SCIENTIFIC COMMENTARY

Running up that pill for amyotrophic lateral sclerosis

This scientific commentary refers to ‘Maiden voyage: induced pluripotent stem cell-based drug screening for amyotrophic lateral sclerosis’ by Ito et al. (https://doi.org/10.1093/brain/awac306).

Amyotrophic lateral sclerosis (ALS) is a devastating neurodegenerative disease characterized by the progressive loss of motor neurons in the cortex and spinal cord. The incidence of ALS is ~1.75 per 100,000 people per year worldwide, whereas the estimated cumulative lifetime risk of developing this disease is up to roughly 1 in 400 people in certain populations.\(^1\) ALS clinically manifests as muscle weakness, spasticity, paralysis and respiratory failure that leads to death typically within 2–5 years after the onset of symptoms. Though ALS is still incurable, the ALS research community has made a tremendous effort to better understand the pathological processes leading to motor neuron dysfunction and loss. In their review in this issue of Brain, Ito and co-workers highlight studies that demonstrate how disease-associated cellular phenotypes can be recapitulated in human stem cell-derived neurons \textit{in vitro} and then used as endpoints for drug screening.\(^2\)

The pathophysiology of ALS is complex and perturbations in a multitude of cellular pathways have been implicated, including altered pre-mRNA splicing, impaired protein homeostasis, nucleocytoplasmic transport defects, glutamate excitotoxicity, mitochondrial dysfunction, increased oxidative stress, impaired axonal transport, and neuroinflammation, each of which has potential for therapeutic development.\(^3\) Additionally, an RNA-binding protein TDP-43, which plays several roles in RNA metabolism and predominantly localizes to the nucleus, is of great interest. In almost all cases of ALS, save for those associated with SOD1 or FUS mutations, cytoplasmic aggregation and concomitant nuclear depletion of TDP-43 are identified in motor neurons upon post-mortem examination. As Ito \textit{et al.} discuss, changes in the localization or solubility of TDP-43 in cell culture can provide a readout to identify compounds that may counter disease pathology.

Many promising therapeutic candidates for ALS have failed in clinical trials, and one possible explanation is that preclinical studies in model organisms may be limited by species-specific...
differences in gene expression within neurons and the functional connectivity of motor system
circuits. This has underscored the importance of developing novel approaches for ALS drug
development. Patient-derived induced pluripotent stem (iPS) cells and their differentiation into
neurons provide a means to carefully examine and manipulate the cellular populations that are
affected in disease but normally inaccessible for study. Applying advances in
neurodevelopmental biology and stem cell technology, pioneering studies by Kevin Eggan and
colleagues demonstrated that patient-derived iPS cells could be differentiated into motor
neurons, and ALS was among the first neurodegenerative diseases to be modelled using patient
stem cells. Of note, about 10% of ALS cases are inherited, often associated with highly
penetrant variants acting in an autosomal dominant manner. Neurons differentiated from patient
iPS cells can be used to examine the effects of these causative variants in a permissive genetic
background, opening the door for translational approaches (Figure 1).

In their review, Ito et al. cover studies that illustrate the mounting advantages of applying human
stem cell-derived neuronal models for drug screening in ALS. They focus mainly on three drugs
that were identified via phenotypic screening of human motor neurons and then evaluated in the
clinic: retigabine, ropinirole and bosutinib. The authors trace their clinical development from the
in vitro experiments that led to their identification as potential ALS treatments to the progress of
recent early-phase clinical trials. They also review the plausible mechanisms of action of
approved drugs and candidate therapies for ALS. Importantly, the authors emphasize that the use
of drug repurposing strategies in human stem cell-based model screening can reduce both the
cost and time to initiation of clinical trials. On the other hand, they also describe areas for
improvement of iPSC-derived neuron drug screening efforts, consistent with calls to increase
technical standards and reduce variability in this field. These concerns will likely remain
applicable as newer stem cell-derived 3D brain organoid models that feature neurons in more
complex and physiological multicellular environments are employed for phenotypic screening
and other translational applications. Further studies in human stem cell-derived neurons as well
as organoid models are poised to uncover new avenues for translational research in ALS and
related neurodegenerative diseases.

It should be noted that mouse models will likely continue to play a significant role in
understanding pathophysiological mechanisms of ALS and providing preclinical toxicology and
pharmacokinetic data for new compounds. Though the translational utility of mouse models for
certain ALS-associated phenotypes may be unresolved, animal models have been critical for providing the preclinical rationale for several candidate therapies. For instance, reduced expression of ataxin-2 improved motor function and extended survival in a TDP-43 transgenic model, prompting the current clinical evaluation of antisense oligonucleotides (ASOs) targeting the genetic modifier ATXN2 in ALS. Rodent studies were instrumental in revealing the neuroprotective effects of sodium phenylbutyrate and taurursodiol. A fixed-dose combination medication of these compounds (AMX0035) recently showed positive clinical results in the multicentre phase II CENTAUR trial for people with ALS, leading to its approval in at least Canada and the United States thus far. Additional ALS animal models continue to be developed, such as adeno-associated virus (AAV)-based models for C9orf72-ALS, and efforts are underway to standardize their usage to help select promising leads for additional studies. The challenges associated with finding treatments for neurodegenerative diseases underscore the importance of putting both feet forward and using complementary approaches while running up that road of ALS drug discovery.

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Competing interests

D.A.M. is an author on a pending patent that describes compounds and methods for treating neurodegenerative diseases (WO2020107037).
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References


**Figure legend**

**Figure 1 Stem cells enable ALS drug discovery.** After reprogramming of ALS patient fibroblasts into induced pluripotent stem cells (iPSCs), the iPSCs are differentiated into motor neurons. In their review, Ito *et al.* discuss two different approaches – targeted and unbiased – used for the identification of novel candidate therapies. Retigabine, an approved anticonvulsant, was tested on ALS patient-derived motor neurons for its electrophysiological current modulating properties. Ropinirole and bosutinib were found through high-throughput screening. These candidates have progressed to clinical trials in patients with ALS. Figure created with BioRender.com.
Figure 1

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