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Review

Advancing cell therapy for neurodegenerative diseases

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SUMMARY

Cell-based therapies are being developed for various neurodegenerative diseases that affect the central nervous system (CNS). Concomitantly, the roles of individual cell types in neurodegenerative pathology are being uncovered by genetic and single-cell studies. With a greater understanding of cellular contributions to health and disease and with the arrival of promising approaches to modulate them, effective therapeutic cell products are now emerging. This review examines how the ability to generate diverse CNS cell types from stem cells, along with a deeper understanding of cell-type-specific functions and pathology, is advancing preclinical development of cell products for the treatment of neurodegenerative diseases.

INTRODUCTION

> Stem cell research is the key to developing cures for degenerative conditions like Parkinson's and motor neuron disease from which I and many others suffer.—Stephen Hawking

Neurodegenerative diseases of the CNS (the brain, retina, and spinal cord) affect all ages. They can range from congenital leukodystrophies that impair the white matter in childhood to those with increased prevalence during aging, such as Alzheimer's disease (AD), Parkinson's disease (PD), and age-related macular degeneration (AMD) (Figure 1). Mental illnesses may have a degenerative component, such as schizophrenia, which is characterized by cortical gray matter loss and signs of accelerated aging in patients.[1] Some neurodegenerative diseases are prevalent, including AD, AMD, and PD, while others are rare, such as Huntington's disease (HD), amyotrophic lateral sclerosis (ALS), frontotemporal dementia (FTD), corticobasal syndrome (CBS), multiple system atrophy (MSA), and progressive supranuclear palsy (PSP). These diseases may have genetic, environmental, or complex etiologies. Many neurodegenerative conditions progress to dementia, which is predicted to affect approximately 150 million people globally in 2050, with an economic burden of $10 trillion[2,3] and untold costs to patients and their families. Although neurodegenerative diseases are currently incurable, for some, treatments are available to alleviate symptoms. A good example is PD, where levodopa (L-DOPA) administration and deep brain stimulation can improve quality of life, although these treatments do not address the underlying disease and its inexorable progression.

Neurodegeneration is characterized by the loss of neurons, but it typically involves multiple interdependent cell types. A landmark discovery showed that ALS resulted from motor neuron defects and non-cell-autonomous "killing" by astrocytes,[4] which could be exacerbated by microglial activation.[5] It is now generally understood that macroglia (astrocytes and oligodendrocytes) and microglia play key roles in disease processes. The multicellular and multisystem involvement that characterizes neurodegenerative diseases has been well documented by neuropathologists. However, through more recent genetic and functional studies, we now appreciate the causal contributions of each cellular player in unprecedented detail. Several excellent reviews have covered the cell therapies for specific neurodegenerative diseases, including PD,[6–8] ALS,[9,10] retinal degeneration,[11] and multiple sclerosis (MS).[12] This review instead takes a cell-type-centric approach. It focuses on some of the major cellular products in development and highlights how greater knowledge of cell subtypes and states can guide preclinical work to assess their feasibility in the treatment of neurodegenerative diseases.

DEVELOPMENT OF CELL PRODUCTS FOR CLINICAL USE

We are now able to generate numerous CNS cell types from human pluripotent stem cells (hPSCs) (Figure 2). Successful cell production is confirmed by comparing the product with human CNS tissue from fetal to adult stages and establishing the degree of similarity. In particular, single-cell and single-nucleus RNA sequencing (sc/nucRNA-seq) approaches have revolutionized our ability to test whether differentiation protocols generate authentic cell products. Small molecules and growth factors guide hPSCs to produce regionally patterned neural progenitor cells (NPCs), then specific neural progeny, including major cell types affected by neurodegenerative disease in the forebrain,[13–18] retina,[19–22] midbrain,[23] spinal cord,[24] and throughout the nervous system[25–30] (Figure 2). Vascular cells are derived from hPSCs by first differentiating into mesoderm and then adding factors to guide production of endothelial cells (ECs) and mural cells, such as pericytes and smooth muscle cells.[26,31] Some brain pericytes arise from the neural crest,[32] which can be achieved *in vitro* through initial neural patterning of hPSCs.[28] Microglia are produced by guiding hPSCs to differentiate into

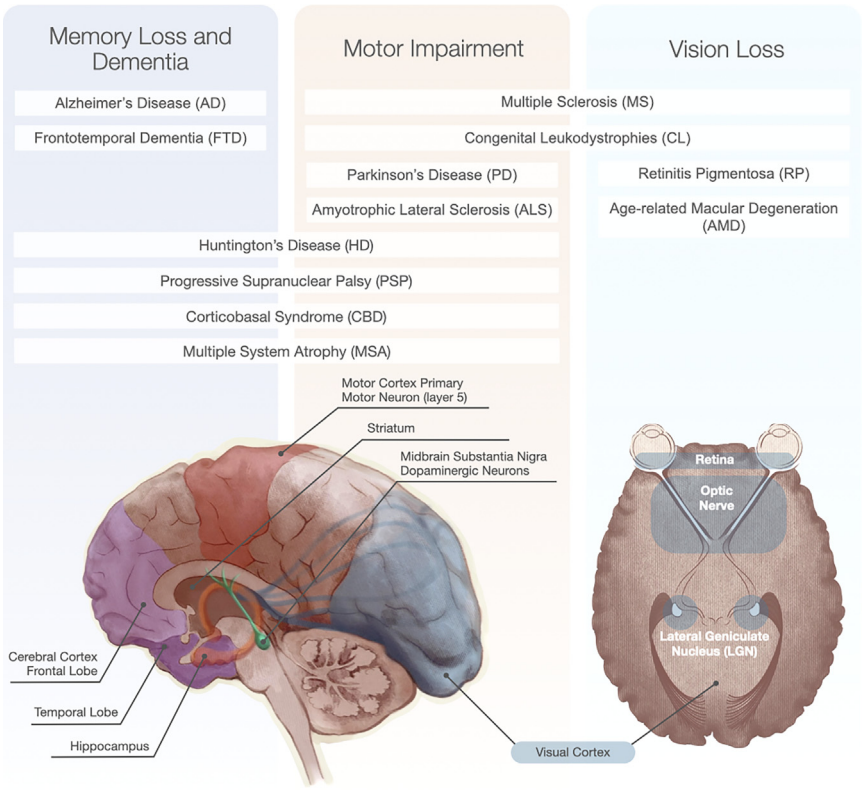


Figure 1. Primary functional impact of neurodegenerative diseases and affected brain regions

Patients experience symptoms that are primarily related to the particular neuronal and glial cell degeneration that occurs in different CNS areas in different diseases.

yolk sac and then mesodermal hematopoietic progenitors, which subsequently produce monocytes and microglia.[33] Although these approaches aim to recapitulate the signals that cells experience throughout development in a condensed time frame, it may still take months to generate desired cell types with reasonable purity. Expression of genetic inducers can rapidly convert hPSCs into target cells. Examples are the overexpression of Neurogenin2 (NEUROG2/NGN2) to make neurons[34] or Nuclear factor 1A (NFIA) to make astrocytes,[35,36] albeit with recognized differences from their *in vivo* counterparts.

Generally, less mature cells integrate and connect better with the host tissue, compared with highly differentiated cells. This applies, for instance, to several neural cell types, such as dopaminergic neurons,[37,38] oligodendrocytes,[39] astrocytes,[40] and retinal pigment epithelial (RPE) cells.[41] Hence, it is important to consider the cell stage when designing the optimal cell therapy for a particular neurodegenerative indication.

Along with improved protocols to generate 2D defined cell products, substantial advances have been made in creating 3D organoids that recapitulate the developmental order and cell composition of brain regions, such as the cerebral cortex.[42] In some cases, these structures reproduce the cytoarchitectural organization, for instance, the multilayered retina.[22,43] The addition of vascular cells and microglia to neural organoids enables the creation of more complex *in vitro* constructs.[44–47] These 3D structures may be part of the manufacturing process to create a desired cell type, such as retinal photoreceptors, or may serve directly as multicell transplant products.

Cell transplantation strategies for neurodegenerative diseases typically aim for long-term cell survival. Therefore, immune rejection is an important consideration. The immune privilege of the CNS affords some protection. Allogeneic transplants have been demonstrated in patients several years post-transplantation, as seen for RPE cells and dopaminergic neurons.[48,49] This immune privilege can be compromised during surgical delivery of the cell product. Hence, a typical clinical regimen will include a period of immunosuppression after transplantation. Another option is to deliver autologous cells, for example, using patient-derived iPSCs, when cell reprogramming, differentiation, and product release testing can occur within a viable therapeutic time window.[50] The autologous approach demands a robust manufacturing process that results in a safe and effective product, reproducibly across different donors.[51,52] Autologous use of iPSCs presents considerable cost challenges, although these can be alleviated by implementing automation and closed, controlled systems during manufacturing.[52,53]

Although many preclinical studies indicate that autologous iPSC products will likely be effective, some have reported an immune response after injection back into the donor, for instance, due to neoepitope generation following mutations in mitochondrial DNA.[50,54] iPSCs designed for protection from the immune system are being developed.[55] Approximately 150 different iPSC lines would be sufficient to provide HLA-matched cells for about 90% of the population in the UK or Japan, although this approach is less effective for highly diverse populations.[55] Alternatively, iPSCs can be engineered to evade immune detection. For example, iPSCs that lack MHC class I and class II genes and overexpress CD47 (an "immune cloaking" anti-phagocytosis molecule) produce hypoimmune cells that are not detected by an MHC-mismatched host.[56] Several approaches to generate hypoimmune cells are being pursued.[55] One challenge is a potential tumorigenic conversion of cells in the hypoimmune transplant, which might then evade immune detection. In such an event, inclusion of a safety switch, such as a suicide gene to kill the transplanted cells, is being contemplated.[57] However, one must consider the negative effects on the CNS of the patient caused by precipitously eliminating integrated neural cells by this mechanism.

The pathway and standards (https://www.isscr.org/standards) for developing stem cell-based products are being refined, including for autologous therapies.[51,58] It is essential to follow rigorous regulatory guidance, such as issued by the U.S. Food and Drug Administration (FDA) (https://www.fda.gov/

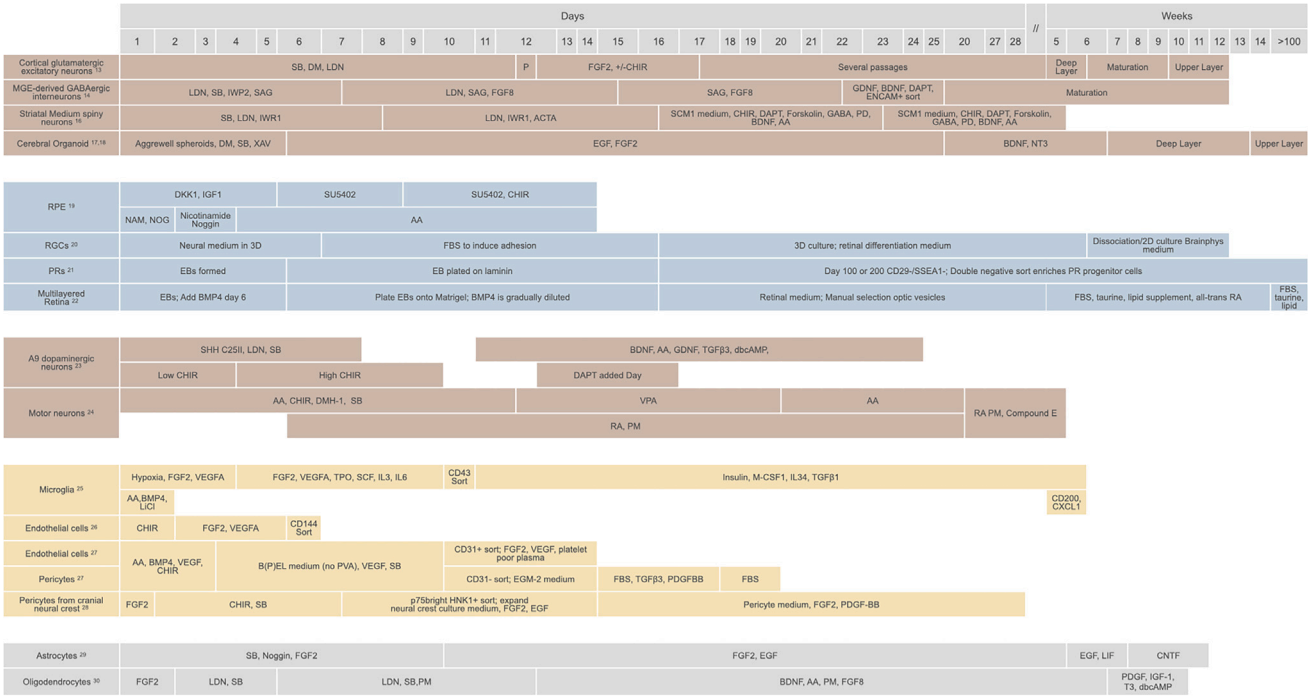


Figure 2. Examples of hPSC protocols to generate the major CNS cell types affected in neurodegenerative diseases

An overview showing the key molecules applied and the approximate time taken to generate the cell products. AA, ascorbic acid; ACTA, activin A; BDNF, brain-derived neurotrophic factor; CHIR, CHIR99021, CNTF (ciliary neurotrophic factor), GSK3 inhibitor, and WNT activator; DAPT, gamma secretase inhibitor and Notch pathway inhibitor; dbcAMP, dibutyryl cyclic AMP; DKK1, dickkopf 1 and WNT inhibitor; DM, dorsomorphin, ALK2, ALK3, and ALK6 inhibitor; DMH-1, ALK1, ALK2, and ALK3 inhibitor; EB, embryoid body; EGF, epidermal growth factor; FGF2, fibroblast growth factor 2; FGF8, fibroblast growth factor 8; FBS, fetal bovine serum; GDNF, glial cell-derived growth factor; IGF1, insulin-like growth factor 1; IL3, interleukin 3; IL6, interleukin 6; IL34, interleukin 34; IWP, WNT production inhibitor; IWR, WNT response inhibitor; LDN, LDN193189, ALK2, and ALK3 inhibitor; LiCl, lithium chloride; LIF, leukemia inhibitory factor; M-CSF1, macrophage colony-stimulating factor; NAM, nicotinamide; NOG, noggin; NT3, neurotrophin 3; P, passage; PD, PD0332991 and CDK4/6 inhibitor; PDGFBB, platelet-derived growth factor BB; PM, purmorphamine; PVA, polyvinyl alcohol; RA, retinoic acid; SAG, sonic hedgehog signaling agonist; SB, SB431542 and ALK4, 5, and 7 inhibitor; SCF, stem cell factor; SSEA-1, stage-specific embryonic antigen 1; SHH, sonic hedgehog; T3, triiodothyronine; TGF-β3, transforming growth factor β-3; TPO, thrombopoietin; VEGFA, vascular endothelial growth factor A; VPA, valproic acid; XAV, XAV-939, tankyrase blocker, inhibits WNT signaling.

vaccines-blood-biologics/biologics-guidances/cellular-gene-therapy-guidances). In developing a cell product for transplantation, a robust GMP-compliant cell manufacturing process is needed. Tests of the final cell product should demonstrate identity, purity, and potency prior to release for functional studies in animals or humans. Cell products contain diverse subpopulations, and their mechanism of action is typically multifactorial. Hence, the critical quality attributes (CQAs), those characteristics that make a cell product therapeutically beneficial for a specific indication, are often difficult to elucidate. To address this challenge, sc/nucRNA-seq is proving highly informative to characterize a cell product's identity and purity pre- and post-grafting. This information can be correlated with clinical outcomes to identify predictive biomarkers of CQAs for use in the manufacture and release of cell products. Recognizing the need for an in-depth characterization of cell products, the NIH in the US supports a regenerative medicine innovation project-linked effort, Catalyst (http://rmidatahub.org/).

An investigational new drug (IND) application made by the FDA to commence a clinical trial includes safety and efficacy testing. Testing is typically done in animal models, although increasingly *in vitro* methods and other alternatives are being pursued, supported by a recent US law rescinding animal testing requirements.[59] Extensive testing is recommended to assure cell products are not tumorigenic or demonstrating undesirable biodistribution after transplantation. In animal testing, the cells should be administered into the same region as intended for use in patients and over a suitable dose range to establish safety. To achieve long-term engraftment of human cells for assessing efficacy and safety in animals, immunosuppression, or the use of immunodeficient animals such as NSG or *rag2*−/− mice, is often employed.

Generation of clinical products at sufficient scale is an important consideration.[60] Although some CNS products have been tested at low doses (for example, 50,000 cells per retina for RPE cells[61]), others require millions of cells per patient to achieve substantial engraftment. Moreover, sufficient product must be made to enable animal testing in the preclinical stage and to supply cells for the clinical trial. Most cell products are cryopreserved during the manufacturing process, and such banks are subject to ongoing stability testing, requiring additional samples. Some CNS cell products are designed for a "thaw and inject" approach, which has substantial manufacturing efficiency and distribution advantages, for example, Lineage Cell Therapeutics' hPSC-derived RPE cell product (NCT02286089). Other cryopreserved cells require some manipulation post-thawing, such as washing to remove freezing medium or a period of cell culture, prior to final formulation and release. As cell manufacturing

technologies are advancing rapidly, opportunities to develop more robust, scaled, and efficient processes are growing. Furthermore, regulators expect more rigorous manufacturing, storage, and release processes, i.e., chemistry, manufacturing, and controls (CMC), to be developed as clinical trials advance. This consideration is important for products eligible for the FDA's regenerative medicine advanced therapy (RMAT) designation, which requires a relatively mature manufacturing process. CMC can be improved by employing quality-by-design principles that optimize product manufacture, holding CQA uppermost.[62] Improvements include, for example, stage-appropriate scale-up; removing animal components, such as fetal bovine serum, from the manufacturing process; or developing a cryopreserved product as the trial progresses.

CELL PRODUCTS FOR THE TREATMENT OF NEURODEGENERATION

Midbrain dopaminergic neurons

In PD, ventral midbrain (VM) substantia nigra A9 dopaminergic neurons that project to the striatum degenerate, leading to motor impairments (Figure 1). Transplantation of human fetal VM neurons into the striatum showed long-term benefit in a subset of PD patients. These pioneering studies also highlighted the importance of defining the cell types in the graft, as contaminating serotonergic neurons could precipitate dyskinesia, debilitating involuntary movements.[63] Using hPSCs solves procurement issues associated with fetal sources and enables manufacture of a more consistent cell product at scale. hPSCs differentiated into VM cells produced A9-enriched dopaminergic neurons that were stable and functional upon transplantation in animals.[64,65] Moreover, optogenetic control of dopamine release from the grafted cells tuned the motor improvement in a PD mouse model, establishing transplant functionality.[66] This preclinical work led to clinical-grade manufacturing and an IND application[23,67] to support a clinical trial of human embryonic stem cell (hESC)-derived dopaminergic cells as treatment option in PD (NCT04802733).

Several protocols have been established that produce dopaminergic neuron-based transplants effective in reversing signs of PD in animal models, including non-human primates.[6,68,69] In general, progenitors produce a more effective graft than mature VM neurons, but the type of progenitor can matter. By correlating the pre-transplantation transcriptome of one cell product with graft outcome, it was shown that enrichment for caudal VM was beneficial.[70] Timed exposure to FGF8b enhanced caudal VM production, enabling development of a clinical-grade process.[70] Globally, a number of cell products for PD are in, or advancing toward, early-stage trials,[6–8] including autologous approaches.[71,72] Recognizing the benefit of working together, the international PD cell transplant community shares information and findings: http://www.gforce-pd.com/.

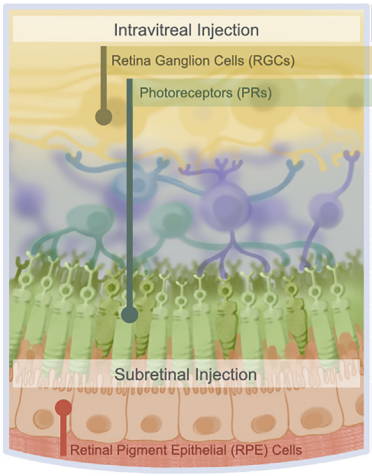
As PD products become more advanced, there are opportunities for product optimization. sc/nucRNA-seq studies cataloging human dopaminergic neurons *in vivo* have identified around 10 distinct populations, including subtypes that are vulnerable or resilient to PD.[73–75] In-depth *in vivo* characterization also benefits analysis of hPSC-derived VM lineages in culture.[76,77] This analysis revealed surface markers, Calsyntenin 2 (CLSTN2) and Protein Tyrosine Phosphatase Receptor Type O (PTPRO), that enriched for dopaminergic progenitors, producing a more stable and efficacious graft with fewer unwanted cell types.[77] Studies of a different PD product identified some unexpected perivascular cells in the graft.[78,79] These findings further illustrate the value of iterative product manufacture informed by deep analysis of the pre-and post-transplant cells, the developing and mature human tissue, and functional outcomes. Even though this approach relies on animal studies in the preclinical phase, ultimately, it should be done in patients. Notably, brain tissue collected from deceased patients several years after fetal VM transplantation showed evidence of Lewy bodies in the graft, aggregates of α-synuclein that are a pathological hallmark of PD.[80] While this occurred rarely and only after more than 10 years post-grafting, it suggests product protection could be improved, perhaps by harnessing knowledge about resilient dopaminergic neuron phenotypes.

Forebrain GABAergic interneurons

A multitude of interneurons, defined by CNS location, morphology, peptide expression, and circuitry, regulates neural activity. Lineage mapping studies in animals revealed that most forebrain interneurons are born in the embryonic ventral forebrain progenitor zones, the medial, lateral, and caudal ganglionic eminences (MGE, LGE, and CGE), then migrate widely. Recent scRNA-seq profiling of the developing human fetal forebrain documents the phenotypes of MGE, LGE, and CGE progenitors and of cortical progenitors that produce some cortical interneurons.[81,82]

Forebrain GABAergic interneurons dampen excitatory neuron activity and fine-tune their output. hPSCs patterned to MGE-type progenitors produced GABAergic interneurons[83,84] that after transplantation into the hippocampus of a mouse epilepsy model migrated extensively, formed synaptic connections with host excitatory neurons, dampened seizures, and improved cognition.[14] A chemically matured GABAergic interneuron transplant shows similar benefit in a mouse epilepsy model, with optogenetic stimulation of graft neurons controlling seizure activity.[85] With this promising therapeutic strategy, an hPSC-derived MGE-type GABAergic interneuron product (NRTX-1001) recently entered a phase I/II trial for patients with intractable temporal lobe epilepsy (NCT05135091). Several other diseases with neurodegenerative elements show impaired GABAergic interneuron functions, including schizophrenia and neuropathic pain.[86–88] Moreover, degenerative diseases affecting the cortex, such as AD and FTD, are associated with epileptiform phenomena and excitotoxicity, in which glutamatergic neurons become overactive and die.[89,90] Hence, in these diverse disorders, increasing GABAergic interneuron function could be therapeutic, and the progress of clinical trial NCT05135091 and similar upcoming trials that seek to increase inhibitory neuron activity will be eagerly followed.

As we understand better how interneuron subtypes contribute to brain function and disease, we can envision many more therapeutic applications.[91] It has, however, proven difficult to generate specific subtypes such as fast-spiking, parvalbumin-expressing GABAergic interneurons in a reasonable number and time frame, which remains an active area of research. Overexpression of LIM Homeobox 6 (LHX6) in human induced pluripotent stem cells (hiPSCs) accelerated production of



Product	Disease Target	Approach	Benefit of Approach	Challenge	Clinical Trial ID	Clinical Phase
hPSC-RGC cells [118-120]	Glaucoma	RGC suspension (Intravitreal)	Restores retina-brain connection	Synapse with retinal neurons; Long axon to LGN targets; Reconstruction of retinotectal map	-	-
PSC-PR precursor [107-112, 114]	RP Advanced AMD	PR suspension (Subretinal)	Restores light detection	Synapse with retinal cells; Develop outer segment; Restore interaction with RPE	-	-
OpRegen© - hESC-RPE	Geographic atrophy secondary to AMD	RPE cell suspension (Subretinal)	Replaces dysfunctional RPE; Supports PR function; Infuse into subretinal space via cannula creating a small self-sealing hole in the retina	Integrate into host RPE monolayer efficiently with tight junction formation	NCT05626114	II
ASP7317 - hESC-RPE					NCT03178149	I
Adult RPE stem cell-RPE RPESC-RPE-4W [41]	Dry AMD				NCT04627428	I/II
Autologous iPSC-derived RPE [101]	Macular Degeneration				NCT05445063	I
hESC-RPE (paralyene scaffold) [95,96]	Acute Wet AMD	RPE monolayer on a Scaffold (Subretinal)	Replaces dysfunctional RPE; Maintains cell junctions and orientation; Scaffold can substitute for impaired Bruch's membrane	Maintain monolayer organization; Requires a specialized surgical device; Surgical complications associated with insertion	NCT02590692	I/II
hESC-RPE (polyester scaffold) [97]					NCT01691261	I
hESC-RPE (amniotic membrane) [100]	Monogenic RP				NCT03963154	I/II
Autologous hiPSC-RPE (Biodegradable poly-(lactic-co-glycolic acid)/PLGA scaffold [99]	Geographic Atrophy Associated With AMD				NCT04339764	I/II
hPSC-derived 3D multilayered neural retina + co-cultured RPE [129-134]	Advanced retinal degeneration	Multi-layered neural retina +/- co-cultured RPE	Replace multi-layered retina loss	Maintain layer organization; Effective integration and connectivity; GMP manufacturing and storage; Requires a specialized surgical device; Surgical complications associated with insertion	-	-

Figure 3. Retinal cell replacement
Examples of ongoing studies and some of the challenges of using stem cell-derived retinal cell products, both single-cell-type and multicell grafts, to replace dysfunctional retinal cells. PR, photoreceptor.

somatostatin and parvalbumin interneurons *in vitro* and enhanced their engraftment *in vivo*.[92] Another hurdle is understanding how interneurons survive and integrate into different brain regions. In mice, survival of transplanted interneurons depends on their gamma protocadherin expression.[93] Transplantation of hPSC-derived MGE-type progenitors into the neonatal rat striatum produced predominantly striatal-type rather than cortical-type interneurons.[94] This result shows the importance of considering the recipient as a selective or instructive environment affecting outcomes. With further advances in hPSC-interneuron manufacture and attaining successful engraftment, the considerable therapeutic potential of this cell type in different neurodegenerative disease settings may be realized.

Retinal neurons and RPE

The retina consists of the neural retina and RPE cells, a crucial support cell that maintains retinal homeostasis, photoreceptor function, and vision. RPE cell dysfunction and death in the central retina, the macula, underlies AMD, the leading cause of blindness in the elderly. Other neurodegenerative diseases primarily affect the neural retina directly, including retinitis pigmentosa that leads to death of photoreceptor cells, and glaucoma that kills retinal ganglion cells (RGCs), the sole neural connection between the retina and brain. Several stem cell-derived retinal cell products are being developed to combat retinal degenerations (Figure 3). Additional approaches aim to augment the environment with other neural cell types or trophic factors to improve retinal cell survival. A human fetal brain-derived neural progenitor product, CNS10-NPC, is being tested as a subretinal injection for patients with retinitis pigmentosa (NCT04284293). JCyte has successfully completed a phase II study of human fetal retinal progenitor cells injected into the vitreous, releasing trophic factors to prevent photoreceptor degeneration in patients with retinitis pigmentosa (NCT03073733). Subretinal injection of a gene therapy expressing a neurotrophic factor to prevent cone photoreceptor cell death in retinitis pigmentosa patients is in clinical trial (NCT05748873).

Ongoing clinical trials for RPE replacement use hESCs, hiPSCs,[11] and adult cadaver-sourced RPE stem cells[41] (NCT04627428) as the starting cell source. Two main subretinal transplant product types are being pursued: an RPE cell suspension or an RPE monolayer on an engineered scaffold, which may be permanent[95–98] or biodegradable.[99,100] The first US clinical trial for autologous hiPSCs uses patient-derived RPE cells on a biodegradable scaffold[99] (NCT04339764). The immune privilege of the subretinal space can be compromised by disease and trauma, so it is important to compare autologous and allogeneic approaches.[99,101] Overall, early-stage RPE transplantation trials are demonstrating that the approach is safe with promising signs of efficacy for patients with AMD.[102]

Although traditionally viewed as a homogeneous monolayer, scRNA-seq analyses of RPE cells acutely isolated from the adult human eye revealed multiple subclusters representing different subtypes and states.[103] Newly identified markers for peripheral and macular RPE provide insight into how these specialized RPE subtypes develop.[103] Artificial intelligence-based image analysis has revealed unique characteristics of RPE cells in macular versus peripheral regions with different disease vulnerabilities.[104] Comparison of healthy and AMD RPE, using scRNA-seq and proteomics, confirmed an involvement of the complement system in AMD and highlighted relevant signaling pathways, such as WNT and prostaglandin signaling.[105] Clearly, the RPE has complex composition and contributions to degenerative retinal diseases, which will inform future therapy development. For example, biasing hPSC-RPE cells toward macular phenotypes may generate a more effective AMD product.

This new understanding of RPE cell diversity is guiding a deeper analysis of existing RPE transplant products. Adult RPE stem cells produce a range of RPE subtypes and states. Tracking their differentiation over time has revealed molecular markers for the progenitor cells that are most effective at visual rescue in animals, including a long non-coding RNA *TREX*, whose expression is positively correlated with RPE cell integration into a pre-formed human RPE monolayer *in vitro*.[41,106] Hence, by defining RPE cell products more completely and correlating with functional outcomes, we can find CQA

biomarkers defining identity, purity, and potency, beyond the canonical RPE markers.

Transplantation of retinal neurons is more challenging than transplantation of RPE cells, primarily due to the need for functional synaptic connectivity. Nevertheless, preclinical studies have documented the benefit of photoreceptor transplantation for vision rescue.[107–109] Intriguingly, mouse photoreceptor cells injected into the mouse retina formed cytoplasmic bridges with existing host photoreceptors,[109–111] providing an unexpected mechanism for transferring cell contents and improving photoreceptor health. However, such fusion events occur less often when using human donor cells in a xenotransplant.[112] Human photoreceptors include rods and cones, each with functional subcategories. scRNA-seq analysis has revealed distinct subtypes of cones spatially organized in the human macula.[113] Human photoreceptor progenitors transplanted into rcd1/PDE6B mutant dogs with advanced inherited retinal degeneration survived with immunosuppression, showed no tumor formation, and differentiated largely into cone photoreceptors with evidence of synaptic contacts.[114] These results represent an encouraging step toward using these progenitors in a clinical trial.

Successful transplantation of RGCs is particularly challenging given their long and precise trajectories,[115,116] primarily to the lateral geniculate nucleus of the thalamus in humans (Figure 1). About 40 subtypes of mouse RGCs have been identified by scRNA-seq,[117] and categorization of human RGCs in healthy and glaucomatous eyes is ongoing. Multiple types of RGCs can be generated from hPSCs, enhancing studies of their specialized functions.[20] hESC-derived RGCs integrated and formed presumptive synaptic contacts in *ex vivo* retinal explants,[118] while cells injected intravitreally in adult rats survived and migrated into the RGC layer.[119] NGN2 induction generates human iRGCs that protected resident retinal cells from neurodegeneration after optic nerve crush injury.[120] Still, efficient integration and connectivity remain substantial hurdles.[121] It will be valuable to determine if different RGC subtypes, such as those resistant to glaucoma or those with more regenerative ability,[122,123] can improve outcomes. Optic nerve repair may also benefit from stimulating a glial progenitor present in the human lamina where RGC axons enter the nerve prior to becoming myelinated.[124]

For advanced retinal degeneration, in which much of the tissue has been lost, multicell-type transplants are being contemplated. Proof of concept has come from pioneering surgeries in which the central retina is translocated over healthier, more peripheral RPE in AMD patients.[125–128] Despite the high degree of operative complications, some patients showed vision benefit. Our ability to produce multilayered retina from hPSCs provides new opportunities for retinal transplantation (Figure 3).[129,130] Several studies have examined the transplantation of hPSC-derived 3D retinal sheets into mice, nude rats, and monkeys. These grafts survived well, although they were frequently disorganized with inclusion of rosette structures rather than well-integrated layers. Younger stage grafts showed evidence of photoreceptor formation, and some studies provided evidence for light detection originating from the graft.[130–134] Incorporating RPE with the neural retinal sheet is challenging, and the tissues frequently separate after transplantation *in vivo*.[135] Nevertheless, these pioneering studies map a path toward a graft that retains effective retinal cell organization and integrates sufficiently to benefit patients with severe vision loss. Concomitantly with this transplantation research, considerable hurdles are being addressed to establish clinical-grade manufacturing of the envisioned 3D retinal products.[136]

Astrocytes

Astrocytes are distributed throughout the CNS and play multiple key roles. They support neurons and synaptic function and regulate brain homeostasis, innate immunity, metabolism, and blood-brain barrier (BBB) integrity.[137,138] In disease or injury, astrocytes become "reactive," exhibiting complex morphological and gene expression changes with altered secretome and cell-cell interactions that may have both positive and negative effects.[137–139]

We have long recognized the heterogeneity of astrocytes, which is related to their specific location, morphology, function, age, and state of disease, and enhanced by recent sc/nucRNA-seq analyses.[140–142] In neurodegenerative disease they may have beneficial roles, such as clearance of pathological tau and α-synuclein, but they also contribute to pathology, for example, by spreading toxic molecules.[139,141,143,144] Specific astrocyte phenotypes can be disease diagnostic, for example, tufted astrocytes with tau pathology in PSP.[145,146] The understanding that astrocytes can be beneficial players is driving development of astrocyte products for several neurodegenerative diseases, including ALS, PD, HD, and AD.[147,148]

During CNS development, astrocytes originate from multipotent NPCs and neural stem cells (NSCs), such as radial glia in the cerebral cortex.[149] NSCs first generate neurons, then later glial progenitor cells (GPCs) that make both astrocytes and oligodendrocytes.[150] GPCs derived from fetal human brain and injected into neonatal and adult mice showed widespread astrocyte integration and improved outcomes in several models of neurodegenerative diseases, such as ALS and MS.[150] Interestingly, human GPCs and their derived astrocytes supported enhanced learning and plasticity, compared with analogous mouse cells, suggesting the human astrocytes conferred greater functionality.[151]

GPCs can be generated from hPSCs and biased toward astrocyte differentiation.[29,152] When produced from disease-derived hPSCs, they model glial contributions to disease, such as neurotoxicity.[144,153,154] For instance, when CD44+ astrocyte progenitors, produced from hESCs carrying the mutant huntingtin gene of HD, were transplanted into the neonatal mouse brain, they impaired motor learning, indicating that glial pathology alone was sufficient to yield aspects of an HD phenotype. Conversely, transplanted wild-type astrocytes improved cognitive and motor outcomes in an HD mouse model.[155] A number of neuropsychiatric disorders, most notably schizophrenia, are accompanied by marked astrocytic pathology, and GPCs and astrocytes produced from patient-derived hiPSCs disrupt normal behavior, cognition, and sleep patterns when transplanted into neonatal mice.[156]

Astrocytes can be generated from hPSCs by first creating NPCs and then treating with CNTF and LIF.[29,157] By guiding NPCs to different rostro-caudal domains, astrocytes were produced that retained regional identity after transplantation.[152]

hESC-derived astrocytes transplanted into an ALS hSOD1G93A mouse model slowed disease onset, although intrathecal administration in this experiment led largely to meningeal engraftment.[158] hiPSC-derived astrocytes directly injected into the adult mouse brain acquired morphologies similar to those in human brain, including the primate-enriched intralaminar and varicose forms. Importantly, the implanted astrocytes responded to AD pathology similarly to endogenous astrocytes in patients with early-stage AD.[159] However, the engrafted cells did not show the degree of migration and integration seen with GPC injection, perhaps indicating that transplantation of more mature astrocytes is not as effective as transplanting progenitor stages.

Astrocyte states are being increasingly explored to promote recovery from neurodegenerative diseases. For example, inactivation of Sirtuin 1 (SIRT1) can switch reactive mouse astrocytes to an anti-inflammatory phenotype that reduces pathology in an animal model of inflammatory demyelinating disease.[160] Studies have defined how interactions with disease-activated microglia can alter astrocytes to exhibit a pathologic "killer" state.[139] In this state, they release neurotoxic saturated lipids, which can be compensated by strategies such as lowering the lipid synthesis enzyme ELOVL Fatty Acid Elongase 1 (ELOVL1), improving recovery in an axonal injury model.[161] In the future, we anticipate the translation of these findings into improved astrocyte-targeting products that may benefit multiple neurodegenerative diseases.

Oligodendrocytes

Oligodendrocytes wrap axons in an insulating, lipid-rich myelin sheath that results in rapid, saltatory conduction of action potentials. Demyelinating diseases cause a failure of neural function due to slowed and interrupted axonal conduction. Leukodystrophies are heritable diseases that primarily affect oligodendrocytes, leading to dysmyelination and often neurodegeneration. They frequently have a childhood onset and are first noted when developmental delays in motor coordination and speech are observed. Examples include Krabbe, Tay-Sachs, and Canavan diseases, among others.[162] Leukodystrophies have diverse underlying genetic causes, many converging on lipid metabolism impairment, which particularly affects oligodendrocytes given their role in myelin production.[163] MS is a more common demyelinating disease that has an immunological component. Several neurodegenerative and psychiatric diseases include prominent oligodendrocyte pathology and white matter loss, such as FTD, AD, PD, HD, MSA, and schizophrenia. In MSA, α-synuclein builds up primarily in oligodendrocytes, leading to widespread degeneration.[164] Some white matter diseases can be treated by blood cell infusion, such as the recently approved therapy for cerebral adrenoleukodystrophy in which a patient's hematopoietic stem cells (HSCs) are engineered to express a normal ATP Binding Cassette Subfamily D Member 1 (*ABCD1)* gene before reinfusion.[165] Several related strategies are in preclinical development for other leukodystrophies.[166] Similarly, patients with MS can benefit from HSC transplantation to "reset" their immune system.[167] However, when there is substantial loss of myelin that is not endogenously repaired, an oligodendrocyte replacement therapy is required.[168,169]

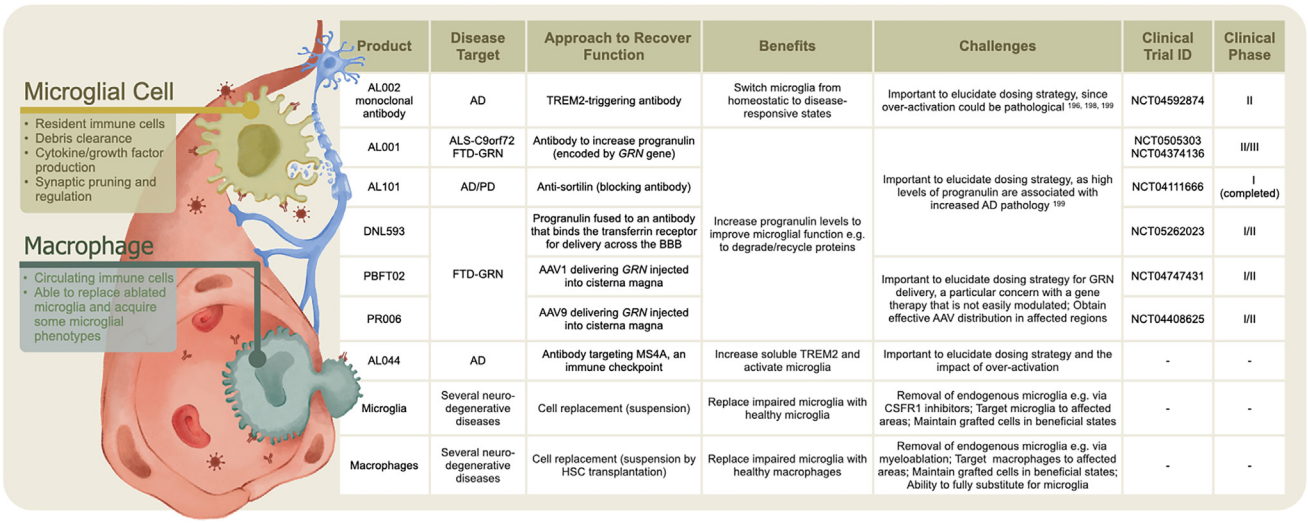
Proof of concept for successful oligodendrocyte replacement has been demonstrated by engrafting human fetal GCPs into animal models of myelin deficiency, such as the immunocompromised shiverer (*shi/shi*)×*rag2*$^{-/-}$ mouse model. Direct injection into the neonatal brain targeting the main white matter tracts led to a remarkable result: widespread replacement of mouse oligodendrocytes with human oligodendrocytes, and significantly extended survival in some recipients.[170] Importantly, human fetal GPCs can also remyelinate in adult mice after chemically induced demyelination and in adult shiverer mice.[171] Similar promising results have been observed using hPSCs as the oligodendrocyte source, with widespread migration, maturation, and remyelination in nude rats after radiation treatment,[30] neonatal (*shi/shi*)×*rag2*$^{-/-}$ mice,[172] adult nude rats after traumatic injury,[173] and in adult mice after spinal cord injury.[174] One of the challenges of manufacturing hPSC-derived oligodendrocytes is their protracted development over months.[30] Direct reprogramming, for example, by expressing SRY-Box Transcription Factor 10 (SOX10), Oligodendrocyte Transcription Factor 2 (OLIG2), and NK6 Homeobox 2 (NKX6.2) in hPSCs, rapidly produces induced oligodendrocyte-like cells that are effective in animal models of demyelinating disease.[175]

During normal development, oligodendrocytes originate in different regions of the CNS and then migrate widely, occupying different domains depending on their origin.[176] Investigations of oligodendrocyte populations in the white matter of human brains using sc/nucRNA-seq analysis has revealed six different subclasses and altered proportions between healthy individuals and MS patients.[177] They also confirmed transcriptional heterogeneity in several neurodegenerative diseases, such as HD, PD, and AD.[178] It will be interesting to learn whether some oligodendrocytes subtypes and states are better at engrafting, self-renewing, and recovering myelin in specific disease settings.

Microglia

Microglia are the resident macrophages in the CNS. They migrate into the early developing CNS, where they are long-lived and sustained by self-renewal.[179] Microglia tile through the CNS, forming an expansive network that maintains homeostasis, surveilles for cellular debris and infectious agents, and participates in response and repair processes.[180] Rather than simplifying microglial behavior into a dichotomous switch between ramified/resting versus amoeboid/reactive states, multiple microglial states are being recognized through single-cell analyses, providing the level of detail needed to better understand their biology.[181]

In neurodegenerative diseases, microglia benefit processes such as debris clearance but also contribute to pathology, for example, by spreading abnormal tau or α-synuclein.[182,183] Impaired microglia can underlie neurodegenerative diseases, such as adult leukodystrophy caused by mutations in the Colony Stimulating Factor 1 (CSF1) receptor (CSF1R), which is important for microglial survival.[184,185] Their critical roles have been brought further into focus through genetic association studies. GWAS analysis has uncovered over 70 loci associated with AD.[186] Surprisingly, most candidate AD risk-modifying genes are strongly expressed by microglia and monocytes, converging on core functions such as efferocytosis.[187,188] Functional genomic and sc/nucRNA-seq analyses across multiple tauopathies in human brain has revealed stage- and disease-specific microglial responses.[189] This understanding and further study



Product	Disease Target	Approach to Recover Function	Benefits	Challenges	Clinical Trial ID	Clinical Phase
AL002 monoclonal antibody	AD	TREM2-triggering antibody	Switch microglia from homeostatic to disease-responsive states	Important to elucidate dosing strategy, since over-activation could be pathological [196,198,199]	NCT04592874	II
AL001	ALS-C9orf72 FTD-GRN	Antibody to increase progranulin (encoded by *GRN* gene)	Increase progranulin levels to improve microglial function e.g. to degrade/recycle proteins	Important to elucidate dosing strategy, as high levels of progranulin are associated with increased AD pathology [199]	NCT0505303 NCT04374136	II/III
AL101	AD/PD	Anti-sortilin (blocking antibody)			NCT04111666	I (completed)
DNL593	FTD-GRN	Progranulin fused to an antibody that binds the transferrin receptor for delivery across the BBB			NCT05262023	I/II
PBFT02		AAV1 delivering *GRN* injected into cisterna magna		Important to elucidate dosing strategy for GRN delivery, a particular concern with a gene therapy that is not easily modulated; Obtain effective AAV distribution in affected regions	NCT04747431	I/II
PR006		AAV9 delivering *GRN* injected into cisterna magna			NCT04408625	I/II
AL044	AD	Antibody targeting MS4A, an immune checkpoint	Increase soluble TREM2 and activate microglia	Important to elucidate dosing strategy and the impact of over-activation	-	-
Microglia	Several neuro-degenerative diseases	Cell replacement (suspension)	Replace impaired microglia with healthy microglia	Removal of endogenous microglia e.g. via CSFR1 inhibitors; Target microglia to affected areas; Maintain grafted cells in beneficial states	-	-
Macrophages	Several neuro-degenerative diseases	Cell replacement (suspension by HSC transplantation)	Replace impaired microglia with healthy macrophages	Removal of endogenous microglia e.g. via myeloablation; Target macrophages to affected areas; Maintain grafted cells in beneficial states; Ability to fully substitute for microglia	-	-

Figure 4. Approaches to target microglia to combat neurodegenerative diseases
Greater understanding of microglial contribution to neurodegenerative pathologies has led to biologics targeting microglial function and to cell replacement strategies using microglia or macrophages.

of AD risk genes enriched in microglia, such as Triggering Receptor Expressed On Myeloid Cells 2 (*TREM2*) and membrane-spanning 4-domains subfamily A (*MS4A*),[190,191] or genes important for microglial regulation such as progranulin (*GRN*)[192,193] are spurring approaches to treat neurodegenerative diseases by manipulating microglia (Figure 4). For example, TREM2-triggering antibodies that promote the transition from a homeostatic to a disease-associated microglial state[194,195] are advancing through clinical trial for AD.[196] Given the association of TREM2 with other neurodegenerative conditions such as ALS, PD, and FTD,[197] it may prove to be a valuable multi-disease approach. Tempering this is the understanding that increased TREM2 could be pathological,[196,198,199] necessitating carefully developed treatment regimens.

Microglia can be generated from hPSCs by mimicking their mesodermal and hematopoietic developmental trajectory[25,200,201] or by direct induction. For example, hiPSCs with dox-inducible cassettes for PU.1, MAFB, CEBPα, CEBPβ, IRF5, and IRF8 produced iTF microglia that resembled other hPSC-derived microglia, albeit with some distinctions from microglia derived from human brain.[202] Subsequent addition of CRISPRi and CRISPRa machinery enabled a large-scale genetic screen for molecular drivers that alter iTF microglial states. For example, microglia expressing the gene encoding osteopontin, Secreted Phosphoprotein 1 (*SPP1*), associated with aging and exposure to amyloid or tau pathology, are increased or decreased in abundance by knocking down Mitogen-Activated Protein Kinase 14 (MAPK14) or CSF1R, respectively.[202] CRISPR-based functional testing has the exciting potential to reveal ways microglial states can be manipulated to promote their therapeutic potential.

Because of their central roles in surveillance, microglia respond chameleon-like to altered environments. One challenge in studying human brain microglia is that they change significantly soon after isolation.[203] Moreover, hiPSC-derived microglia in culture are highly variable between individuals,[204] as seen in primary microglia.[205] Transplanting human microglia into the murine brain circumvents some of these issues. hPSC-derived microglia injected into the perinatal mouse forebrain integrated widely and adopted phenotypes similar to human microglia *in vivo*. Their survival depends on hCSF1 that can be incorporated into the model, e.g., in *Rag2−/− Il2rγ−/− hCSF1KI* or MITRG mice.[206,207] Microglia injected into a MITRGx5xFAD model showed acquisition of human disease-associated microglial signatures, including upregulation of TREM2.[207] Fascinatingly, in an AD mouse model that lacks microglia, pathology was shifted from cerebral plaques to cerebral amyloid angiopathy, with brain calcification and cerebral hemorrhage.[208] This pathology was reversed by a single injection of wild-type microglia.[208]

Hence, xenotransplantation can shed light on microglial contributions to pathology and provide preclinical evidence supporting safety and efficacy. For a transplantation to be successful, endogenous microglia may need to be reduced (for instance, using the CSF1R inhibitors PLX3397 or PLX647) to provide open niches for new cells to occupy.[209] Preclinical studies of PLX3397 and PLX647, which cross the BBB, demonstrate that microglial removal is safe in adult mice and non-human primates, without significant changes in inflammation or cognition, and show benefits in AD, PD, ALS, and prion disease models.[209,210] PLX3397 is an FDA-approved drug for patients with brain cancer.[211] Excitingly, human microglia engineered to resist CSF1R inhibitors show more widespread integration in the adult murine brain than wild-type microglia after PLX3397 treatment, potentially revealing an effective way to achieve replacement in patients.[212] In a different approach, mice treated with the myeloablation agent busulfan followed by HSC transplantation showed extensive microglial clearing and replacement with peripheral macrophages.[213] A similar macrophage replacement strategy slowed progression of neurodegeneration caused by prosaposin deficiency.[214] Future studies will determine the extent of human macrophage replacement of microglial functions, as transplanted cells exhibit persisting differences compared with brain

microglia.[214,215] These studies encourage further assessment of the potential benefits of microglial replacement in multiple neurodegenerative indications, including genetic deficiencies, such as lysosomal storage diseases. They also hint at a potential to prevent diseases, such as AD, from progressing to a state of widespread, multicell loss.

Vascular cells

The brain vasculature delivers essential nutrients and helps to recycle interstitial and cerebrospinal fluids. ECs in the brain vasculature are firmly connected by tight junctions, forming the BBB that regulates the exchange of circulating molecules. Vascular involvement is common across neurodegenerative diseases.[216] The majority of AD patients have brain blood vessel defects, sometimes predating symptom onset by decades.[216,217] Vascular abnormalities that contribute to neurodegeneration include reduced cerebral blood flow, BBB breakdown, vascular stiffening, endothelial dysfunction, and pericyte loss. For this reason, vascular repair is being examined as a potential therapeutic option for several neurodegenerative conditions, including traumatic brain injury,[218] ischemia,[219] and ALS.[220]

hPSCs can be differentiated efficiently into mesoderm-derived ECs, pericytes, and smooth muscle cells, albeit with lower trans-endothelial electrical resistance than in the brain.[26,31] Methods to generate brain ECs with high trans-endothelial electrical resistance from hPSCs require additional research to determine the factors regulating this aspect of brain vessel function. Studies demonstrate that some approaches to produce brain ECs from hPSCs actually produce epithelial cells with high trans-endothelial electrical resistance that lack canonical EC markers.[221,222] However, these markers can be gained by enforcing expression of ETS Variant Transcription Factor 2 (ETV2), ETS Transcription Factor ERG (ERG), and Fli-1 Proto-Oncogene, ETS Transcription Factor (FLI1).[222] hiPSC-derived vascular cells transplanted into a rat model of white matter infarct improved hind limb movement and remyelination in the infarct region.[219] hPSCs differentiated into cranial neural crest generate pericyte-like cells that promote BBB repair and reduce neuronal loss after transplantation into a mouse model of stroke.[28]

Hence, both ECs and mural cells are important in neurodegenerative pathogenesis. sc/nucRNA-seq analyses provide a deeper understanding of the heterogeneity of different vascular beds[223] and the responses of large and small vessel cells to neurodegenerative diseases, such as AD,[224–226] PD,[227] and HD.[228] sc/nucRNA-seq analysis of white matter in patients with vascular dementia has revealed a subset of disease-associated ECs expressing genes implicated in cell death and protein folding and a subset expressing genes associated with angiogenesis and oligodendrocyte maturation.[229] For AD, sc/nucRNA-seq analysis of the prefrontal cortex highlighted ECs from patient samples with increased expression of angiogenic growth factors and receptors (such as EGF Like Domain Multiple 7 (*EGFL7*), Fms Related Receptor Tyrosine Kinase 1 (*FLT1*), and Von Willebrand Factor (*VWF*)) and genes associated with antigen presentation (such as Beta-2-Microglobulin (*B2M*) and *HLA-E*), implying functions in vessel regrowth and immune responses.[225] A method to improve sc/nucRNA-seq analysis of vascular cells (VINE-seq) has revealed selective vulnerability of a subset of pericytes specialized to maintain the extracellular matrix, which may contribute to the loss of BBB integrity seen in AD.[224] Notably, this study also showed that 30 of the top 45 genes linked to AD by GWAS are vascular cell-associated. sc/nucRNA-seq analysis of carriers of the APOE4 isoform of Apolipoprotein E, which increases risk for AD, shows APOE and Nuclear Factor Of Activated T-Cells (NFAT) dysregulation in pericytes and that targeting of calcineurin/NFAT signaling reduces APOE-associated cerebral amyloid angiopathy pathology in model systems.[226] Hence, these exciting studies are highlighting promising targets for vascular cell-focused therapies.

Neural stem cells

NSCs build the CNS, create multiple types of neurons and glia, and seem perfectly suited for replacement therapies for disorders in which a variety of cells are lost.[230] Pioneering clinical studies tested human fetal-derived NSCs for neurological conditions, such as Batten disease, Pelizaeus-Merzbacher disease, spinal cord injury, AMD, and AD.[231] NSC transplantation proved safe with signs of positive efficacy. A human fetal-derived NSC line transduced to express GDNF improved motor neuron survival and animal lifespan in a Superoxide Dismutase 1 (SOD1) rat model of ALS[232,233] and demonstrated safety in an early-phase clinical trial (NCT05306457).[234] Cell delivery is a challenge for any neurodegenerative disease. Typically, direct cell injection is the envisioned initial path. Several studies indicate that intravascular administration can be effective using NSCs with high $\alpha4\beta1$-integrin expression, with brain penetration and improved outcomes demonstrated in an ALS mouse model.[235]

NSCs are highly diverse regionally and temporally, which biases their ability to produce specific types of progeny.[236] Mouse NSCs patterned to the visual cortex can survive and extend appropriate connections when transplanted into the visual but not the motor cortex of adult mice.[237] Hence, matching the NSC product may be an important factor to accomplish effective multicell replacement in different CNS regions. In HD, the loss of striatal medium spiny neurons progresses to a devastating degeneration of the basal forebrain and cerebral cortex. Transplanting hESC-derived NSCs into the brains of a P6 mouse model of HD showed improved histology and motor and cognitive functions.[238] In a similar approach, transplanting hiPSC-derived NPCs produced neurons, astrocytes, and oligodendrocytes and improved cognitive and motor outcomes in an HD mouse model, with overall benefits greater than those obtained with human GCPs.[239] These studies demonstrate the value of using multipotent progenitors to replace lost CNS cell types. Moreover, incorporating developing vascular cells along with NPCs improved engraftment and vessel structure in a mouse model of stroke, supporting the feasibility of yet more complex, mixed cell products for extensive CNS repair.[240] hPSC-derived cortical organoids engrafted into the neonatal rat brain received thalamocortical and corticocortical inputs, extended axons, and responded to optogenetic stimulation, driving reward-seeking behavior.[241] In adult mouse cortex, human cerebral organoids also engraft, become vascularized, and show microglial colonization, rhythmic electrical activity, and optogenetic-stimulated graft-host connectivity.[242] Excitingly, cortical organoids transplanted into adult mice equipped with electrodes to monitor brain activity integrate and show

electrophysiological cortical responses to visual stimuli.[243] Successful transplantation of organoids into the monkey cerebral cortex has also been demonstrated.[244] These studies suggest that in the future, hPSC-derived 3D neural grafts could potentially treat important aspects of the multicellular, regional neural cell loss associated with complex neurodegenerative diseases.

SUMMARY OF PROGRESS AND FUTURE PERSPECTIVES

Active clinical evaluation is ongoing for stem-cell-derived dopaminergic neurons for PD, RPE for AMD, and GABAergic interneurons for epilepsy. Progress in these areas includes a deeper characterization of the cell products and graft outcomes. The goals are to better define CQA biomarkers and to produce improved or refined products for specific indications. Technologies such as sc/nucRNA-seq benefit these preclinical studies and may in the future be employed widely in manufacturing and release criteria, aided by the implementation of cross-platform benchmarking.[245] Several years of preclinical research indicate that astrocyte and oligodendrocyte transplantation might be beneficial for a variety of demyelinating and neurodegenerative diseases, and we anticipate clinical evaluation shortly. Exciting progress in achieving effective microglial replacement in animal models opens new opportunities for cross-disease therapies, given their broad immunomodulatory role. However, maintaining microglia in a beneficial state could be challenging. In cases where the goal is widespread cell replacement after microglial or macroglial transplantation, the effects of such a drastic change on human CNS function are yet to be determined, although animal studies are encouraging. Restoring neuronal connectivity, both local and long-range, remain substantial hurdles. However, there are positive signs from retinal and cerebral transplants that despite disrupted organization, connections with host cells can improve functionality. Finally, the impact of the human host environment, and how it changes with disease stage, is an important consideration for successful grafting. Looking forward, we anticipate that the preclinical pipeline for neurodegenerative diseases will include cell products that are genetically manipulated to be resilient, to slow disease progression, and to enhance cell function or control cell states for specific disease indications.

Conclusions

Increased knowledge of individual brain cell phenotypes and states, through in-depth characterization of tissue and stem-cell-derived cells, is guiding development of neural cell therapeutics. With brisk translation, there is a good reason to think that human stem-cell-based products will produce disease-altering therapies to improve the lives of patients with currently incurable neurodegenerative diseases. Although there are challenges, we are encouraged by progress to date, and take inspiration from Steven Hawking's last message to spur us to take care of our world and future: "*Be brave, be determined, overcome the odds. It can be done*."

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DECLARATION OF INTERESTS

S.T. is co-founder of Luxa Biotech developing an RPE therapy for AMD and has patents related to RPE cell therapy: retinal pigment epithelial stem cells, patent number: 8481313; methods of treating a retinal disease by retinal pigment epithelial stem cells, patent number: 10034916. S.T. has advised BlueRock Therapeutics, Vita Therapeutics, and SANA Biotechnology.

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