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Can astrocytes be a target for precision medicine?

Chloe Allen, Pamela J Shaw and Laura Ferraiuolo

Contents

Can astrocytes be a target for precision medicine? ................................................................. 1

Abstract ........................................................................................................................................ 2

1. Astrocyte function and dysfunction in the central nervous system ....................................... 2
   1.1 Brain homeostasis ................................................................................................................. 2
   1.2 Neuronal development and support ....................................................................................... 3
   1.3 Blood Brain Barrier ............................................................................................................... 4
   1.4 Inflammation and immune response ...................................................................................... 5

2. Current application of personalised medicine in neurological disorders ............................... 6
   2.1 Alzheimer’s disease ............................................................................................................... 7
   2.2 Parkinson’s disease .............................................................................................................. 7
   2.3 Amyotrophic Lateral Sclerosis ............................................................................................. 8

3. Astrocytes driving the future of precision medicine ................................................................. 9
   3.1 Sources of Astrocytes .......................................................................................................... 9
   3.2 Astrocytes in Cellular Transplantation Therapy ................................................................. 11
   3.3 Astrocytes in Genomics and Transcriptomics ..................................................................... 13
   3.4 Astrocytes in Diagnostic and Prognostic Biomarkers .......................................................... 15
   3.5 Astrocytes in Drug Screening ............................................................................................. 17

4. Conclusion ................................................................................................................................ 20

5. Bibliography ............................................................................................................................ 22

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Abstract
Astrocytes are the most abundant non-neural cell type residing within the central nervous system (CNS) displaying tremendous heterogeneity depending on their location. Once believed to be ‘passive support cells for electrically active neurons’, astrocytes are now recognised to play an active role in brain homeostasis by forming connections with the surrounding neurons, microglia and endothelial cells. Most importantly, they provide an optimum microenvironment for functional neurons through regulation of the blood brain barrier, energy supply and removal of debris and toxic waste.

Their dysfunction has been identified as a potential contributing factor for several neurodegenerative disorders, from Alzheimer’s Disease to Amyotrophic Lateral Sclerosis.

In this chapter, we will explore the implications of astrocyte dysfunction in neurodegenerative diseases and how these cells can be used as therapeutic targets in precision medicine.

1. Astrocyte function and dysfunction in the central nervous system
Current research is discovering that many neurological diseases have shared pathological mechanisms; for example, neuroinflammation and neuronal death in concomitance with accumulation of misfolded and oxidised protein aggregates are common features of Alzheimer’s disease (AD), Parkinson’s disease (PD) and Amyotrophic Lateral Sclerosis (ALS)\(^3\). Most of these phenomena have been associated not only with motor neuron degeneration, but also with astrocyte dysfunction. In the next few sections, some of the studies providing evidence for astrocytes role in maintaining CNS function will be summarised along with studies highlighting how failure in those same functions is involved in neurological diseases.

1.1 Brain homeostasis
Astrocytes maintain brain homeostasis through strict regulation of ion distribution, osmotic balance and recycling of glutamate, the main excitatory neurotransmitter in the CNS\(^4\). Glutamate uptake by astrocytes is an essential process as a high extracellular concentration of glutamate causes over-activation of neurons which could lead to excitotoxicity or cell death. Glutamate is taken into the astrocytes by sodium-dependent glutamate transporter proteins which are present on the cell membrane. Within the astrocyte, glutamate is
converted to glutamine by the enzyme glutamine synthase and is then converted back to glutamate at the synaptic terminal\(^5\). This uptake of glutamate stimulates glycolysis within astrocytes, resulting in the secretion of lactate to the surrounding neurons\(^6\), which is an important source of energy.

The function of glutamate transporters can be impaired either by altered function, reduced expression or disrupted RNA synthesis of the protein; this leads to increased synaptic glutamate levels which cause excitotoxicity and neuronal death\(^7\) (Figure 1). Studies performed by Rothstein\(^8\) and Bruijn\(^9\) documented that astrocytes from the motor cortex and spinal cord of patients with sporadic or familial amyotrophic lateral sclerosis (ALS), as well as mutant SOD1 (mSOD1) mouse models, had a reduced expression of the glutamate transporter EAAT2 (GLT-1 in mouse). Rothstein\(^10\) discovered that complete knockdown of the glutamate transporter caused paralysis and motor neuron degeneration. In agreement with the finding that glutamate handling is impaired in ALS, the only treatment currently available for this disease is the antiglutamatergic drug Riluzole, the main function of which is to reduce the pre-synaptic release of glutamate to protect neurons from excitotoxicity\(^7\).

1.2 Neuronal development and support
Astrocytes regulate neurite outgrowth through the production of growth promoting molecules, such as laminin, fibronectin, N-cadherin and neural cell adhesion molecule (NCAM), which guide the direction of growth during development or after injury\(^11\). They secrete growth factors essential for normal brain function, including neuronal growth factor (NGF), glial cell line-derived neurotrophic factor (GDNF), brain-derived growth factor (BDNF), glial maturation factor (GMF) and vascular endothelial growth factor (VEGF)\(^12\). Astrocytes have an important role in the protection of neuronal cells from oxidative damage and neurotoxins. Healthy astrocytes will increase their metabolic activity after brain injury or during disease to promote regenerative processes\(^12\).

Studies using chimeric mice for mSOD1 discovered that there was a higher loss of motor neurons when they were surrounded by mSOD1 astrocytes than wild type astrocytes\(^13\). Multiple in vitro studies using post-mortem astrocytes from patients\(^14\) or reprogrammed astrocytes from fibroblasts\(^15\) have shown that astrocytes from ALS patients induce death of
healthy motor neurons. Several pathways have been associated to this toxicity, but many aspects are still unknown. The study by Ferraiuolo\textsuperscript{16} demonstrated that astrocytes derived from mSOD1 mouse models have altered lactate and nerve growth factor (NGF) processing which increases neuronal death signalling and vulnerability (Figure 1).

On the other hand, human and mouse studies appear to support the theory that astrocytes have a neuroprotective role in Parkinson’s disease (PD). Depletion of nitric oxide and glutathione are common pathological hallmarks of PD. Exposure of astrocytes to nitric oxide seems to stimulate glutathione production in the astrocytes and it is thought that glutathione availability makes neurons less susceptible to reactive nitrogen species\textsuperscript{7}. Data from patients with PD support this theory as the dopaminergic neurons were preserved when surrounded by glutathione-containing cells\textsuperscript{17}. However, the role of astroglia in PD is still controversial and unexplained.

1.3 Blood Brain Barrier
The blood brain barrier (BBB) is a physical barrier of cells that prevents the exchange of molecules of a specific size and charge between the brain and the blood. The astrocytes form this barrier with the endothelial cells and pericytes of the brain microvessels\textsuperscript{18}. They also regulate cerebral blood flow in response to neuronal activity by releasing potassium ions through their end feet onto the blood vessels\textsuperscript{4}. Permeability of the BBB is regulated by tight junctions which are activated by NF-kB upregulation in astrocytes; astrocytes also regulate blood vessel dilation through the release of prostaglandins which stimulate calcium influx\textsuperscript{18}.

High-density lipoproteins move through the BBB to transport lipids into the brain tissue\textsuperscript{19}. Astrocytes are responsible for the synthesis of the most abundant apolipoproteins in the CNS; ApoE and ApoJ\textsuperscript{20}. Halliday\textsuperscript{21} demonstrated that AD patients that were carriers for the APOE4 gene displayed accelerated pericyte degeneration at the BBB. APOE4 also contributed to the activation of the LRP1-dependent CypA–MMP-9 BBB-degrading pathway in the endothelial cells and pericytes, causing extensive damage to the BBB\textsuperscript{21}.
1.4 Inflammation and immune response

Since the brain has a limited capacity for regeneration, astrocytes must control inflammation and immune responses to prevent neuronal damage. They release neuroprotective factors towards endothelial cells to increase permeability of the BBB, including cytokine IL-1 and IL-6, macrophage inflammatory protein, endothelin-1 and tumour necrosis factor (TNF)\(^{12}\). Astrocytes regulate inflammation through the release of pro-inflammatory cytokines (IL-1, IL-6, and TNF) or anti-inflammatory molecules (prostaglandin E2 and transforming growth factor)\(^{12}\). They suppress the activity and induce apoptosis of T lymphocytes which are a source of neuroinflammation toxic to the CNS\(^{22}\).

When astrocytes detect lesions in the brain, they undergo ‘reactive astrogliosis’; the astrocytes become hypertrophic and rapidly proliferate, increasing glial fibrillary acidic protein (GFAP) expression which is the main marker for the astrogliotic response (Figure 1). Reactive astrogliosis is often seen in the later stages of disease and can be triggered by either the deposition of toxic amyloid-β protein aggregates or dysfunctional neuronal signalling\(^4\). Transgenic AD animal models have demonstrated altered calcium signalling in astrocytes associated with these plaques\(^{23}\). These activated astrocytes undergo apoptosis, forming more amyloid plaques positive for common astrocyte markers GFAP and S100\(^\beta\)\(^4\).

Neuroinflammation is also a classic hallmark of ALS seen across both mouse models and human patients with familial and sporadic cases. Astrocytes and microglia positive for mSOD1 have been shown to express a multitude of pro-inflammatory genes\(^{24}\). Haidet-Phillips\(^{14}\) found that the NF-kB pathway was the master regulator of inflammation in ALS astrocytes derived from post-mortem tissue using microarray analysis. Frakes\(^{25}\) demonstrated that NF-kB signalling is activated in glia during disease progression in a mSOD1 mouse model, suggesting that NF-kB signalling regulates microglial activation in ALS.
2. Current application of personalised medicine in neurological disorders

Since many neurological disorders share common pathological hallmarks, identifying the shared etiopathological mechanisms between diseases will allow the development of stratified therapeutics for more than one neurological disorder\textsuperscript{26}. For example, cytoplasmic accumulation of TAR DNA-binding protein 43 (TDP-43) is seen in multiple neurodegenerative disorders; from ALS to frontotemporal dementia (FTD), thus creating a new spectrum of diseases called TPD43 proteinopathies\textsuperscript{27}. The study by Tan et al\textsuperscript{28}, where the authors assessed the severity of TDP-43 pathology in selected brain regions of patients with ALS, FTD and AD, also proposed that the regional concentration of TDP-43 could potentially characterise these distinct clinical disorders. Advances in genetics have allowed medical professionals to make a start at personalising treatment for neurological disorders; however, these are still early days. To date the main efforts towards precision medicine have focused on a better classification of the patient population through the identification of disease-specific biomarkers, as highlighted in the following sections.
2.1 Alzheimer’s disease
AD is caused by a complex interplay of genetic, epigenetic and environmental factors that result in neuronal shrinkage and cell death, leading to progressive loss of cognition and memory. The main diagnostic hallmark is the accumulation of extracellular amyloid plaques which consist of amyloid-β and aggregated tau protein\textsuperscript{29}. Due to the aging population, disease prevalence of AD is predicted to triple by 2050\textsuperscript{30}.

Neurons that are surrounded by reactive astrocytes develop intracellular protein aggregates and axonal pathology which makes them more susceptible to cell death. In AD, it has been proposed that amyloid-β aggregates interfere with the gap junctions between astrocytes which could alter calcium signalling and glial communication\textsuperscript{29}. Studies suggest that astrocytes are responsible for the clearance of the Aβ peptide to prevent the accumulation of these plaques\textsuperscript{31}.

Positron emission tomography (PET) technology is being used by clinicians to visualise these amyloid plaques in AD patients to assist in diagnosis and treatment\textsuperscript{32}. Radioactive PET ligand 1 ([\textsuperscript{18}F]florbetapir) is used in the brain imaging of patients with cognitive defects to confirm a diagnosis of AD or other forms of dementia\textsuperscript{33}, while PET ligands 2 ([\textsuperscript{18}F]flutemetamol) and 3 ([\textsuperscript{18}F]florbetaben) detect the presence of amyloid plaques\textsuperscript{34,35}. These ligands have a great potential to improve patient treatment through the monitoring of patients undergoing therapy, patient-risk analysis and patient selection for Aβ-targeting therapy. However, recent studies suggest that the abnormal aggregation of the tau protein has a greater contribution to neurodegeneration in AD, therefore detecting the severity of tau pathology may be a more effective biomarker than amyloid plaques\textsuperscript{36}. One PET tau imaging agent has been clinically validated\textsuperscript{37} and three more have reached clinical trials\textsuperscript{38,39,40}.

2.2 Parkinson’s disease
The cognitive impairment and dysfunctional control of movement in PD arises as the result of progressive dopaminergic neuron degeneration in the substantia nigra pars compacta and striatum\textsuperscript{36}. On average, by the time of diagnosis patients will have already lost 60-70%
of dopaminergic neurons$^{41}$, hence the need for biological indicators of disease progression early on.

In PD, microRNAs are essential for neuron survival and previous studies have witnessed miRNA downregulation in the substantial nigra, frontal cortex and cerebellum of PD patients$^{42}$. The study by Khoo and colleagues$^{41}$ identified 9 pairs of miRNAs that are predictive of PD and 13 differentially expressed miRNAs which could be potential biomarkers for PD. After qPCR, replication and validation steps, this number was cut down to three candidate markers that showed the highest predictive biomarker performance: k-TSP1(miR-1826/miR-450b-3p), miR-626, and miR-505. Although the role of these miRNAs in PD pathogenesis is still unknown, previous studies have witnessed an upregulation of miR-1826 in the blood plasma of MS patients$^{43}$, miR-626 is overexpressed in the blood of glioblastoma patients$^{44}$ and miR-505 has been called a potential ‘informative’ biomarker present in the CSF of AD patients$^{45}$. These diagnostic biomarkers are desperately required seeing as current diagnosis of PD relies on the clinical assessment of symptoms which occur once the neurons are heavily damaged$^{41}$. Biofluids such as blood plasma and CSF are great resources for biomarkers however they have yet to be fully validated for use in clinical assessment$^{26}$.

2.3 Amyotrophic Lateral Sclerosis

ALS is associated with the progressive loss of motor neurons from the motor cortex, brain stem and spinal cord. It is estimated that 5-10% of patients have the hereditary form, known as familial ALS (fALS), while most patients are sporadic (90-95%). Devastatingly, 60% of patients die within 3 years of diagnosis normally due to respiratory failure and there is still only one treatment available, Riluzole, which provides only a modest benefit. Current research has discovered 23 genes associated with ALS; these account for approximately 2/3 of fALS and 10% of sporadic cases$^{46}$.

Mutant genes associated with ALS pathology, including TDP-43 and FUS, are directly involved in messenger RNA (mRNA) processing, indicating that miRNAs may play a role in the disease. The study by Butovsky$^{47}$ demonstrated that the inhibition of pro-inflammatory
miR-155 in mSOD1 mice rescued mice from the disease phenotype through restoration of abnormal microglia, hinting the therapeutic potential of miRNAs in ALS.

Interestingly, MiR-125b is expressed by microglia and has been shown to activate NF-kB signalling in the CNS through targeting the tumour necrosis factor alpha-induced protein 3 (TNFAIP3) A20. This microRNA has also been shown to regulate NF-κB-dependent inflammatory and oxidative stress pathways in both models of AD\textsuperscript{48} and ALS\textsuperscript{49}, thus indicating that miR-125b may be a potential biomarker and therapeutic target in both diseases.

3. Astrocytes driving the future of precision medicine
As summarised in the previous section, astrocytes contribute to a series of toxic mechanisms affecting neuronal function and survival. Therefore, these cells are vital in the development of precision medicine either for cell replacement, genomics, biomarker identification and drug discovery.

3.1 Sources of Astrocytes
Studies show that transplanted astrocyte progenitor cells can survive and differentiate within the host brain, and have even been shown to slow down disease progression in ALS and Alzheimer animal models\textsuperscript{50,51}. However, these protocols require the isolation of astrocyte progenitor cells from the neonatal brain from which there is only a small supply. These cells also have a different immunoprofile to the host, causing rejection and immune response to transplantation, meaning that they have limited use in cell therapies\textsuperscript{52}.

In 2006, history was made when Takahashi and Yamanaka\textsuperscript{53} demonstrated how to reprogram adult somatic cells into pluripotent stem cells (iPSCs) using four transcription factors; Klf4, Oct3/4, Sox2 and cMyc. This opened up a world of opportunities in the derivation of a huge supply of stem cells directly from diseased patients\textsuperscript{4}. For example, the study by Chestkov\textsuperscript{54} derived iPSC lines from ALS patient fibroblasts carrying the SOD1 mutation using either Sendai or lentivirus and further differentiated these lines into motor neurons.
Many studies have been able to reprogram astrocytes from human derived iPSCs\textsuperscript{55,56}. However, the problem is that these protocols are time-consuming, complex and are highly variable in the maturation time of the astrocytes (Table 1). Therefore, a promising alternative to iPSC resources is the direct reprogramming of fibroblasts into astrocytes from an immuno-matched host\textsuperscript{57}.

### Table 1. Protocols for astroglial differentiation from stem cells

<table>
<thead>
<tr>
<th>Cell Source</th>
<th>Method of Differentiation</th>
<th>Key transcription/growth factors</th>
<th>Astrocyte Outcome</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>hESCs</td>
<td>Neurospheres</td>
<td>Heparin: 2(\mu)g/ml FGF2: 20ng/ml BDNF/GDNF: 10ng/ml cAMP: 1(\mu)M Ascorbic acid: 200(\mu)M</td>
<td>Astrocytes appeared after 9 weeks</td>
<td>\cite{58}</td>
</tr>
<tr>
<td>hESCs and iPSC</td>
<td>Embryoid bodies</td>
<td>Retinoic acid: 0.5(\mu)m FGF8: 50ng/ml SHH: 500ng/ml EGF/FGF2: 10ng/ml CNTF/LIF: 10ng/ml</td>
<td>Populations of immature astrocytes</td>
<td>\cite{59,60}</td>
</tr>
<tr>
<td>hESCs and hiPSC</td>
<td>Embryoid bodies</td>
<td>SB43152: 10(\mu)M Noggin: 500ng/ml</td>
<td>55-70% GFAP+ cells after 5 weeks</td>
<td>\cite{61}</td>
</tr>
<tr>
<td>hiPSC</td>
<td>Neurospheres/ EZ spheres</td>
<td>EGF/FGF2: 20ng/ml CNTF: 10(\mu)l/ml</td>
<td>90% GFAP+ cells after 9 weeks</td>
<td>\cite{66}</td>
</tr>
<tr>
<td>mESC, hESC and hiPSC</td>
<td>Monolayer</td>
<td>LDN193189: 0.2(\mu)M SB43152: 10(\mu)M Ascorbic acid: 0.4(\mu)g/ml Retinoic acid: 1(\mu)M BDNF/GDNF: 10ng/ml</td>
<td>100% S100\beta+ and 70% GFAP+ cells after 80 days</td>
<td>\cite{55}</td>
</tr>
<tr>
<td>mEF, hfibroblast</td>
<td>Direct reprogramming</td>
<td>Lentiviral vectors: NFIA, NFIB, SOX9 TFs</td>
<td>Astrocytes derived from fibroblasts after 2-3 weeks</td>
<td>\cite{52}</td>
</tr>
<tr>
<td>hfibroblast</td>
<td>Direct reprogramming</td>
<td>Retroviral vectors: Klf4, Oct3/4, Sox2 and c-Myc FGF2/EGF: 20ng/ml Heparin: 5(\mu)l/ml DMEM: 10% FBS &amp; 0.3% N2</td>
<td>iAstrocytes generated from patients in less than 4 weeks</td>
<td>\cite{15}</td>
</tr>
</tbody>
</table>

Instead of generating IPSCs, direct reprogramming involves the use of cell-lineage transcription factors to convert adult somatic cells into another cell type\textsuperscript{15}. This technology has been used to generate sub-specific neural lineages such as cholinergic, dopaminergic and motor neurons\textsuperscript{62–64}. Meyer\textsuperscript{15} decided to use direct reprogramming technology to derive
astrocytes from ALS patient fibroblasts. Using the protocol from Kim\textsuperscript{65}, they generated tripotent induced neural progenitor cells (iNPCs) from ALS patients and controls within one month. When these cells were differentiated into astrocytes, they displayed similar toxicity towards motor neurons in co-cultures as autopsy-derived astrocytes\textsuperscript{14}, making them useful tools in the development of drug screens.

3.2 Astrocytes in Cellular Transplantation Therapy
Further effort is being invested in the development of cellular replacement therapy for neurodegenerative disease as it can provide therapeutic benefit through not only cell replenishment but also by reducing inflammation and protein aggregates\textsuperscript{57}.

Both microglia and astrocytes are capable of phagocytosing and degrading amyloid-\(\beta\) deposits in the brain\textsuperscript{31,66}. In the study by Pihlaja\textsuperscript{51} et al, the authors transplanted both mature and neonatal mouse astrocytes into the brains of AD mouse models and ex vivo human AD brain sections. In these brain sections, only adult astrocytes were able to internalise the amyloid-\(\beta\) deposits, however both adult and neonatal astrocytes were able to remove aggregates in vivo within 1-7 days of transplantation. This study suggests that therapeutic strategies looking into the transplantation of amyloid-\(\beta\)-clearing astrocytes or promoting endogenous astrocytes to degrade these toxic aggregates\textsuperscript{51} might lead to positive results.

Experimental therapies for PD are focused on preventing dopaminergic neuron loss using pharmacological compounds or transplantation of new dopaminergic neurons\textsuperscript{67}. The transplantation of astrocytes derived from glial-restricted precursor cells exposed to bone morphogenetic protein (GDA\textsuperscript{BMP}) into injured spinal cord have promoted the survival of multiple neuron populations\textsuperscript{68}. Proschel\textsuperscript{67} demonstrated that the delayed transplantation of rat or human GDA\textsuperscript{BMP} cells into an experimental model of PD rescued parvalbumin-positive GABAergic interneurons (a population that has not been rescued by any other experimental treatment) and restored synaptophysin expression which is essential for synaptic function. Unlike previous cell transplantation methods in PD, GDA\textsuperscript{BMP} do not require genetic modification prior to transplantation since they intrinsically produce multiple therapeutic molecules against PD symptoms, including BDNF, GDNF\textsuperscript{68}, neurotrophic factors,
synaptogenic modulators and the antioxidant glutathione. The ability of these cells to target multiple problems within the PD model indicate the potential of using astrocytes as a vehicle for restoration of the CNS. Human derived neural stem/progenitor cells (NSC/NPC) are a promising tool for cell replacement due to their plasticity and the ability to differentiate into neurons, astrocytes and oligodendrocytes. The study by Das injected young and aged rats with human NPCs derived from the foetal brain to investigate if cell replacement therapy could rescue motor neuron function in aging. There was a short-term motor neuron rescue seen in young rats receiving NPC injections, although there was a much more robust NPC survival and migration towards the aged motor neurons. Unfortunately, the implantation of NPCs had no positive effect on motor neuron function as they were unable to provide protection to neuromuscular junction (NMJ) innervation, preventing motor neurons from innervating and stimulating the muscle.

Human IPSCs represent an alternative source to human derived-NSCs. Transplantation of human IPSC-derived neural progenitor cells into mSOD1 mice have been shown to differentiate into healthy astrocytes, upregulate expression of neurotrophic factors and increase survival of mice. A second study by Nizzardo also found that the transplantation of neural stem cells enhanced neuronal survival and maintained NMJ integrity in mSOD1 mice. The authors suggest that the implanted NSCs secrete growth factors that inhibit the GSK3β pathway, preventing motor neuron death. Cellular transplantation of NSC might directly protect motor neurons from degeneration and indirectly by antagonising the toxic effects of astrocytes.

One question that arises is why did the IPSC-derived NPCs in Nizzardo’s study improved motor function and the human-derived NSCs in Das’s study did not? Das suggests that the dysfunctional microenvironment in ALS is more susceptible to NSC survival than the wildtype environment due to the higher availability of space, more permissible factors or the weakened immune system of the model, indicating the NSCs are more likely to survival and exert beneficial effects within the ALS model.
The promising results from these studies in rodents have led to attempts to translate this into clinical patients for patients (https://clinicaltrials.gov/ct2/home). Clinical trials using transplantation of glia progenitors for ALS and other neurological diseases are listed in Table 2.

Table 2. Current clinical trials using transplantation of glia progenitors for ALS and other neurological diseases (https://clinicaltrials.gov/ct2/home)

<table>
<thead>
<tr>
<th>Study name</th>
<th>Condition</th>
<th>Intervention</th>
</tr>
</thead>
<tbody>
<tr>
<td>Safety of the Injection of Human Glial Restricted Progenitor Cells Into Subjects With ALS</td>
<td>ALS</td>
<td>Transplantation of human glia progenitor cells</td>
</tr>
<tr>
<td>Dose Escalation and Safety Study of Human Spinal Cord Derived Neural Stem Cell Transplantation in ALS Patients</td>
<td>ALS</td>
<td>Transplantation of human spinal cord stem cells</td>
</tr>
<tr>
<td>Transplantation of Human Neural Progenitor Cells Secreting GDNF for the Treatment of ALS</td>
<td>ALS</td>
<td>Transplantation of human glia progenitor cells</td>
</tr>
<tr>
<td>Safety and Tolerability of Fetal Mesencephalic Dopamine Neuronal Precursor Cells for Parkinson’s Disease</td>
<td>PD</td>
<td>Transplantation of mesencephalic neuronal precursor cells</td>
</tr>
<tr>
<td>Embryonic Dopamine Cell Implants for Parkinson’s Disease</td>
<td>PD</td>
<td>Transplantation of embryonic dopamine cell implants</td>
</tr>
<tr>
<td>Infusion of Recombinant-Methionyl Human GDNF for the Treatment of Idiopathic PD</td>
<td>PD</td>
<td>Administration of recombinant-methionyl human GDNF</td>
</tr>
<tr>
<td>Infusion of Recombinant-Methionyl Human GDNF to Treat Progressive Supranuclear Palsy (PSP)</td>
<td>PSP</td>
<td>Administration of recombinant-methionyl human GDNF</td>
</tr>
</tbody>
</table>

3.3 Astrocytes in Genomics and Transcriptomics

As highlighted in the previous sections, astrocytes have been identified as key players in disease development in several neurodegenerative disorders. Gene expression profiling has greatly contributed to uncover the molecular mechanisms underlying the progression of neurodegenerative diseases, helping us identify specific biochemical pathways and cellular processes that are altered by disease. Gene expression profiles of the CNS are difficult to comprehend due to the complex interplay of microglia and astrocytes interspersed between different neuronal subtypes. This means that cell specific gene expression changes cannot be detected when analysing the whole brain tissue72.

To overcome this problem, several studies have used laser capture microdissection (LCM) in order to isolate single cells from brain or spinal cord and obtain a highly enriched cell population for transcriptomic analysis16,73–75. These studies led to the identification of pathways specifically activated/altered in astrocytes during disease.
In a longitudinal study analysing astrocytes isolated from the spinal cord of mSOD1 mice throughout disease, the lactate shuttle between astrocytes and neurons was identified as altered at the pre-symptomatic stage of ALS for the first time. As disease progresses, inflammatory pathways and cytokine production increase, along with astrocytic lysosomal and phagocytic activity.

Simpson et al, by analysing different post-mortem brain areas from AD patients, identified dysregulation of genes associated with cell proliferation, apoptosis, and ubiquitin-mediated proteolysis at low Braak stages, while they found that altered regulation of intracellular signaling pathways, including insulin, phosphatidylinositol 3-kinase (PI3K)/Akt, and mitogen-activated protein kinase (MAPK) pathways were primarily associated with high levels of Alzheimer-type pathology, and occurred at lower Braak stages in individuals with the APOEε4 allele. These studies identified valuable therapeutic targets, either based upon timing of disease or brain regions associated with specific genetic mutations.

One of the limitations of LCM is the length of the process as well as the potential contamination with surrounding cells. Srinivasan and colleagues generated a method of isolating neuronal and non-neuronal cell types simultaneously from brain tissue without these limitations. All enzymatic incubations were performed at 4°C or on ice and specific cell types were immunolabelled, for example, anti-GFAP label for astrocytes, anti-CD11b for microglia and anti-NeuN for neurons. The authors generated sequencing data from specific cell type populations derived from the brain tissue of mouse models and human patients with FTD, ALS and AD. From this data, they determined that the different patterns of gene expression in disease is due to the altered cellular composition of the brain tissue rather than changes in transcriptional regulation patterns. In ALS and FTD, there was a recurring trend of lower neuronal RNA expression and higher levels of astrocytic and microglia RNA which reflected the altered neuron to glia ratio due to neuronal loss. However, the authors were unsure as to how representative the population of purified cells are to the tissue as transcripts were only derived from the cell body; dendrites and axons were lost during dissociation.

Gene expression analysis is often used to identify therapeutic targets, however, an interesting approach has been undertaken by Aronica et al. The authors performed whole-genome expression analysis of post-mortem cortex tissue from sALS patients and
normal controls to explore the entire spectrum of genetic and molecular pathways in ALS pathology. Using these data, they were able to distinguish between patient and control samples, and then further subdivide the patients into two groups which had similar expression profile changes. However, the authors argue that a much larger sample size is required in order to categorise patients by clinical characteristics\(^77\). Molecular taxonomy of patients through gene expression analysis has been used in cancer to diagnose and develop personalised therapies\(^78\). This taxonomy could also be used in sALS patients to uncover hidden pathogenic mechanisms and develop personalised treatment\(^77\).

A similar approach was also taken by Wang and colleagues\(^79\). The authors analysed the transcription profile of 19 cortical regions from 125 individuals with a severity spectrum of dementia and neuropathology of AD. Comparing their data with single-cell RNA-sequencing data, the authors determined that the cells mainly contributing to the transcriptional alterations were neurons, astrocytes and oligodendrocytes. Their contribution was different in different brain regions, thus suggesting that pathology is driven by selective regional vulnerability. By analysing the data in terms of degree of severity, the authors observed that most of the transcriptional changes occur early in the disease and astrocytes and oligodendrocyte play a crucial role in neuronal death.

### 3.4 Astrocytes in Diagnostic and Prognostic Biomarkers

Biomarkers are proteins, lipids or mutant transcripts associated with disease that can be used to tract normal or abnormal biological processes\(^80\). They can be divided into three sub-categories; prognostic (determine stage of disease), therapeutic (determine the right drug at the right dose) and predictive (the effectiveness of treatment)\(^81\). A good biomarker is tested on the sensitivity, specificity and positive predictive value; new technology such as genomics, proteomics and bioinformatics can be used to help develop more accurate biomarkers\(^82\). This is important for the advancement of medicine as novel biomarkers increase treatment efficiency and safety and reduce the cost of diagnostic methods and treatments\(^81\).

An effective way of monitoring neurological disease would be using serum biomarkers. This minimally invasive technique is able to detect brain-specific pathology and is reflective of
the health status of the glial cells\textsuperscript{83}. Previous studies have detected GFAP in the blood serum both before and after traumatic brain injury\textsuperscript{84}. Using GFAP as a clinical biomarker could be a cost-effective replacement to expensive imaging scans while retaining diagnostic sensitivity\textsuperscript{83}. Another astrocyte marker S100\(\beta\) has also been detected in the blood of patients with neurological disorders\textsuperscript{85}. This gene is mainly expressed by mature astrocytes and is responsible for regulation of calcium signalling and apoptosis in the surrounding glial cells\textsuperscript{83}. A comparison study between S100\(\beta\) and GFAP found that S100\(\beta\) rose and peaked within the serum at 2 hours post-injury while GFAP rose more steadily over the first 4 hours after injury\textsuperscript{84}. Shepheard et al.\textsuperscript{86} validated the use of the extracellular domain of the neurotrophin receptor p75 (p75NTR\textsuperscript{ECD}) as a candidate marker for ALS; there were increased levels of p75NTR\textsuperscript{ECD} detected in the urine of human ALS patients and mSOD1 mice. This links back to the study by Ferriauolo\textsuperscript{16} which demonstrated that the increased activation of pro-nerve growth factor and p75 signalling were important components of astrocyte toxicity in ALS.

During reactive astrogliosis, there is a wide variety of gene expression changes which could be used as medical biomarkers to tract disease progress. PET tracers can detect neuroinflammation and microglial activation through the expression of the translocator protein (TSPO) on the outer mitochondrial membrane\textsuperscript{87}. Studies show that TSPO expression is globally non-existent in a healthy brain, but the expression rapidly increases during astrocytic/microglial activation and neuroinflammation\textsuperscript{88}, making TSPO an attractive biomarker for targeting reactive gliosis in cerebral inflammation and imaging microglial activation\textsuperscript{87}.

Another potential biomarker is the type 2 cannabinoid receptor (CB2R) which is virtually undetectable in healthy tissue but greatly expressed in activated astrocyte and microglial cells\textsuperscript{89}. This receptor is also found in macrophages and peripheral T lymphocytes as it is involved in central and peripheral inflammatory responses\textsuperscript{90}. Previous studies have discovered PET ligands of CB2 receptors that could be potential biomarkers for multiple sclerosis (MS)\textsuperscript{91,92}.

Monoamine oxidase type B, located the outer mitochondrial membrane, is also greatly expressed in reactive astrocytes in neurological disease\textsuperscript{93}. It is responsible for the modulation of neurotransmitter concentrations, making it a major drug target for
movement disorders\textsuperscript{94}. Gulyas et al.\textsuperscript{95} reported increased monoamine oxidase type B expression in reactive astrocytes of AD patients and it is currently beginning investigated in MS\textsuperscript{94}.

3.5 Astrocytes in Drug Screening

Over the previous decades, research has been focusing on how genetic differences between individuals can lead to variations in patient drug response, giving rise to pharmacogenetics which aims to tailor drug choice and dosage based on the individual patient’s genome for optimal therapeutic benefit. Neurodegenerative diseases demonstrate widespread genetic variability, hence there is a broad range of patient responses to prescribed medications, in both terms of efficiency and adverse reactions\textsuperscript{96}. The acetylcholinesterase inhibitor Donepezil is the main prescribed drug treatment for AD, providing a modest benefit on cognitive function, behaviour and disease progression in both moderate and severe AD patients\textsuperscript{97}. Approximately 15-20\% of patients demonstrate abnormal metabolism of the drug, depending on the function of CYP-related enzymes CYP2D6, CYP3A4, and CYP1A2. The CYP2D6 locus has more than 100 different polymorphisms which define whether a patient is a poor, normal or ultra-rapid metaboliser of the drug\textsuperscript{97}. In PD, 80\% of patients treated with levodopa demonstrate positive benefits to initial therapy, but 45\% of these patients develop levodopa-induced dyskinesias within 5 years of treatment\textsuperscript{98}. Genetic research has identified multiple genetic polymorphisms as strong candidates for determination of safety and efficiency of levodopa treatment, see table, however many of these are still controversial\textsuperscript{96}.

**Table 3. Patient response to L-dopa mediation variation based on individual genetic mutations.**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Polymorphism</th>
<th>Drug Response to L-dopa</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>BDNF</td>
<td>rs6265 (Val66Met)</td>
<td>Significantly higher risk of developing dyskinesias early on course</td>
<td>\textsuperscript{99}</td>
</tr>
<tr>
<td>ACE</td>
<td>rs4646994</td>
<td>Risk of L-dopa-induced psychosis</td>
<td>\textsuperscript{100}</td>
</tr>
<tr>
<td>APOE</td>
<td>rs429358 rs7412 (e2, e3, e4)</td>
<td>No association with L-dopa-induced dyskinesias</td>
<td>\textsuperscript{101}</td>
</tr>
<tr>
<td>OPRMI</td>
<td>rs1799971 (118A&gt;G, Asn40Asp)</td>
<td>Increased risk of earlier onset of dyskinesia</td>
<td>\textsuperscript{102}</td>
</tr>
<tr>
<td>MAOB</td>
<td>rs1799836 intron 13</td>
<td>No association with L-dopa dosage.</td>
<td>\textsuperscript{96}</td>
</tr>
<tr>
<td>GBA</td>
<td>Various mutations</td>
<td>Higher risk of L-dopa induced dyskinesias</td>
<td>\textsuperscript{103,104}</td>
</tr>
</tbody>
</table>
CNS drug development has slowed down since the 1990’s as the candidate drugs often fail at the later stages of clinical trials. Almost all experimental drugs for ALS have failed clinical trials, potentially due to the unreliability and species differences of current animal models\textsuperscript{105} or the heterogeneity of the patient population included in the trial. To overcome this problem, Isobe\textsuperscript{106} used hESC-derived motor neurons with identical genetic backgrounds but differing mutations in SOD1 to investigate whether different SOD1 mutations might lead to different drug response. Surprisingly, through this model, they discovered mutant-specific morphological alterations within the motor neurons and differential drug responses. This result indicates that most likely heterogeneous patient populations will not benefit from the same drug treatment, thus indicating that there is a great need for targeted precision medicine.

Consistently, the study by Shichinohe\textsuperscript{107} found that the compound MCI-189 only provided neuroprotective effects to motor neurons expressing the G93A-SOD1 variant. This suggests that neuroprotective drugs may be effective at treating ALS phenotypes with specific SOD1 mutations. There are currently no reports that astrocytes also display these SOD1 mutation-dependent drug responses but this question should be addressed if we are to attempt to classify ALS into treatment-responsive categories.

Studies using transgenic AD mouse models have found that GFAP expression changes in astrocytes between different regions in the brain and at different stages of disease progression\textsuperscript{108,109}, reinforcing that subpopulations of astrocytes play separate roles in disease. Barbeito and collaborators\textsuperscript{110} isolated an astrocyte subpopulation from the spinal cord of transgenic mSOD1 rats, which he referred to as ‘aberrant astrocytes’ due to their fierce proliferation capacity. These aberrant cells were morphologically different to primary neonatal astrocytes, lacked detectable GLT-1 expression and their condition medium was specifically toxic to motor neurons\textsuperscript{110}. These astrocytes are considered a distinct subpopulation of highly toxic astrocytes which could represent an additional cellular target for future treatment of ALS\textsuperscript{111}.

Drug screening in cellular cultures is a process traditionally mainly used by pharmaceutical companies to identify candidate compounds for further investigation but with the development of new automated systems and widespread availability of screening facilities, academic sites have also embarked in high-throughput drug screening\textsuperscript{112}. 

18
Most drugs have a targeted approach; companies develop drugs that target only one gene or biological pathway which selectively helps to treat the disease while avoiding adverse effects. However, drugs with a selective target do not always deliver an effective treatment, as target engagement and phenotypic effect do not always match, due to the complexity of neurological diseases. For this reason, more effort has been invested in developing phenotypic screenings with a disease-relevant readout.

These cell culture models normally consist of 2D cultures containing neuronal-like cells such as neuroblastoma or patient derived IPS cells. However, 3D cultures of interacting cell types from different tissues are needed to be fully representative of the organ system, as the establishment of cell-cell and cell-extracellular matrix interactions in in vitro 3D models tries to mimic the tissue microenvironment; reducing the gap between animal models and human trials.

Whilst high content imaging, combined with high-throughput drug screening, has been applied to primary neurons, iPSC-derived neural progenitors and motor neurons, very few studies so far have focused on astrocytes and neurons co-cultures and only one used cells from ALS patients.

Rinaldi and colleagues described a robust 96-well assay to identify drugs that can dampen ALS astrocyte toxicity against motor neurons Z-score (0.679), thus supporting the idea that astrocytes and co-culture screenings can be used for precision medicine.

The identification of pharmacological agents for PD has proved slow going due to the limited availability of human cell-based neuronal models. The study by Efremova combined immortalized mesencephalic neuronal precursors differentiated into post-mitotic dopaminergic neurons and immortalized murine astrocytes to create a potential new co-culture model to test experimental neuroprotective compounds against the toxic compound MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine). The most striking finding from this study was the different efficacy of the neuroprotective compounds between mono- and co-cultures. This lack of protection in the co-culture assays could be explained by compounds being metabolised or modified by the astrocytes or that neuronal metabolism and cell death mechanisms might be altered in the presence of astrocytes, potentially indicating that astrocytes response differently to different drugs.
4. Conclusion
More personalised approaches to medicine are being investigated in neurological disease because of the gap between patient disease variability and categorisation, possibly resulting in overall failure of clinical trials. The desperate need for better patient classification and effective drugs converge in the concept of precision medicine, where patients with a certain “profile” can be identified as responders or non-responders to a treatment. An overwhelming amount of evidence has indicated that glial cells\textsuperscript{79,124,125} contribute to the early phases of neurodegeneration.

Astrocytes are the cellular backbone of the CNS, providing support to the neurons through formation of the blood brain barrier, glutamate regulation, cellular communication, inflammation and the immune response. Therefore, it is not surprising that neurodegenerative diseases occur in concomitance with astrocytes dysfunction. In this book chapter we have tried to highlight the many pathways controlled by astrocyte and how their failure contributes to neurological disease in a wide variety of ways; dysfunction of glutamate transport, reactive astrogliosis and neuroinflammation, altered metabolism and growth factor secretion to mention a few.

In terms of precision medicine, astrocytes could be used to predict disease susceptibility, monitoring disease progression and developing the right drug at the right dose for treatment of patients (Figure 2). The technology generated by Takahasi and Yamanaka\textsuperscript{53} has opened up a world of opportunities in the derivation of a huge supply of stem cells directly from diseased patients which could help treat and understand disease. Cellular reprogramming could be used to derive patient fibroblasts into astrocytes and these astrocytes would behave uniquely to that patient phenotype. Patient astrocytes could be used in drug screening to identify new target compounds and understand the underlying mechanisms of drug treatment\textsuperscript{122} (Figure 2). These methods could be used to either individualise drug treatment or stratify it to a subgroup with similar mutations or genetic setup. To provide further evidence to back up the drug screening analysis, astrocytes could be used to study what genes the patient expresses, identify possible drug treatments and discover drug mechanisms through RNA sequencing post drug treatment (Figure 2). Biomarkers allow us to tract the abnormal biological processes that arise during disease.
Identification of these protein aggregates in fibroblast-derived patient astrocytes could be used as diagnostic biomarkers or prognosis in response to treatment.

Figure 2. Astrocytes as a target for personalised medicine. Astrocytes and NPCs can be harvested from mice or human brain or spinal cord or derived from fibroblasts or stem cells using differentiation and reprogramming technologies. They have a wide variety of uses in cellular transplantation therapy, drug screening, genomic expression and biomarker studies.

Precision medicine is moving medicine away from the ‘one size fits all’ therapy, which is important for complex mainly sporadic disorders like ALS, AD and PD where the patient gene expression profile is as unique as a fingerprint. Further experimental investigation of patient-derived astrocytes is required to explore their potential as a tool for precision medicine, but their overpowering number and varied functions make them the ideal therapeutic target. Astrocyte manipulation can, in fact, lead to a complete modification of the environment surrounding injured neurons, thus potentially halting degeneration.
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