDa antisense oligonucleotide delivered via lumbar intrathecal injection approved in 2016 for spinal muscular atrophy [307–310], and (iii) cerliponase alfa, a 66 kDa enzyme delivered via intracerebroventricular infusion approved in 2017 for late infantile neuronal ceroid lipofuscinosis type 2 (CLN2) [311]. The latter disorder is unarguably a whole-brain disease, for which delivery of the enzyme to every cell is desired; however, the actual distribution of this enzyme throughout the brain is has not been fully described. The lack of published studies describing detailed distribution profiles for intracerebroventricularly and

intrathecally applied macromolecules is something that needs to be addressed in the future; indeed, there is an urgent need for preclinical studies focused on the mechanisms governing macromolecule transport to and distribution within the brain so that the potential translatability of different methods can be better understood across different therapeutic classes (e.g., peptide versus oligonucleotide versus larger proteins).

CSF administration to target drugs to the brain relies on communication between the CSF and the brain interstitial fluid (ISF), the governing physiology of which is an area of great interest but also a topic with significant questions and some recent controversies [122,312] Modern studies have confirmed that diffusion appears to hinder the transport of substances between the CSF and bordering brain extracellular spaces, for a variety of macromolecules, including antibodies [122]; this finding is somewhat in line with older work where CSF-administered macromolecule delivery into the brain was previously thought to be minimal [313,314]. However, the low-resistance pathways (the cerebral perivascular spaces and white matter tracts, discussed above) have increasingly been appreciated to play perhaps a key role in rapid exchange between the CSF and brain ISF [122,291–295]. Transport along the perivascular space (defined here as the fluid-filled vascular connective tissue space of the vessel adventitia and also possibly the extracellular space associated with the smooth muscle basement membrane of the tunica media) has been suggested to occur in part due to vessel pulsatility driving convective flow along the vessel wall [290,295,315] or alternatively, dispersion [316]. Most importantly, substantial entry into the perivascular compartment from the CSF may theoretically provide access to the whole brain (by reaching down to the level of the capillaries) so it is critically important to better understand what factors (physiological and physicochemical) govern this access and how to tune it for better delivery [122].

The intranasal route of administration has also received recent attention as a potentially non-invasive method to deliver biologics to the CNS [126,127,317–321]. Several groups have demonstrated that intranasal administration of specific full length IgG antibodies [209–211], and smaller antibody fragments [212], may result in CNS delivery sufficient to show efficacy in rodent models of AD. Recent published work from our laboratory shows that intranasally administered IgG can rapidly access the CNS at therapeutically relevant levels via transport along extracellular perineural and perivascular pathways associated the olfactory and trigeminal nerves with further widespread distribution within the brain via the perivascular spaces of cerebral blood vessels [213]. Evidence supporting access to olfactory and trigeminal pathways in the nasal lamina propria and subsequent transport to and distribution within the CNS following intranasal delivery has now been demonstrated for untargeted antibodies [213], and targeted antibodies [209], as well as other protein [127,321] and dextran tracers [319]. This accumulating evidence suggests (i) the transport pathways from the nasal mucosa to the CNS are unique to the nasal route of administration and (ii) that these unique anatomical pathways themselves do not appear to vary considerably for different intranasally administered macromolecules. However, specific binding (e.g., binding to antigens or Fc receptors) or non-specific binding (e.g., binding to extracellular matrix components) interactions will likely affect the efficiency of transport of different antibodies along these nose-to-brain pathways. Intranasal delivery to the CNS may be influenced by several factors: formulation [322,323], molecular size [319,324], use of nasal epithelial permeability enhancers [319,325], and body position [326,327] among others. Size-dependent aspects of intranasal

antibody delivery to the CNS can now be addressed due to significant advances in protein engineering and the rise of antibody fragment-based therapies [10]. Molecular size may potentially influence intranasal delivery to the CNS at several stages of the transport process. Intranasally administered molecules must first cross the nasal epithelium via transcellular or paracellular pathways [126,318], prior to accessing perineural and perivascular pathways to the CNS. However, tight junction proteins expressed at the nasal epithelium (e.g., occludin, claudins-1, −3, and −5, and zonula occludens-1 and −2 expressed in the apical olfactory epithelium [328]) impose a size-dependent barrier to paracellular transport [126,318,319]. Smaller molecules are thus able to cross the nasal epithelium to reach the underlying lamina propria and access pathways to the CNS more efficiently than larger molecules; such a size-dependence has been demonstrated using fluorophore-labeled 3 and 10 kDa dextrans [319]. Preliminary studies from our laboratory also suggest a similar trend for intranasally administered antibodies, with better brain delivery for smaller sdAbs (hydrodynamic diameter (d_H) ~ 4.5 nm) and Fab fragments (d_H ~ 6.5 nm) than for full length IgG (~ 10 nm) ([213] & unpublished observations). It has been suggested that IgG transport across the nasal epithelium following intranasal administration may also be attributed to FcRn-dependent transcytosis [213,329,330]. Once in the nasal lamina propria, intranasally administered molecules would need to escape clearance into the sink of the systemic circulation or nasal lymphatics in order to access perineural and perivascular pathways to the CNS [317]. Larger hydrophilic molecules are more likely to escape clearance into the systemic circulation and lymphatics than smaller molecules [317]. Entry of intranasally administered macromolecules into the CSF has also been shown to be size-dependent [324]. As with nasal epithelial transport, IgG access to the CSF following intranasal delivery may partly be attributed to FcRn-dependent transport processes at lining cells of nerves and leptomeningeal vessels [122,213,287], although this will require further study. Our work has shown that intranasally administered IgG accesses the brain more efficiently than the CSF compartment, suggesting that some degree of CNS entry/distribution can occur without access to the CSF first [213]. Taken together, the size-dependent entry of intranasally administered antibodies to the CNS appears most likely attributed to size-dependent transport across the nasal epithelium, although other mechanisms influenced by molecular size may also play important roles.

Our work investigating the distribution of antibodies within the CNS following intraparenchymal [289], intrathecal [122], and intranasal administration [213] (Figure. 11) emphasizes that drug access to perivascular spaces and subsequent distribution along these spaces into the brain may likely govern whole brain delivery and thus therapeutic efficacy [122,236,287]. Several physiological factors may influence the capacity of antibodies to access the perivascular compartments of cerebral blood vessels and subsequently diffuse from the perivascular space and into the brain parenchyma. First, size-dependent entry of protein tracers into the perivascular spaces of cerebral blood vessels has been shown to play a large role in brain distribution from the CSF, with smaller sdAbs having greater access to the perivascular spaces compared to full length IgG [122]. Size-dependent access to the perivascular spaces likely serves additional physiological roles; e.g., insulin-like growth factor 1 (IGF1, 7.6 kDa) binding to insulin-like growth factor binding protein 2 (IGFBP-2; 32 kDa) in the CSF may prevent substantial brain access of IGF-1 under some conditions, and release of IGF-1 from this same binding protein may allow free IGF-1 to enter the brain

more easily [331], possibly via non-saturable perivascular pathways [332]. Second, binding of immunoglobulin G to Fc receptors (e.g., the Fcγ receptor 2b) in the brain at key interfaces (e.g., the pial surfaces, the glia limitans, and around cerebral blood vessels) may impede the ability of IgG to enter the brain from the CSF and hinder its exit from the perivascular compartment by diffusion. Surprisingly, very little information is available on Fc receptor distribution in the CNS so such a 'binding' barrier cannot yet be fully appreciated. Third, the astrocyte basement membrane may also pose a barrier to substances attempting to enter the extracellular space from the perivascular spaces [288]. Indeed, previous studies have demonstrated that full-length IgG exhibits limited diffusion out of the perivascular spaces and into the surrounding neuropil [122,288]. Finally, the impact of administration parameters, e.g., infusion rates, delivery volumes [269], and body positioning [326,333], etc. may ultimately prove quite significant when carefully studied across the different routes.

Pathological changes that occur with the progression of CNS disorders may also have an important, disease-specific impact on the ability of centrally administered drugs to access the perivascular compartment of cerebral blood vessels and diffuse within the brain target regions. For example, brain tumors often have an astrocytic border that likely serves to hinder diffusion between surrounding brain and the tumor. Increased laminin content in the tumor extracellular matrix may also further impede antibody diffusion within the tumor microenvironment [334]. Perivascular access and transport may also be physically blocked by cancer cells that have been shown to reside and disperse along cerebral perivascular compartments [335–338], the subpial spaces [335], nerves [335], and white matter tracts [335]; remarkably, and perhaps not coincidentally, such pathways are likely the same ones important for drug transport to and from deeper brain regions following central delivery [292–294]. The disease pathology in Alzheimer's disease and related dementias has been shown to influence the architecture of cerebral perivascular compartments due to either deposition of hyperphosphorylated tau or Aβ40 within the perivascular compartment [339], sometimes accompanied by enlargement of the perivascular space [340]. Similar abnormally enlarged cerebral perivascular spaces have also been demonstrated in patients with traumatic brain injuries [341], acute ischemic stroke [342], and certain lysosomal storage disorders [343]. Whether such a physiologically enlarged perivascular space affects perivascular drug transport remains an open question.

A final important consideration is systemic exposure, which is often relatively low with intra-CSF administration compared to systemic or intranasal routes of administration [122,126,318]. However, CSF-administered substances do eventually drain from the central compartment via arachnoid granulations into the blood of the dural sinuses [344] as well as via lymphatic pathways that ultimately drain into systemic circulation as well [200,201].

CONCLUSIONS AND FUTURE PERSPECTIVES

The development of passive immunotherapies for CNS disorders has lagged behind that of other non-CNS indications [345]. The reasons for this development lag for CNS indications are varied but they are thought to include insufficient mechanistic understanding of the brain, a paucity of reliable biomarkers to monitor disease progression and drug efficacy, and perhaps most significantly, the tremendous challenge of effective drug delivery to target brain regions

[12]. However, this review emphasizes that we have nonetheless made significant strides toward better understanding the challenges in overcoming the various physiological and pathological barriers that may impede therapeutic antibody delivery to the CNS. Creative strategies that harness the capacity of pre-existing anatomical pathways and mechanisms to deliver antibodies to their target site, coupled with rapid advances in antibody engineering that facilitate customization of antibody target selectivity, effector functions, and distribution properties, provide a promising outlook for the future of passive immunotherapies to treat CNS disorders.

In this review, we primarily focused on passive immunotherapies for the treatment of proteinopathies (e.g., PD, AD, and HD) and brain cancers. However, passive immunotherapies are also being investigated and applied for treating other CNS disorders such as multiple sclerosis, stroke, and traumatic brain injury among others. Multiple sclerosis (MS), an autoimmune neuroinflammatory disorder of unknown etiology causes demyelination of axons within the CNS. There are currently no treatments that can entirely stop disease progression or reverse existing disabilities in MS patients [346–348]. Acute inflammatory episodes in MS are typically treated with intravenously administered corticosteroids that cause immunosuppression; however, the symptomatic relief and response to corticosteroids diminishes with repeated use [346].

Several passive immunotherapies that largely target immune responses in the periphery have been approved for treatment in MS [346,348]: (i) natalizumab (Tysabri; Biogen/Élan; FDA approval – 2004) targets the cell adhesion molecule α4-integrin and blocks lymphocyte migration into the CNS [348–351]; (ii) alemtuzumab (Lemtrada; Genzyme/Sanofi; FDA approval – 2014) depletes lymphocytes via antibody-mediated cell cytotoxicity (ADCC) and by activating the complement system [348,352–354]; (iii) daclizumab (Zinbryta; Biogen/Abbvie; FDA approval – 2016) targets the IL2 receptor [348,355] but was recently withdrawn (April, 2018) due to complex and significant adverse events; and (iv) ocrelizumab (Ocrevus; Genentech/Roche; FDA approval – 2017) targets CD20+ B cells and is the first and only therapy currently that has been approved for the treatment of both primary and progressive forms of MS [348,356–58]. However, therapies that directly target the inflammation in the central compartment in MS could potentially be more effective [347]. The BBB appears to be intact during the first 6 weeks of lesion formation in MS when activation of microglia and macrophages begins with very few peripheral lymphocytes infiltrating the brain at this stage [347,359]. Thus, strategies targeting passive immunotherapies to the CNS at the earliest stages of MS to prevent steps that lead to lesion formation could potentially alter the course of the disease but face the challenge of delivering antibodies across the BBB. Systemic immunosuppressive therapies that deplete lymphocyte populations also pose a risk of CNS infections [360] and investigating unique passive immunotherapy targets that prevent pathological infiltration of lymphocytes into the CNS without hampering CNS immune surveillance is crucial.

Ischemic strokes have the highest incidence among all cases of stroke and are caused due to obstruction of blood flow within cerebral vessels that may be attributed to one or more factors, e.g., fatty deposits along vessel walls that narrow the vessel lumen (atherosclerosis), elevated blood pressure, diabetes, and genetic predisposition. [361,362] The obstruction in the

cerebral blood vessel may be caused either due to the formation of a blood clot (thrombus) within the brain itself (referred to as cerebral thrombosis) or a blood clot that migrates from the periphery into the brain (referred to as a cerebral embolism) [363]. Arterial occlusion in stroke triggers a sequence of events leading to cell death and subsequent cognitive damage due to ischemia and neuroinflammatory responses in the brain [361]. Elimination of the cerebral blood clot either by dissolving it using a thrombolytic agent such as tissue plasminogen activator (tPA) or by surgical removal (embolectomy) are currently the most effective treatments available for ischemic stroke [362]. However, due to the narrow therapeutic time window (e.g., less than 4.5 hours post-stroke for tPA treatment) and invasiveness (e.g., embolectomy), currently available treatment options only benefit a small number of stroke patients [362]. Passive immunotherapies are an attractive means to modulate neuroinflammation and neuroplasticity in stroke patients both as a means to widen the therapeutic window of current revascularization strategies and to minimize brain damage [362]. For example, antibodies against myelin-associated proteins that inhibit neurite growth (such as Nogo-A and its receptor Nogo-66) are being explored as a way to increase neuroplasticity following stroke [364–366]. tPA therapy has side-effects such as cerebral edema and hemorrhage that may partly be attributed to its interaction with the N-methyl-D-aspartate (NMDA) receptor, resulting in Ca^{2+} ion influx and the triggering of cell death signaling cascades [367]. Antibodies that block the interaction between tPA and NMDA receptors are being investigated as a neuroprotective strategy in stroke [362]. Since the BBB often appears compromised following ischemic stroke [368], it may pose less of a barrier for therapeutic antibody delivery to brain target sites. However, it may very well be crucial to administer the antibody-based therapy at specific points in the time course of post-stroke pathological events for central delivery and efficacy. For instance, systemic administration of anti-Nogo-A antibodies immediately post-stroke during the hyperacute phase (when tissue damage pathways are more active than tissue repair) may result in deleterious rather than beneficial effects [364].

Traumatic brain injuries are typically grouped based on severity of the impact/injury as mild, moderate, or severe. Mild traumatic brain injury (TBI), commonly referred to as a concussion, is often caused by a blunt non-penetrating trauma and its repeated occurrence (often seen in athletes playing high impact contact sports) has been linked to neurodegenerative conditions such as chronic traumatic encephalopathy (CTE) [369,370]. Although primary mechanical injury in TBI may cause hematoma, edema, hemorrhage, and axonal injury, it is becoming apparent that secondary injury due to sterile neuroinflammation, excitotoxicity, and oxidative stress may ultimately result in more progressive long-term detrimental effects [371,372]. Passive immunotherapies that can inhibit neurotoxic innate and adaptive immune responses without disrupting physiological CNS immune surveillance are therefore being investigated as potential treatment strategies for TBI [372]. These strategies include intravenous immunoglobulin (IVIg) to ‘normalize’ the immune environment and monoclonal antibodies that can inhibit lymphocyte trafficking to the CNS (e.g., anti-CD20 and anti-CXCL10 antibodies) [372]. Finally, it bears noting that BBB disruption observed in TBI correlates with adverse neurological effects, so restoration of normal BBB function is also being investigated as a therapeutic strategy [373].

An important physiological distinction between proteinopathies such as AD, PD, and HD versus conditions such as MS, stroke, and TBI appears related to differences in the intactness of the CNS barriers. For example, work with experimental models and clinical studies have demonstrated that the BBB may be profoundly compromised over the course of disease progression in conditions like MS but remain relatively intact during the course of pathological processes associated with AD [274]. Future endeavors to translate passive immunotherapies for the treatment of CNS disorders will therefore have to take into consideration the spatial and temporal heterogeneity in the extent the BBB and the BCSFBs restrict antibody transport to brain target sites in different diseases and stages of pathology.

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CONFLICTS OF INTEREST:

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ABBREVIATIONS:

CNS	central nervous system
Ig	immunoglobulin
IgG	immunoglobulin G
Fc	crystallizable fragment
Fab	antigen binding fragment
FcRn	neonatal Fc receptor
sdAb	single domain antibody
BBB	blood-brain barrier
BCSFBs	blood-cerebrospinal fluid barriers
GBM	glioblastoma multiforme
VEGF	vascular endothelial growth factor

MMP-9	matrix metalloproteinase-9
FDA	Food and Drug Administration
PFS	progression free survival
BELOB	bevacizumab and lomustine for recurrent GBM
BTB	blood-tumor barrier
TCR	T cell receptor
MHC	major histocompatibility complex
APC	antigen presenting cell
Treg	regulatory T cell
CTLA-4	cytotoxic T-lymphocyte-associated protein 4
PD-L1	programmed cell death ligand-1
PD-1	programmed cell death-1
CD	classification determinant
CSF	cerebrospinal fluid
PCNSL	primary CNS lymphoma
HD-MTX	high dose methotrexate
WBRT	whole brain radiotherapy
ADC	antibody drug conjugate
EGFR	epithelial growth factor receptor
MMAF	cytotoxin monomethyl auristatin F
IL	interleukin
HER2	human epidermal growth factor receptor 2
CED	convection-enhanced delivery
ICV	intracerebroventricular
PE38	*Pseudomonas* exotoxin
AD	Alzheimer’s disease
Aβ	amyloid β-peptide
APP	amyloid precursor protein
sAPP	secreted amyloid precursor protein

CTF	carboxy-terminal fragment
AICD	amino-terminal APP intracellular domain
BACE1	beta-site APP-cleaving enzyme 1
CAA	cerebral amyloid angiopathy
PHF	paired helical filament
NFT	neurofibrillary tangle
APOE	apolipoprotein E
DIAN	Dominantly Inherited Alzheimer Network
ARIA	amyloid-related imaging abnormalities
TNF-α	tumor necrosis factor alpha
EVVP	external vertebral venous plexus
PD	Parkinson’s disease
GDNF	glial-derived neurotrophic factor
LAG3	lymphocyte-activation gene 3
PFF	preformed fibril
ECS	extracellular space
HD	Huntington’s disease
HTT	huntingtin gene
mHTT	mutant huntingtin protein
SEMA4D	semaphorin 4D protein
TrkB	Tropomyosin receptor kinase B
BDNF	brain-derived neurotrophic factor
SOD	superoxide dismutase
RMT	receptor-mediated transport
MiNDS	miniaturized neural drug delivery system
CLN2	ceroid lipofuscinosis type 2
ISF	interstitial fluid
IGF1	insulin-like growth factor 1
IGFBP-2	insulin-like growth factor binding protein 2

PVS	perivascular space
IT	intrathecal
IN	intranasal
RECA-1	rat endothelial cell antigen-1
GFAP	glial fibrillary acidic protein
tPA	tissue plasminogen activator
DAPI	4', 6-diamidino-2-phenylindole
NMDA	N-methyl-D-aspartate

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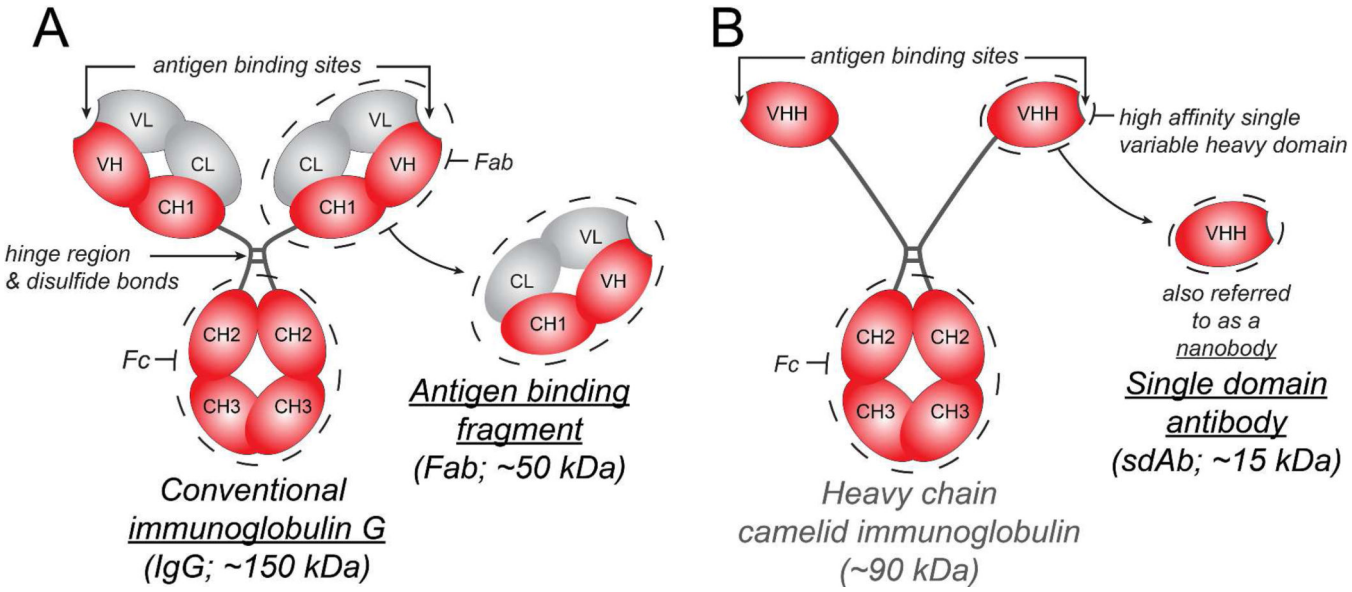

Figure 1.

Summary of IgG, Fab, and sdAb structure and sizes. (A) Full length IgG is a Y shaped molecule made up of four polypeptide chains – two heavy chains (red) and two light chains (grey) that are linked by disulfide bonds. Each polypeptide chain has constant domains (C) and variable domains (V). There are two Fab arms, each containing an antigen-binding site made up of the variable domains of the heavy and light chains, which can recognize antigens with high specificity. The crystallizable fragment or Fc arm can interact with Fc receptors. (B) Camelids, sharks and other cartilaginous fish (Chondrichthyes) produce a unique IgG molecule consisting of heavy chains alone. A camelid IgG molecule is depicted here. A single heavy chain variable domain is also referred to as a single domain antibody or nanobody. Unlike the antibody variable domains in other species, camelid and cartilaginous fish variable domains do not aggregate when isolated and retain their antigen binding capacity; this has generated interest in their use as therapeutics when a smaller size and no Fc interactions are desired [10].

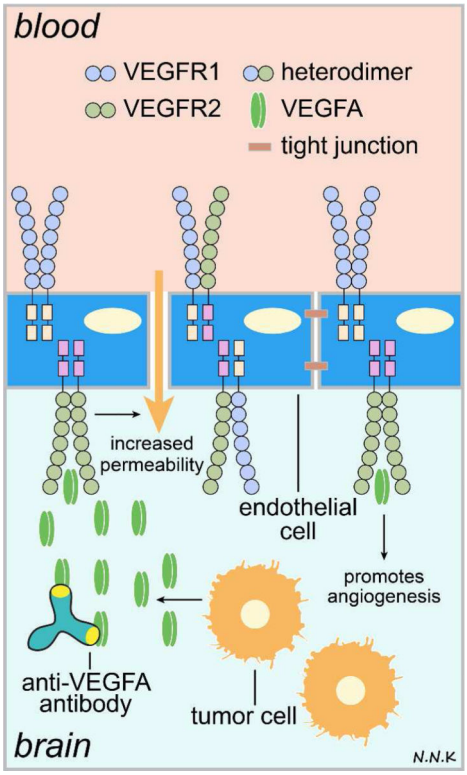

Figure 2. Passive immunotherapy strategies for brain cancer using anti-angiogenic antibodies.

VEGFA binding to VEGFR2 triggers an increase in paracellular permeability, downregulation of tight junctional proteins, and the promotion of angiogenesis. Anti-VEGFA antibodies can bind to VEGFA and prevent angiogenesis thus inhibiting tumor growth and survival. Adapted from: [51]. Abbreviations: VEGF – vascular endothelial growth factor; VEGFR – VEGF receptor.

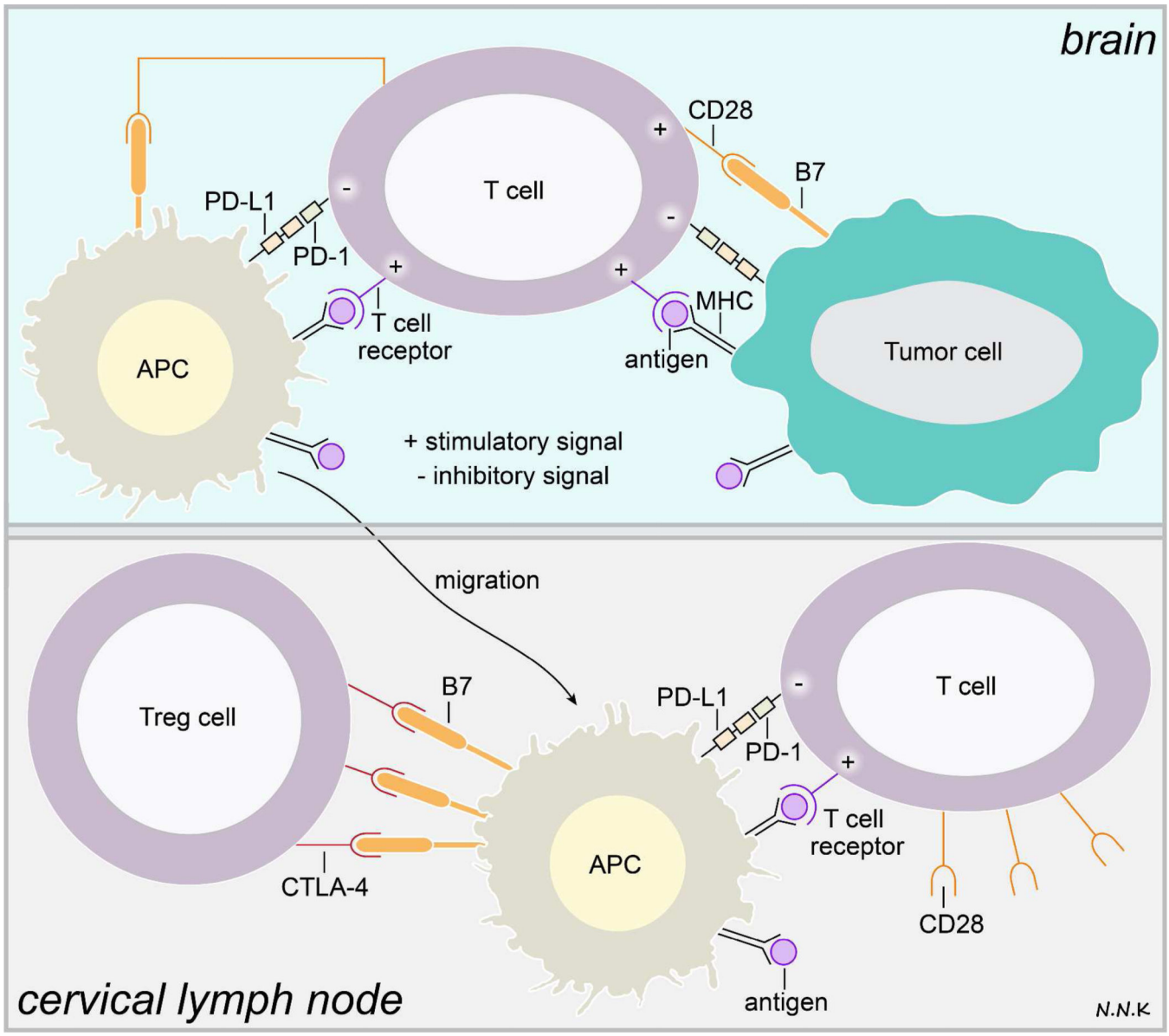

Figure 3. T cell immune response and immune checkpoints in brain cancer.

T cells may recognize tumor antigen peptides presented via MHC class I/II molecules on tumor cells or antigen presenting cells (APCs) via the TCR, resulting in a weak immune stimulatory signal. Interaction between the TCR and tumor antigen peptide/MHC complex can only activate the T cell in the presence of other co-stimulatory immune signaling. However, tumor cells and APCs in the tumor microenvironment express high levels of programmed cell death-ligand-1 (PD-L1), a ligand for the programmed cell death - (PD-1) receptor expressed by T cells, which inhibits T cell activation. APCs presenting the tumor antigen peptide/MHC complex may migrate to the cervical lymph nodes where T cells recognizing the tumor antigen may be activated and directed to the tumor. In addition to the TCR-tumor antigen/MHC interaction, the T cell must receive co-stimulatory signals in order to be activated. This co-stimulatory signal is typically received when the classification

determinant 28 (CD28) receptor on T cells interacts with the B7 ligand expressed by APCs. However, regulatory T cells (Treg cells) express high amounts of cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) – a receptor that mimics CD28 and has an even higher affinity for the B7 ligand. Thus, CTLA-4-B7 interaction can compete with the CD28-B7 interaction, resulting in the lack of appropriate co-stimulatory signaling to activate tumor antigen recognizing T cells. Adapted from: [53–55]. Abbreviations: PD-1 – programmed cell death protein-1; PD-L1 – programmed cell death protein ligand-1; CTLA-4 – cytotoxic T-lymphocyte-associated protein 4; Treg – regulatory T cells; CD28 – classification determinant 28.

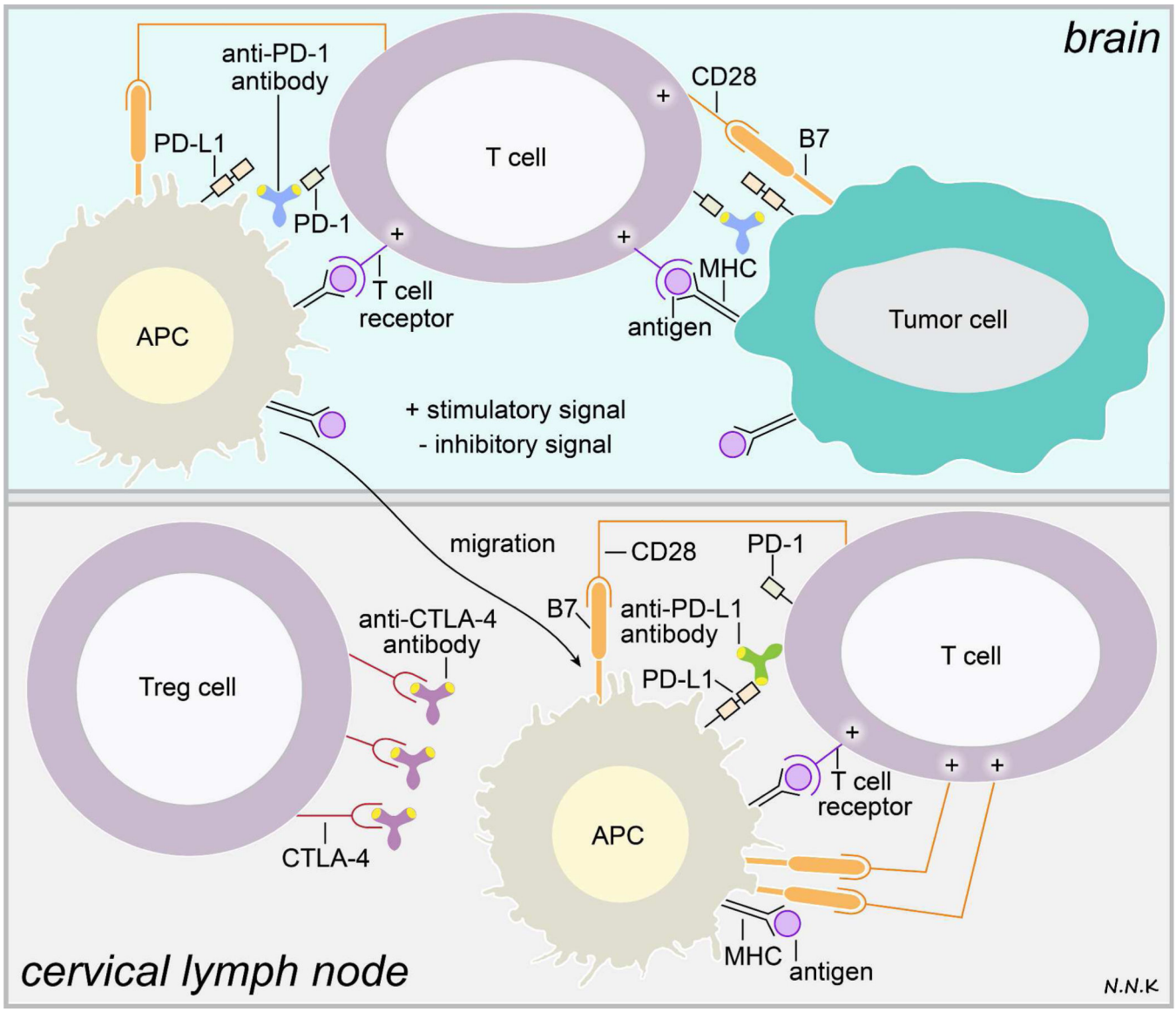

Figure 4. Passive immunotherapy strategies for brain cancer using immune checkpoint inhibitory antibodies.

Interactions between T cells, antigen presenting cells (APCs), and tumor cells that inhibit appropriate activation of T cell cytotoxic immune responses may be modulated via passive immunotherapy. For example, anti-PD-1 antibodies can bind to the PD-1 receptor that is expressed by T cells and disrupt PD-1's interaction with its ligand PD-L1, which is highly expressed on tumor cells and APCs in the tumor microenvironment. Alternatively, anti-PD-L1 antibodies can neutralize the PD-L1 ligand's ability to bind to PD-1. Anti-CTLA-4 antibodies may be used to block the interaction between the CTLA-4 receptor on Treg cells and the B7 ligand on tumor cells and APCs; this would subsequently allow B7 interaction with the CD28 receptor on T cells, which provides a stimulatory signal for T cell activation. Adapted from: [53–55]. Abbreviations: PD-1 – programmed cell death protein-1; PD-L1 – programmed cell death protein ligand-1; CTLA-4 – cytotoxic T-lymphocyte-associated protein 4; Treg – regulatory T cells; CD28 – classification determinant 28.

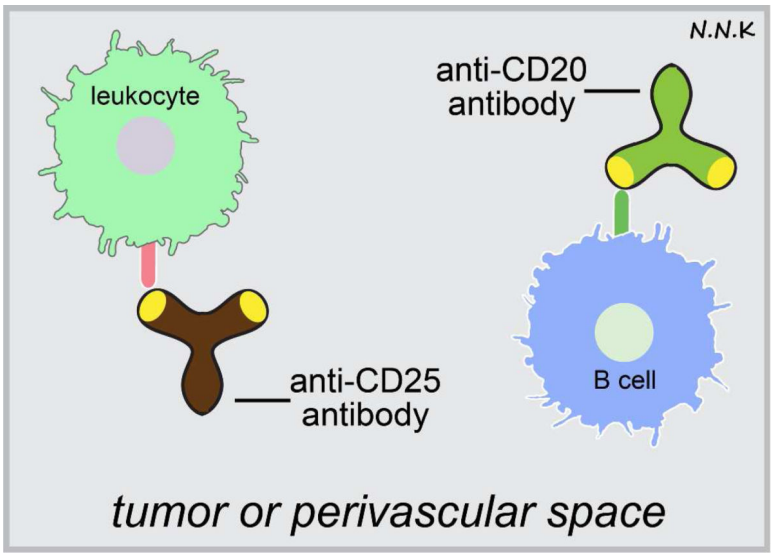

Figure 5. Passive immunotherapy strategies for antibodies recognizing lymphocyte antigens.
Antibodies that recognize molecules expressed by malignant infiltrating lymphocytes may be used to treat certain CNS lymphomas. Adapted from: [74,78].

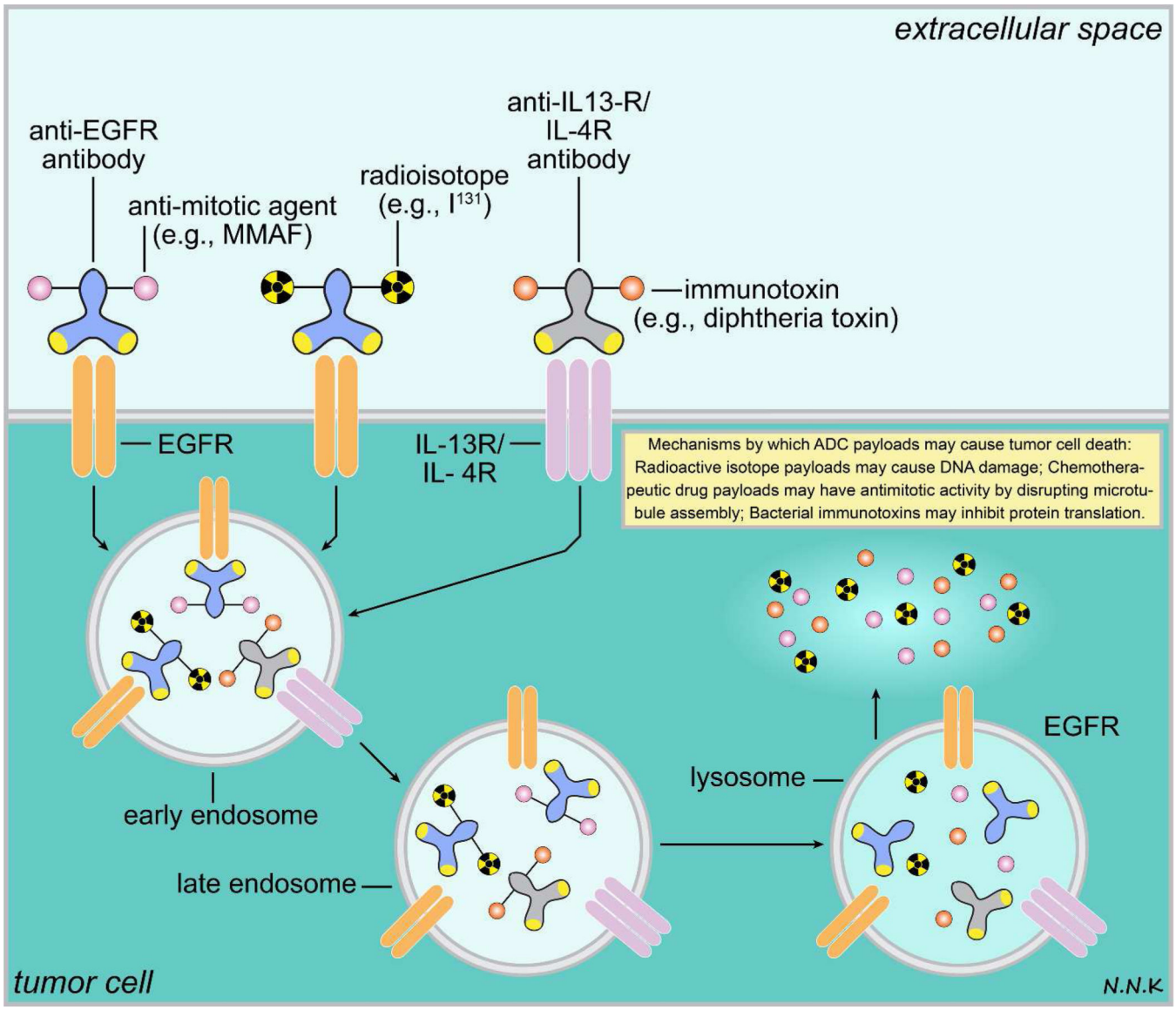

Figure 6. Passive immunotherapy strategies for brain cancer using antibody drug conjugates (ADCs).

ADCs combine the ability of antibodies to recognize specific antigens overexpressed by tumor cells (e.g., EGFR, IL-13R, or IL-4R) and the ability to deliver a cytotoxic payload that can lead to tumor cell death or arrest tumor growth. Typically an ADC has 3 main components – an antibody that can recognize a tumor antigen, a linker, and a cytotoxic payload. The cytotoxic payloads may be radioisotopes (e.g., I^{131}) that can cause DNA damage within the tumor cell, bacterial immunotoxins (e.g., diphtheria toxin) that may interfere with microtubule assembly or protein translation, or anti-tumor chemotherapeutic drugs (e.g., MMAF). Since ADCs can deliver a cytotoxic payload to the tumor target with high specificity they minimize off-target effects. Adapted from: [81]. Abbreviations: EGFR – epidermal growth factor receptor; IL – interleukin; I^{131} – iodine radioisotope; MMAF – monomethyl auristatin F

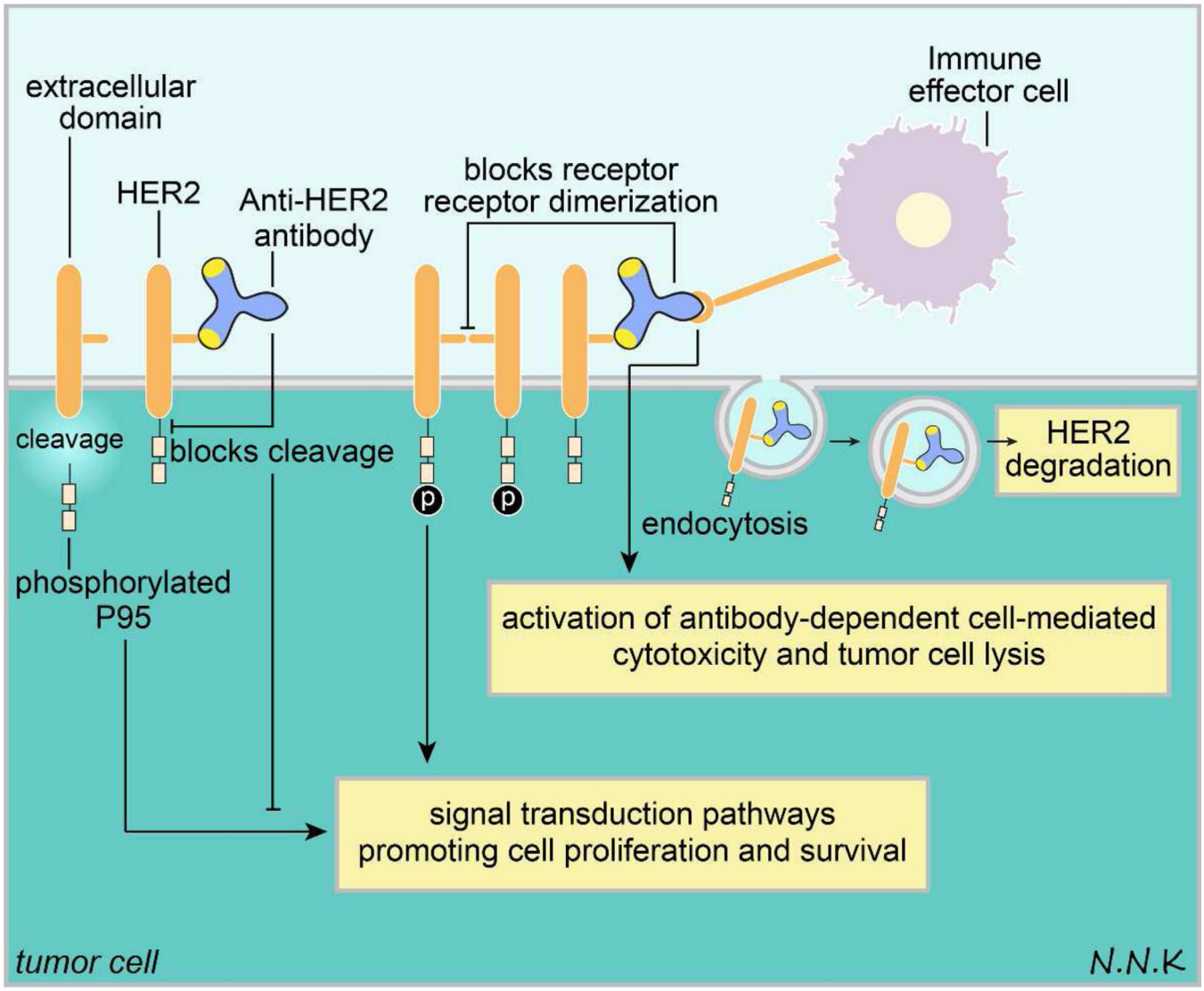

Figure 7. Passive immunotherapy strategy to treat breast cancer brain metastases using anti-HER2 antibodies.

HER2 overexpressing breast cancer brain metastases may be treated with anti-HER2 antibodies. Anti-HER2 passive immunotherapy may have several effects. First, HER2 homo or hetero dimerization that drives downstream signaling that promotes tumor cell survival may be disrupted using anti-HER2 antibodies. Second, the extracellular domain of HER2 is typically shed in tumor cells, leaving behind a phosphorylated P95 that is membrane bound and can drive downstream signaling promoting tumor cell growth and survival; anti-HER2 antibodies can bind to the HER2 extracellular domain and prevent its cleavage. Third, anti-HER2 antibodies may bind to HER2 expressed on tumor cell surfaces and initiate an Fc-mediated immune effector function that targets tumor cells. Fourth and finally, anti-HER2 antibodies may bind to HER2 and cause its internalization by endocytosis, resulting in HER2 degradation. Adapted from: [91]. Abbreviations: HER2 – human epidermal growth factor receptor 2.

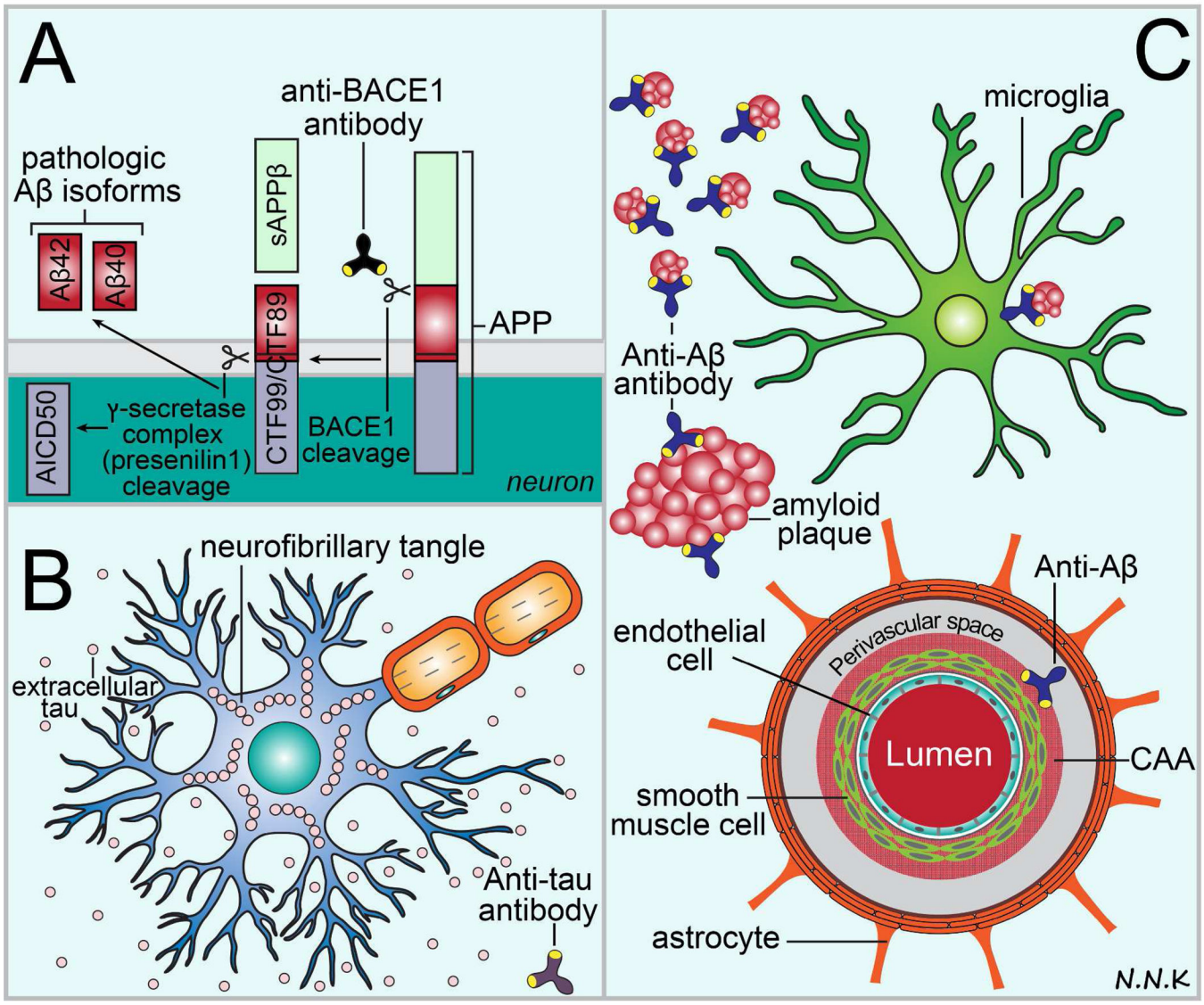

Figure 8.

Passive immunotherapy strategies to treat Alzheimer's disease (AD). Some of the major hallmarks of pathology in AD include: (i) excess production of amyloid β-peptide (Aβ) fragments catalyzed by the beta-site APP-cleaving enzyme 1 (BACE1) and γ-secretase enzyme complex cleavage of the amyloid precursor protein (APP); (ii) accumulation and aggregation of hyperphosphorylated tau within neurons leading to cell death and cell-to-cell transmission of extracellular tau; and (iii) accumulation and aggregation of Aβ within the brain parenchyma (Aβ42) and the perivascular compartments of cerebral arteries (Aβ40). Passive immunotherapy may be used to target these different features of AD pathology. (A) Anti-BACE1 antibodies can be used to block the BACE1 cleavage of APP and thus minimize abnormal and excess production of Aβ fragments. (B) Anti-tau antibodies that target hyperphosphorylated tau can be used to block intracellular tau aggregation (likely using intrabodies [169]) and prevent the extracellular cell-to-cell transmission of pathologic tau (conventional antibodies [163,164]). (C) Anti-Aβ42 antibodies can be used to target Aβ42 in

the brain parenchyma and halt or reverse disease pathology by aiding microglia mediated Aβ42 clearance via Fc interactions, binding to monomers and oligomers and preventing their aggregation, and resolving plaques via serine protease activity. Anti-Aβ40 antibodies may be used to target Aβ40 accumulation in the perivascular compartment of cerebral arteries (also referred to as cerebral amyloid angiopathy or CAA) in a similar manner. Adapted from: [136,183–185].

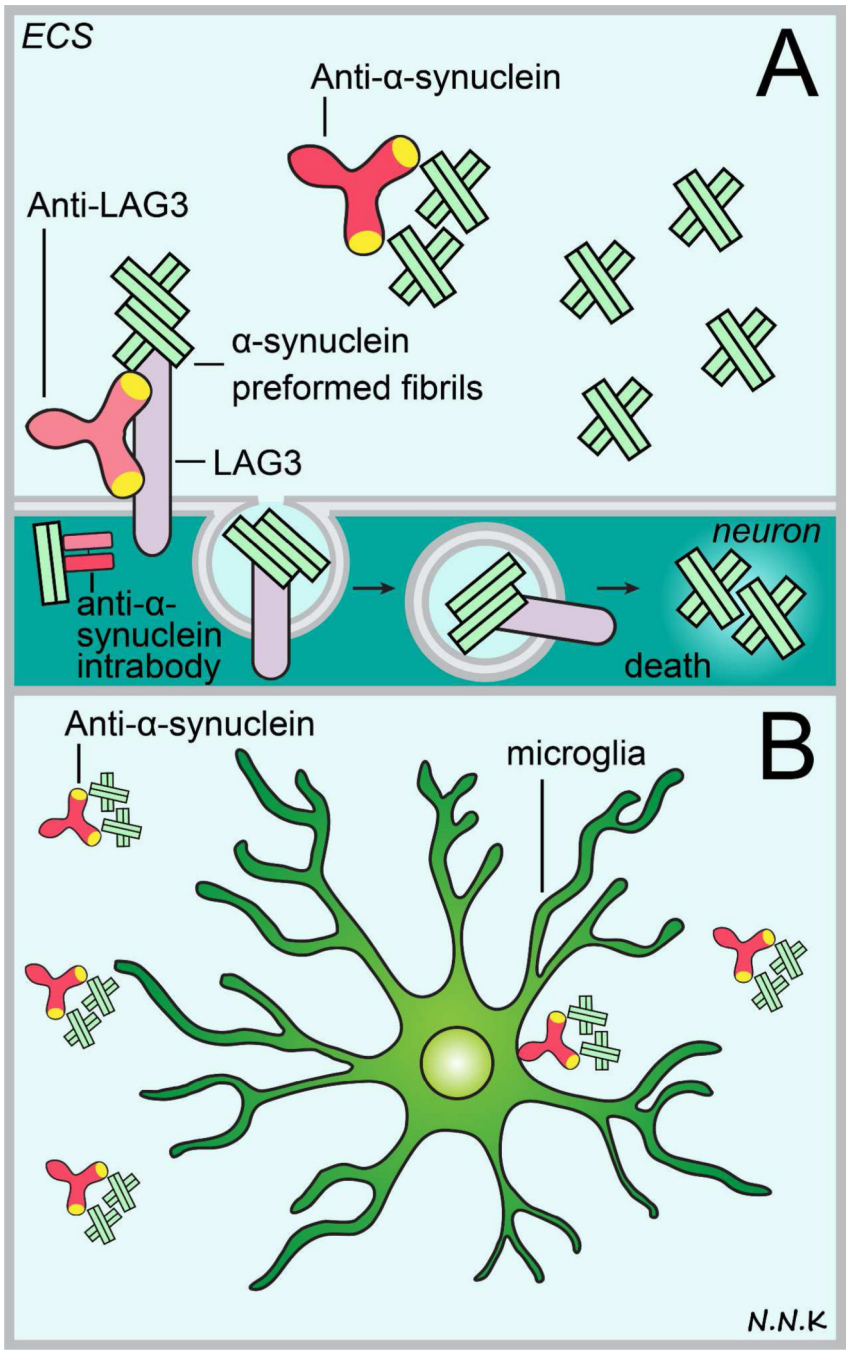

Figure 9. Passive immunotherapy strategies to treat Parkinson's disease.

Disease pathology in Parkinson's disease typically entails the accumulation and aggregation of abnormal alpha synuclein protein, subsequently leading to neuronal cell death and cognitive decline. Anti-alpha synuclein antibodies may be used to block the intracellular aggregation of abnormal alpha synuclein which typically leads to the formation of intracellular Lewy bodies (thus the most likely strategy would be to use intrabodies) or prevent the cell-to-cell transmission of extracellular abnormal alpha synuclein (using conventional antibodies). Extracellular anti-alpha synuclein antibodies may prevent

abnormal alpha synuclein monomers and oligomers from aggregating further and may recruit microglia to phagocytose abnormal protein via Fc mediated interactions. Lymphocyte activation gene 3 (LAG3) protein was recently implicated in the internalization of pathologic alpha synuclein during cell-to-cell transmission so an anti-LAG3 antibody strategy may therefore be promising to prevent the spread of alpha synuclein pathology. Abbreviations: ECS – extracellular space. Adapted from: [217,231].

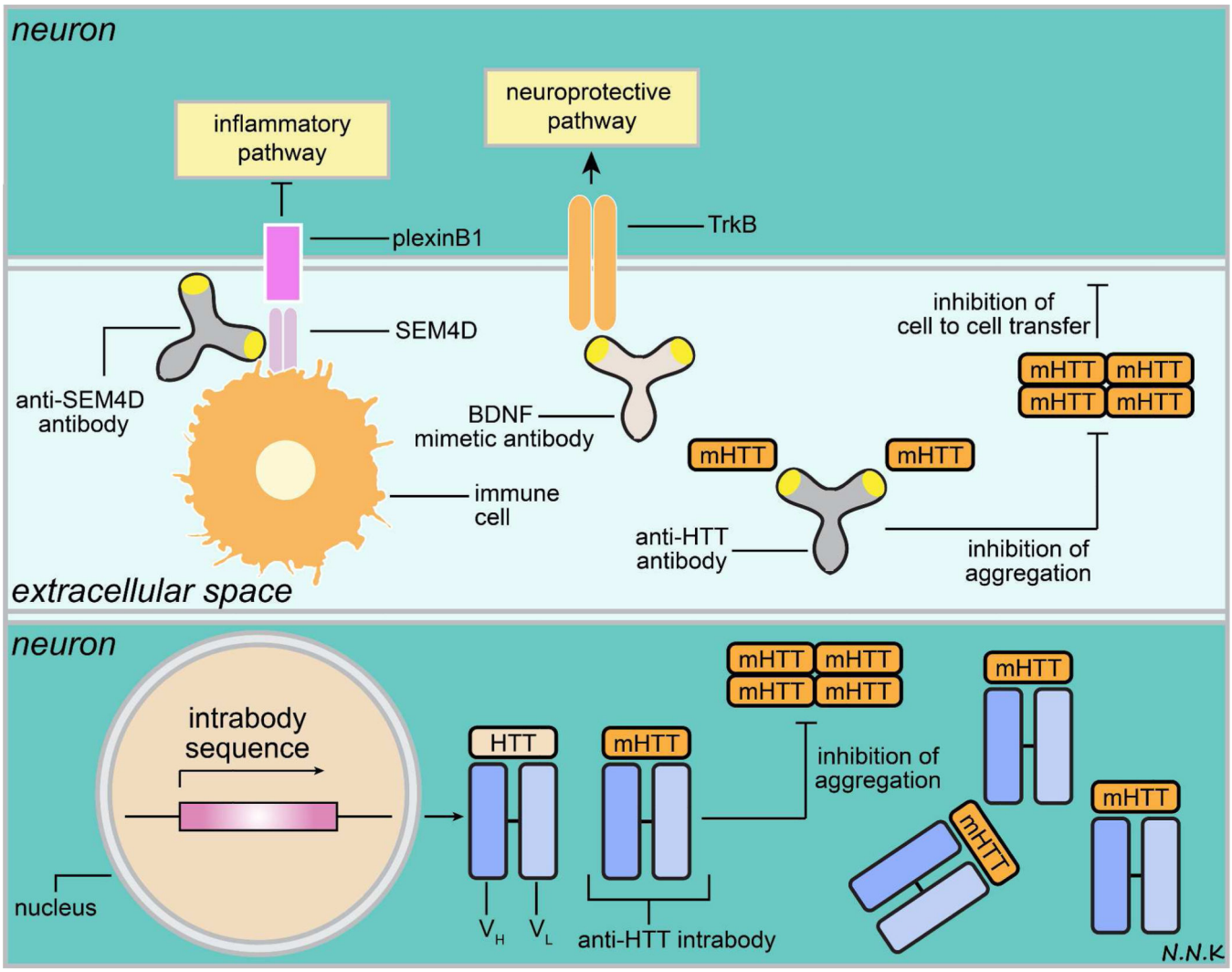

Figure 10. Passive immunotherapy strategies to treat Huntington's disease.

Huntington's disease (HD) pathology is characterized by the intracellular accumulation and aggregation of the mutant huntingtin protein (mHTT), which results in subsequent cell death, and the spread of pathology due to cell-to-cell transmission of extracellular mHTT. Other hallmarks of HD pathology include pro-inflammatory signals (e.g., SEM4D/plexinB1 signaling pathway), and down-regulation of cell survival/neurotrophic signals (e.g., BDNF/TrkB signaling pathway). HD progression may potentially be blocked by passive immunotherapy strategies that target one or more aspects of this pathology. For example, anti-HTT antibodies (e.g., intrabodies) may be used to target intracellular mHTT. It is important to note that anti-HTT intrabodies typically bind to both normal HTT and mHTT. The ratio of mHTT to normal HTT is indicative of HD pathology and mHTT mRNA transcripts were found to exceed normal HTT in the cortex and striatum of nearly 75% patients in an HD clinical study [257]. The increase in mHTT compared to normal HTT may be attributed to increased transcription of the mHTT allele, or decreased clearance of mHTT, or both [257]. Therefore, engineering antibodies that recognize and bind with higher affinity to mHTT than normal HTT may be important since an equimolar inhibition of mHTT and

normal HTT may increase the mHTT to normal HTT ratio [257]. Additionally, normal HTT is thought to play a role in promoting cell survival and depleting it may further exacerbate disease pathology and clinical outcome [258].

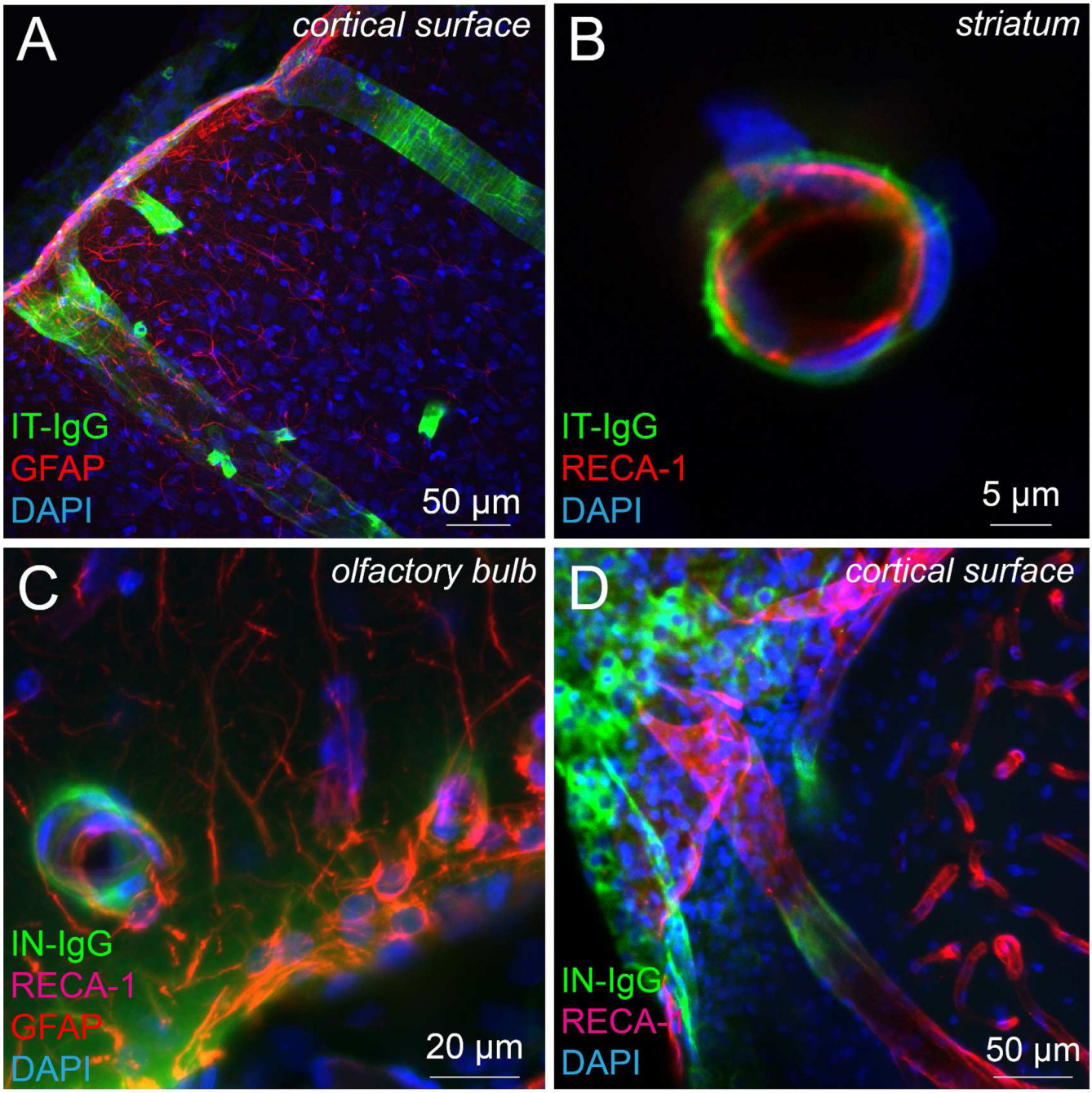

Figure 11. Immunoglobulin G (IgG) access to the perivascular space (PVS) surrounding cerebral blood vessels following intrathecal and intranasal delivery.

(A, B) Examples of blood vessels at the rat the cortical surface and in the striatum respectively showing intrathecally administered rat IgG accessing the PVS. (C, D) Examples of blood vessels in the rat olfactory bulb and at the cortical surface respectively showing intranasally administered rat IgG accessing the PVS. Abbreviations: IT – intrathecal; IN – intranasal; RECA-1 – rat endothelial cell antigen-1 (endothelial cell marker); GFAP – glial

fibrillary acidic protein (astrocyte marker); DAPI – 4', 6-diamidino-2-phenylindole (cell nucleus marker).

Table 1:

Strategies for delivering passive immunotherapies to the CNS (adapted from [263]).

Delivery strategy for CNS penetration/distribution	Advantages	Disadvantages
Parenteral systemic administration (IV infusion, SC/ IM injection)		
Passive systemic delivery: passive transport across the BBB and BCSFBs and subsequent diffusion within the brain parenchyma	Well characterized and commonly used route of administration; global brain delivery; conventional antibody production	Less than 0.1% of systemically administered exogenous antibody penetrates the brain parenchyma [156]; high doses required; possible side-effects (microhemorrhages, edema); pleiotropic effects/no targeted region specific delivery; neutralizing anti-drug antibodies may affect pharmacokinetics and diminish effects with chronic dosing
Receptor-mediated transport (RMT): bispecific antibodies and fusion proteins targeting transcytosis receptors expressed at the BBB allow the antibody/ fusion protein to be shuttled across the BBB 282,285	Well characterized and commonly used route of administration; global brain delivery; enhancement of delivery compared to passive transport across CNS barriers	Antibody engineering and selection of an appropriate RMT pathway is required; expression of transcytosis receptors in the periphery poses a risk of off-target side effects [106,286]; antibodies may need to have a low affinity to the transcytosis receptor to escape the endothelial compartment and enter the brain parenchyma consequently requiring high doses [190]; pleiotropic effects/no targeted region specific delivery; neutralizing anti-drug antibodies may affect pharmacokinetics and diminish effects with chronic dosing
BBB disruption strategies (MRI-guided focused ultrasound with microbubbles; hyperosmolar mannitol)	Enhancement of delivery compared to passive transport across CNS barriers; global brain delivery (hyperosmolar mannitol infusion) or region specific delivery (MRIguided focused ultrasound with microbubbles)	Requires expertise and/or additional equipment to apply and monitor BBB disruption strategies; BBB disruption poses a risk since it allows non-specific entry of plasma proteins/ macromolecules into the CNS which may subsequently cause neurotoxicity [280]
Central administration (bypassing BBB and BCSFBs)		
Intraparenchymal administration (infusion via convection enhanced delivery; implantation of polymer release system)	Relatively targeted delivery at site of administration (e.g., potentially advantageous while targeting brain tumors); limited peripheral side effects	Limited diffusion away from the site of administration prevents global delivery; Surgically invasive; restricted volume of administration necessitates reloading for chronic dosing; risk of infection
Intracerebroventricular (ICV) administration	Duration and rate of administration into the CSF can be controlled using a device (e.g., a Rickham reservoir or Ommaya reservoir) that allows isovolumetric and chronic dosing into the ventricles via a port implanted under the scalp in the subgaleal space [304]; limited peripheral exposure; antibody-based therapeutic access to cerebral perivascular spaces may allow more global and rapid distribution within the brain relative to diffusion alone [122]	Surgically invasive; therapeutic needs to cross the ependyma, perivascular lining cells, and/or pia and glia limitans to access the brain parenchyma; reservoir reloading runs a risk of infection; possibility of device failure; diffusion out of the cerebral perivascular compartment and into the brain parenchyma may be restricted and dependent on several factors such as antibody size and interactions with receptors and extracellular matrix components, among others
Intrathecal administration	Duration and rate of administration into the CSF can be controlled using an infusion pump (e.g., Synchromed, Medtronic); antibody-based therapeutic access to cerebral perivascular spaces may allow more global and rapid distribution within the brain relative to diffusion alone [122]	Surgically invasive; therapeutic needs to cross the perivascular lining cells and/or pia and glia limitans to access the brain parenchyma; diffusion out of the cerebral perivascular compartment and into the brain parenchyma may be restricted and dependent on several factors such as antibody size and interactions with receptors and extracellular matrix components, among others; device failure and risk of infection
Intranasal administration	Non-invasive; some targeting to the CNS via direct nasal mucosae-to-brain pathways [213]; rapid distribution within the brain via access to cerebral perivascular compartment; lower costs due to ease of self-administration	Limited transport across the nasal epithelial barriers, rapid clearance from the nasal mucosae, inter- and intra-patient variability in CNS delivery efficiency; enzymatic degradation in the nasal passages; therapeutic needs to cross the perivascular lining cells and/or pia and glia limitans to access the brain parenchyma; diffusion out of the cerebral perivascular compartment and into the brain parenchyma may be restricted and dependent on several factors such as antibody size and interactions with receptors and extracellular matrix components, among others; novel route of administration with

Delivery strategy for CNS penetration/distribution	Advantages	Disadvantages
Parenteral systemic administration (IV infusion, SC/ IM injection)		
		preliminary characterization; pleiotropic effects/no targeted region specific delivery; potential immunogenicity