This is a repository copy of *Mini-review: Induced pluripotent stem cells and the search for new cell-specific ALS therapeutic targets*.

White Rose Research Online URL for this paper:
https://eprints.whiterose.ac.uk/173757/

Version: Published Version

Article:

Ferraiuolo, L. orcid.org/0000-0001-9118-5714 and Maragakis, N.J. (2021) Mini-review: Induced pluripotent stem cells and the search for new cell-specific ALS therapeutic targets. Neuroscience Letters, 755. 135911. ISSN 0304-3940

https://doi.org/10.1016/j.neulet.2021.135911

Reuse
This article is distributed under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs (CC BY-NC-ND) licence. This licence only allows you to download this work and share it with others as long as you credit the authors, but you can't change the article in any way or use it commercially. More information and the full terms of the licence here: https://creativecommons.org/licenses/

Takedown
If you consider content in White Rose Research Online to be in breach of UK law, please notify us by emailing eprints@whiterose.ac.uk including the URL of the record and the reason for the withdrawal request.

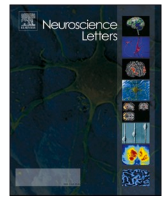
eprints@whiterose.ac.uk
https://eprints.whiterose.ac.uk/

Neuroscience Letters 755 (2021) 135911

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet



Mini-Review: Induced pluripotent stem cells and the search for new cell-specific ALS therapeutic targets

Laura Ferraiuolo [a], Nicholas J. Maragakis [b,*]

[a] Sheffield Institute for Translational Neuroscience, University of Sheffield, Sheffield, UK
[b] Johns Hopkins University, Department of Neurology, Baltimore, MD, USA

A R T I C L E I N F O

Keywords:
Motor neuron disease
Screening
Models
3D
Therapeutics
Precision medicine

A B S T R A C T

Amongst the most important discoveries in ALS pathobiology are the works demonstrating that multiple cell types contribute to disease onset and progression. However, a significant limitation in ALS research is the inability to obtain tissues from ALS patient brain and spinal cord during the course of the disease. *In vivo* modeling has provided insights into the role of these cell subtypes in disease onset and progression. However, *in vivo* models also have shortcomings, including the reliance on a limited number of models based upon hereditary forms of the disease. Therefore, using human induced pluripotent stem cells (iPSC) reprogrammed from somatic cells of ALS patients, with both hereditary and sporadic forms of the disease, and differentiated into cell subtypes of both the central nervous system (CNS) and peripheral nervous system (PNS), have become powerful complementary tools for investigating basic mechanisms of disease as well as a platform for drug discovery. Motor neuron and other neuron subtypes, as well as non-neuronal cells have been differentiated from human iPSC and studied for their potential contributions to ALS pathobiology. As iPSC technologies have advanced, 3D modeling with multicellular systems organised in microfluidic chambers or organoids are the next step in validating the pathways and therapeutic targets already identified. Precision medicine approaches with iPSC using either traditional strategies of screening drugs that target a known pathogenic mechanism as well as "blind-to-target" drug screenings that allow for patient stratification based on drug response rather than clinical characteristics are now being employed.

1. Introduction

One of the most significant limitations in understanding disease mechanisms in ALS and, therefore, in the investigation of potential ALS therapeutics, lies in our inability to obtain tissues from ALS patient brain and spinal cord during the course of the disease. Autopsy tissues from ALS patients are important in providing a window into the disease, but tissues obtained at endstage do not reflect disease mechanisms as they evolve. Access to tissues during the critical time periods of early diagnosis would allow for a more detailed understanding of disease progression by offering a window into how disease-relevant pathways are activated temporally and anatomically. Animal models of ALS have proven valuable in our understanding of how different cell subtypes contribute to disease but because these models are largely based upon uncommon ALS gene mutations, are not particularly predictive of the disease as a whole nor has their study resulted in broad therapeutic success. Therefore, the identification first of human embryonic stem cells (ESC) by Thomson et al. in 1998 [1] and later the development of techniques for reprogramming from somatic cells to induced pluripotent stem cells (iPSC) by Takahashi et al. in 2007 [2], has offered investigators the capacity to utilize these cells for modeling disease mechanisms in real time. Since that time, the field of iPSC biology has progressed at an impressive pace and a number of other methods for reprogramming somatic cells have been identified—each with its own advantages and limitations [3–5].

Induced pluripotent stem cells can be differentiated into all somatic cell types and, for the purposes of ALS research, cell subtypes of both the central nervous system (CNS) and peripheral nervous system (PNS). Motor neuron and other neuron subtypes, astrocytes, oligodendrocytes, microglia, muscle, and Schwann cells have all been differentiated from human iPSC and studied for their potential contributions to ALS pathobiology. Perhaps most importantly, because iPSC can be reprogrammed from somatic cells (usually peripheral blood mononuclear cells or skin fibroblasts) during adulthood, they can be obtained from ALS

* Corresponding author at: Johns Hopkins University, Department of Neurology, 855 North Wolfe Street, Room 248, 2nd Floor, Baltimore, MD, 21205, USA.
E-mail address: nmaragak@jhmi.edu (N.J. Maragakis).

https://doi.org/10.1016/j.neulet.2021.135911
Received 30 September 2020; Received in revised form 5 April 2021; Accepted 17 April 2021
Available online 20 April 2021
0304-3940/© 2021 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

patients with genotypically and phenotypically heterogeneous ALS presentations; offering insights into the spectrum not only of familial ALS but of the larger sporadic ALS population as well.

In this review, we discuss the different cell types that have been proposed as playing a role in ALS pathogenesis and progression based upon human observations and animal modeling. This is highlighted by the observations that pathologies in a number of neuronal cell subtypes, particularly in the context of ALS/FTD, continue to redefine the breadth of neural cell involvement. The second section highlights how these multiple cell types derived from human iPSC can be utilised to identify new therapeutic targets for drug treatment. Creative phenotypic readouts using iPSC have begun to facilitate drug discovery that is cell-specific or genotype-specific. Precision medicine approaches using iPSC have been applied to ALS to allow for patient stratification based on drug response rather than clinical characteristics and we conclude with an assessment of future advances beyond our current strategies including multicellular 2D and 3D-modeling of these cultures that may be used to further refine therapeutic approaches.

2. Cell subtype involvement in ALS pathogenesis

Amongst the most important discoveries in ALS pathobiology are the works demonstrating that multiple cell types contribute to disease onset and progression. These observations, detailed in a growing body of literature, are not only important in understanding disease mechanisms but are exciting in their prospects for translational research. Since numerous cell types contribute to disease, the proposition that numerous pathways could be involved in different stages of the disease offers hope for the identification of several therapeutic targets.

While traditionally thought of as a motor neuron disease primarily involving corticospinal and spinal motor neurons, the evolution in our understanding of ALS pathobiology now suggests that other neuronal cell subtypes (sometimes producing ALS with Frontotemporal Dementia (FTD)) are susceptible as well.

2.1. Frontotemporal Dementia and ALS—beyond motor neurons

Frontotemporal dementia, manifested by cognitive and behavioral changes, is found in 5–15 % of ALS patients [6]. The cognitive dysfunction that occurs along with ALS are notable in suggesting that pathologies are not limited to motor neurons but a heterogeneous pattern of association between motor and cognitive impairment. ALS/FTD pathologically share the presence of TDP-43 deposition that begins in the corticospinal motor neuron (CSMN) and spinal motor neurons and then progresses to involve neurons of the frontal and anterior parietal regions, and brainstem reticular formation. This is followed by pathology in the anterior frontal and basal forebrain, lateral thalamus and substantia nigra and finally, in the most advanced cases, TDP-43 deposition in neurons of anterior temporal lobe and hippocampus [7].

Several genes have been identified as part of the ALS/FTD spectrum including those associated with autophagy (SQSTM1, UBQLN2, VCP, OPTN), mitochondrial function (CHCHD10), and RNA metabolism (C9orf72, HNRNPA1, HNRNPA2B1) [8]. However, the C9orf72 hexanucleotide expansion (HRE) in an intronic region of this gene has been identified as the most common mutation in familial ALS/FTD patients [9–11].

To model ALS associated with the HRE in the C9orf72 gene, Chew et al. used an AAV vector to induce robust expression of the HRE delivered intracerebroventricularly (ICV) into neonatal mice. This resulted in the development of nuclear RNA foci, inclusions of DPRs, as well as TDP-43 pathology. This was accompanied by the loss of cortical neurons suggesting that, as with seen with mutations in SOD1, alsin, and TDP-43, the expression of the C9orf72 HRE is also associated with cortical neuron pathology [12]. Similarly, BAC transgenic mice carrying the C9orf72 HRE construct developed pathology in layer V of the cortex associated with CSMN loss as well as a behavioral phenotype that included reduced survival and/or motor abnormalities [13]. However, other C9orf72 models expressing the HRE have not shown evidence of neurodegeneration [14,15]. The discrepancies in some of these findings likely reflect methodological differences in the *in vivo* approaches to expressing the C9orf72 HRE as well as the complexities of modeling an *in vivo* network. Given some of the complexities of C9orf72 HRE modeling *in vivo*, induced pluripotent stem cells from C9orf72 HRE patients have been used extensively as an adjunct for modeling ALS as discussed in the sections below.

2.2. Corticospinal and spinal motor neurons-hallmarks of ALS pathobiology

Corticospinal motor neuron (CSMN) dysfunction and loss is a well-known clinical and pathological feature in ALS patients [16–18]. However, CSMN biology has presented challenges for ALS modeling, possibly because human cortex-spinal cord connectivity is *via* monosynaptic connections while the mouse has different patterns of connectivity [19], resulting in less dramatic pathological and phenotypically-observable changes that could be attributed to corticospinal motor neuron dysfunction in mouse models. There are, however, *in vivo* studies demonstrating CSMN dysfunction in ALS models and, therefore, highlight an underappreciated pathology amenable to study [20–23].

Spinal motor neuron dysfunction is often recognized as the hallmark feature of ALS manifesting as weakness, muscle atrophy, hypotonia, muscle fasciculations and electrophysiological features of muscle denervation [24,25]. Because of the prominent spinal motor neuron loss present in the transgenic mutant SOD1 (mSOD1) mouse, much of our knowledge about motor neuron pathobiology has leaned heavily on this model. Moreover, since this was the first mouse model of ALS [26], numerous potential therapeutic approaches have been aimed at neuroprotection and positive influences on behavioral outcomes. While the vast majority of therapeutics studied in this model have not proven translatable to human therapies [27], in the right context it remains a useful model of motor neuron disease.

Additionally, in several genetic models of ALS (SOD1, TDP-43, FUS, UBQLN2, Profilin), using promoters known to drive gene expression in neurons (Thy1, PrP), the incorporation of ALS genes with known mutations is sufficient to produce varying degrees of motor neuron disease or motor coordination impairment in some form—conceptually highlighting that spinal motor neuron dysfunction by itself is a central feature associated with motor phenotypes [28]. Furthermore, deletion of mSOD1 only in motor neurons using Cre/Lox strategies has contributed to the widely held belief that motor neuron dysfunction and/or death is the initiating event in ALS whereas disease progression is subsequently modulated by other non-neuronal cell subtypes [29].

2.3. Astrocytes

Reactive astrocytosis is a hallmark of ALS and other neurodegenerative diseases, and long thought to be a pathological byproduct of neuron loss rather than a significant contributor to disease itself. Therefore, the assertion, in the 1990s by Rothstein and colleagues, that ALS astrocytes could contribute to ALS was important in highlighting the non-cell autonomous contributions of these cells to ALS pathogenesis. Their focus was on the demonstration that ALS astrocytes had a reduction in the astrocyte-specific glutamate transporter EAAT2 [30,31]. This phenomenon was demonstrated in human ALS brain and spinal cord tissues as well as animal models of ALS and provided an important bridge for further therapeutic study [32]. Since then, astrocytes are known to be important in the interplay amongst other ALS-relevant cell subtypes.

In animal models of ALS, the transgenic overexpression of mSOD1 in astrocytes alone was not sufficient to produce motor neuron loss, although the glial fibrillary acidic protein (GFAP) promoter used to

overexpress mSOD1 in astrocytes may not have been sufficient to initiate an ALS phenotype or produce pathology in motor neurons [33]. A number of studies have since provided evidence to suggest that ALS astrocytes are mediators of disease progression *in vivo* [34–37] including a recent study by Guttenplan et al. that broadens the role of astrocytes through the demonstration that neuroinflammatory reactive astrocytes, responding to microglially released IL-1α, TNFα, and C1q, are responsible for disease progression in ALS and suggests that gliosis itself may be a therapeutic target for neurodegeneration [38]. However, perhaps the most versatile studies linking ALS astrocytes to motor neuron toxicity have come from elegant *in vitro* studies using iPSC (as discussed below).

2.4. Oligodendrocytes

Oligodendrocytes are primarily recognized for their myelin production and their capacity for wrapping around axons to improve conduction. Therefore, disorders primarily affecting myelin, like multiple sclerosis, have received the most attention as demyelination is evident radiographically and its sequelae have unique phenotypic representations. However, oligodendrocyte pathology with patchy regions of demyelination has also been observed in ALS autopsy tissues [39]. These findings are also supported in mSOD1 mouse models of ALS where oligodendrocyte pathology and death were accompanied by proliferation of oligodendrocyte precursor cells (OPC). This phenomenon is accompanied by the loss of the monocarboxylate 1 (MCT1) transporter which appears to also play a role in oligodendrocyte-mediated motor neuron support by providing lactate under conditions of metabolic stress [39–41]. The effect of oligodendrocyte dysfunction in ALS models is quite robust, since deleting mSOD1 specifically from oligodendrocytes has resulted in a significant improvement in motor neuron pathology and mSOD1 mouse survival [39].

2.5. Microglia and macrophages

As the CNS's resident immune cell, microglia are typically observed in a resting state unless perturbed by an insult in which they become activated [42]. In patients with ALS, techniques using [^{11}C]-PBR28, a PET radiotracer that selectively binds to the translocator protein (TSPO), and is upregulated in activated microglia and reactive astrocytes shows a correlation of reactive microgliosis in regions of pathology in ALS patients [43,44] and the involvement of microglia as part of the neuroinflammasome in ALS, has been the focus of intense research and the target of several ALS-relevant therapeutic trials [45].

Historically, a somewhat simplified nomenclature has identified two types of microglia: M1, which are believed to promote toxicity through the release of pro-inflammatory cytokines and chemokines and M2, identified as having neuroprotective qualities through the release of anti-inflammatory chemokines and other functions including promoting phagocytosis of debris or promoting tissue repair [46–48]. However, a sophisticated molecular and genetic interrogation has identified a novel microglial type (enriched in Trem2 expression), Disease Associated Microglia (DAM), in neurodegeneration [49]. These DAM have been noted to increase in spinal cords of SOD1^{G93A} mice as well as motor cortices of ALS patients [50] and may have differing roles in neuroprotection early in the disease course. The implications of DAM activation as disease progresses remains under investigation [51].

In ALS mouse models, activated microglia are a consistent feature of spinal cord pathology. The potential importance of microglia in mediating neuroinflammatory responses has been demonstrated through the genetic manipulations [52] of key microglially-mediated factors as well as drugs that target microglial-mediated neuroinflammatory cascades. Important early work using LoxSOD1G37R/CD11b-Cre mice to selectively reduce SOD1 in microglia resulted in a prolonged disease progression thus suggesting that microglia are mediators of disease progression after onset [29]. However, one limitation of these studies is that most of these *in vivo* manipulations have taken place in mSOD1 mice and therefore the relevance of this pathway to sporadic ALS, while clearly important, may be difficult to extrapolate from these studies [53]. Interestingly, microglia that have decreased C9orf72 expression show aberrations in microglial function including defects in lysosomal trafficking and profiles in both human C9orf72 ALS patients and C9orf72-/- mice suggesting enhanced neuroinflammation [54].

In a recent set of experiments, Chiot et al. dissected the contribution of peripheral macrophages to ALS progression in two ALS mouse models. Interestingly, modifying peripheral macrophages from an ALS phenotype to a neuroprotective phenotype, downregulated inflammation along peripheral nerves and resulted in neuroprotection and prolonged survival. This study elegantly demonstrated that peripheral macrophages do not infiltrate the CNS as previously hypothesized but emphasized that macrophages in the periphery can have an effect on motor neuron loss occurring centrally [55].

2.6. Other non-neuronal cellular contributions to ALS

Although not discussed more extensively in this review, animal modeling has also demonstrated a role for T-lymphocytes [56–58], myocytes [59–62], Schwann cells [63–66], NG2 glia [67], and pericytes [68–70] offering the potential to use iPSC modeling for the discovery of additional cell-specific contributions throughout the CNS and PNS.

In vivo ALS modeling has provided us with enormous insights regarding cell-specific etiologies of ALS but is somewhat limited in its reliance on modeling genetic forms of ALS, the complexity of the interactions amongst numerous cell types and networks, expense, and duration of study. Therefore, in light of these relative limitations, there are opportunities for complementing *in vivo* modeling by using iPSC-based platforms in the investigation of ALS mechanisms and therapeutics.

3. Using somatic cell reprogramming for therapeutic approaches

The first report of iPSC-derived MNs harbouring a TDP-43 mutation (TDP-43 M337 V) [71] showed mild changes in TDP-43 localization or aggregate formation and increases in cell death compared to controls under stress conditions. Since the early studies, the field has seen a remarkable improvement in differentiation protocols yielding purer and more mature MN cultures. These have, in fact, improved from a yield of 20 % [72] to 80–90 % HB9^{+} cells [73] with mature electrophysiological properties [74]. At the same time, key cues for neuronal patterning have been identified, specifically resulting in limb-innervating MNs [75]. In a disease where different neuronal populations and cell types are affected in different ways and others are spared, *in vitro* culture conditions can substantially affect the resulting phenotype and, therefore, reproducibility across studies. Regardless of these limitations, iPSC-derived MNs carrying mutations in ALS-linked genes have highlighted the importance of some key pathological pathways that have been recently reviewed [76] and have led to new approaches for drug discovery and patient stratification, reviewed in the following sections.

3.1. Drug discovery

The heterogeneity of ALS in terms of site of disease onset, duration and severity might underlie the activation of different disease mechanisms all converging in MN death. Our ability to understand which cell populations and pathways are involved in neurotoxicity is likely to inform effective therapeutic interventions. While recent efforts are concentrating on multicellular co-culture systems, present drug screenings have mainly focused on targeting MNs and using phenotypic readouts consistent across various laboratories and common to a variety of ALS patient genotypes: TDP-43 proteinopathy, protein homeostasis impairment and hyperexcitability (Table 1).

Table 1
Therapeutic agents identified using iPSC-derived MNs.

Pathway targeted.	Cell type	Mutation	Therapeutic agent	Therapeutic stage	Reference
RNA metabolism	MN	TDP-43	anacardic acid	*in vitro*	Egawa et al. [77]
Stress granule formation	MN	TDP-43	nucleic acid intercalating molecules and cardiac glycosides	*in vitro*	Fang et al. [78]
Stress granule formation	MN	sALS	cardiac glycosides	*in vitro*	Burkhardt et al. [79]
Autophagy	MN and astrocytes	TDP-43	fluphenazine and methotrimeprazine	*in vitro*	Barmada et al. [82]
Autophagy	MN	FUS	torkinib and PQR309	*in vitro*	Marrone et al. [84]
Proteasome activation	MN	C9orf72	rolipram	*in vitro*	Khosravi et al. [83]
Hyperexcitability	MN	SOD1	ezogabine	Phase II clinical trial	Wainger et al. [74]
Src/abl inhibition	MN	SOD1, TDP-43, C9orf72, sALS	bosutinib	Phase I clinical trial	Imamura et al. [90]
Dopamine D2 receptor agonism	MN	FUS, TDP-43, sALS	ropinirole	Phase I/IIa clinical trial	Fujimori et al. [92]

3.2. TDP-43 proteinopathy

One of the first drug screening studies using iPSC-derived MNs was conducted by Egawa et al. [77], who found that MN from ALS patients carrying mutant TDP-43 formed cytosolic aggregates similar to those seen in postmortem tissues and exhibited shorter neurites. In addition, MNs displayed increased mutant protein aggregates bound to the spliceosomal factor SNRPB2 and widespread dysregulation of splicing and transcription. This prompted the authors to perform a small drug screening focusing on molecules targeting histone modification or RNA splicing, which led to the identification of a histone acetyltransferase inhibitor called anacardic acid. Treatment with this drug rescued the abnormal ALS MN phenotype reported in the study, thus providing the first proof of principle that iPSC-derived cells can be effectively used to identify therapeutic compounds [77]. Another approach to target TDP-43 mislocalization was identified by Fang et al. who used puromycin treatment to obtain TDP-43 cytoplasmic puncta in mutant TDP-43 iPS MNs. Excitingly, molecules with planar moieties, which act as nucleic acid intercalating molecules, prevented stress granule localization of TDP-43 and reduced its cytoplasmic accumulation [78].

Due to the presence of TDP-43 proteinopathy in sALS, Burkhardt et al. used this as readout for patient stratification and drug screening in sporadic disease [79]. The authors reprogrammed 16 sporadic fibroblast cell lines and differentiated them into MNs, they then characterised the cells for TDP-43 aggregates and found that only 3 out of the 16 lines displayed abnormal staining for this marker. Although variability in the cell model cannot be excluded, this finding could be descriptive of a subgroup of sporadic patients where TDP-43 proteinopathy is involved, for example, in the early stage of disease. This pathogenic phenotype was then utilised to screen for FDA approved compounds that can lower the amount of TDP-43 aggregates in both familial and sporadic cases [79].

3.3. Proteostasis

Protein aggregation is a hallmark of several neurodegenerative disorders and, as such, proteostasis is considered an appealing therapeutic target [80]. The link between ALS and proteostasis dysregulation is highlighted by the number of ALS-linked genes which are involved in this pathway including SQSTM1, OPTN, TBK1 and VCP.

Small molecules activating autophagy and the proteasome have been investigated in the context of ALS as promising therapeutics to decrease accumulation of misfolded proteins [81]. Once more, iPSC MNs displaying TDP-43 mislocalization through endogenous or induced mechanisms have been used to identify potential drug candidates where TDP-43 proteinopathy was reduced through manipulation of proteostasis rather than the RNA-related mechanisms as discussed above.

Barmada et al. demonstrated that in primary cortical neurons the amount of TDP-43 proteinopathy was proportional to neurotoxicity and that activation of autophagy *via* fluphenazine and methotrimeprazine successfully increased TDP-43 aggregate clearance and rescued neuronal survival in primary rodent neurons, as well as patient-derived mutant TDP-43 MNs and astrocytes [82].

Similarly, a recent study by Khosravi et al. has demonstrated that expression of poly-GA in transgenic cell models, as well as patient cells, promotes TDP-43 aggregation by inhibiting the proteasome in a cell-autonomous fashion as well as in a non-cell autonomous paradigm of cell-to-cell dipeptide repeat (DPR) transmission. The authors found that protein aggregation can be prevented by boosting proteasome activity with rolipram [83]. Consistent with these reports, MNs derived from patients carrying mutant FUS and displaying cytoplasmic protein aggregates were preserved by treatment with autophagy-inducing drugs, such as the mTOR inhibitors torkinib and PQR309, currently in clinical trial for cancer treatment [84].

The above-mentioned studies collectively suggest that activation of autophagy or the proteasome are promising therapeutic approaches for the treatment of ALS, however, *in vivo* studies indicate that several factors should be considered. Firstly, the stage of the disease when treatment commences and duration of treatment. Genetic inhibition of autophagy in motor neurons of mutant SOD1 mice revealed that autophagy can be protective early in disease progression, but detrimental at later stages [85]. In addition, most autophagy activators act through mTOR inhibition, which is likely to affect several other pathways beyond autophagy, including protein synthesis and immunosuppression. In conclusion, future studies targeting proteostasis *in vitro* should be complemented with *in vivo* tests, which are more likely to highlight the limitations of these therapeutic approaches, as they give the opportunity to observe the effect of drugs in a complex system over time and in various disease stages.

3.4. Electrophysiological abnormalities

Similar targeted approaches have been applied to other consistent pathophysiological characteristics, such as electrophysiological defects, which occur early in disease development in patients [86]. Indeed, electrophysiological alterations have been detected in iPSC-derived MNs from patients carrying various mutations, thus demonstrating impairment in cell functionality. Studies from various groups have reported that patient MNs are initially hyperexcitable and then they become hypoexcitable, potentially due to a progressive decrease in voltage-activated Na^{+} and K^{+} currents causing a progressive loss of synaptic activity [87]. These electrophysiological changes were recorded in iPSC-derived MNs from both mutant TDP-43 and C9orf72 cells, even in absence of decreased viability [87]. Due to the relevance of these

early alterations in ALS pathology and thanks to the ability in monitoring these changes in patients throughout disease, therapeutic approaches targeting these functional alterations are particularly appealing. One recent example is the identification of retigabine/ezogabine, a Kv7 channel activator, able to rescue mSOD1 iPSC-MN hyperexcitability and improve MN survival [74]. Impairment of this ion channel was subsequently validated in a study using a high-throughput all-optical screening approach, where single MNs can be recorded and analyzed within a complex network [88]. Indeed these findings were recently confirmed in a phase 2 randomized clinical trial in which a total of 65 ALS participants were randomized to placebo (23), 600 mg/d of ezogabine (23), and 900 mg/d of ezogabine (19 participants)(ClinicalTrials.gov Identifier:NCT02450552). Patients treated with ezogabine displayed a decrease in cortical and spinal MN excitability as predicted by the *in vitro* results [89]. This latest study is an example of how, even if with limitations, iPSC-derived cells can guide target identification and inform therapeutic approaches.

3.5. Precision medicine approaches

Recently, a different approach, based on “blind-to-target” drug screenings, has been applied to ALS to allow for patient stratification based on drug response rather than clinical characteristics. These approaches use the underlying concept that we can reliably and consistently measure disease-relevant pathological hallmarks in patient-derived cells and these can be used to screen drugs that correct a certain phenotype, thus allowing us to identify a target and, potentially, a therapeutic compound at the same time.

Such efforts often focus on drug repurposing, using libraries of FDA approved drugs, with the aim to streamline the drug approval process. One of the main targets identified with this approach is the Src/c-Abl pathway identified by Imamura et al. [90]. The authors developed an inducible, high-throughput MN differentiation protocol that allowed screening more than 1400 compounds, targeting various pathways, in one iPSC patient-derived mSOD1 line. The screening returned 27 hits, highly enriched for molecules targeting the Src/c-Abl pathway, which improved MN survival. Selected hits, amongst which bosutinib (currently in clinical trial UMIN:000036295) [91], were then screened on 11 additional ALS iPSC lines from sporadic patients or carrying SOD1, TDP-43 and C9orf72 mutations to verify neuroprotection.

As new cell reprogramming and differentiation protocols for high-throughput screenings are developed, as well as the ability to optimise and scale up reliable readouts, we might be able to stratify patients more accurately based on drug responses against several parameters. An elegant example of such an approach is the study by Fujimori et al. [92], where a staged high-throughput drug screening assessed more than 1200 molecules for their effect on 6 different pathological readouts, from cell death to stress granule formation and protein aggregates, in one FUS iPSC line. Ninety-five selected hits were then taken forward in a TDP-43 line, where 9 were confirmed as neuroprotective and, after scoring the hits for brain permeability, potential side-effects and efficacy, ropinirole was selected as top hit. This compound was then tested in 24 out of 32 different sporadic ALS lines that were stratified according to the 6 pathological readouts assessed for the screening. The 24 iPSC MN lines were selected based on their *in vitro* “disease severity”. Interestingly, the authors found that only 16 of the 24 MN lines displayed improvement in cell death and protein aggregation upon ropinirole treatment. Whether this patient-specific drug response translates in the patient population and what specific characteristics and biomarkers could be used to stratify the population ‘a priori’ are still open questions and present challenges that we will have to be resolved in order to advance this precision medicine approach, as other fields, such as cancer and asthma, have achieved.

4. The future of disease modeling and drug discovery

The progress achieved in the past 5 years has led to new clinical trials testing drugs identified using iPSC-derived patient MNs. The complex multifactorial nature of ALS, however, indicates that therapies targeting only MNs might have a limited effect. As highlighted in the first part of this review article, in fact, several other cell types play a key role in MN death. Hence, effective disease-modifying therapies are likely to require investigation of pathogenic mechanisms and therapeutic targets in patient-derived models that mirror the complex interaction between different cell types.

4.1. Co-culture systems

Reports implicating non-neuronal cells and, in particular, astrocytes in the pathology of ALS have prompted a number of studies aiming to identify the impact of these cells on motor neuron survival. As previously discussed, *in vivo* studies have been crucial in highlighting the role of glia in disease progression [29,34] and, potentially disease onset [93].

In vitro co-culture studies have been important in uncovering astrocyte neurotoxicity initially from mSOD1 mouse models [94,95] and subsequently from post-mortem human samples harbouring SOD1 mutations or isolated from sporadic ALS cases [96,97]. These studies showed for the first time that astrocyte toxicity is a widespread disease mechanism affecting familial as well as sporadic ALS patients and that astrocyte toxicity is associated with soluble factors [96,97].

Whereas opposing results have emerged when investigating the neurotoxic properties of iPSC-derived astrocytes harbouring TDP-43 mutations *in vitro* [98], astrocyte toxicity is a well-established and consistent phenotype in other familial forms of disease, including C9orf72 [99,100] and VCP-linked [101] ALS, as well as sporadic disease [36,99]. One of the most pressing questions in the field focuses around the origin of this toxicity and the factors mediating MN death, with the aim of developing therapeutic approaches. A plethora of mechanisms have been proposed [102], but the role of astrocytes in MN death is likely to be related to multiple factors, some of which exert toxicity, while others underlie overall astrocyte dysfunction, leading to a toxic lack of support. Recent studies have, in fact, reported widespread changes in astrocyte transcriptome [100] and secretome [103], as well as their ability to maintain calcium homeostasis due to Connexin 43 dysregulation [104], indicating that these cells are likely to be failing in several physiological functions essential to support MN survival. Amongst these, recent studies have reported severe metabolic dysfunction in C9orf72 patient-derived astrocytes [105,106], particularly linked to defects in adenosine deaminase, thus leading to healthy MN death in a co-culture system. Neuroprotection in this system was achieved *via* supplementation of inosine. Besides providing metabolic support to neurons, astrocytes are also known to be essential in supporting neurite growth and maintenance, as well as dampening oxidative stress through secreted factors [107]. Recent studies have shown that C9orf72 astrocytes are defective in secreting anti-oxidant enzymes [108], as well as miRNAs supporting neurite maintenance in co-cultured MNs [103]. Neurite growth and MN survival could both be rescued by supplementation of miRNA 494−3p in the co-cultures, thus identifying this miRNA as a potential therapeutic target. Future studies should focus on understanding the impact of therapies targeting these pathways *in vivo*.

In addition, patient-derived astrocytes seem to be able to induce in healthy MNs a number of pathological features we have previously described as cell-autonomous [82,87]. For example, C9orf72 patient-derived astrocytes can induce electrophysiological defects in healthy MNs by triggering loss of action potential output through a decrease in the magnitude of voltage-activated Na^+ and K^+ currents [109]. Similarly, Madill et al. [110], report that conditioned medium from C9orf72 patient astrocytes can induce accumulation of p62 in MNs, which is, in turn, alleviated by rapamycin treatment. Interestingly, Rajpurohit et al. propose activation of the opposite mechanism in

mutant SOD1 astrocyte, which trigger hyperactivation of autophagy, thus resulting in MN stress and death [111]. Such a complex neurotoxic interaction could be unravelled through recent co-culture blind--to-target drug screening approaches [112], where neuroprotective drugs could shed light on the pathways involved in the toxic interaction between two cell types.

An alternative approach to unravelling the nature of this toxic interaction has recently been taken by Mishra et al. [113], where the authors specifically focused on ligand-mediated interactions between MNs and astrocytes. By using an integrative analysis approach that combines proteomics and regulatory network analysis, the authors identified the interaction between astrocyte secreted Amyloid Precursor Protein (APP) and death receptor-6 (DR6) on MNs the top predicted ligand-receptor pair responsible for astrocyte-mediated MN death. This toxic interaction was confirmed using murine mutant SOD1 astrocytes as well as human sporadic astrocytes.

Although clear evidence from post-mortem tissue and mouse models of ALS has uncovered the contribution of oligodendrocytes and microglia/macrophages to disease as presented in previous sections, *in vitro* studies from patient-derived cells are still scarce, probably due to the length and complexity of the protocols that lead to functional and mature cells.

Consistent with *in vivo* data [39], oligodendrocytes derived from ALS patients did not present any maturation impairment [114,115], however, they induced MN death *in vitro* and displayed impaired lactate release in the medium, associated with decreased expression of MCT1 [115]. Surprisingly, lactate supplementation was not sufficient to rescue MN survival in co-culture, thus indicating that metabolic defects are only one aspect of cell dysfunction. Interestingly, oligodendrocyte-mediated MN death could be rescued in SOD1 and sporadic ALS cases *via* SOD1 knockdown only if protein suppression was achieved in OPCs [115], thus suggesting that functional impairment might have a short therapeutic window in these cells. Recently, a high-throughput platform including iPSC-derived MNs and myelinating oligodendrocytes has been generated [116], thus opening new opportunities for future drug screening efforts.

Reliable and efficient protocols to study the properties of patient-derived microglia and macrophages are still under development. As highlighted earlier in this review, *in vivo* studies and co-culture systems from the mSOD1 mouse models have shown that microglia mainly contribute to disease through inflammation [117], thus focusing therapeutic efforts towards anti-inflammatory approaches. Exciting new findings showed that iPSC-derived M2 macrophages from C9orf72 and sALS patients exert an anti-inflammatory effect on M1 macrophages and boost ALS Tregs [118], thus demonstrating the potential for immune-cell-based therapy to mitigate inflammation in ALS.

4.2. 3D disease modeling

Multicellular systems organised in microfluidic chambers or organoids are the next step to validate the pathways and therapeutic targets already identified and are a potential platform for further discovery. Such models are being developed not only to study the interaction between neurons and glia, but also to model the neuromuscular junction (NMJ), considering that failure in this specialised synapse is one of the first signs of disease.

Recently, 3D NMJ models have been developed with the aim to characterize this complex system and interrogate the interaction between MNs and muscles. In 2018 Osaki et al. [119] successfully obtained formation of motor units by using microfluidic chambers where MN spheroids and muscle bundles were plated in different compartments of the chamber. This system recapitulated the motor unit loss observed in ALS patients [120] and recovery was obtained by boosting autophagy or inhibiting the Src/c-Abl pathway, thus leading to decreased TDP-43 proteinopathy. Further insight has been gained through these initial NMJ systems with regards to the selective vulnerability of MNs compare to sympathetic neurons (SNs) [121], which are mainly spared in ALS. Healthy NMJs displayed a higher level of stationary mitochondria in MNs compared with SNs, consistent with the finding that the NMJ is highly dependent upon mitochondrial respiration, while SN synapses rely on both mitochondria and glycolysis for ATP production. This indicates that mitochondrial dysfunction might have a higher impact on NMJ function compared to SN synapse.

Alongside microfluidics and bioprinting, which provide structure and better system control, organoids have emerged as a developing resource to study cell-to-cell interaction under basal pathological conditions in diseases like AD, where organoids seem to have reliably retained specific profiles of protein aggregation [122,123] and HD [124].

Interesting future avenues for ALS are the emerging spinal cord spheroids, fabricated using magnetic nanoparticles. These are positioned in a three-dimensional hydrogel construct using magnetic bioprinting, thus resulting in structures that provide both localized cell–cell interactions and long-distance projections that mimic *in vivo* structure [125].

5. Conclusions

Twelve years after the generation of the first iPSC-derived MNs from ALS patients, significant progress has been made in disease modeling and therapeutics discovery thanks to the development of more efficient and reliable differentiation protocols. In addition, our ability to model not only MN pathology, but also the pathological changes affecting non-neuronal cells, will allow for the identification of new and potentially more effective therapeutic targets. These advancements have already led to the identification of compounds in clinical trials including: bosutinib, ropinirole [91], and ezogabine. The use of patient-derived cells, however, is not limited to mechanistic and drug discovery studies, but they have also supported biomarker studies. In fact, iPSC-derived MNs have also been used to show that there is a correlation between intracellular and extracellular poly-GP [126], thus supporting that secreted CSF poly-GP is a surrogate of intracellular protein and, therefore, a promising pharmacodynamic marker for trials of antisense oligonucleotides in ALS clinical trials.

Moreover, the generation of patient-derived cellular models has offered unprecedented opportunities to overcome the limitations presented by models driven by transgene overexpression, including the possibility of obtaining insight into sporadic disease. This is either determined by complex genetic factors that we are still far from unravelling and modeling, or epigenetic changes accumulated over a lifetime. Unfortunately, however, some reprogramming techniques do not preserve epigenetic changes, thus limiting our ability to model sALS. Direct reprogramming methodologies overcome, at least in part, this limitation and allow the production of neurons [127] and astrocytes [128] that retain some epigenetic features and, therefore, are likely to be more suitable for modeling aging and disease. Other challenges include reproducing the phenomenon of aging cells in their embryonic phenotype [129–131], consistently reproducing results amongst laboratories using iPSC, and overcoming the costs of producing ALS iPSC on a scale that allows for the implementation of drug screening strategies [132]. Indeed, achieving consistency and reproducibility between different laboratories will be crucial in developing effective neuroprotective compounds. One of the challenges in evaluating and comparing the findings from different studies over the past twelve years, in fact, partly lies in the lack of details regarding iPSC differentiation efficiency, culture purity and maturity, as well as quantification of other neuronal and non-neuronal cell types in each study.

As these challenges are addressed, the use of human iPSC to model cell-specific contributions to ALS offers the potential for versatility by providing nearly limitless combinations of culture conditions that can be analyzed in a relatively efficient manner for understanding disease mechanisms and screening therapeutic compounds. This is

complemented by *in vivo* modeling using a number of different animal models that will continue to help inform about the complexity of these interactions for ALS therapeutic development.

CRediT authorship contribution statement

Laura Ferraiuolo: Conceptualized the review, wrote the original manuscript, and was responsible for reviewing and editing the revised submission. **Nicholas Maragakis:** Conceptualized the review, wrote the original manuscript, and was responsible for reviewing and editing the revised submission.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

References

[1] J.A. Thomson, et al., Embryonic stem cell lines derived from human blastocysts, Science 282 (5391) (1998) 1145–1147.
[2] K. Takahashi, et al., Induction of pluripotent stem cells from adult human fibroblasts by defined factors, Cell 131 (5) (2007) 861–872.
[3] M. Brouwer, H. Zhou, N. Nadif Kasri, Choices for induction of pluripotency: recent developments in human induced pluripotent stem cell reprogramming strategies, Stem Cell Rev. Rep. 12 (1) (2016) 54–72.
[4] G. Liu, et al., Advances in pluripotent stem cells: history, mechanisms, technologies, and applications, Stem Cell Rev. Rep. 16 (1) (2020) 3–32.
[5] F. Gonzalez, S. Boue, J.C. Izpisua Belmonte, Methods for making induced pluripotent stem cells: reprogramming a la carte, Nat. Rev. Genet. 12 (4) (2011) 231–242.
[6] E.V. Hobson, C.J. McDermott, Supportive and symptomatic management of amyotrophic lateral sclerosis, Nat. Rev. Neurol. 12 (9) (2016) 526–538.
[7] J.R. Burrell, et al., The frontotemporal dementia-motor neuron disease continuum, Lancet 388 (10047) (2016) 919–931.
[8] R. Guerreiro, J. Bras, J. Hardy, SnapShot: genetics of ALS and FTD, Cell 160 (4) (2015), p. 798-798 e1.
[9] M. DeJesus-Hernandez, et al., Expanded GGGGCC hexanucleotide repeat in noncoding region of C9ORF72 causes chromosome 9p-linked FTD and ALS, Neuron 72 (2) (2011) 245–256.
[10] A.E. Renton, et al., A hexanucleotide repeat expansion in C9ORF72 is the cause of chromosome 9p21-linked ALS-FTD, Neuron 72 (2) (2011) 257–268.
[11] E. Majounie, et al., Frequency of the C9orf72 hexanucleotide repeat expansion in patients with amyotrophic lateral sclerosis and frontotemporal dementia: a cross-sectional study, Lancet Neurol 11 (4) (2012) 323–330.
[12] J. Chew, et al., Neurodegeneration. C9ORF72 repeat expansions in mice cause TDP-43 pathology, neuronal loss, and behavioral deficits, Science 348 (6239) (2015) 1151–1154.
[13] Y. Liu, et al., C9orf72 BAC mouse model with motor deficits and neurodegenerative features of ALS/FTD, Neuron 90 (3) (2016) 521–534.
[14] J.G. O'Rourke, et al., C9orf72 BAC transgenic mice display typical pathologic features of ALS/FTD, Neuron 88 (5) (2015) 892–901.
[15] O.M. Peters, et al., Human C9ORF72 hexanucleotide expansion reproduces RNA foci and dipeptide repeat proteins but not neurodegeneration in BAC transgenic mice, Neuron 88 (5) (2015) 902–909.
[16] K.M. Rosler, et al., Quantification of upper motor neuron loss in amyotrophic lateral sclerosis, Clin. Neurophysiol. 111 (12) (2000) 2208–2218.
[17] H.G. Stewart, et al., Corticomotoneuronal dysfunction in ALS patients with different SOD1 mutations, Clin. Neurophysiol. 117 (8) (2006) 1850–1861.
[18] N. Geevasinga, et al., Pathophysiological and diagnostic implications of cortical dysfunction in ALS, Nat. Rev. Neurol. 12 (11) (2016) 651–661.
[19] R.N. Lemon, Descending pathways in motor control, Annu. Rev. Neurosci. 31 (2008) 195–218.
[20] P.H. Ozdinler, et al., Corticospinal motor neurons and related subcerebral projection neurons undergo early and specific neurodegeneration in hSOD1G(9)(3)A transgenic ALS mice, J. Neurosci. 31 (11) (2011) 4166–4177.
[21] H.X. Deng, et al., Distal axonopathy in an alsin-deficient mouse model, Hum. Mol. Genet. 16 (23) (2007) 2911–2920.
[22] D. Fil, et al., Mutant Profilin1 transgenic mice recapitulate cardinal features of motor neuron disease, Hum. Mol. Genet. 26 (4) (2017) 686–701.
[23] J. Kim, et al., Changes in the excitability of neocortical neurons in a mouse model of amyotrophic lateral sclerosis are not specific to corticospinal neurons and are modulated by advancing disease, J. Neurosci. 37 (37) (2017) 9037–9053.
[24] M. de Carvalho, et al., Electrodiagnostic criteria for diagnosis of ALS, Clin. Neurophysiol. 119 (3) (2008) 497–503.
[25] B.R. Brooks, et al., El Escorial revisited: revised criteria for the diagnosis of amyotrophic lateral sclerosis, Amyotroph. Lateral Scler. Other Motor Neuron Disord. 1 (5) (2000) 293–299.
[26] M.E. Gurney, et al., Motor neuron degeneration in mice that express a human Cu, Zn superoxide dismutase mutation [see comments], Science 264 (5166) (1994) 1772–1775.
[27] M. Benatar, Lost in translation: treatment trials in the SOD1 mouse and in human ALS, Neurobiol.Dis. 26 (1) (2007) 1–13.
[28] F. De Giorgio, et al., Transgenic and physiological mouse models give insights into different aspects of amyotrophic lateral sclerosis, Dis. Model. Mech. 12 (1) (2019).
[29] S. Boillee, et al., Onset and progression in inherited ALS determined by motor neurons and microglia, Science 312 (5778) (2006) 1389–1392.
[30] L.A. Bristol, J.D. Rothstein, Glutamate transporter gene expression in amyotrophic lateral sclerosis motor cortex, Ann.Neurol. 39 (1996) 676–679.
[31] C.G. Lin, et al., Aberrant RNA processing in a neurodegenerative disease: the cause for absent EAAT2 a glutamate transporter, in amyotrophic lateral sclerosis, Neuron 20 (3) (1998) 589–602.
[32] J.D. Rothstein, Excitotoxicity and neurodegeneration in amyotrophic lateral sclerosis, Clin. Neurosci. 3 (6) (1995) 348–359.
[33] Y.H. Gong, et al., Restricted expression of G86R Cu/Zn superoxide dismutase in astrocytes results in astrocytosis but does not cause motoneuron degeneration, J. Neurosci. 20 (2) (2000) 660–665.
[34] K. Yamanaka, et al., Astrocytes as determinants of disease progression in inherited amyotrophic lateral sclerosis, Nat.Neurosci. 11 (3) (2008) 251–253.
[35] S.T. Papadeas, et al., Astrocytes carrying the superoxide dismutase 1 (SOD1G93A) mutation induce wild-type motor neuron degeneration in vivo, Proc. Natl. Acad. Sci. U. S. A. 108 (43) (2011) 17803–17808.
[36] K. Qian, et al., Sporadic ALS astrocytes induce neuronal degeneration in vivo, Stem Cell Reports 8 (4) (2017) 843–855.
[37] K.D. Foust, et al., Therapeutic AAV9-mediated suppression of mutant SOD1 slows disease progression and extends survival in models of inherited ALS, Mol. Ther. 21 (12) (2013) 2148–2159.
[38] K.A. Guttenplan, et al., Knockout of reactive astrocyte activating factors slows disease progression in an ALS mouse model, Nat. Commun. 11 (1) (2020) 3753.
[39] S.H. Kang, et al., Degeneration and impaired regeneration of gray matter oligodendrocytes in amyotrophic lateral sclerosis, Nat. Neurosci. 16 (5) (2013) 571–579.
[40] Y. Lee, et al., Oligodendroglia metabolically support axons and contribute to neurodegeneration, Nature 487 (7408) (2012) 443–448.
[41] T. Philips, et al., Oligodendrocyte dysfunction in the pathogenesis of amyotrophic lateral sclerosis, Brain 136 (Pt 2) (2013) 471–482.
[42] J.D. Cherry, J.A. Olschowka, M.K. O'Banion, Neuroinflammation and M2 microglia: the good, the bad, and the inflamed, J. Neuroinflammation 11 (2014) 98.
[43] N.R. Zurcher, et al., Increased in vivo glial activation in patients with amyotrophic lateral sclerosis: assessed with [(11)C]-PBR28, Neuroimage Clin. 7 (2015) 409–414.
[44] M.J. Alshikho, et al., Glial activation colocalizes with structural abnormalities in amyotrophic lateral sclerosis, Neurology 87 (24) (2016) 2554–2561.
[45] S.G. Crisafulli, et al., Therapeutic strategies under development targeting inflammatory mechanisms in amyotrophic lateral sclerosis, Mol. Neurobiol. 55 (4) (2018) 2789–2813.
[46] X. Hu, et al., Microglial and macrophage polarization-new prospects for brain repair, Nat. Rev. Neurol. 11 (1) (2015) 56–64.
[47] Y. Tang, W. Le, Differential roles of M1 and M2 microglia in neurodegenerative diseases, Mol. Neurobiol. 53 (2) (2016) 1181–1194.
[48] L. Du, et al., Role of microglia in neurological disorders and their potentials as a therapeutic target, Mol. Neurobiol. 54 (10) (2017) 7567–7584.
[49] H. Keren-Shaul, et al., A unique microglia type associated with restricting development of alzheimer's disease, Cell 169 (7) (2017) 1276–1290, e17.
[50] O. Dols-Icardo, et al., Motor cortex transcriptome reveals microglial key events in amyotrophic lateral sclerosis, Neurol. Neuroimmunol. Neuroinflamm. 7 (5) (2020).
[51] A. Deczkowska, et al., Disease-associated microglia: a universal immune sensor of neurodegeneration, Cell 173 (5) (2018) 1073–1081.
[52] D.R. Beers, et al., Wild-type microglia extend survival in PU.1 knockout mice with familial amyotrophic lateral sclerosis, Proc. Natl. Acad. Sci. U. S. A. 103 (43) (2006) 16021–16026.
[53] M.C. Geloso, et al., The dual role of microglia in ALS: mechanisms and therapeutic approaches, Front. Aging Neurosci. 9 (2017) 242.
[54] J.G. O'Rourke, et al., C9orf72 is required for proper macrophage and microglial function in mice, Science 351 (6279) (2016) 1324–1329.
[55] A. Chiot, et al., Modifying macrophages at the periphery has the capacity to change microglial reactivity and to extend ALS survival, Nat. Neurosci. 23 (11) (2020) 1339–1351.
[56] J.S. Henkel, et al., Regulatory T-lymphocytes mediate amyotrophic lateral sclerosis progression and survival, EMBO Mol. Med. 5 (1) (2013) 64–79.
[57] D.R. Beers, et al., ALS patients' regulatory T lymphocytes are dysfunctional, and correlate with disease progression rate and severity, JCI Insight 2 (5) (2017) e89530.
[58] D.R. Beers, et al., CD4+ T cells support glial neuroprotection, slow disease progression, and modify glial morphology in an animal model of inherited ALS, Proc. Natl. Acad. Sci. U. S. A. 105 (40) (2008) 15558–15563.
[59] T.M. Miller, et al., Gene transfer demonstrates that muscle is not a primary target for non-cell-autonomous toxicity in familial amyotrophic lateral sclerosis, Proc. Natl. Acad. Sci. U. S. A. 103 (51) (2006) 19546–19551.

[60] M. Wong, L.J. Martin, Skeletal muscle-restricted expression of human SOD1 causes motor neuron degeneration in transgenic mice, Hum. Mol. Genet. 19 (11) (2010) 2284–2302.

[61] G. Dobrowolny, M. Aucello, A. Musaro, Muscle atrophy induced by SOD1G93A expression does not involve the activation of caspase in the absence of denervation, Skelet. Muscle 1 (1) (2011) 3.

[62] G. Dobrowolny, et al., Muscle expression of SOD1(G93A) triggers the dismantlement of neuromuscular junction via PKC-Theta, Antioxid. Redox Signal. 28 (12) (2018) 1105–1119.

[63] B.J. Turner, et al., Dismutase-competent SOD1 mutant accumulation in myelinating Schwann cells is not detrimental to normal or transgenic ALS model mice, Hum. Mol. Genet. 19 (5) (2010) 815–824.

[64] C.S. Lobsiger, et al., Schwann cells expressing dismutase active mutant SOD1 unexpectedly slow disease progression in ALS mice, Proc. Natl. Acad. Sci. U. S. A. 106 (11) (2009) 4465–4470.

[65] D. Arbour, et al., Early and persistent abnormal decoding by glial cells at the neuromuscular junction in an ALS model, J. Neurosci. 35 (2) (2015) 688–706.

[66] D. Arbour, C. Vande Velde, R. Robitaille, New perspectives on amyotrophic lateral sclerosis: the role of glial cells at the neuromuscular junction, J. Physiol. (Paris) 595 (3) (2017) 647–661.

[67] S.H. Kang, et al., NG2+ CNS glial progenitors remain committed to the oligodendrocyte lineage in postnatal life and following neurodegeneration, Neuron 68 (4) (2010) 668–681.

[68] F. Laredo, J. Plebanski, A. Tedeschi, Pericytes: problems and promises for CNS repair, Front. Cell. Neurosci. 13 (2019) 546.

[69] E.A. Winkler, et al., Blood-spinal cord barrier breakdown and pericyte reductions in amyotrophic lateral sclerosis, Acta Neuropathol. 125 (1) (2013) 111–120.

[70] Z. Zhong, et al., ALS-causing SOD1 mutants generate vascular changes prior to motor neuron degeneration, Nat.Neurosci. 11 (4) (2008) 420–422.

[71] B. Bilican, et al., Mutant induced pluripotent stem cell lines recapitulate aspects of TDP-43 proteinopathies and reveal cell-specific vulnerability, Proc. Natl. Acad. Sci. U. S. A. 109 (2012) 5803–5808.

[72] J.T. Dimos, et al., Induced pluripotent stem cells generated from patients with ALS can be differentiated into motor neurons, Science (New York, N.Y.) 321 (2008) 1218–1221.

[73] X. Sun, et al., Modeling hallmark pathology using motor neurons derived from the family and sporadic amyotrophic lateral sclerosis patient-specific iPS cells 11 Medical and Health Sciences 1109 Neurosciences, Stem Cell Res. Ther. 9 (1) (2018) 315.

[74] Brian J. Wainger, et al., Intrinsic membrane hyperexcitability of amyotrophic lateral sclerosis patient-derived motor neurons, Cell Rep. 7 (1) (2014) 1–11.

[75] M.W. Amoroso, et al., Accelerated high-yield generation of limb-innervating motor neurons from human stem cells, J. Neurosci. 33 (2) (2013) 574–586.

[76] A. Zhao, Y. Pan, S. Cai, Patient-specific cells for modeling and decoding amyotrophic lateral sclerosis: advances and challenges, Stem Cell Rev. Rep. 16 (3) (2020) 482–502.

[77] N. Egawa, et al., Drug screening for ALS using patient-specific induced pluripotent stem cells, Sci. Transl. Med. 4 (2012) 145ra104.

[78] M.Y. Fang, et al., Small-molecule modulation of TDP-43 recruitment to stress granules prevents persistent TDP-43 accumulation in ALS/FTD, Neuron 103 (5) (2019) 802–819, e11.

[79] M.F. Burkhardt, et al., A cellular model for sporadic ALS using patient-derived induced pluripotent stem cells, Mol. Cell. Neurosci. 56 (2013) 355–364.

[80] O. Corti, et al., Autophagy in neurodegeneration: new insights underpinning therapy for neurological diseases, J. Neurochem. 154 (4) (2020) 354–371.

[81] A. Amin, et al., Amyotrophic lateral sclerosis and autophagy: dysfunction and therapeutic targeting, Cells 9 (11) (2020) 2413.

[82] S.J. Barmada, et al., Autophagy induction enhances TDP43 turnover and survival in neuronal ALS models, Nat. Chem. Biol. 10 (8) (2014) 677–685.

[83] B. Khosravi, et al., Cell-to-cell transmission of C9orf72 poly-(Gly-Ala) triggers key features of ALS / FTD, EMBO J. 39 (8) (2020).

[84] L. Marrone, et al., FUS pathology in ALS is linked to alterations in multiple ALS-associated proteins and rescued by drugs stimulating autophagy, Acta Neuropathol. 138 (1) (2019) 67–84.

[85] N.D. Rudnick, et al., Distinct roles for motor neuron autophagy early and late in the SOD1G93A mouse model of ALS, Proc. Natl. Acad. Sci. 114 (39) (2017) E8294–E8303.

[86] S. Vucic, G.A. Nicholson, M.C. Kiernan, Cortical hyperexcitability may precede the onset of familial amyotrophic lateral sclerosis, Brain 131 (Pt 6) (2008) 1540–1550.

[87] A.-C. Devlin, et al., Human iPSC-derived motoneurons harbouring TARDBP or C9ORF72 ALS mutations are dysfunctional despite maintaining viability, Nat. Commun. 6 (2015) 5999.

[88] E. Kiskinis, et al., All-optical electrophysiology for high-throughput functional characterization of a human iPSC-Derived motor neuron model of ALS, Stem Cell Reports 10 (6) (2018) 1991–2004.

[89] B.J. Wainger, et al., Effect of Ezogabine on cortical and spinal motor neuron excitability in amyotrophic lateral sclerosis: a randomized clinical trial, JAMA Neurol. (2020).

[90] K. Imamura, et al., The Src/c-Abl pathway is a potential therapeutic target in amyotrophic lateral sclerosis, Sci. Transl. Med. (2017) 9.

[91] H. Okano, et al., Ropinirole, a new ALS drug candidate developed using iPSCs, Trends Pharmacol. Sci. 41 (2) (2020) 99–109.

[92] K. Fujimori, et al., Modeling sporadic ALS in iPSC-derived motor neurons identifies a potential therapeutic agent, Nat. Med. 24 (10) (2018) 1579–1589.

[93] A.E. Frakes, et al., Additive amelioration of ALS by co-targeting independent pathogenic mechanisms, Ann. Clin. Transl. Neurol. (2017) 4.

[94] F.P. Di Giorgio, et al., Non-cell autonomous effect of glia on motor neurons in an embryonic stem cell-based ALS model, Nat. Neurosci. 10 (5) (2007) 608–614.

[95] M. Nagai, et al., Astrocytes expressing ALS-linked mutated SOD1 release factors selectively toxic to motor neurons, Nat. Neurosci. 10 (5) (2007) 615–622.

[96] A.M. Haidet-Phillips, et al., Astrocytes from familial and sporadic ALS patients are toxic to motor neurons, Nat. Biotechnol. 29 (9) (2011) 824–828.

[97] D.B. Re, et al., Necroptosis drives motor neuron death in models of both sporadic and familial ALS, Neuron 81 (5) (2014) 1001–1008.

[98] A. Serio, et al., Astrocyte pathology and the absence of non-cell autonomy in an induced pluripotent stem cell model of TDP-43 proteinopathy, Proc. Natl. Acad. Sci. U. S. A. 110 (2013) 4697–4702.

[99] K. Meyer, et al., Direct conversion of patient fibroblasts demonstrates non-cell autonomous toxicity of astrocytes to motor neurons in familial and sporadic ALS, Proc. Natl. Acad. Sci. U. S. A. 111 (2) (2014) 829–832.

[100] A. Birger, et al., ALS-related human cortical and motor neurons survival is differentially affected by Sema3A, Cell Death Dis. 9 (2018) 256.

[101] C.E. Hall, et al., Progressive motor neuron pathology and the role of astrocytes in a human stem cell model of VCP-Related ALS, Cell Rep. 19 (9) (2017) 1739–1749.

[102] L. Ferraiuolo, The non-cell-autonomous component of ALS: new in vitro models and future challenges, Biochem. Soc. Trans. 42 (2014) 1270–1274.

[103] A. Varcianna, et al., Micro-RNAs secreted through astrocyte-derived extracellular vesicles cause neuronal network degeneration in C9orf72 ALS, EBioMedicine 40 (2019) 626–635.

[104] A.A. Almad, et al., Connexin 43 in astrocytes contributes to motor neuron toxicity in amyotrophic lateral sclerosis, Glia (2016).

[105] S. Allen, et al., Astrocyte adenosine deaminase loss increases motor neuron toxicity in amyotrophic lateral sclerosis, Brain (2018).

[106] S.P. Allen, et al., C9orf72 expansion within astrocytes reduces metabolic flexibility in amyotrophic lateral sclerosis, Brain (2019).

[107] A. Verkhratsky, et al., Neurological diseases as primary gliopathies: a reassessment of neurocentrism, ASN Neuro 4 (2012) 131–149.

[108] A. Birger, et al., Human iPSC-derived astrocytes from ALS patients with mutated C9ORF72 show increased oxidative stress and neurotoxicity, EBioMedicine 50 (2019) 274–289.

[109] C. Zhao, et al., Mutant C9orf72 human iPSC-derived astrocytes cause non-cell autonomous motor neuron pathophysiology, Glia 68 (5) (2019) 1046–1064.

[110] M. Madill, et al., Amyotrophic lateral sclerosis patient iPSC-derived astrocytes impair autophagy via non-cell autonomous mechanisms, Mol. Brain 10 (1) (2017).

[111] C.S. Rajpurohit, et al., Mechanistic insights of astrocyte-mediated hyperactive autophagy and loss of motor neuron function in SOD1L39R linked amyotrophic lateral sclerosis, Mol. Neurobiol. 57 (10) (2020) 4117–4133.

[112] M. Stopford, S. Allen, L. Ferraiuolo, A high-throughput and pathophysiologically relevant astrocyte-motor neuron Co-culture assay for amyotrophic lateral sclerosis therapeutic discovery, BIOPROTOCOL (2019).

[113] V. Mishra, et al., Systematic elucidation of neuron-astrocyte interaction in models of amyotrophic lateral sclerosis using multi-modal integrated bioinformatics workflow, Nat. Commun. 11 (1) (2020).

[114] M.R. Livesey, et al., Maturation and electrophysiological properties of human pluripotent stem cell-derived oligodendrocytes, Stem Cells (2016).

[115] L. Ferraiuolo, et al., Oligodendrocytes contribute to motor neuron death in ALS via SOD1-dependent mechanism, Proc. Natl. Acad. Sci. 113 (42) (2016) E6496–E6505.

[116] J.A. García-León, et al., Generation of oligodendrocytes and establishment of an all-human myelinating platform from human pluripotent stem cells, Nat. Protoc. 15 (11) (2020) 3716–3744.

[117] A.E. Frakes, et al., Microglia induce motor neuron death via the classical NF-κB pathway in amyotrophic lateral sclerosis, Neuron 81 (2014) 1009–1023.

[118] W. Zhao, et al., Immunosuppressive functions of M2 macrophages derived from iPSCs of patients with ALS and healthy controls, iScience 23 (6) (2020) 101192.

[119] T. Osaki, S.G.M. Uzel, R.D. Kamm, Microphysiological 3D model of amyotrophic lateral sclerosis (ALS) from human iPS-derived muscle cells and optogenetic motor neurons, Sci. Adv. (2018) 4.

[120] W.A. Boekestein, et al., Motor unit number index (MUNIX) versus motor unit number estimation (MUNE): a direct comparison in a longitudinal study of ALS patients, Clin. Neurophysiol. (2012).

[121] T. Altman, et al., An in vitro compartmental system underlines the contribution of mitochondrial immobility to the ATP supply in the NMJ, J. Cell. Sci. 132 (23) (2019) jcs234492.

[122] Y. Pomeshchik, et al., Human iPSC-Derived hippocampal spheroids: an innovative tool for stratifying alzheimer disease patient-specific cellular phenotypes and developing therapies, Stem Cell Reports 15 (2020) 256–273.

[123] C. Arber, et al., Familial Alzheimer's disease patient-derived neurons reveal distinct mutation-specific effects on amyloid beta, Mol. Psychiatry (2019).

[124] Correction for Conforti, et al., Faulty neuronal determination and cell polarization are reverted by modulating HD early phenotypes, Proc. Natl. Acad. Sci. 115 (9) (2018) p. E2148-E2148.

[125] D.A. Bowser, M.J. Moore, Biofabrication of neural microphysiological systems using magnetic spheroid bioprinting, Biofabrication (2019).

[126] T.F. Gendron, et al., Poly(GP) proteins are a useful pharmacodynamic marker forC9ORF72-associated amyotrophic lateral sclerosis, Sci. Transl. Med. 9 (383) (2017) eaai7866.

[127] J. Mertens, et al., Directly reprogrammed human neurons retain aging-associated transcriptomic signatures and reveal age-related nucleocytoplasmic defects, Cell Stem Cell 17 (6) (2015) 705–17718.

[128] N. Gatto, et al., Directly converted astrocytes retain the ageing features of the donor fibroblasts and elucidate the astrocytic contribution to human CNS health and disease, Aging Cell (2020).

[129] M. Arbab, S. Baars, N. Geijsen, Modeling motor neuron disease: the matter of time, Trends Neurosci. 37 (11) (2014) 642–37652.

[130] R. Ho, et al., ALS disrupts spinal motor neuron maturation and aging pathways within gene co-expression networks, Nat. Neurosci. 19 (9) (2016) 1256–1267.

[131] J.D. Miller, et al., Human iPSC-based modeling of late-onset disease via progerin-induced aging, Cell Stem Cell 13 (6) (2013) 691–705.

[132] M.A. Myszczynska, et al., Applications of machine learning to diagnosis and treatment of neurodegenerative diseases, Nat. Rev. Neurol. (2020).