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Translational roadmap of BBB-targeted nanoparticle strategies for neuroregenerative therapy in neurodegenerative diseases

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Neuroregeneration has drawn scientific attention due to its therapeutic potential for neurodegenerative diseases such as Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), and traumatic brain injury (TBI). A major obstacle in delivering neuroregenerative and neuroprotective drugs is crossing the blood–brain barrier (BBB)—a selective, physiological barrier that protects the central nervous system (CNS) from circulating toxins and pathogens. While this protective role is essential for maintaining CNS homeostasis, it also limits therapeutic efficacy and increases the risk of systemic side effects due to off-target accumulation. To overcome these challenges, recent advances in nanoparticle engineering have focused on enhancing BBB transcytosis by employing biologically inspired surface modifications. In this review, we highlight three mechanistically distinct approaches: (1) transporter-mediated transcytosis (TMT), which uses glucose or amino acid conjugation; (2) receptor-mediated transcytosis (RMT) *via* ligands such as transferrin or angiopep-2; and (3) adsorptive-mediated transcytosis (AMT), utilizing cationic polymer coatings or cell-penetrating peptides (CPPs).

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1. Introduction

As the prevalence of neurodegenerative diseases continues to increase, the lack of therapies that can restore neuronal function underscores the critical importance of developing regenerative approaches. Since current therapeutic strategies are centered on symptom management rather than neuronal restoration, the need for neuroregenerative approaches has become increasingly critical.[1] Neuroregeneration has drawn scientific attention due to its therapeutic potential for neurodegenerative diseases such as Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), and traumatic brain injury (TBI). The blood–brain barrier (BBB), while physiologically essential for protecting the central

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nervous system from toxins and pathogens, paradoxically restricts the entry of therapeutic agents, creating a major obstacle.[2,3] Among the different approaches explored so far, transcytosis-based medication delivery has emerged as one of the most promising approaches for overcoming this obstacle. By utilizing the BBB's own transport mechanisms, this approach offers a potentially safer and more targeted alternative than invasive or disruptive methods. In this perspective review, we provide not only an overview of the three main transcytosis methods for neuroregeneration but also a framework for prioritizing their applicability. In addition, we discuss the translational factors that need to be considered for successful clinical application. While bio-derived carriers, such as exosomes and cell-based delivery systems, have shown promise for BBB crossing, they present distinct translational challenges, including batch-to-batch heterogeneity, limited standardization in characterization and cargo loading, and evolving regulatory considerations. In this perspective, rather than providing a comprehensive catalogue of BBB delivery strategies, we propose a decision-oriented framework that reframes BBB transcytosis as a context-dependent process. Specifically, we argue that the translational value of transporter-, receptor-, or adsorptive-mediated pathways critically depends on disease type, disease stage, downstream parenchymal barriers, and clinical feasibility, rather than BBB permeability alone.

2. Comparative analysis of BBB-crossing strategies: strengths and weaknesses for neuroregeneration

Although transporter-, receptor-, and adsorptive-mediated transcytosis are often discussed as parallel or interchangeable strategies, as schematically illustrated in (Fig. 1), we posit that these mechanisms represent fundamentally different translational trade-offs rather than equivalent options. In this section, we compare these pathways not by delivery efficiency alone but by their susceptibility to disease-associated BBB alterations, off-target distribution, mechanistic uncertainty, and clinical scalability. This comparison forms the mechanistic foundation for the translational roadmap proposed later in this perspective.

2.1 Transporter-mediated transcytosis (TMT)

Transporter-mediated transport (TMT) utilizes endogenous carrier proteins, such as GLUT1 and LAT1, to facilitate the directional movement of therapeutics across the BBB.[4–7] A recent related example is the use of mannose-modified PLGA–PEG nanoparticles designed to target GLUT1 recycling.[8] In an AD mouse model, oral administration of this formulation enhanced brain delivery of the immunomodulatory drug Fingolimod (FTY720), leading to therapeutic effects such as microglial polarization toward an anti-inflammatory phenotype, normalization of reactive astrocytes, and improved amyloid-β clearance. However, the study could not directly demonstrate GLUT1-mediated transport due to methodological limitations. Moreover, according to other research studies, GLUT1 expression is reduced in AD patients,[9–14] and increased in the liver, which may lead to off-target specificity. Another study utilized the LAT1 pathway to deliver an antisense oligonucleotide (ASO) inhibiting miR-485-3p.[15] A PEG–PLL copolymer nanoparticle modified with phenylalanine ligands was developed to engage LAT1, forming polyion complexes that self-assemble into nanoparticles. The system was able to penetrate the BBB with up to 7.0% ID g^{-1} accumulation in the brain—64-fold higher than naked ASO. However, LAT1 is widely expressed in peripheral organs such as the liver, kidneys, and lungs, raising concerns about non-specific distribution. The study was not conducted in models of neurodegenerative disease, limiting its translational relevance, and also the exact molecular mechanism of LAT1-mediated nanoparticle transport remains uncertain.[16]

In conclusion, LAT1 has a high capacity for uptake and GLUT1 has the possibility for non-invasive oral administration; yet, both have challenges of specificity, disease-context variability, and mechanistic uncertainty. Nevertheless, TMT still has distinct advantages in its capacity not only to penetrate the BBB but also to deliver therapeutic reagents directly to brain parenchymal cells such as neurons, astrocytes, and microglia. For example, as compared to parent drugs, LAT1-based designs that conjugate amino acid-mimetic structures to drugs have significantly better brain cell uptake.[7]The BBB also exhibits high levels of GLUT1 expression, and glucosylated nanocarriers have been shown to enhance brain accumulation by 56-fold.[17] Additionally, systemic glucose levels can regulate GLUT1 distribution and activity, indicating that BBB penetration may be improved by physiological modulation (*e.g.*, fasting-glucose challenge).[17] However, because these strategies are based on single transporters, they are susceptible to endogenous substrate competition. For instance, the presence of competitive substrates like methylammonium significantly

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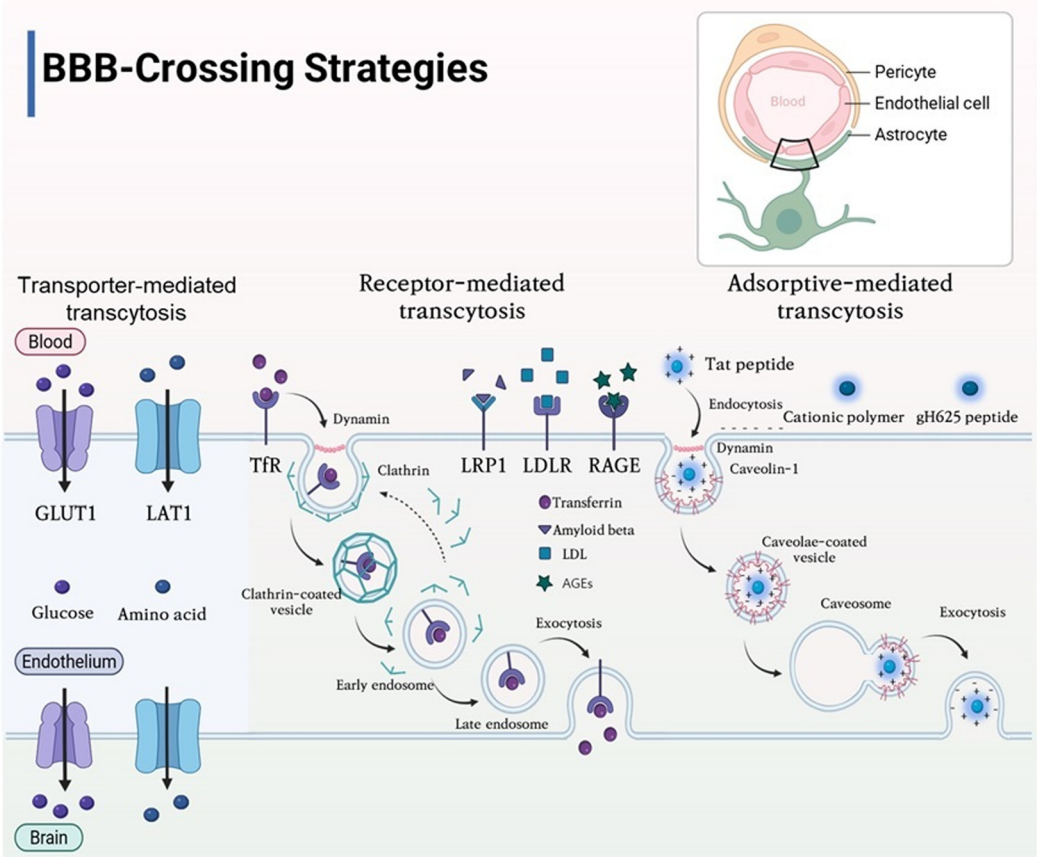

Fig. 1 Schematic illustration of drug transport across the BBB *via* distinct transcytosis mechanisms, including transporter-mediated (TMT), receptor-mediated (RMT), and adsorptive-mediated (AMT) pathways. Adsorptive-mediated transcytosis (AMT) is illustrated as a charge-driven pathway that often involves HSPG-mediated endocytosis, including lipid raft- and caveolae-associated transcytosis mechanisms, rather than direct membrane penetration. Figure created using BioRender (https://biorender.com/).

decreased transport efficiency.[18] This shows a fundamental drawback of TMT: while it can be a route for BBB penetration, maintaining selectivity and overcoming substrate competition remain critical obstacles for further development.

2.2. Receptor-mediated transcytosis (RMT)

Receptor-mediated transcytosis (RMT) relies on receptor–ligand interactions and enables the transport of macromolecules such as transferrin and insulin across the BBB.[19,20] Among the available targets, the transferrin receptor (TfR) has been investigated most extensively.[21] In a TBI model, researchers evaluated a TfR-targeted polypeptide liposome (TPL) as a carrier for the anti-inflammatory agent fluvoxamine (Flv). Intravenous administration of TPL-Flv facilitated BBB penetration in a TfR-dependent manner, as uptake was attenuated by the TfR inhibitor Ferristatin II. Behavioral assessments further demonstrated that TPL-Flv treatment improved BBB integrity and promoted functional recovery *in vivo*. Despite these promising findings, TfR-based approaches are limited by competition with endogenous transferrin, which is abundant under physiological conditions. At higher dosages, receptor saturation may occur, and the extent of competition between therapeutic ligands and native transferrin *in vivo* remains incompletely understood.[22] Another example of an RMT-based BBB penetration method is using LRP1 (low-density lipoprotein receptor-related protein 1). PLGA-PLL nanoparticles were conjugated with angiopep-2 ligand encapsulated simvastatin, and they were delivered into an AD mouse model. The nanoparticles were able to reduce soluble Aβ 40/42 levels and suppress astrocyte and microglial activation. Also, *in vitro* assays validated LRP1-mediated uptake.[23] However, LRP1 is expressed not just at the BBB but is also abundant in peripheral tissues such as the liver, kidneys, and lungs, so the challenge of non-specific distribution still remains. RMT strategies hold broad potential since they can be applied to diverse therapeutic modalities, including antibodies, enzymes, peptides, small molecules, and nanoparticles. By exploiting receptor binding, RMT allows versatile chemical or structural modifications, facilitating tailored design. Unlike passive diffusion or non-specific permeability enhancement, RMT provides active, receptor-specific transport, enabling precise targeting of brain endothelial cells and downstream parenchymal targets. Clinical safety is further supported by the results that RMT does not interfere with the BBB itself, making repeated or long-term

administration possible.[24–26] Furthermore, after 52 weeks of once-weekly intravenous administration, clinical studies have shown the safety of the anti-TfR-IDS fusion protein for patients with mucopolysaccharidosis II (MPS II).[25] The translation potential of preclinical findings is further enhanced by the high expression of receptors including TfR, INSR (insulin receptor), LRP1, and CD98hc in the BBB and their conservation in rodents, non-human primates, and humans.[27] Beyond single-target approaches, these features also create opportunities for multireceptor targeting approaches. However, because RMT depends on specific receptors, it has inherent limitations. First, there is limited receptor expression and insufficient BBB selectivity. Major receptors like TfR, LDLR, and LRP1 are abundantly distributed in peripheral tissues and the vascular system, which might result in systemic adverse effects and off-target uptake. Receptor saturation may happen at high dosages, which would restrict linear dose–response relationships. Additionally, cellular dysfunction and toxicity can occur from unintentional binding in nonbrain tissues, such as reticulocytes in the case of TfR. This kind of on-target toxicity reduces the effectiveness of treatment.[21] RMT efficiency is further restricted by endogenous ligand competition. For instance, transferrin directly competes with engineered TfR ligands, and the degree of competition may change under pathological conditions that disrupt iron homeostasis, thereby increasing translational barriers.[28] Finally, ligands with high binding affinities carry the risk of being delivered to lysosomes instead of transcytosis, which would lower the effectiveness of BBB penetration. These limitations emphasize the necessity of meticulous ligand engineering to avoid inefficient intracellular transport and maintain balanced binding affinity.[29]

2.3. Adsorptive-mediated transcytosis (AMT)

Adsorptive-mediated transcytosis (AMT) is commonly described as a charge-driven transport process in which cationic nanomaterials interact with the negatively charged surface of brain endothelial cells.[30,31] Accumulating evidence, however, indicates that this interaction is frequently mediated by heparan sulfate proteoglycans (HSPGs), which function as autonomous endocytic receptors for cationic ligands, including cell-penetrating peptides (CPPs). In this context, CPP-mediated BBB transport categorized under AMT predominantly occurs *via* energy-dependent endocytic pathways, such as lipid raft- or caveolae-associated mechanisms, rather than direct membrane translocation, as comprehensively reviewed by Christianson and Belting.[32] Cationic polymer coatings and the use of cell-penetrating peptides (CPPs) are common strategies. One instance is the delivery of PSD-95 inhibitors like NR2B9c and N-dimer (AVLX-144) *via* the Tat peptide, which is derived from HIV.[33] Despite the fact that the conjugation of these drugs to Tat enhanced endothelial internalization, their BBB penetration efficiency was still moderate. Tat-NR2B9c showed excellent membrane binding but low stability, whereas Tat-N-dimer was more stable but showed limited transport. These results highlight how difficult it is to find a balance between stability and uptake in CPP-based delivery systems. Another example is the development of chitosan-based nanocapsules containing donepezil, a cholinesterase inhibitor used to treat AD.[34] In a mouse model of chemically induced amnesia, the cationic surface increased interaction with endothelial cells and improved memory function. The study did not, however, explicitly validate BBB penetration, raising concerns over the delivery mechanism and its translational relevance. AMT's main advantage over receptor-mediated techniques is that it does not rely on specific receptors and therefore can facilitate the uptake of a variety of positively charged proteins, peptides, and nanoparticles. The process is comparatively fast and allows diverse cargos to enter brain endothelial cells with fewer structural restrictions.[35] CPPs are highly attractive because they are short, amphipathic, and positively charged peptides that serve as cargo carriers and can overcome intricate physiological barriers in a non-invasive, receptor-independent manner.[31] However, conventional CPPs are frequently trapped in endosomes, which restricts their effective intracellular release. In contrast, gH625, a cell penetrating peptide that originates from the herpes simplex virus's glycoprotein H, is internalized through a non-endocytic process that avoids endosomal entrapment. This facilitates effective cytosolic release and has been demonstrated to cross the BBB both *in vitro* and *in vivo*. Notably, some research has shown gH625 in the brain parenchyma as well as the cerebral vasculature.[31,36] Furthermore, without affecting the mitochondrial function of cell viability, conjugation of gH625 with multiple nanosystems (liposomes, dendrimers, nanofibers, *etc.*) has been shown to improve the delivery efficiency of chemotherapeutics and neuroprotective agents.[31,37,38] Additionally, a recent study reported that gH625 interacts with biomimetic membranes, selectively fusing with and penetrating BBB-mimicking membranes at low doses, and disrupts liver-mimicking membranes at high concentrations. This indicates that gH625 provides a degree of selectivity for the BBB, making it a suitable CPP for AMT-based strategies.[31] Nevertheless, gH625's neurodegenerative disease model validation is still limited, and more research is needed. However, the intrinsic non-specificity of AMT remains a significant drawback: limited target selectivity frequently results in reduced precision and widespread systemic distribution. Furthermore, there are opportunities to increase delivery efficiency by structural modification of drug molecules because AMT is fundamentally controlled by electrostatic interactions.[39] AMT's rapid clearance is yet another drawback. Fast blood clearance, aggregation, and high accumulation in the liver and lungs are common characteristics of cationic nanoparticles.[31] Therefore, circulation time, biodistribution, and the final CNS delivery efficiency are all significantly influenced by the number and type of surface charges on the nanosystem. In general, AMT-based strategies in the context of neuroregeneration highlight both possibilities and risks. While receptor-independent uptake can lead to rapid access during phases of inflammation or injury, its low specificity constrains sustained, cell-type-precise delivery required for circuit repair.

Practically, AMT may be most beneficial as an initial-entry strategy to deliver trophic factors or anti-inflammatory payloads under acute or inflammatory conditions (*e.g.*, TBI). This is followed by receptor or transporter-guided platforms (RMT/TMT) for cell-type specific support of synaptic repair and axonal regrowth. The challenges related to transporter-, receptor-, and adsorptive-mediated transcytosis represent not merely mechanistic or efficiency issues but crucial translational barriers that must be addressed in the progression from preclinical proof-of-concept to clinical application. Each strategy highlights distinct limitations—such as substrate competition, receptor saturation, and non-specific biodistribution—that ultimately converge as determinants of clinical feasibility and safety Table 1. Therefore, these limitations collectively define the key considerations that must be resolved for successful clinical transition. In the following section, we integrate these discussions to summarize disease-specific alterations of the BBB and propose the corresponding delivery strategies for each pathological context, thereby establishing the foundation for the clinical transition roadmap presented later in this review.

3. Disease- and stage-specific BBB alterations and delivery strategies

Rather than treating BBB dysfunction as a uniform barrier defect, we interpret disease-specific BBB alterations as dynamic variables that actively reshape the feasibility and desirability of different transcytosis strategies. Below, we synthesize quantitative and mechanistic evidence to highlight how identical delivery pathways may yield divergent translational outcomes across AD, PD, HD, and TBI. As the neurodegenerative disease progresses, the BBB undergoes structural and functional changes that have a significant impact on the effectiveness of several transcytosis pathways (Fig. 2). Therefore, it is crucial to adjust delivery methods based on the stage of the disease.

In mild cognitive impairment (MCI) and early Alzheimer's disease (AD), BBB leakage begins to emerge and reduced glucose transport *via* GLUT1 has been observed,[12,13] which reduces the efficacy of glucose-based TMT strategies. BBB disruption has been quantitatively documented using multiple human-relevant metrics. Dynamic contrast-enhanced MRI studies report region-specific increases in BBB permeability (K^{trans}), with increases of approximately 24–53% in patients with MCI and up to ~107% in hippocampal CA1 regions compared to the age-matched controls. Consistently, the CSF/plasma albumin ratios are increased by ~30% in MCI,[40] and MRI-detected microbleeds are observed in 25% of MCI and up to 45–78% of early AD patients, indicating that BBB dysfunction emerges early and persists throughout disease progression.[41]

On the other hand, elevated FATP1 expression has been documented in preclinical AD models, suggesting potential opportunities to enhance the endothelial-to-parenchymal

Table 1 Summary of transport mechanisms across the BBB: targets, strengths, and weaknesses for neuroregenerative applications

Type	Mechanistic targets and tools	Strengths	Weaknesses	Ref.
TMT	GLUT1	• Enables direct delivery to brain parenchymal cells (neurons, astrocytes, microglia) • High expression and physiological regulation at the BBB • Potential for oral administration (*e.g.*, GLUT1-targeted systems)	• Reduced transport efficiency due to substrate competition (*e.g.*, methylammonium) • Risk of off-target distribution (liver, kidney, lung) • Decreased transporter expression under disease conditions (*e.g.*, GLUT1 in AD)	4–7, 9 and 15–18
	LAT1	• Strong brain uptake (*e.g.*, LAT1 NP: 64× higher *vs.* naked ASO) • Utilizes endogenous transport mechanisms	• Unclear or incomplete understanding of molecular transport mechanisms • Dependency on a single transporter increases vulnerability	
RMT	TfR	• Applicable to diverse payloads (antibodies, peptides, enzymes, NPs) • Suitable for repeated administration (non-disruptive to the BBB) • Strong clinical translational linkage	• Limited BBB specificity due to peripheral receptor expression • Receptor saturation at high doses • Ligand competition with endogenous molecules (*e.g.*, transferrin)	21–23, 25 and 27–29
	LRP1	• Receptor expression conserved across species (rodents to humans) • Potential for multireceptor targeting strategies	• On-target toxicity risks (*e.g.*, reticulocyte binding) • Overbinding may lead to lysosomal degradation instead of transcytosis	
AMT	Cationic polymers (*e.g.*, chitosan NPs)	• Broad applicability due to receptor-independent uptake • Fast cellular entry *via* electrostatic interactions	• Low targeting specificity → systemic off-target distribution • Limited options for rational drug design	31 and 33–39
	CPPs (*e.g.*, Tat and gH625)	• Compatible with diverse nanocarriers (liposomes, dendrimers, *etc.*) • gH625 enables non-endocytic internalization and endosomal escape	• Rapid blood clearance and potential lung/liver accumulation • *In vivo* BBB penetration remains incompletely validated in many cases	

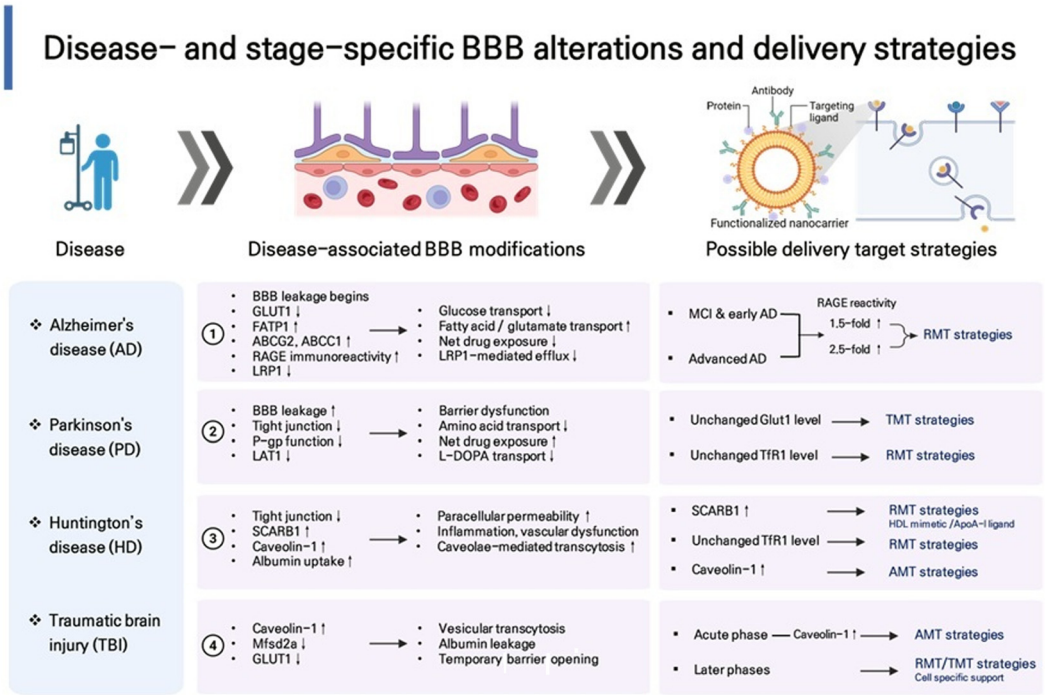

Fig. 2 Schematic illustration of disease-specific BBB alterations and potential delivery target strategies in major neurological disorders, including Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), and traumatic brain injury (TBI). Figure created using BioRender (https://biorender.com/).

release of fatty acid-mimetic or glutamate analogue cargos following endothelial uptake. At the same time, upregulation of efflux transporters such as ABCG2 and ABCC1 may reduce the net brain exposure of drugs given through these pathways, thus counteracting therapeutic entry.[42] According to reports, RAGE immunoreactivity in the brain endothelium increases gradually with RMT; it is approximately 1.5-fold higher in early AD and 2.5-fold higher in advanced stages when compared to the controls.[43,44] In contrast, AD patients and transgenic models have lower levels of LRP1 expression,[44–47] which limits the effectiveness of LRP1-mediated efflux of amyloid-β and other therapeutic payloads. Altogether, these results suggest that AD-related BBB changes present serious obstacles to RMT and TMT methods, but they also highlight the potential for specialized strategies that align transporter selection to pathology and disease stage.

In Parkinson's disease (PD), BBB alterations are more subtle and progressive than those observed under acute neuroinflammatory conditions but can nevertheless be quantitatively detected. PET imaging using [^{11}C]-verapamil has demonstrated an approximately 18% increase in tracer uptake in the midbrain, reflecting impaired P-glycoprotein (P-gp) efflux function.[48] In advanced stages, postmortem and imaging studies further reveal pronounced vascular leakage, with extravascular erythrocytes and plasma-derived proteins such as hemoglobin and fibrinogen increasing by approximately 7- to 9-fold compared with the controls.[49] Importantly, these BBB changes are minimal or absent in early PD but become more evident with disease progression, indicating a stage-dependent and cumulative BBB dysfunction rather than an abrupt barrier breakdown. Overall, in PD, increased BBB leakage, lower expression of tight junction, pericyte alterations, and a general decline in P-glycoprotein (P-gp) function occur.[50–52] There is no consistent evidence of either upregulation or downregulation of TMT in human illness or animal models, suggesting that GLUT1 expression is mostly unchanged in PD.[53] This suggests that glucose transporter-based strategies may be applicable in PD without significant disease-related restrictions. On the other hand, LAT1 expression in the capillary endothelium is decreased in PD models.[54,55] Since LAT1 is responsible for transporting L-DOPA across the BBB, its downregulation is thought to underlie impaired dopamine replenishment in PD. This finding indicates that LAT1-dependent nanocarrier approaches might not work as well in this particular illness setting. The data are less clear with RMT. In PD patients or in PD models, TfR1 and CD98hc have not consistently displayed disease-associated changes. The accumulation of iron in the brain and increased serum ferritin or transferrin saturation are commonly reported in clinical and postmortem studies.[56,57] However, it is unclear if they are due to changes in peripheral iron metabolism or BBB TfR1 expression. Therefore, while TfR1 does not seem to provide PD-specific selectivity, its relative stability across disease states and extensive prior validation in human trials suggest that it may still be an applicable RMT target in PD. Taken together, BBB changes in PD shows that P-gp reduction and general barrier dysfunc-

tion may increase CNS exposure to treatments, but decreased LAT1 restricts the use of amino acid transporter-based methods. In contrast, GLUT1 and TfR1 seem to be less impacted and could be more reliable delivery systems for the development of treatments for PD.

In Huntington's disease (HD), weakened tight junctions and increased paracellular permeability are early signs of BBB dysfunction.[58,59] Both HD patients and controls exhibit measurable levels of key RMT receptors, including TfR (TFRC/TFR1), LRP1, and LDLR, with no consistent indication of significant downregulation in HD. Also, in patients, CSF profiles show increased levels of blood-associated proteins when normalized to albumin (*e.g.*, prothrombin/Alb and ApoA-IV/Alb), and some measures correlate with clinical severity, supporting quantitatively altered barrier/CSF exchange under disease conditions.[60] Furthermore, in R6/2 mice, transcriptional dysregulation of tight junction components (occludin, claudin-5, ZO-1) emerges from the pre/early disease stages and becomes more pronounced with progression, consistent with an early onset of barrier vulnerability.[59] Research using iBMEC models derived from HD patients reported similar levels of TfR expression but a 2–5-fold increase in transferrin transcytosis.[61] This implies that enhanced TfR-mediated transport in HD may be facilitated by altered tight junction integrity or other trafficking pathways rather than receptor upregulation. SCARB1 (SR-BI), a receptor for HDL/ApoA-I that has been shown to be markedly upregulated in HD models, is another potentially relevant target.[61] Targeting strategies based on SCARB1 may be promising because of its link to inflammation and vascular dysfunction. Ligands produced from ApoA-I or HDL-mimetic nanoparticles may exhibit improved binding and uptake in the HD context. Furthermore, HD IPSC-BMEC models show improved albumin uptake and elevated caveolin-1 expression, suggesting a shift toward caveolae-mediated transcytosis.[62] Although this might give nanoparticles more physical access through AMT-like entry pathways, such pathways are inherently non-selective and present issues with off-target uptake and peripheral sequestration. Therefore, delivery *via* a combination of methods is suggested by BBB changes in HD. While conventional RMT targets like TfR are still viable, caveolae activation and SCARB1 overexpression may create alternative delivery pathways for nanoparticles. At the same time, the low specificity of these alternative routes highlights the need to carefully design strategies to achieve a balance between efficiency and safety.

In traumatic brain injury (TBI), BBB disruption is characterized by a pronounced but temporally dynamic increase in permeability. Quantitative imaging studies report marked elevations in BBB permeability metrics such as K^{trans} during the acute and subacute phases, accompanied by increased CSF/plasma albumin ratios indicative of macromolecular leakage.[63] Notably, these abnormalities can persist for days to weeks following injury, with albumin ratios often remaining elevated beyond one-week post injury before gradually normalizing.[64] This prolonged yet reversible BBB dysfunction underscores the importance of temporal considerations when designing BBB-targeted interventions for TBI, as therapeutic windows may extend well beyond the initial insult. Furthermore, increased caveolin-1 vesicles and decreased expression of Mfsd2a (major facilitator superfamily domain-containing protein 2) are acute changes in the BBB that occur within hours to days after injury. These changes result in enhanced vesicular transcytosis, albumin leakage, and temporary barrier opening.[65–67] This implies that AMT/caveolae pathways might offer more access for therapeutic delivery during the acute phase. However, TMT-based methods are complicated by changes in glucose transport. Patchy loss of GLUT1 expression in injured microvessels was reported in human TBI surgical samples (7–8 hours post injury), while the surrounding regions displayed mixed normal or increased expression.[68] AMT/caveolae entry can expedite the delivery of neuroprotective or proregenerative drugs during the acute phase, yet, RMT/TMT strategies may be useful in subsequent phases to maintain cell-specific support. In general, BBB changes should be considered as disease-specific conditions that present both opportunities and challenges for drug delivery, rather than only as obstacles. In AD, elevated efflux transporters and decreased expression of GLUT1 and LRP1 provide significant hurdles. On the other hand, shifts in iron metabolism and the relative stability of TfR expression in PD might offer beneficial prospects for receptor-mediated targeting. Increases in permeability caused by inflammation and injury in TBI may allow it to be less difficult for drugs to enter the BBB, but they also raise important questions about how to preserve target specificity when the widespread barrier is disrupted. How to include disease- and stage-specific BBB alterations into the development of drug delivery is the primary concern, not just how to get past the BBB. Recognizing the BBB as a dynamic and context-dependent system, rather than a uniform barrier, may provide a more realistic framework for improving the translational relevance of BBB transcytosis approaches.

4. Expanding evaluation beyond permeability

Most BBB-delivery studies have relied on *in vitro* permeability assays (*e.g.*, BMEC–astrocyte transwell co-culture with tracer readouts) and *in vivo* biodistribution as primary endpoints.[69,70] These are essential for feasibility, but they do not establish whether BBB crossing translates into neuroregenerative benefit. So, in this perspective review, we propose to expand evaluation from barrier crossing to functional and clinically relevant outcomes. First, functional neuroregenerative studies should be incorporated into the evaluation. Some examples are (i) axonal regrowth (*e.g.*, neurite length, GAP-43, and tract tracing),[71] (ii) synaptic structure/function (*e.g.*, Ca^{2+} transient assay and synapsin),[72,73] and (iii) electrophysiological recovery (*e.g.*, patch clamp and multi-electrode array (MEA)[74,75]). These results show practical attributes for neuron restoration and regeneration. Second, include behavioral recovery as complementary evidence in disease-appropriate models—*e.g.*,

Morris water maze or novel object recognition for cognition;[76] rotarod or open-field for motor function—implemented with randomization, blinding, *a priori* power calculations, and longitudinal follow-up. Third, adopt human-relevant platforms to improve translational fidelity: BBB-on-a-chip and iPSC-based multicellular models (endothelial cells, pericytes, astrocytes, microglia, neurons) under physiological shear.[77,78] In particular, organ-on-chip systems recreate the dynamic microenvironment of the BBB by introducing microfluidic flow, enabling physiological shear stress and concentration gradients.[79] Such systems enable simultaneous measurement of permeability (TEER, tracer flux), post-BBB neuronal delivery, synaptic connectivity, and electrophysiology.[80,81] Patient-derived iPSCs can capture genetic background and disease-stage variability.[82] Altogether, BBB penetration should be treated as a necessary precondition, not the therapeutic endpoint. Integrating functional, behavioral, and human-relevant assays will support more reliable down-selection of delivery strategies with genuine translational potential.

Importantly, the limitation of permeability-focused evaluation extends beyond the endothelial barrier itself. Even after successful BBB transcytosis, nanoparticles encounter additional biological barriers within the brain parenchyma that critically influence their distribution and therapeutic efficacy. The dense and heterogeneous extracellular matrix of brain tissue restricts nanoparticle diffusion, often confining the delivered systems to perivascular regions. In parallel, non-specific uptake by resident glial cells, particularly microglia and astrocytes, can act as a sequestration sink that reduces nanoparticle availability to target neuronal populations. Furthermore, intracellular trafficking pathways such as endosomal and lysosomal routing may prevent effective cytosolic delivery of therapeutic payloads, thereby limiting functional benefit despite apparent BBB penetration. Importantly, BBB-targeting strategies alone do not ensure selective engagement of pathological cell populations in neurodegenerative diseases, where diseased and healthy cells coexist. Therefore, effective neurotherapeutic delivery requires consideration not only of BBB crossing but also of post-BBB transport and disease-specific cellular targeting within the brain microenvironment.

5. Translational roadmap for clinical application

Unlike existing BBB delivery reviews that implicitly equate successful BBB penetration with translational promise, the roadmap proposed here explicitly separates endothelial crossing from therapeutic efficacy and clinical viability. We propose this roadmap not as a linear checklist but as a filtering framework designed to eliminate delivery strategies that fail at critical translational bottlenecks, even if they demonstrate robust BBB permeability in preclinical models.

Although BBB penetration methods have frequently demonstrated promising results in preclinical models, they have not translated effectively in clinical trials. For instance, a clinical trial on MCI and AD showed respectable safety profiles but did not result in significant improvements in cognitive outcomes.[83] Likewise, clinical studies attempting to deliver GDNF for PD have been proved safe but were unable to produce meaningful efficacy.[84] These challenges highlight the point that clinical translation cannot be ensured by simply proving BBB penetration. Therefore, understanding the requirements for clinical entry and establishing comprehensive validation methods are crucial.

1. For the concept validation of BBB-penetrating drugs, emerging computational approaches may complement cargo selection. Artificial intelligence-based models could assist in predicting ligand–receptor interactions relevant to BBB targeting,[85,86] prediction of BBB permeability,[87] and generating new (*de novo*) BBP (blood–brain barrier penetrating peptide) candidate peptide sequences by learning the sequence distribution of actual BBPs.[88–90] Such tools are expected to function as enabling technologies rather than replacements for mechanistic validation, and their integration with experimentally grounded BBB models may further enhance efficient drug selection in the future.

2. Beyond BBB penetration, verification of neurological impact is essential. For instance, in AD, neuronal viability, synaptic signaling, and cognitive improvement must be demonstrated in combination with decreases in amyloid-β plaques or tau phosphorylation.

3. A crucial prerequisite for neuroregenerative therapies, which frequently necessitate chronic or high-dose treatment, is repeated and safe administration. Viral vectors, for example, face obstacles due to immunological reactions and limited redosing capability (ClinicalTrials.gov Identifier: NCT00087789 (https://clinicaltrials.gov)).

4. In addition to biological efficacy, translational feasibility must account for manufacturing scalability and regulatory complexity. Cell-based or bio-derived delivery platforms, while attractive for BBB crossing, may introduce additional regulatory hurdles related to product heterogeneity, characterization, and long-term safety.[91–93] As such, careful target selection and early regulatory consideration are essential when evaluating these approaches within a translational framework.

5. Non-invasive delivery techniques are recommended. Intraparenchymal infusion restricts the therapeutic reach to small brain regions and necessitates craniotomy. It has also demonstrated limited diffusion in clinical trials for both AD and PD. Although these methods verify direct brain entrance, they are not appropriate for repeated administration and place a significant strain on patients.[94,95]

Recent preclinical studies illustrate these translational barriers. For example, a GLUT1-targeted nanocarrier (Gal-NP@siRNA) loaded with BACE1 siRNA was reported to transiently increase brain accumulation in AD mouse models by leveraging GLUT1 upregulation under fasting conditions.[96] However, the distribution of the drug moved to the peripheral organs within 24 hours, limiting the durability of the effect. Furthermore, mechanistic validation was incomplete, as GLUT1 knockdown/knockout or competitive inhibition experiments were not performed to confirm transporter dependence. Importantly, the protocol required repeated intravenous

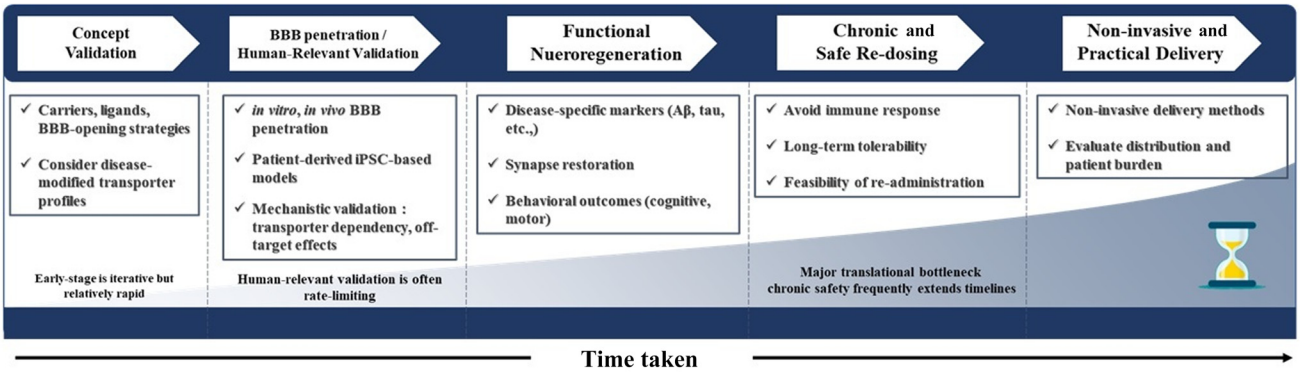

Fig. 3 Roadmap for translational neurotherapeutic development. Each stage is associated with distinct and often overlapping time scales in practice. Early concept validation is typically iterative and relatively rapid, whereas human-relevant BBB validation and chronic safety assessment frequently represent rate-limiting steps in translational neurotherapeutic development.

administration following 24 hours of fasting—a regimen with low feasibility and limited relevance for human translation. This case highlights how mechanistic uncertainty and impractical protocols can undermine the clinical applicability of otherwise promising strategies. Finally, a detailed validation in human-relevant models is essential. Since GLUT1 expression has been reported to be reduced in AD patients, strategies relying on this transporter must be evaluated in patient-derived or humanized BBB models to ensure relevance. Establishing clear mechanistic evidence and verifying absorption in human-based systems will be critical steps to improve the translational predictability of BBB-crossing approaches. As shown in Fig. 3, classic drug development frameworks and historical success rates highlight the challenges of clinical translation, and BBB-targeted neurotherapeutics face additional rate-limiting steps that are not captured by conventional timelines. In particular, human-relevant BBB validation and chronic safety assessment often dominate translational timelines, irrespective of success in early-stage concept validation.

6. Conclusions

This perspective challenges the prevailing assumption that improving BBB penetration is sufficient to advance neurotherapeutic translation. Instead, we argue that BBB transcytosis should be evaluated as one component within a broader, disease-specific translational ecosystem that includes parenchymal transport, functional recovery, safety, and feasibility of chronic administration.

Although BBB transcytosis has demonstrated promise in preclinical studies for CNS drug delivery, its translation into clinical applications remains limited. Several contributing factors must be systematically addressed before substantial progress can be made. Evaluation only of BBB penetration *in vitro* or *in vivo* provides insufficient evidence. Ultimately, the clinical significance will depend on whether such approaches can promote neuroregeneration, enable synaptic repair, and restore both cognitive and motor functions. Moreover, disease-specific alterations of the BBB necessitate tailoring therapeutic strategies to the underlying pathological context. The distinct pathological alterations in BBB integrity and transporter expression observed in AD, PD, HD and TBI directly impact transporter-mediated, receptor-mediated, or adsorptive-mediated methods. A wide range of targeting methods are therefore unlikely to be productive. Instead, administration techniques must be tailored to the unique BBB environment of each disease, taking into account its stage of development. The following three priorities are crucial at the end. First, evaluation should include both BBB permeability and neurological improvement to show the therapeutic value of BBB-crossing therapies. Plus, to bring preclinical findings closer to the clinic, models that more closely resemble human biology —such as BBB-on-a-chip systems and iPSC-derived BBB platforms—should be prioritized.

At the same time, manufacturing and regulatory aspects cannot be treated as afterthoughts. Long-term safety, reproducibility, and tolerance to repeated dosing all have to be tested early. Otherwise, even the most promising platform will fail. What ultimately determines clinical significance is not simply BBB permeability but the capacity of this process to yield measurable functional improvement in patient outcomes. Approaching the problem from this angle creates a more realistic foundation for therapeutic development in neuroregeneration and neurovascular disease.

Author contributions

Conceptualization: S. L.; original draft preparation, review and editing: S. L., J. L.; project administration, funding acquisition, and supervision: Prof. K. L; all authors read and approved the final manuscript.

Conflicts of interest

There are no conflicts to declare.

Data availability

This review article does not contain any primary data, and all data discussed are available in the cited literature.

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