Trends in Pharmacological Sciences

CellPress REVIEWS

Review

Ropinirole, a New ALS Drug Candidate Developed Using iPSCs

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Induced pluripotent stem cells (iPSCs) are increasingly used in the study of disease mechanisms and the development of effective disease-modifying therapies for neurodegenerative diseases, including amyotrophic lateral sclerosis (ALS). Recently, three candidate anti-ALS drugs – ropinirole (ROPI), retigabine, and bosutinib – have been identified in iPSC-based drug screens and are now being evaluated in clinical trials for safety and effectiveness. We review the preclinical data, clinical research design, and rationale for ROPI as an anti-ALS drug candidate compared with those of the other two drugs. We also discuss the use of iPSCs for understanding and monitoring treatment response as well as for new insights into the development of new drugs and therapeutic interventions for major neurodegenerative diseases.

Introduction of iPSC-Based Drug Screening

Amyotrophic lateral sclerosis (**ALS**, see Glossary) is a devastating neurodegenerative disease characterized by loss of upper **motor neurons (MNs)** and/or lower MNs (LMNs) [1,2]. In its clinical presentation, ALS is a progressive disease characterized by muscle atrophy and weakness caused by selective vulnerability of MNs. While its clinical course is highly variable, the average time from the onset of symptoms to death or use of respiratory support is 3–5 years [1,3]. At present, there is no established effective treatment for ALS; riluzole (an antagonist of glutamate neurotransmission) [4] and edaravone (a superoxide scavenger) [5] are the only drugs approved for use in the treatment of ALS and both produce only slight beneficial effects in a limited population of ALS patients.

Human induced pluripotent stem cell (iPSC)-based techniques offer new opportunities for disease modeling and the development of new drugs, especially for conditions such as neurological and psychiatric diseases in which access to the affected cells and pathogenic sites is limited [6]. Combinatorial approaches involving iPSC-based phenotypic screening and repositioning of approved drugs may facilitate the drug development process. In the case of ALS, patient-derived iPSCs can serve as a source of spinal MNs, a disease-relevant cell type, and a series of studies have demonstrated disease-relevant phenotypes using ALS-derived iPSCs [7–11]. Three drugs –ROPI [a dopamine D2 and D3 receptor (D2R/D3R) agonist used as an antiparkinsonian drug] [12], retigabine (a neuronal Kv7 channel opener used as an antiepileptic) [7], and bosutinib [a dual Src/Abl tyrosine kinase inhibitor used as an anti-chronic myelocytic leukemia (CML) drug] [8] – have been identified as potential anti-ALS drugs using iPSC-based technologies and all are currently being investigated in clinical trials. In this review, we describe iPSC-based disease modeling, drug development, and clinical trials for ALS and discuss the rationale of ROPI and other drugs as a candidate therapies for ALS (Table 1).

Clinical Presentation and Pathogenesis of ALS

Of all ALS cases, approximately 5–10% are heritable [familial ALS (FALS), in which ALS is caused by a mutation of a single gene]. The remaining 90% are classified as sporadic ALS (SALS),

Highlights

iPSC-based drug discovery is a promising technology for developing novel therapeutics for neurodegenerative diseases lacking useful disease models, such as amyotrophic lateral sclerosis (ALS).

Ropinirole, retigabine, and bosutinib were identified as candidate therapeutic agents for ALS by the combination of iPSC-based drug discovery and drug repositioning.

The potential anti-ALS mechanism of ropinirole is independent of antioxidant activity, rescue of mitochondria, reduction of stress granules, and abnormal proteins such as phosphorylated TDP-43 and FUS, and dopamine D2 receptor (D2R) agonism.

Retigabine inhibits the hyperexcitability of motor neurons in ALS and bosutinib prompts autophagy and reduces abnormal proteins such as SOD-1 and phosphorylated TDP-43 via the Src/c-Abl pathway in motor neurons in ALS.

Stratification of ALS, personalized medicine strategies, and the identification of common mechanisms with other neurodegenerative diseases are key aspects in the development of ALS therapies.

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Table 1. Comparison of the Three Therapeutic Candidates for ALS Developed by iPSC Drug Discovery

	Known drug target	Potential mechanism	Targeted ALS subtype
ROPI	Dopamine receptor agonist	Suppressing oxidative stress Inhibiting TDP-43 and FUS aggregation Improving mitochondrial function Suppressing neurite retraction and cell death (sporadic, *TDP-43*, *FUS* mutation)	Most of sporadic *TDP-43* mutation *FUS* mutation NOT *SOD1* mutation
Retigabine	Kv7 or KCNQ voltage-gated potassium channel activator	Inhibiting motor neuronal excitability Decreasing activation of endoplasmic reticulum (ER) stress pathway Suppressing cell death (*SOD1* mutation)	*SOD1* mutation *C9orf72* mutation *FUS* mutation
Bosutinib	Src/c-Abl inhibitor	Inducing autophagy Inhibiting misfolded SOD1 aggregation Suppressing cell death (*SOD1*, *TDP-43*, *C9orf72* mutation, a part of sporadic)	*SOD1* mutation *TDP-43* mutation *C9orf72* mutation A part of sporadic

in which the underlying genetic background and etiological factors are highly variable and remain for the most part unknown. To date, there have been few clues to the disease mechanisms of SALS. However, characterization of gene products involved in FALS are providing insights into the disease mechanisms underlying SALS. At least 25 genes have now been reproducibly implicated in FALS, SALS, or both [1,13], including **superoxide dismutase 1 (*SOD1*)** [14], *ALS2* [15], **TAR DNA-binding protein of 43 kDa (*TDP-43*, *TARDBP*)** [16,17], **fused in sarcoma (*FUS*)** [18,19], and **optineurin (*OPTN*)** [20]. Four genes have been implicated in the form of the disease involving frontotemporal deterioration [**frontotemporal dementia (FTD)**-ALS)], including ***C9orf72*** [21]. The gene products of these genes can be functionally classified, with some overlaps [1,2], into those involved in: (i) protein homeostasis (including degradation and quality control); (ii) RNA metabolism; (iii) axonal transport and cytoskeletal dynamics; and (iv) non-cell-autonomous toxicity, including glial cell activity.

Many of these disease mechanisms, such as impaired protein homeostasis, which leads to the abnormal aggregation/accumulation of pathogenic proteins, are common to other neurodegenerative diseases. However, impaired RNA metabolism is relatively distinct to ALS. Investigating FALS and SALS with a focus on impaired RNA metabolism may thus be a fruitful approach to identify new therapeutic candidates. For example, *FALS-6*, *FALS-10*, and *FALS-20* encode **RNA-binding proteins (RBPs)** [FUS, TDP-43, and heterogeneous nuclear ribonucleoprotein A1 (hnRNPA1), respectively]. These three ALS-related RBPs belong to members of the hnRNP family and commonly possess a low-complexity (LC) domain, which is involved in protein–protein aggregation and fibrillization, and an RNA-binding domain (RBD). The co-occurrence of these domains suggests that these RBPs have an endogenous propensity to form aggregates comprising both RNAs and proteins, leading to stress granules (i.e., membraneless organelles generated by liquid–liquid phase separation [22]), which are characteristic pathological hallmarks observed in ALS MNs.

iPSCs can be efficiently induced into MN precursor cells (MPCs) and MNs (Figure 1A), which provided a useful tool for analyzing the pathogenesis of human MN disorders as well as for drug screening and monitoring responses to treatment [12]. MNs differentiated from iPSCs derived from patients with FALS carrying missense mutations in the *FUS* and *TDP-43* (*TARDBP*) genes recapitulated several neurodegenerative phenotypes, including mislocalization

Glossary

Amyotrophic lateral sclerosis (ALS): a neurodegenerative MN disease characterized by muscular atrophy, muscle weakness, difficulty swallowing, and respiratory dysfunction. Prognosis is poor with death typically following the onset of symptoms within 3–5 years.
C9orf72: hexanucleotide repeat expansion in the intron of *C9orf72* is a frequent cause of ALS and FTD.
Frontotemporal dementia (FTD): a group of neurodegenerative disorders characterized by progressive changes in behavior, executive dysfunction, and language impairment owing to degeneration of the frontal and temporal cortices. Four clinical subtypes have been identified: semantic dementia, progressive nonfluent aphasia, behavioral variant FTD, and right temporal lobar atrophy.
Fused in sarcoma (FUS): FUS/translated in liposarcoma (TLS) is a DNA-binding protein/RBP with a prion-like domain in ALS that leads to aggregation and the formation of cytoplasmic inclusions in MNs. *FUS* is a causative gene of ALS.
Induced pluripotent stem cell (iPSC)-based drug discovery: the use of patient-derived iPSCs to generate various differentiated human cells, such as neurons, muscles, and epithelial cells, and to screen for novel therapeutic compounds.
Motor neuron (MN): a specialized type of neuron located in the spinal cord and the precentral gyrus in the brain. They come in two main subtypes – namely, the upper MNs in the precentral gyrus and the LMNs in the spinal cord.
Optineurin (OPTN): *OPTN* is a causative gene of normal-tension glaucoma, adult-onset primary open-angle glaucoma, and ALS. OPTN is reported to regulate the function of MNs via the NF-κB signaling pathway.
Pre-Investigational New Drug Application (Pre-IND): the Pre-IND meeting can be helpful in developing a strategy for drug development by identifying studies that will support the initiation of clinical trials and discussing available methods to enhance development.
RNA-binding proteins (RBPs): a group of proteins that bind to double-stranded or single-stranded RNA and form ribonucleoprotein complexes. RBPs contain many structural motifs, such as the RNA recognition motif

of FUS/TDP-43 into cytosolic and stress granules under stress conditions, and cellular vulnerability [9,12].

Drug Screening of Potential Anti-ALS Drugs Using ALS iPSC Models

As described above, the following three drugs have been identified as anti-ALS therapeutic candidates using iPSC-based screening; all are currently being investigated in clinical trials.

ROPI was identified from a panel of 1232 FDA-approved drugs in a drug screening analysis conducted at Keio University, which examined *FUS*- and *TDP-43* (*TARDBP*)-ALS iPSC-derived MNs for suppression of ALS-related phenotypes *in vitro*, such as mislocalization of FUS/TDP-43, stress granule formation, MN death/damage, and neurite retraction (Figure 1B) [12].

Retigabine (known as an antiepileptic) was identified as a drug that suppresses the hyperexcitability of ALS iPSC-derived MNs based on electrophysiological analysis using a multielectrode array (MEA). That study reported that the spontaneous neuronal excitability of MNs derived from iPSCs from a patient with FALS carrying a *SOD1* mutation ($SOD1^{A4V/+}$) was increased [7]. The group found that $SOD1^{A4V/+}$ ALS patient-derived MNs showed reduced delayed-rectifier K^+ current amplitudes relative to those of the control MNs, which may lead to neuronal hyperexcitability. They also found that the Kv7 channel activator retigabine (a known antiepileptic) both blocks hyperexcitability and improves MN survival *in vitro* when tested in FALS cases with *SOD1* mutation, as well as those with *C9orf72* repeat expansions and *FUS* mutations [7].

Bosutinib (an anti-CML drug with Src/c-Abl kinase activity) was identified in a screen of approved drugs as a promoter of autophagy and was shown to rescue ALS MN degeneration [8]. Through screening of an existing drug library, inhibitors of Src/c-Abl kinases were shown to promote autophagy and rescue ALS MN degeneration [8].

From Drug Candidate Identification to Clinical Trials in ALS Patients

Prior to initiating clinical trials with anti-ALS candidate drugs identified by iPSC-based screening, several points need to be addressed in the **Pre-Investigational New Drug Application (Pre-IND)** process: (i) the drug's mechanism of action in ALS; (ii) potential advantages of the drug compared with existing anti-ALS drugs (riluzole and edaravone); (iii) implementation of a placebo-controlled trial; (iv) determination of whether the candidate drug is effective in SALS patients using iPSC-based assays; and (v) determination of whether responses to treatment can be distinguished in advance using iPSC-based assays.

We evaluated these questions in conducting the Pre-IND process for ROPI as an anti-ALS drug candidate and determined the following: (i) *in vitro* anti-ALS actions of ROPI are mediated by D2R-dependent and D2R-independent mechanisms (see the next section for details) [12]; (ii) compared with the existing drugs, such as riluzole [4] and edaravone [5], and investigational drugs, such as ceftriaxone and dexpramipexole, previously studied in clinical trials for ALS [23], ROPI significantly improves multiple ALS phenotypes *in vitro*; (iii) the active drug and placebo of ROPI extended-release tablets for a placebo-controlled trial are to be provided by the manufacturer [GlaxoSmithKline plc (GSK)]; (iv) an *in vitro* assay suggested that ROPI is also effective in approximately 73% of patients with SALS – however, LMNs of the FALS patients with *SOD1* mutations did not show sufficient improvement after treatment with ROPI [12]; and (v) cytoplasmic mislocalization and aggregation of ALS-related RBPs (TDP-43 and FUS) in patient-derived MNs were shown to be indicators for the ROPI responder group.

(RRM), double-stranded RNA-binding domains, prion-like domains, and zinc-finger domains. Although they are located in the cytoplasm and nucleus, most mature RNAs are exported from the nucleus quickly, so almost all RBPs in the nucleus exist as complexes of protein and pre-mRNA called hnRNPs.

Superoxide dismutase 1 (SOD1): binding to Cu and Zn, an enzyme that catalyzes the disproportionation of superoxide to hydrogen peroxide and dioxygen. Mutations in *SOD1* are the most common cause of FALS.

TAR DNA-binding protein of 43 kDa (TDP-43, TARDBP): a DNA-binding protein/RBP with a prion-like domain in ALS that leads to aggregation and the formation of cytoplasmic inclusions in MNs. *TDP-43* is a causative gene of ALS.

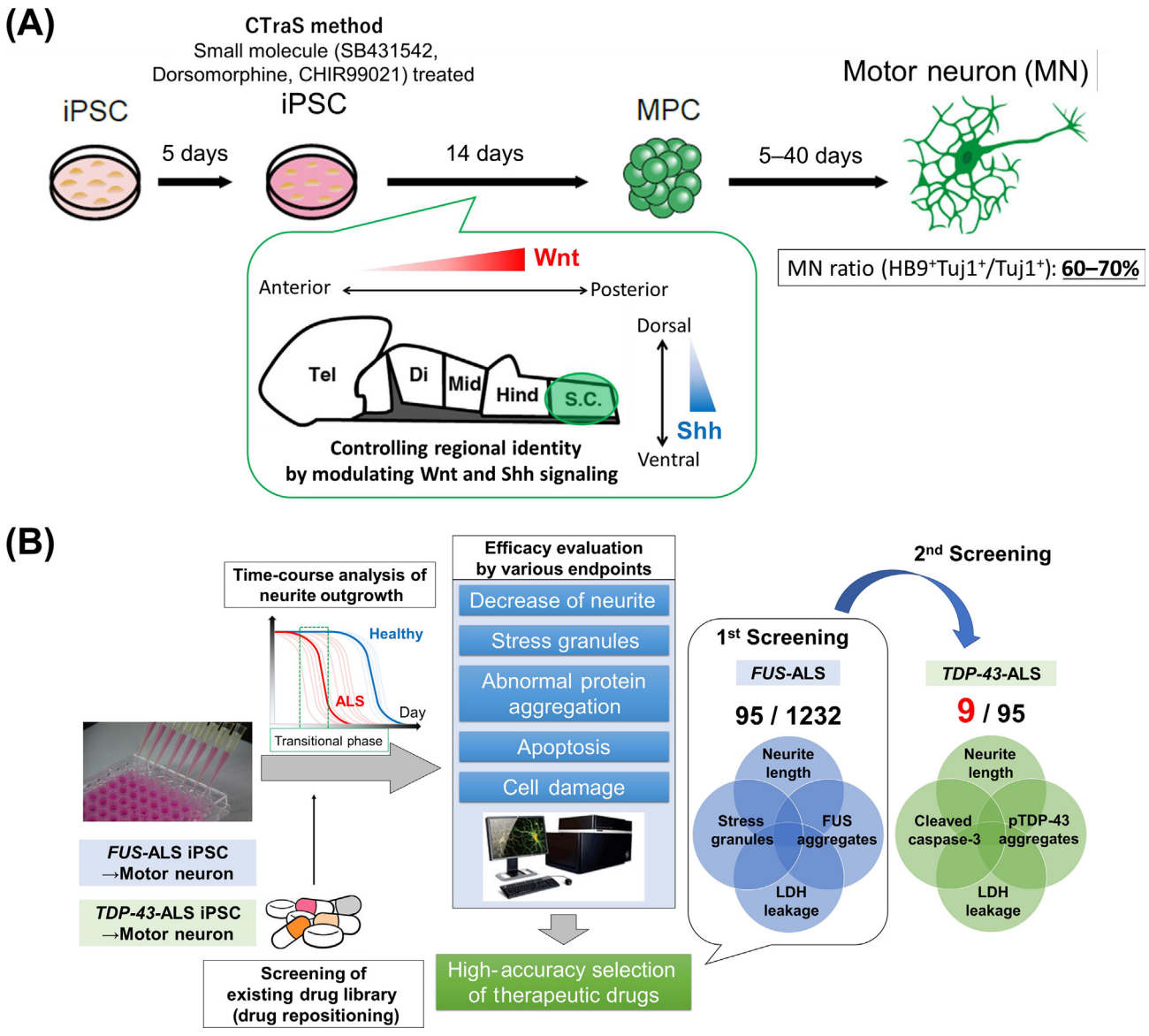

Figure 1. Induced Pluripotent Stem Cell (iPSC)-Based Modeling of Amyotrophic Lateral Sclerosis (ALS) Motor Neuron (MN) Phenotypes and Drug Screening with an FDA-Approved Drug Library. (A) Rapid and efficient induction of MN precursor cells (MPCs) from iPSCs. Modified from a previous report [12]. Human iPSC cultures are rapidly induced into neural progenitor cells by the chemically transitional embryoid-body-like state (CTraS) method [45] using three chemical compounds: transforming growth factor (TGF)β signal inhibitor, bone morphogenetic protein (BMP) signal inhibitor, and glycogen synthase kinase (GSK) 3β inhibitor. Subsequent to this, positional information was adjusted by activating the Wnt, retinoic acid, and Sonic hedgehog (Shh) signaling pathways so that they are adapted from undifferentiated human iPSCs to MPCs within 20 days *in vitro*. Then, the MPCs are transferred from the floating culture to the matrix-coated dish. Next, maturation of the MNs was further induced for 5–40 days [9,12]. (B) High-content FDA-approved drug library screening using familial ALS (FALS) iPSCs. We screened 1232 compounds from the existing drug library to explore drugs that suppress the ALS-related phenotypes {neurite retraction, stress granule formation, FUS/TDP-43 mislocalization, and cell damage [lactate dehydrogenase, (LDH) leakage} using 96-well plates and a high-content screening system. The first screening was performed using FALS patient-derived MNs with *FUS* mutation, and 95 of the 1232 drugs were identified. Subsequently, the second screening was performed using FALS patient-derived MNs with *TDP-43* (*TARDBP*) mutation and nine drugs that suppressed ALS-related phenotypes were identified. Thus, nine drugs suppressed ALS-related phenotypes of FALS patient-derived MNs with *FUS* and *TDP-43* (*TARDBP*) mutations.

Retigabine

The Eggan group at Harvard University used MEA recordings to show the hyperexcitability of MNs-derived from iPSCs generated from patients with FALS carrying the C9orf72 hexanucleotide repeat expansion. In addition to sodium current block, such as by riluzole, they focused on the enhancement of potassium currents to prevent hyperexcitability-induced degeneration of MNs and identified retibabine, a Kv7 current enhancer, which suppressed the hyperexcitability and subsequent degeneration of MNs derived from FALS iPSCs *in vitro* [7].

Bosutinib

The Inoue group at Kyoto University developed a drug screening of ALS MNs using FALS iPSCs with *SOD1* mutation. They performed high-throughput screening of 1416 compounds from an existing drug library to identify compounds that promote ALS MN survival as the readout. They found that that more than half of the hits targeted the Src/c-Abl signaling pathway. Among these compounds, they found that bosutinib increased the survival *in vitro* of ALS iPSC-derived MNs, boosted autophagy, reduced the amount of misfolded mutant SOD1 protein, and attenuated the altered expression of mitochondrial genes [8].

Potential Anti-ALS Action of ROPI, Retigabine, and Bosutinib

ROPI was developed as a non-ergot dopamine receptor agonist. The anti-ALS actions of ROPI can be interpreted from both its D2R-dependent and -independent mechanisms. Recent reports suggest that autophagy may be activated by D2R/D3R agonists, possibly through a Beclin-1-dependent pathway [24,25]. It would be interesting to determine whether ROPI-induced D2R/D3R activation induces autophagy leading to degradation or disassembly of abnormal RNA–protein complexes in ALS MNs. Dopamine D2R activation by ROPI additionally stimulates the Gi pathway, thereby repressing neuronal hyperexcitation, which is toxic to MNs [26]. Such suppression of neuronal hyperexcitation would be neuroprotective to MNs, which is relevant to the action of retigabine.

D2R-independent anti-ALS actions of ROPI are likely to result from its unique structural and chemical characteristics. Before ROPI was first reported in 1985 [27], 7-hydroxyropinirole was identified as a highly potent dopamine agonist [28,29]. 7-Hydroxyropinirole has also been identified as a metabolite of ROPI in humans. Structurally, both ROPI and 7-hydroxyropinirole have an oxindole (indoline-2-one) skeleton and an *N*,*N*-di-*n*-propylethylamine moiety. Because the oxindole skeleton is a bioisostere of phenol (Figure 2), 7-hydroxyropinirole is regarded as a structural analog of *N*,*N*-di-*n*-propyl dopamine and a structurally simpler dopamine receptor agonist [30]. The oxindole skeleton of ROPI is considered to be a chemically equivalent form of phenol, a general antioxidant. Although oxindole itself does not have high antioxidant activity compared with that of other antioxidants, such as uric acid [31], it has been reported that ROPI scavenges reactive oxygen species (ROS) [32]. Other dopamine receptor agonists such as pramipexole, rotigotine, bromocriptine, and pergolide do not have an oxindole skeleton. This structural difference may be involved in ROPI's ROS-suppressing, mitochondrion-protective, and therapeutic effect against ALS. ROPI shows a more potent protective effect on mitochondria in iPSC-derived ALS MNs than pramipexole or dexpramipexole [12], which suggests that ROPI is also a mitochondrion-targeted antioxidant. Moreover, its tertiary amine moiety exists as a lipophilic cation under physiological pH conditions [32], which allows ROPI to readily cross the cell membrane and localize to the strongly negatively charged mitochondrial inner membrane [33]. As is the case with other mitochondrion-targeted antioxidants, ROPI also readily localizes to mitochondria and is likely to scavenge ROS to protect mitochondria and prevent apoptosis (Figure 3). Collectively, the structural features of the oxindole skeleton and *N*,*N*-di-*n*-propylethylamine moiety appear to make ROPI an attractive candidate for use in ALS treatment.

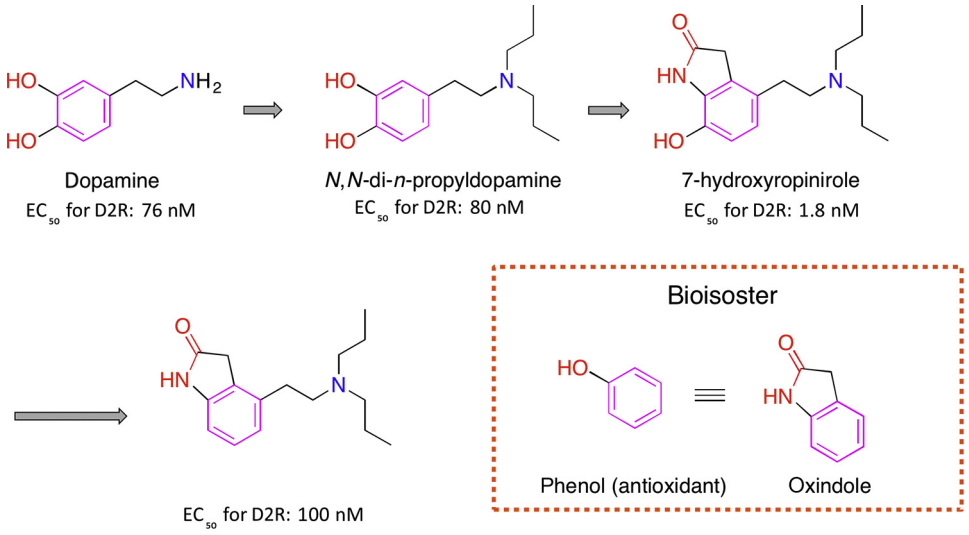

Figure 2. Structural Expansion from Dopamine to Ropinirole (ROPI). ROPI was developed by structural expansion from dopamine according to the concept of bioisosterism. The EC_{50} value of each compound was reported by Weinstock *et al.* [29] as the relaxation activity against an electrically stimulated rabbit ear artery. *N*,*N*-di-*n*-Propyldopamine is a lipophilic derivative of dopamine. By conversion of the monocyclic ring of *N*,*N*-di-*n*-propyldopamine to a bicyclic oxindole skeleton (bioisostere of phenol), 7-hydroxyropinirole was developed. Surprisingly, compared with that of *N*,*N*-di-*n*-propyldopamine, the EC_{50} of 7-hydroxyropinirole for the dopamine D2 receptor (D2R) was greatly improved. ROPI does not have a 7-hydroxy group and its EC_{50} for D2R is higher than that of *N*,*N*-di-*n*-propyldopamine; nevertheless ROPI was ultimately selected as a clinical drug candidate. Phenol is an antioxidant and its structural analog oxindole could also have antioxidant activity. It was reported that ROPI scavenged reactive oxygen species [27].

Retigabine

MN cell death in ALS is thought to be associated with enhanced excitability of the axonal membrane of the same MNs. Retigabine, a Kv7 channel activator, inhibits the hyperexcitability and improves MN survival *in vitro*, which provides a rationale for the use of this antiepileptic drug in a clinical trial in ALS [7].

Bosutinib

iPSC-derived MNs of ALS with *SOD1* mutation showed increases in p62 and the LC3-II:LC3-I ratio, accumulation of misfolded SOD1, and reduction of ATP production [8]. Bosutinib (an Src/c-Abl inhibitor) promoted autophagy, reduced the amount of misfolded SOD1 and TDP-43, and rescued ATP production. Furthermore, bosutinib enhanced the survival of iPSC-derived MNs of ALS with *SOD1* and *TDP-43* mutations and SALS. Additionally, treatment with bosutinib led to delay of onset and prolonged survival time in *SOD1*-mutant mice [8].

Pharmacokinetics (PK), Pharmacodynamics (PD), and Rationale for Use in ALS Patients

The achievable concentration of ROPI in the central nervous system (i.e., brain and spinal cord) is an important issue when ROPI is administered to ALS patients. The protective effects of ROPI against ALS-related MN damage were observed at concentrations of 0.1–10 μmol/l [12]. Such concentrations cannot be achieved in the brain and spinal cord with an oral intake of between 2 and 16 mg daily (permitted doses for Parkinson's disease in Japan) of the extended-release tablets ReQuip® CR [34,35]. When ReQuip® CR is repeatedly administered once daily, the concentration of ROPI in plasma rises gradually, reaches the peak concentration at approximately 6 h after the last administration, and then gradually decreases via excretion in urine [36]. When 2 and 16 mg of ReQuip® CR were administered, the trough concentrations of ROPI in plasma were

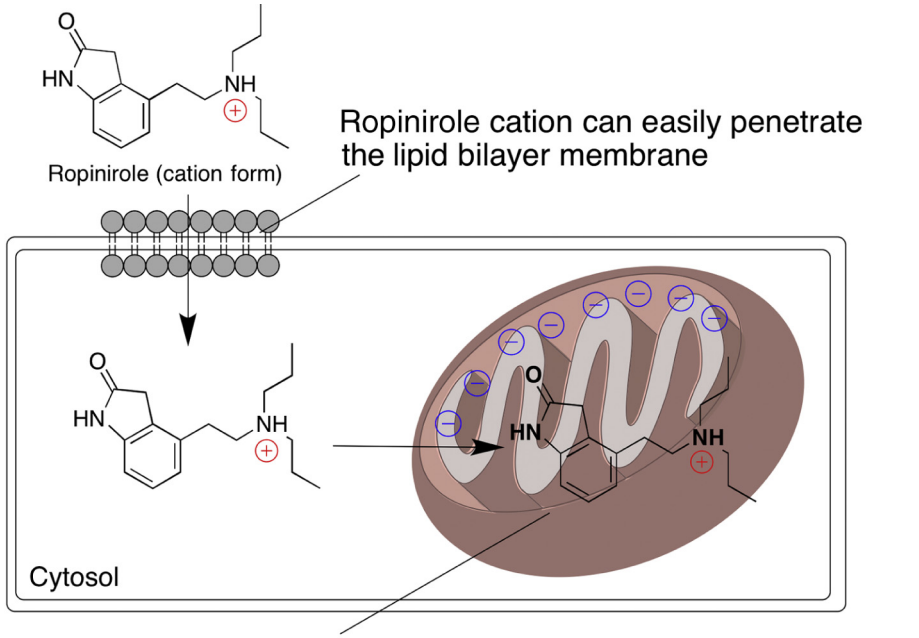

Figure 3. Schematic Model of the Accumulation of Ropinirole (ROPI) in Mitochondria. ROPI exists in the lipophilic cation form under physiological pH conditions due to its basic tertiary amine moiety. This allows it to easily penetrate into the cytosol and reach the mitochondrial inner membrane, which has a high negative charge. Since mitochondria are also a production source of reactive oxygen species, mitochondrion-targeted cationic molecules with antioxidant activity such as ROPI are most likely to be suitable mitochondrion-protective agents in amyotrophic lateral sclerosis (ALS). This phenomenon is a possible mechanism of the dopamine D2 receptor (D2R)-independent therapeutic effect of ROPI against sporadic amyotrophic lateral sclerosis (SALS).

1.81 ± 1.76 ng/ml and 15.14 ± 8.29 ng/ml, respectively [34]. Based on the formula weight of ROPI (296.84), the trough concentration in plasma ranges between 5 and 50 nmol/l. Importantly, ROPI in plasma rapidly crosses the blood–brain barrier (BBB). Overall, the minimal achievable concentration of ROPI in the brain and spinal cord is assumed to be between 5 and 50 nmol/l when patients with ALS receive 2–16 mg of ReQuip® CR daily.

In the current clinical trial (the ROPALS trial) [3], therefore, the initial dose of ReQuip® CR will be 2 mg and, provided no adverse effects are observed, it will be gradually increased by 2 mg/day weekly to a maximum dose of 16 mg/day. The highest tolerated dose of ROPI for each the patient will be continued.

Retigabine

POTIGA® (retigabine) tablets are a potassium channel opener indicated for adjunctive treatment of partial-onset seizures (not available in Japan). The optimized effective dosage is between 200 mg three times daily (600 mg per day) and 400 mg three times daily (1200 mg per day) [37].

In a clinical trial of retigabine (ezogabine) in ALS Subjects (clinicaltrials.gov identifier: NCT02450552) completed in August 2019, 600 or 900 mg per day of retigabine was administered. After both single and multiple oral doses, retigabine is rapidly absorbed, with median time to actual plasma

concentration (Tmax) values generally between 0.5 and 2 h. The precise concentrations in the serum of ALS patients have not been published. According to the previous publication [38], the maximum plasma concentration (Cmax) of retigabine was 604 ng/ml (271–997 ng/ml), when subjects received a single dose of 300 mg retigabine as encapsulated tablets or matching placebo capsules. Based on the molecular weight of ezogabine of 303.3, concentrations of 0.89–3.29 μmol/l were presumably obtained. Another report [7] showed a dose–response curve for retigabine on suppression of spontaneous action potentials in MEA recordings and the Hill plot fit of the mean data with an EC_{50} of 1.5 ± 0.8 μmol/l, suggesting that oral administration of retigabine 600 or 900 mg per day effectively inhibits motor neuronal excitability based on the high BBB permeability of retigabine, as an antiepileptic drug.

Bosutinib

BOSULIF® (bosutinib) exhibits dose-proportional increases in area under the curves (AUC) and Cmax over the oral dose range of 200–800 mg (0.33–1.3 times the maximum approved recommended dosage of 600 mg).

In a clinical trial using bosutinib in ALS subjects (UMIN000036295), the safety and tolerability of BOSULIF® (bosutinib) tablets (100 mg/day, 200 mg/day, 300 mg/day, or 400 mg/day) were evaluated to determine the maximum tolerated dose and a recommended Phase II dose of bosutinib for treatment of ALS patients. According to the data shown in an Interview Form [39], Cmax of 88.02 ± 23.05 ng/ml was obtained when 400 mg of bosutinib was administered to healthy adults. Based on a molecular weight of 548.46 for bosutinib, concentrations of 0.16 ± 0.04 μmol/l with Tmax of 6 h were reached. A previous report indicated that bosutinib inhibited motor neuronal death at concentrations of 0.1–10 μmol/l, suggesting the efficacy of oral administration of 400 mg of bosutinib [8]. Unfortunately, this drug does not seem to cross the BBB. In an experiment using ^{14}C-labeled compound, the autoradiogram exhibited no accumulation of bosutinib in the rat brain after a single administration of ^{14}C-labeled bosutinib. The detailed PK and PD will be evaluated in this clinical trial.

Summary of Ongoing Clinical Trial Protocols

Three clinical trials using pre-existing drugs (ROPI, retigabine, and bosutinib) identified through iPSC-based drug screening are ongoing (Table 1; [2,3,6]).

The ROPALS trial is a randomized, double-blind, placebo-controlled, single-center, and open-label continuation Phase I/IIa clinical trial for ALS using ROPI [3]. Subjects groups were allocated as follows: 15 patients for ReQuip® CR up to 16 mg/day and five patients for the matching placebo. This trial's objectives are to assess the safety, tolerability, and efficacy of ROPI for an initial 24 weeks (double-blind phase) and a subsequent 24 weeks (open-label continuation phase), as measured by delay in the progression of ALS, after oral treatment in ALS patients. As a feature of this trial, we planned the following exploratory endpoints to evaluate the utility of **iPSC-based drug discovery** and to identify novel biomarkers for the pathophysiology of ALS and the responder group (Figure 4, Key Figure): (i) comparison of *in vitro* drug effect evaluation and clinical effect using patient iPSC-derived LMNs (iPSC-based companion diagnostics); (ii) exploration of new biomarkers [neurofilament light (NfL) protein [40], TDP-43 protein [41], and exosomal miRNA [42] in plasma and cerebrospinal fluid (CSF)] for diagnosis, pathology, and drug effect evaluation in ALS; (iii) genome analysis of known FALS genes [43]; and (iv) implementation of the Zarit caregiver burden interview [44].

Key Figure

Using Induced Pluripotent Stem Cell (iPSC)-Based Assays to Explore and Predict Patient Response: The ROPALS Trial *In Vitro*

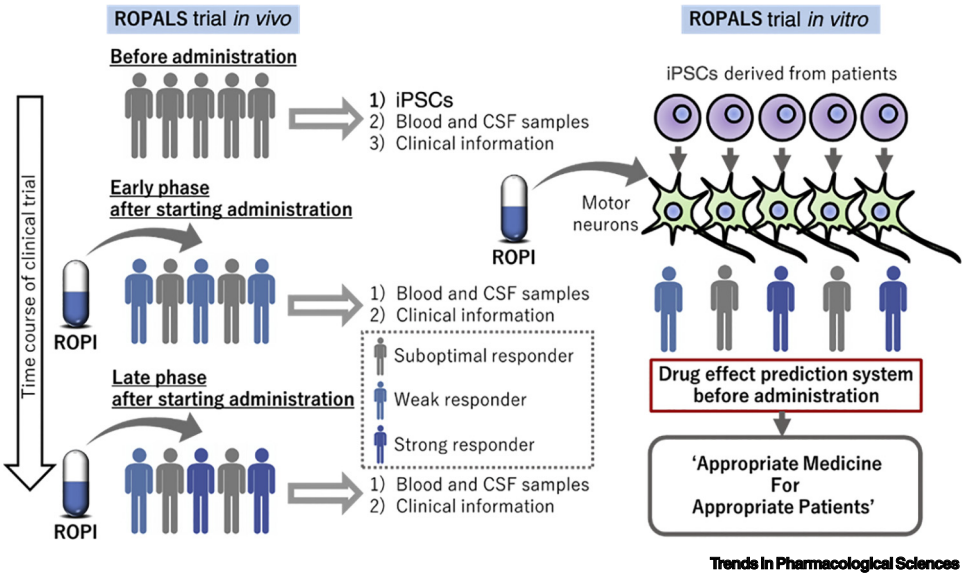

Figure 4. The ROPALS trial derived from iPSC-based drug discovery includes two trials; namely, a 'clinical trial *in vivo*' and a 'laboratory trial *in vitro*'. The *in vivo* trial will reveal whether ropinirole (ROPI) improves the phenotypes of sporadic and/or familial amyotrophic lateral sclerosis (ALS) patients clinically. In addition, sequential biological samples such as blood and cerebrospinal fluid (CSF) from the subjects will provide useful information on the effects of the investigational agent on disease mechanisms. The *in vitro* trial will aim to develop a drug effect prediction system before administration to discriminate the responder group and suboptimal responder group and to explore biomarkers that reflect the effect of ROPI on ALS and disease progression. As a result, we may be able to administer appropriate medicines to appropriate patients in the future.

Retigabine

Based on the findings described above, the ALS Association, Harvard University, and Massachusetts General Hospital collaborated with GSK and conducted a clinical trial for ALS using retigabine [A Phase II Pharmacodynamic Trial of Ezogabine (Retigabine) on Neuronal Excitability in Amyotrophic Lateral Sclerosis; clinicaltrials.gov identifier: NCT02450552) from 2015 to 2018]. Subjects groups were allocated as follows: 19 patients for ezogabine (retigabine) 900 mg/day, 23 patients for ezogabine (retigabine) 600 mg/day, and 23 patients for the matching placebo. The evaluation period for the primary endpoint is 8 weeks.

Bosutinib

A new Phase I clinical trial of the drug bosutinib for ALS [Phase I Dose Escalation Study of Bosutinib in Patients with Amyotrophic Lateral Sclerosis (ALS); unique ID issued by UMIN: UMIN000036295] was recently initiated at Kyoto University Hospital (KUH) in Japan on March 18 2019. The study comprises a 12-week observation period, a 1-week (5–9 days) transitional period, a 12-week study treatment period, and a 4-week follow-up period. Three to six ALS patients will be enrolled in each of the four bosutinib dose levels (100 mg/day, 200 mg/day, 300 mg/day, or 400 mg/day).

Outstanding Questions

What kind of phenotypic screening has been done to develop new therapies for ALS using an iPSC-based approach?

How do you evaluate three anti-ALS candidate drugs that were identified through drug screening using iPSC-derived cells by independent groups?

What are common features shared by major neurodegenerative diseases, such as Alzheimer's disease, Parkinson's disease, and ALS?

How can we develop new drugs that are broadly effective for various neurodegenerative diseases?

How do we maximize the advantages of iPSC technologies in drug discovery and personalized medicine?

Concluding Remarks and Future Perspectives

Through a series of investigations, we found that an iPSC-based approach to the identification of new therapies for ALS and modalities for monitoring and predicting response are effective once appropriate evaluation of *in vitro* systems of ALS-related phenotypes, such as stress granule formation, neurite retractions, hyperexcitability, and reduced autophagy, were established. Drug screening using iPSC-derived cells identified three anti-ALS candidate drugs with different mechanisms of action. In the future, it will be necessary to clarify whether these three drugs share common pathways for their mechanisms of action and how much responder groups overlap for each drug. For these purposes, it is important to subclassify ALS based on clinical features, biomarkers, and epigenomic information and to identify the most suitable drugs for each subclass.

Similar to ALS, other major neurodegenerative diseases, such as Alzheimer's disease and Parkinson's disease, occur in familial form in <10% of cases, with >90% occurring in sporadic form. Since the sporadic forms of these neurodegenerative diseases are highly variable due to complex genetics/low penetrance and the contribution of environmental and age-related epigenetic modifications, single drugs are unlikely to be effective across all sporadic cases of each disease. Clustering of sporadic forms and precision medicine approaches will thus be crucial. Notably, abnormal protein accumulation and distribution, reduced mitochondrial activity, enhanced inflammation by astrocytes/microglia, and impaired neuron–glia interactions are commonly observed in all of these neurodegenerative diseases. Thus, drugs that suppress such common phenotypes may be broadly effective as new drugs for neurodegenerative diseases (see Outstanding Questions).

To maximize the advantages of iPSC technologies in drug discovery and personalized medicine, we suggest 'iPSC-based companion diagnostics', in which the effect of a medicine can be estimated prior to administration through the use of patient-derived iPSCs, making it possible to deliver the appropriate medicine to responder patients. Additionally, iPSCs may be used as biomarkers of disease progression and the effects of a medicine and to stratify ALS patients with various phenotypes.

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Disclaimer Statement

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