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## EXPERT INSIGHT

# Blood–brain barrier models in pre-clinical assessment of CNS-targeting cell and gene therapies

Danica B Stanimirovic & Anna Jezierski

The central nervous system (CNS) diseases are among the most difficult to treat; the brain is thoroughly shielded by the blood–brain barrier (BBB), making many systemically administered therapies ineffective. Many rare, progressive and debilitating CNS diseases are caused by specific and variously inherited mutations in the DNA sequence of a single gene which can be modified or cured by correcting the faulty gene. Recent rapid advances in gene therapy and genome editing technologies are making this lofty goal much closer to reality. CNS is a major disease focus of key industry players in gene and cell-gene therapy. In this article, we will discuss how the emerging human BBB models derived from induced pluripotent stem cell (iPSC) have emerged as an important tool for discovery and preclinical evaluation, including CNS toxicity, of brain-targeting viral and non-viral gene and cell-gene therapies.

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## PROMISE OF GENE THERAPY FOR NEUROLOGICAL DISEASES

Advances in viral vector engineering and precision genome editing,

spurred a resurgence of gene therapy as a viable option for monogenic diseases, culminating in a recent approval and commercialization of adeno associated virus (AAV)-based

gene therapy (Luxturna™) for a rare form of genetic childhood blindness and Zolgensma™ for pediatric spinal muscular atrophy. The clinical pipeline of AAV-based gene

therapies is growing, with a strong focus on musculoskeletal and neurological diseases. Monogenic neurological diseases include, among others, a spectrum of debilitating and progressive childhood epilepsy syndromes, lysosomal storage diseases, Huntington's disease, amyotrophic lateral sclerosis (ALS) and various forms of inherited ataxias and dementia, where disease outcomes can be modified by restoring the functional gene product or by genome editing specific mutations. Beyond monogenic neurological syndromes, current clinical trials of AAV-based therapies are attempting to also address major neurodegenerative diseases such as Parkinson's and Alzheimer's disease by restoring trophic support for damaged neurons and by eliminating genetic risk factors, respectively. The promise of gene therapies for neurological diseases has been reinforced by the recent approval of an anti-sense oligonucleotide (ASO) treatment (Spiranza<sup>TM</sup>), another way to modify 'faulty' genes, for spinal muscular atrophy (SMA) as well as by positive effects of ASO strategies in advanced clinical trials for Huntington's disease [1] and ALS [2]. The rapidly growing toolkit of genome editing nucleases (CRISPER/cas9, TALENs, ZFN and others) is expanding the applications of AAV-based gene delivery into precise editing of genes [3], RNA [4] and single base pairs [5].

However, in current clinical trials and treatment paradigms, both ASO and AAV therapies targeting neurological diseases, are administered by intrathecal injection or infusion, or via surgical brain injections or implantation of convection-enhanced diffusion (CED) pumps [6]. Whereas intrathecal,

intraventricular or intracerebral delivery of AAV in rodent models often achieves a widespread transduction of spinal cord or brain, or diffusion of secreted transgene to broader brain regions, it is unlikely that similar broad brain tissue coverage can be achieved in species with bigger brain volumes, including humans. These approaches typically limit the access of large molecules (including viruses) to superficial cortical layers or specific brain regions, and may not be optimal or sufficient to modify diseases affecting deep brain regions or 'whole brain', which is ultimately the case with the majority of neurodegenerative diseases.

This article summarizes current and future applications of emerging human pre-clinical models of the blood-brain barrier (BBB) *in vitro* that can facilitate selection, development and translation of emerging gene- and cell/gene therapies developed for diseases of the central nervous system (CNS).

## BRAIN BEYOND BARRIERS

The human brain is a complex and difficult-to-access compartment, with the BBB preventing systemic delivery of gene therapies into the CNS.

An immense surface area (~25 m<sup>2</sup> in human) of densely-packed brain capillary networks, as well as close proximity of neurons to brain capillaries (distance of ~25 μm<sup>2</sup>) provide an opportunity to both target the brain endothelial cell (BEC) compartment for transgene expression and to deliver gene therapies to essentially all brain regions via a transvascular (cross-BBB) route. The blood-spinal cord barrier (BSCB) is the functional equivalent of the

## Blood–brain barrier

The blood–brain barrier is formed by brain endothelial cells lining brain capillaries and micro-vessels, tightly sealed together by tight junctions. Brain endothelial cells regulate exchange of water and nutrients between blood and brain via polarized transporters and pumps. Barrier properties of brain endothelial cells are induced by neighboring pericytes and astrocytes. Blood–brain barrier prevents most biologic and gene therapies from accessing the brain from systemic circulation.

BBB that shares the same principal building blocks, namely a specialized endothelium linked by tight junctions. Although some morphological and functional differences exist among these barriers, both are highly restrictive for systemically delivered biotherapeutics. To enable systemic delivery of gene therapies into the brain, spinal cord or peripheral nerves, specific barrier targeting strategies have to be engineered into gene therapy delivery vectors. Additional challenges of this approach include systemic instability, potential adverse effects resulting from distribution of viral vectors in peripheral organs and immunogenicity – often necessitating encapsulation of gene therapies into variously engineered and targeted nanocarriers.

Recent advances in understanding receptor-mediated vesicular transport across the BBB, which can accommodate antibodies, viruses and nanocarriers (20–50 nm), led to the development and engineering of molecular ligands, mostly antibodies, against BBB target receptors capable of transmigration and delivering ‘therapeutic payloads’ into the brain. At present time, the field has at its disposal a pipeline of antibody-based BBB carriers capable of delivering pharmacologically relevant amounts of biotherapeutics across the BBB in preclinical models [7–10]. Notably, single-domain antibodies, including broadly species cross-reactive FC5 [11,12], owing to their compact size, may be

particularly suitable for re-targeting of viral vectors or gene therapy-loaded nanoparticles for CNS access.

In preclinical studies, transgenes encoding therapeutic proteins, microRNAs, antibodies or gene-editing machinery have been delivered to the CNS via non-systemic routes (intrathecal, intraventricular and intracerebral) using natural or engineered viral capsids [13,14] and cell-specific (neuronal or glial) promoters [15,16]. Advances in optimizing brain tropism of viral vectors propelled AAV9 serotype as a rapidly emerging gene therapy platform for the treatment of neurological diseases. A variant further engineered to enhance BBB- and CNS tropisms, AAV-PHP.B, delivered 40-fold more AAV into the CNS than AAV9 after systemic administration in mice, with widespread transduction of neuronal and astrocyte populations [17,18]. However, the CNS tropism of AAV-PHP.B demonstrated in rodent studies [19,20] could not be replicated in non-human primates [21] limiting its translational potential. The mechanisms involved in BEC internalization and BBB transmigration of various AAV serotypes are not well understood; in particular, species differences in interacting receptor abundance or glycosylation may limit application of viral vectors selected/optimized in rodent models to humans. These caveats emphasize the need for deployment of human BBB models in

pre-clinical selection and evaluation of CNS-targeting gene therapy vectors and systemic delivery strategies

### BBB MODELS *IN VITRO*

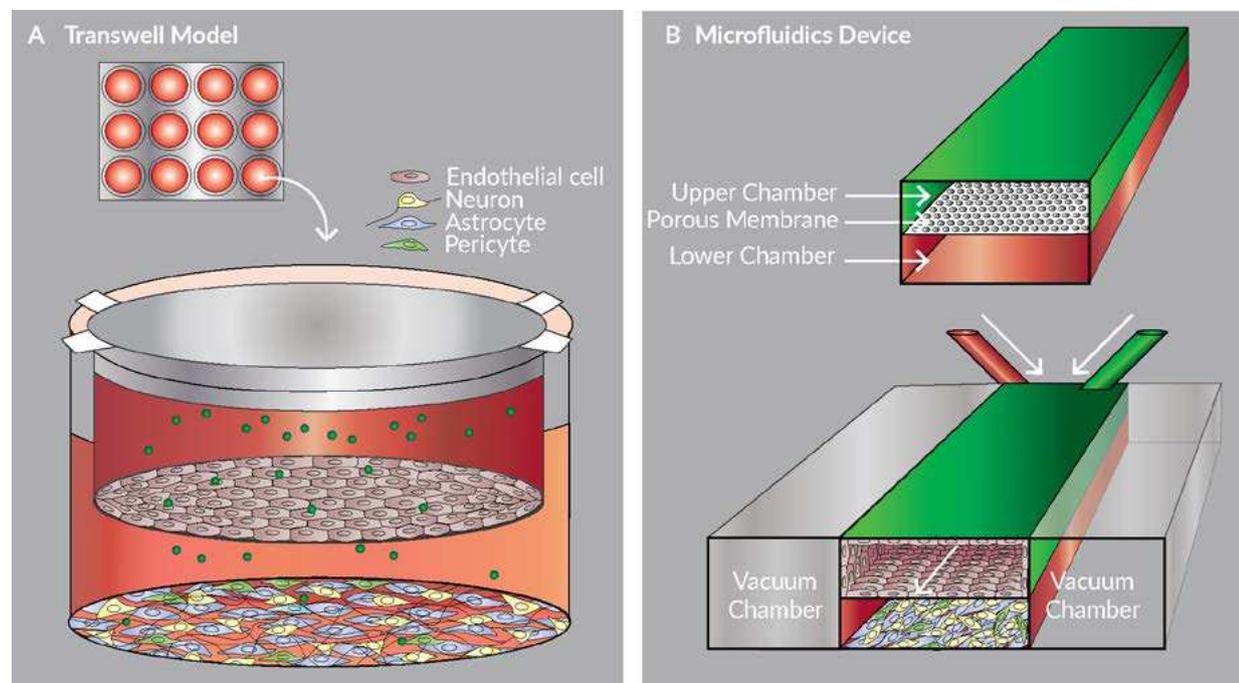
The need to de-risk CNS discovery pipelines and improve translation has accelerated the development of more robust preclinical BBB models *in vitro* for early assessment of biotherapeutic leads.

The BBB field has achieved a quantum leap in this area by developing methods and protocols to differentiate brain endothelial cells (BECs) from a renewable source of human induced pluripotent stem cells (iPSCs) [22]. In addition, differentiation of pericytes, astrocytes,

and neurons from isogenic iPSCs [23,24] enabled assembly of human BBB models *in vitro* that closely resemble anatomical organization and physiological/molecular phenotype of the BBB *in vivo* [25,26]. These models exhibit near-physiological tightness, measured as transendothelial electrical resistance (TEER) ( $>5000 \Omega\text{cm}^2$ ) [27], polarization of transport and immunological barrier [28]. They have been used to evaluate transport of synthetic molecules, species-specific receptor-mediated transcytosis of antibodies [29], response to inflammatory stimuli [30], interactions with and infiltration of inflammatory cells and mechanism of CNS entry of Zika virus [31]. Patient derived iPSC-based BBB models have been

## ► FIGURE 1

Schematic representation of human BBB models *in vitro* based on Transwell (A) or microfluidic-flow (B) configurations.



(A) Tight-junction sealed brain endothelial cell differentiated from iPSCs (banked lines or patient-derived) are cultured in Transwell inserts dividing two liquid compartments and are often co-cultured with isogenic pericytes or astrocytes/neurons in the basolateral (brain) compartment. (B) Microfluidic-based BBB models re-create vessel lumen and introduce flow and shear stress, modeling anatomical and physiological features of the neurovascular unit.

## Induced pluripotent stem cells

Induced pluripotent stem cells (iPSCs) are re-programmed somatic cells that can give rise to any cell type in the body through specific differentiation methods. An iPSC from a patient can be differentiated into multiple cell types carrying the same genotype (including disease-causing mutations). The human BBB models *in vitro* are constructed from brain endothelial cells differentiated from iPSCs to unique barrier phenotype, and are sometimes co-cultured with other brain cells (astrocytes/neurons, pericytes) derived from the same iPSC source (isogenic).

generated from Huntington's disease [32] and patients harboring inactivating mutations in the thyroid hormone (TH) transporter monocarboxylate transporter 8 (MCT8) that causes severe psychomotor retardation in children [33]. The later study demonstrated that the BBB defect in TH transport was responsible for disease manifestations and could be corrected by targeting gene therapy/genome editing to brain endothelial cells themselves.

iPSC-derived human BBB models could be assembled in a 'static' Transwell model (Figure 1A), with co-culture of 'inducing' or target cells in the receiving compartment – often used as an *in vitro* bioassay to demonstrate both BBB crossing and target cell engagement in a single assay. Recently, microfluidic and bioengineered devices have enabled designs of BBB *in vitro* that incorporate flow and shear stress, and can be used as serially-linked units to multiplex evaluation of therapeutic transport or cell interactions with the BBB (Figure 1B).

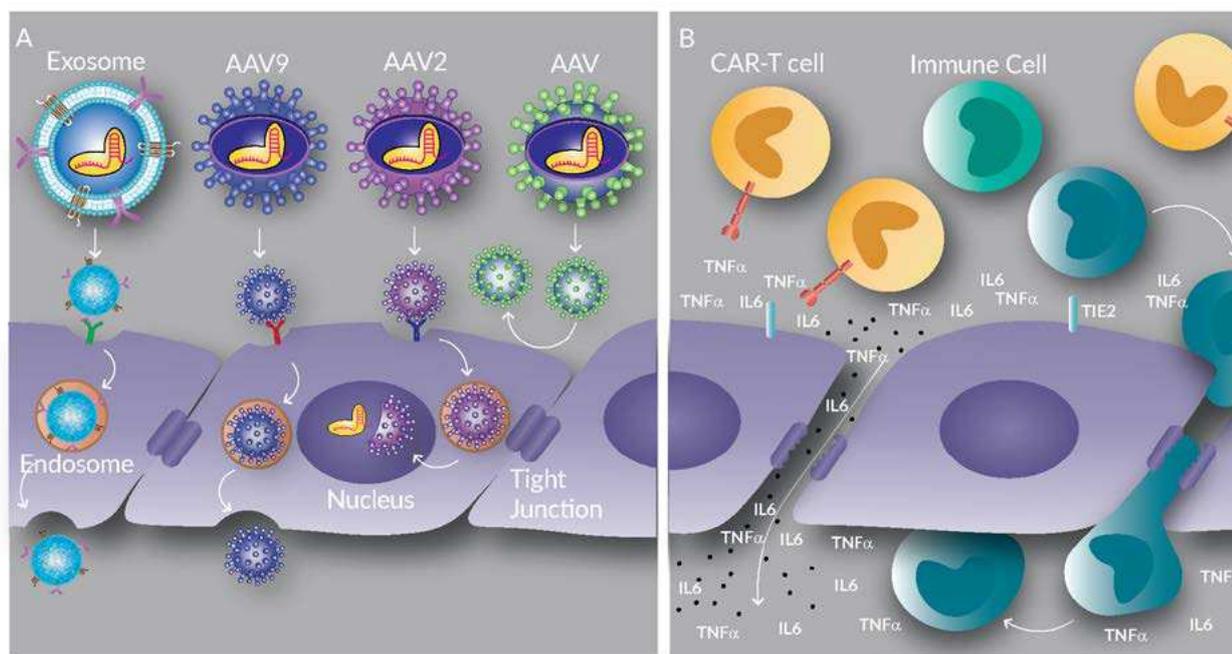
### BBB MODELS *IN VITRO* CAN FACILITATE DISCOVERY & DE-RISKING OF CNS-TARGETING GENE THERAPIES

The preclinical to clinical translation of antibody therapeutics and

targeted gene therapies necessitates humanized preclinical models to circumvent species-related differences in immune systems, receptor expression and antibody cross-reactivity. For CNS-targeted therapies, human BBB models that recapitulate essential molecular and physiological features of the BBB *in vivo* are on the critical path for selecting lead candidates and de-risking their development. Such models can add value in all stages of therapy development, from discovery to translational PK-PD modeling. Recently, BBB model *in vitro* was used to identify a short linear residue of the AAVrh.10 capsid that triggers transcytosis of an otherwise non-penetrant AAV1 [34] and to discriminate the ability of AAV2 and AAV9 serotypes to transduce and transmigrate the BBB, respectively [35]. Iterative selection (panning) of engineered AAV libraries in human BBB models *in vitro*, could facilitate future discovery of capsid variants suitable for vascular- or neuronal-targeting of gene therapies with human specificity. Understanding AAV-host interactions [36], novel cellular AAV receptors, host restriction factors for AAV entry and AAV capsid determinants that mediate viral vector transduction, trafficking or transmigration across BEC (Figure 2A), would expand the arsenal of vectors optimized for gene delivery to the CNS [37–39]. These BBB models *in*

## ► FIGURE 2

Schematic representation of human BBB model application in discovery and pre-clinical testing of CNS-targeting gene therapies (A) and neurotoxicity of CAR-T therapies (B).



(A) Systemic delivery of gene therapy to CNS requires targeting to and transport across the BBB. BBB models *in vitro* have been used to select crossing AAV serotypes from libraries of engineered AAV capsids. In particular, using BBB models *in vitro*, AAV9 has been shown to cross the BBB via a (still uncharacterized) receptor-mediated transcytosis pathways, whereas AAV2 was 'trapped' within brain endothelial cells. BBB models *in vitro* are also useful in evaluating delivery of genome editing nucleases encapsulated into targeted nanoparticles, including exosomes, or incorporated into viral vectors. (B) Human iPSC-derived BBB models could be particularly useful in pre-clinical assessment and/or prediction of neurotoxicity and brain edema in response to CAR-T therapy. Evaluation of BBB disruption (TEER measurement), BEC activation and modified T-cell transmigration (in the presence or absence of target cancer cells) across the model, could all become indicators of potential neurotoxicity of a particular CAR-T construct.

*in vitro*, with some limitations, could be used as 'surrogates' for evaluating gene therapy penetration across BSCB.

Syngenic (human) or isogenic (from the same iPSC source) multi-cellular BBB models can be used to evaluate BBB-crossing and efficiency of transduction/target gene modification in reporter neuronal/glial cells in the same (bio) assay. These types of bioassays are suitable as a screen to select lead candidates, as well as an iterative assay in optimization cycles. In addition, or in combination, quantitative assays determining rate of transport, BBB permeability coefficients, and

required 'loading' dose to achieve desired levels of viral vectors in the brain compartment, can all be incorporated into PK-PD models to estimate dosing in translational studies or clinical trials. These multi-cellular models could also be applied to evaluate efficiency and selectivity of cell-specific promoters targeting cellular elements of the neurovascular unit, or target cells (e.g., cultures of glial and neuronal cells) co-cultured in the basolateral compartment.

Human BBB models *in vitro* have been instrumental in identifying BEC-enriched targets, and validating transcytosis of BBB antibody

carriers targeting receptor mediated transcytosis (RMT) [29,40,41]. Non-viral gene delivery systems such as exosomes [42] and lipid nanocarriers [43], capable of encapsulating AAV, ASO or gene editing therapeutic payloads, are an emerging brain delivery strategy (Figure 2A) [44,45]. Functionalization of nanocarriers with BBB-crossing antibodies [46], enhances the versatility of delivery options for gene therapy modalities. Human BBB models *in vitro*, in conjunction with animal pre-clinical studies, could be used to systematically evaluate BEC binding, transduction and transcytosis of these novel formulations to support their clinical translation.

### APPLICATIONS OF BBB MODELS IN ASSESSING NEUROTOXICITY OF GENE-MODIFIED CELL THERAPIES

In some cases, genes can be introduced into cells *ex-vivo*; modified cells are then used as a means to deliver gene therapy to patients – this is known as gene-modified cell therapy. Chimeric antigen receptor (CAR)-T cell therapy is a revolutionary form of gene-modified cell immunotherapy for hematological malignancies. However, a potential limitation to the use of CAR-T include two relatively common adverse effects: cytokine release syndrome (CRS) and neurotoxicity [47–49]. The symptoms of the neurotoxicity range from mild confusion to cerebral edema with coma and, potentially, death [50]. The mechanisms of CAR-T-induced neurotoxicity are not well understood, nor can they be reliably predicted; however, recent studies

point to the central role of the BBB damage and disruption. High levels of inflammatory cytokines (IL6, TNF $\gamma$  and TNF $\beta$ ) lead to BEC activation, a loss of BBB functional integrity and subsequent vasogenic brain edema [50–52]. A subsequent influx of inflammatory cytokines and immune cells into the CNS initiates a feedback loop of continued endothelial activation resulting in encephalopathy syndrome (Figure 2B). “Considering the high frequency of neurotoxicity events, randomized prospective studies of treatment algorithms are urgently needed to improve patient monitoring and management”, suggested a recent article evaluating 25 adult patients who presented with neurotoxic syndromes after CAR-T cell therapy at the Massachusetts General Hospital [53].

There are no established preclinical models for predicting neurotoxicity after CAR-T cell immunotherapy; such models will need to be human(ized) and amenable to ‘personalized’ testing. We suggest that iPSC-derived human BBB models *in vitro* could be of significant value in deciphering the mechanisms of CAR-T-induced BBB disruption and accompanying neurotoxicity. BBB model miniaturization and advancements in iPSC-derived BEC cryopreservation methods, bring preclinical screens or clinical titration of CAR-T products based on BEC-induced toxicity (Figure 2B) to predict/mitigate clinical neurotoxicity, much closer to reality. In addition, such models could be used to evaluate vasculo- or neuro-protective strategies to minimize/manage neurotoxic effects of CAR-T. In another paradigm, human BBB models *in vitro* could support the development of systemically-delivered CAR-T

designs that target brain tumors, in particular glioblastomas [54].

## TRANSLATION INSIGHT & OPPORTUNITIES

The development of iPSC-derived human BBB models has been a milestone achievement for CNS drug discovery, de-risking and translational studies of biotherapeutics. Recent advancements in viral vector engineering, as well as an expanding genome editing toolbox of nucleases capable of precision editing single base-pairs, brings therapies for monogenic brain diseases a leap-step closer to reality. The accompanying development of delivery strategies, including trans-BBB delivery is on the critical path to clinical success of these pipeline programs. Preclinical evaluation of these delivery strategies requires reliable, scalable and reproducible human iPSC-derived BBB models that recapitulate molecular make-up and physiological features of the BBB *in vivo*. Such models can also be valuable for assessing safety and adverse event mitigation strategies to

reduce brain edema and neurotoxicity, in particular of gene-modified cell therapies, such as CAR-T. BBB models *in vitro* have been benchmarked for their ability to predict *in vivo* brain exposure in animals using panels of synthetic molecules [55] and antibodies [29,40]. The ultimate ability of human iPSC-derived BBB models to predict human brain exposure or toxicity, including that of gene therapies, can be assessed only through future clinical trials.

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