**Protein kinase inhibitors for Amyotrophic Lateral Sclerosis therapy**

Valle Palomo,1,2 Vanesa Nozal,1 Elisa Rojas-Prats,1 Carmen Gil,1,2 Ana Martinez1,2,\*

1 Centro de Investigaciones Biológicas-CSIC, Ramiro de Maeztu 9, 28040 Madrid (Spain)

2 Centro de Investigación Biomédica en Red de Enfermedades Neurodegenerativas (CIBERNED), Instituto Carlos III, 28031 Madrid (Spain).

Running title:**Kinase inhibitors for ALS therapy**

Words: 8441

Figures: 4

References: 199

**Correspondence**

Prof Ana Martinez

e-mail: ana.martinez@csic.es

**ABSTRACT**

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disorder that causes the progressive loss of motoneurons, and unfortunately, there is no effective treatment to stop the disease. Multiple pathological mechanisms are interconnected in the neuropathology of this disorder, including abnormal aggregation of proteins, neuroinflammation and dysregulation of the ubiquitin proteasome system. Such complex mechanisms, together with the lack of reliable animal models of the disease, have hampered drug discovery in the last decades. Protein kinases, key pharmacological targets in several diseases, have been linked to ALS, as they play a central role in numerous of these pathological mechanisms. Therefore, several inhibitors are currently in their way to achieve a clinical proof of concept in ALS patients. In this review we recapitulate the protein kinase inhibitors currently in development for this disease together with their molecular targets and their involvement in the pathobiology of ALS.

(145 words)

**Introduction**

Protein kinase inhibitors represent the major family of drugs recently approved by the regulatory agencies such as FDA and EMA. Protein kinases, with critical roles in cellular signaling, have emerged as valuable pharmacological targets in the last decades and oncological pathologies have witnessed the intensive efforts from academic and industrial research to discover new therapies for patients (Cohen, 2002; Kannaiyan & Mahadevan, 2018). As protein kinases are involved in several cellular signaling processes, different therapeutic approaches have been pursued in the development of their inhibitors. Nowadays, small molecules, usually orally bio-available, and predominantly multi-targeting different tyrosine kinases and receptor tyrosine kinases have entered in clinical use. In fact, 52 protein kinase inhibitors have been approved up to now by the FDA (Roskoski, 2020). The great majority of these new drugs has been registered for cancer treatments, while only seven reached the market for the therapy of other diseases, usually with a chronic inflammatory component such as Chron’s disease or rheumatoid arthritis, among others (Simmons, 2013). Until now, none of these protein kinase inhibitors have been approved for the treatment of neurological and/or neurodegenerative diseases although there are several candidates in clinical and preclinical trials for these unmet disorders (Chico, Van Eldik et al., 2009; Cohen & Alessi, 2013). In this area, there are some initial technical challenges that must be overcome, including the crossing of the blood-brain barrier (BBB), the selectivity of the protein kinase inhibitors and the emergence of drug resistances. New innovative solutions using different approaches are now being found, such as allosteric or covalent modulation drug design, together with various strategies to overcome BBB. The main goal is to be able to finally validate protein kinase inhibitors as valuable drugs for central nervous system disorderss (Moura, Pacheco et al., 2020; Shi & Mader, 2018; Weisner, Gontla et al., 2015; Wu, Clausen et al., 2015).

Amyotrophic lateral sclerosis (ALS) is a devastating and fatal neurodegenerative disease with a current lack of effective treatments (Taylor, Brown et al., 2016). However, recent advances in its molecular pathobiology have provided new insights in potential drug targets for effective drug design and discovery (Liscic, 2017). Etiology of ALS is mixed between genetic and environmental factors, being 90% of the patients of sporadic origin. However, several genes have been found in the familial form of the disease that accounts for 5-10% of the overall cases. Of the more than 25 genes discovered, four of them account for 70% of the familial cases of the disease, being C9orf72, TARDBP, SOD1 and FUS (Hardiman, Al-Chalabi et al., 2017). These genes have helped in the understanding of some pathological mechanisms and in the development of animal models of disease (Mathis, Goizet et al., 2019). However, they do not fully represent the human pathology and the validation of animal models for ALS disease has encountered many difficulties.

Although the underlying mechanisms of ALS are not well understood, there are several neuropathological hallmarks that are characteristic of the disorder. The most representative one is the presence of inclusions of aggregated proteins in the cytoplasm of motoneurons, mainly formed by the nuclear TAR DNA binding protein of 43 KD (TDP-43), both in familial and sporadic cases (Neumann, Sampathu et al., 2006). This protein is encoded by the *TARDBP* gene and regulates several mRNA processes, such as mRNA transport and transcription. In more than 97% of ALS patients, TDP-43 shows an imbalance in motoneurons, in which a decrease of nuclear TDP-43 is observed together with the accumulation of cytoplasmic aggregates with multiple posttranslational modifications, including hyperphosphorylation, truncation and ubiquitination (Palomo, Tosat-Bitrian et al., 2019). Other relevant mechanisms that have been described for ALS etiology include a dysregulation in autophagy and ubiquitin proteasome system, neuroinflammation, excitotoxicity, oxidative stress, alteration of RNA metabolism, nuclear and vesicle transport, and mitochondrial dysfunction.

It is currently believed that the disease is a result of several interacting pathological processes which are difficult to separate (Hardiman et al., 2017). The heterogeneity found from patient to patient, and the lack of validated animal models makes research in drug development extremely challenging since deciphering the contribution of each pathological mechanism to the final motoneuron death is very complex. With this landscape, protein kinases are emerging as relevant therapeutic options for pharmacological intervention in ALS (Guo, Vandoorne et al., 2020), and currently there are several drug candidates in advanced phases of clinical trials (Martinez, Palomo Ruiz et al., 2017). Furthermore, multiple candidates are being evaluated in preclinical or early research projects representing a ray of hope for patients, families and society. In the present review, we have collected small molecules targeting protein kinases that are in the development or discovery phase to be disease-modifying agents with a future real impact on ALS pathology.

**Protein kinase inhibitors in clinical trials for ALS**

Several compounds with different protein kinase inhibition properties are now in clinical trials. They have reached this advanced stage of development mainly through a repurposing strategy, taking into account the recent advances on the molecular pathobiology of ALS. The preclinical models currently used to advance compounds to clinical phases are very diverse, being one of the main obstacles for ALS drug development: the lack of validated animal and cellular models of disease that mimic the human pathology.

*Masitinib (c-KIT receptor inhibitor)*

Masitinib (AB1010) was described as an oral, novel phenylaminothiazole-type tyrosine kinase inhibitor targeting c-KIT receptor with improved selectivity with respect to other tyrosine kinase inhibitors and subsequently, less adverse effects (Figure 1) (Dubreuil, Letard et al., 2009). The first indication of masitinib was for the treatment of canine mast cell tumors because of the activation of the c-KIT receptor in this kind of skin tumors (Hahn, Ogilvie et al., 2008). The c-KIT receptor in humans has mainly been related to cancer and inflammatory processes (Reber, Da Silva et al., 2006; Stankov, Popovic et al., 2014). In fact, a number of clinical trials to evaluate the efficacy of masitinib in various advanced or metastatic solid human cancers are ongoing (Marech, Patruno et al., 2014). With regard to non-oncologic indications, the ability of this enzyme to modulate inflammatory and immunological response has motivated the investigation of employing masitinib for the treatment of some neurological diseases (Gagalo, Rusiecka et al., 2015). In the case of ALS, masitinib has demonstrated promising preclinical activity in the SOD1G93A mutant rat model after oral administration (30 mg/kg/day) starting after paralysis onset and during 20 days. The observed control of microgliosis and subsequently, the neuroinflammation, together with the significantly prolonged survival of the animals, supported the evaluation of its efficacy in ALS patients (Trias, Ibarburu et al., 2016). Moreover, it has been reported that the pharmacological action of masitinib in mast cells and neutrophils may be involved in preventing motoneuron degeneration, contributing to the potential beneficial effect in ALS (Trias, Ibarburu et al., 2017; Trias, King et al., 2018).

Thus, considering the efficacy in the SOD1 model and the good safety profile showed in clinical trials for different human diseases (Marech et al., 2014; Piette, Belmin et al., 2011; Vermersch, Benrabah et al., 2012), masitinib was selected to carry out a controlled phase II/III trial with 394 ALS patients (ClinicalTrials.gov Identifier: NCT02588677) (Mora, Genge et al., 2020). The trial was multicentre, randomised and the patients received riluzole (100 mg/day) plus placebo or masitinib at 4.5 or 3.0 mg/kg/day during 48 weeks. The primary endpoint was to measure changes in patients’ scores on the ALS functional rating scale-revised (ALSFRS-R) at week 48.

Masitinib was well tolerated without any severe effects, as expected due to the known risk profile. Remarkably, it showed a significant benefit in ALSFRS-R over placebo, suggesting a possible benefit from receiving a higher masitinib dose. All these data support a confirmatory phase III study to confirm these findings (ClinicalTrials.gov Identifier: NCT03127267) sponsored by AB Science.

*Fasudil (ROCK inhibitor)*

Rho-associated coiled-coil-containing protein kinase (ROCK) is a Ser/Thr protein kinase that has shown an important potential as therapeutic target for different diseases (Feng, LoGrasso et al., 2016). In fact, two isoquinoline ROCK inhibitors, fasudil and ripasudil are approved for clinical use. Fasudil (Figure 1) is approved in China and Japan for the treatment of cerebral vasospasm, while ripasudil is approved in Japan for the treatment of glaucoma (Honjo & Tanihara, 2018).

Fasudil has been broadly studied in different models of neurodegenerative diseases showing great potential for clinical translation (Bowerman, Murray et al., 2012; Li, Yasumura et al., 2013; Tatenhorst, Eckermann et al., 2016; Tönges, Frank et al., 2012), due to the association of ROCK with the pathogenesis of neuronal damage (Mueller, Mack et al., 2005). Safety data of the drug licensed for clinical use in 1995 shows good tolerability after intravenous administration. Moreover, it has also been administered orally in different clinical trials (Koch, Tatenhorst et al., 2018). Of interest for the treatment of neurodegenerative diseases is its good blood-brain barrier penetration (Taniguchi, Seki et al., 2014).

With regard to the potential of fasudil for the treatment of ALS, it has been tested in different *in vitro* and *in vivo* models of the disease. *In vitro*, it was able to protect motoneuron (NSC34) cells of SOD1G93A induced neurotoxicity (Takata, Tanaka et al., 2013) and primary mice motoneurons damaged with kainic acid (Tönges, Günther et al., 2014). *In vivo*, the results from two different research groups were also convergent. One group administered fasudil at 30 and 100 mg/kg dissolved in drinking water to SOD1G93A mice during 150 days. In these animals, fasudil slowed disease progression and increased animal survival at both doses, protecting motoneurons against degeneration (Takata et al., 2013). Other group administered fasudil to the same mouse model both at a presymptomatic disease stage (Tönges et al., 2014) and after disease onset, showing therapeutic potential as symptomatic treatment (Günther, Balck et al., 2017). The observed effects in the animals were improved motor function, extended survival and protection of spinal cord motoneurons. Fasudil was more effective in the animals treated in the asymptomatic phase, although it is of utmost importance that therapeutic benefits were also observed in animals treated after disease onset (Günther et al., 2017). Remarkably, the beneficial effects of the drug are mediated not only by its direct action on motoneurons, but also through regulation of the surrounding astroglia and microglia (Tönges et al., 2014).

Because of the beneficial effects of fasudil in cell culture and animal models of ALS, a clinical phase IIa trial named ROCK-ALS is currently ongoing (ClinicalTrials.gov Identifier: NCT03792490) (Lingor, Weber et al., 2019). The trial is multicentre, double-blind, randomized and placebo controlled. 120 patients in early-stage ALS will receive fasudil at 30 or 60 mg/kg/day or placebo in two intravenous administrations during a total of 20 days. Patients included in the study will be followed up for 6 months after treatment. Population taking either riluzole or edaravone will be considered in the study. Primary endpoints of the ROCK-ALS trial are safety and tolerability and secondary, efficacy. If successful, this trial will yield important data with regard to this novel therapeutic option for ALS and pave the way to a future IIb trial to explore safety, tolerability and efficacy after oral administration.

*Rapamycin (mTOR inhibitor)*

mTOR is a family of Ser/Thr protein kinases called mammalian target of rapamycin with a known role in regulating cell growth as well as lipid and glucose metabolism (Yang, Rudge et al., 2013). The main indications of mTOR inhibitors are related to their anticancer and immunosuppressant effects, being rapamycin in clinical use to prevent solid organ transplant rejection. Additionally, important neuroprotective properties have been described for this kind of inhibitors in different neurodegenerative diseases, including ALS (Lipton & Sahin, 2014; Pignataro, Capone et al., 2011).

mTOR enzyme has two functionally different protein complexes, mTORC1 and mTORC2. Rapamycin (Figure 1) targets mTORC1 selectively as an allosteric inhibitor (Mukhopadhyay, Frias et al., 2016), modulating mechanisms that play an important role in ALS, including autophagy and neuroinflammation. Autophagy is particularly important due to the required degradation of misfolded protein aggregates associated with ALS pathology. Rapamycin induces autophagy and has been reported to decrease TDP-43 cytoplasmatic accumulation in N2A and SH-SY5Y cell lines (Caccamo, Majumder et al., 2009) and FUS-positive stress granules in neurons expressing FUS under oxidative stress (Ryu, Jun et al., 2014). This compound has also shown pathology amelioration in TDP-43 related animal models. On one hand, it rescued and alleviated memory deficits of a mouse model with TDP-43 proteinopathy due to the autophagy activation (Wang, Guo et al., 2012). On the other, it increased the diminished lifespan and locomotive defects of *Drosophila* with transgenic expression of TDP-43 (Cheng, Lin et al., 2015). By contrast, rapamycin has not shown any beneficial effect in the widely used SOD1 model (Zhang, Li et al., 2011). However, since SOD1 mutations are present in a reduced number of cases while the vast majority of ALS patients present TDP-43 inclusions (Mackenzie, Bigio et al., 2007), rapamycin was nevertheless considered as a promising drug for the treatment of neurodegenerative diseases with TDP-43 proteinopathies, including ALS. Moreover, ALS patient iPSC-derived astrocytes have been described to mediate an imbalance in the autophagy pathway, which may result in the accumulation of ALS-related proteins responsible of pathological effects (Madill, McDonagh et al., 2017).

Based on these evidences, a clinical trial II named RAP-ALS with 63 ALS patients in Italy (Eudra CT Identifier: 2016-002399-28) has started (Mandrioli, D'Amico et al., 2018). The trial is multicentre, randomized, double-blind and placebo controlled. The patients (sporadic and familial excluding those with SOD1 mutations) will receive a daily oral dose of rapamycin at 1 mg/m2 or 2 mg/m2 during 18 weeks, being followed up for further 36 weeks. Patients included in the study must have been treated during 1 month at least with 100 mg/day of riluzole. Primary endpoint of this clinical trial will be the change from baseline to week 18 in regulatory T lymphocytes number in treated patients with respect to the ones receiving placebo. Secondary endpoints include safety and tolerability, required dose to show levels in cerebrospinal fluid, changes in immunological and inflammatory markers, clinical score and quality of life according to well-known scales.

*Bosutinib (Src/c-Abl inhibitor)*

The discovery of the importance of the Src/c-Abl pathway in ALS was reported after a phenotypic screening of 1416 compounds carried out to repurpose drugs for ALS. Compounds were tested in iPSCs motoneurons derived from ALS patients with SOD1 mutation. 27 hits emerged from this study as capable to increase survival of motoneurons. Remarkably, 14 of these hits targeted the Src/c-Abl pathway. The relationship between this pathway and the observed effect was confirmed after Src/c-Abl knockdown with siRNA. Among the Src/c-Abl inhibitors tested, bosutinib (Figure 1) was selected for advanced studies due to its additional effects such as promotion of autophagy, reduction of misfolded SOD1 protein and restoration of energy homeostasis in ALS motoneurons. Bosutinib also promoted survival in iPSCs-derived motoneurons from ALS patients with *TDP-43* or *C9orf72* mutations, and in SOD1G93A mice after i. p. administration of 5 mg/kg five times per week for 5 weeks (Imamura, Izumi et al., 2017).

The studies of bosutinib *in vitro* and *in vivo* described above (Imamura et al., 2017) support the potential use of Src/c-Abl inhibitors as new drugs for the treatment of ALS (Riancho, Gil-Bea et al., 2018). Bosutinib is a potent Src/c-Abl inhibitor (Remsing Rix, Rix et al., 2009) approved by the FDA in 2012 for the treatment of chronic myeloid leukemia. However, this drug showed manageable adverse effects that must be taken in consideration in future clinical trials (Cortes, Apperley et al., 2018). Bosutinib is orally bioavailable and blood-brain barrier permeable. Based on these facts, a clinical trial II dose escalation study of bosutinib for ALS patients (iDReAM) has been approved by the Japanese Pharmaceuticals and Medical Devices Agency (Trial registration number UMIN000036295) (Imamura, Izumi et al., 2019). The number of patients included in the study will be limited because, although bosutinib is already in clinical use, as mentioned before, it is necessary to evaluate safety and tolerability in ALS patients. Furthermore, the study design combines the safety study of oncology and the efficacy study of neurology. Groups of 3-6 ALS patients will receive bosutinib for 12 weeks in four dose ranges (100, 200, 300 or 400 mg/day). An internal committee of oncologists, hematologists and neurologists will determine dose escalation effects and maximum tolerated doses. The primary endpoint is the dose-limiting toxicity after the first 4 weeks of treatment and during the whole treatment period. Moreover, ALSFRS-S scores will allow a preliminary evaluation of the efficacy of bosutinib in ALS patients. The results from this exploration study will lead to a future phase II trial to evaluate the efficacy of the resulting recommended dose.

*Lithium (GSK-3 inhibitor)*

Glycogen synthase kinase 3 (GSK-3) is a Ser/Thr kinase involved in numerous biological pathways including cancer, inflammation and neurodegenerative diseases.

In ALS patients, an up-regulation of GSK-3 in spinal cord and frontal and temporal cortex has been described (Hu, Zhang et al., 2003; Yang, Leystra-Lantz et al., 2008). Furthermore, GSK-3 is also expressed in peripheral blood mononuclear cells of patients with ALS and these levels of active kinase are directly related to clinical symptoms (González-Muñoz, I. Rodríguez-Mahillo et al., 2013). Thus, inhibitors of GSK-3 have been proposed as a new therapy for ALS in the last decade (Palomo, Perez et al., 2011).

Through different studies, it has been shown how small molecule GSK-3 inhibitors were able to slow disease progression in different animal models of ALS, including the well-known SOD1G93A and the L-BMAA excitotoxicity model (Ahn, Kim et al., 2012; de Munck, Palomo et al., 2016; Koh, Kim et al., 2007). A more recent study, has shown how GSK-3 is required for the motoneuron degeneration produced by TDP-43, establishing a further link between these two targets (Sreedharan, Neukomm et al., 2015).

Lithium is the only clinically approved direct GSK-3 inhibitor, currently used to treat bipolar disorder. Lithium inhibits GSK-3 directly with an IC50 of 2.0 mM, and indirectly through the Akt pathway (Freland & Beaulieu, 2012). The efficacy of lithium in ALS mouse models has been controversial, as some studies showed no clinical benefits in SOD1 mice (Pizzasegola, Caron et al., 2009), while others did find neuroprotection effects associated with an increase of mice lifespan and a delayed disease onset (Fornai, Longone et al., 2008). In this sense, clinical studies have also been controversial.

A 15-month randomized clinical study with lithium was performed in 44 sporadic ALS patients, in which the treated group received two daily doses of 150 mg of lithium carbonate plus riluzole. The primary endpoint of the study was survival and the secondary was measurement changes in ALSFRS and Norris ALS scales. The study showed how the lithium treated group presented an increase in the survival rate, and the outcomes based on ALS scales revealed improved benefits for the treated group (ClinicalTrials.gov Identifier: NCT00790582)(Fornai et al., 2008; Vanacore & Galeotti, 2008). However, subsequent clinical trials did not find any evidence of clinical benefit in the group treated with lithium (ClinicalTrials.gov Identifier: NCT00818389; Eudra CT Identifier: 2008-006891-31, 2008-002110-22) (Aggarwal, Zinman et al., 2010; U KMND-LiCALS Study Group, Morrison et al., 2013; Verstraete, Veldink et al., 2012).

In other preclinical studies, it was shown that lithium in combination with valproic acid, an histone deacetylase inhibitor, that also inhibits GSK-3 but indirectly (Hall, Brennan et al., 2002), showed clinical benefits in the SOD1 mice (Feng, Leng et al., 2008). Motivated by these results there is an ongoing phase II clinical trial that aims to find whether a combination of lithium and valproic acid would show clinical benefits in ALS patients (ClinicalTrials.gov Identifier: NCT03204500). The study is composed by 40 patients, and the treatment group will receive 600 mg of valproate and 600 mg of lithium carbonate per day during 21 months. The primary endpoint will be disease progression based on the ALSFRS score, and the secondary outcomes will measure change in score on ALSAQ scale and biomarkers from basal conditions. However neither updates nor results of this clinical trial have been published so far. Treatment only with valproic acid had already proven a decrease in disease progression in SOD1 mutant mice (Sugai, Yamamoto et al., 2004), however it did not show any clinical benefit in a previous clinical trial with ALS patients (ClinicalTrials.gov Identifier: NCT00136110) (Piepers, Veldink et al., 2009). This clinical study of phase III finished in 2007 and included 163 ALS patients, where the treated group received 1500 mg of valproate, and the primary endpoint was survival.

As several GSK-3 inhibitors have entered in clinical trials for Alzheimer’s disease (AD), in which its upregulated expression and exacerbated phosphorylation of tau validated this kinase as a potential target for this pathology, repurposing these compounds for ALS therapy may be a good strategy to follow in the near future. Such is the case of tideglusib, that has showed good safety and tolerability in AD patients (Lovestone, Boada et al., 2015), plus a significant decrease of BACE1 in cerebrospinal fluid (CSF) in a small subgroup of patients and significant responses on cognitive tests in a group of patients with mild AD, being a good candidate for repurposing (Martinez-Gonzalez, Porras et al., 2019).

*GDC-0134 (DLK inhibitor)*

Dual leucine zipper kinase (DLK, MAP3K12), highly expressed in neuronal cells, plays a key role in the progressive neurodegeneration that occurs in different severe and unmet diseases such as ALS and AD (Villanueva, 2017). DLK acts upstream of stress-responsive c-Jun N-terminal kinase (JNK) being a key player in neuronal response to different acute and traumatic injuries (Jin & Zheng, 2019). Thus, inhibitors of DLK represent a good opportunity to alleviate neurodegeneration (Siu, Sengupta Ghosh et al., 2018). Several pharmacological strategies have been used to discover and design DLK inhibitors to treat neurodegenerative diseases such as scaffold-hopping (Patel, Harris et al., 2015), e-pharmacophore screening (Langeswaran, Jeyaraman et al., 2019), and structure-based drug discovery (Patel, Cohen et al., 2015) resulting in different heterocyclic scaffolds with privileged DLK inhibitory activity (Oetjen & Lemcke, 2016). DLK is required for the initiation of JNK signaling, and has been found to be upregulated in mouse models of ALS (SOD1G93A and TDP-43A315T) as well as in lumbar spinal cord lysates from patients with sporadic ALS (Le Pichon, Meilandt et al., 2017). Indeed, pharmacological inhibition of DLK with two different compounds, GNE-8505 and GNE-3511, showed efficacy in the SOD1G93A model of ALS. They reduced the activation of JNK and after the administration of GNE-3511 in food for 5 weeks, the neuromuscular junction denervation was reduced by ~10% compared with vehicle-treated mice. These data moved Genentech to advance a DLK inhibitor (GDC-0134, previously known as RG6000) (structure not disclosed) into clinical development for the treatment of ALS. The phase I clinical trial to explore safety, tolerability, and pharmacokinetic (PK) properties of this first-in-man drug was launched in 2017 (ClinicalTrials.gov Identifier: NCT02655614), being data completion forecast for the summer of 2020.

*DNL747 (RIPK1 inhibitor)*

Receptor‐interacting Ser/Thr protein kinase 1 (RIPK1) regulates inflammation and necroptotic cell death (Yuan, Amin et al., 2019). RIPK1 inhibitors have been developed as potential treatment for chronic inflammatory diseases (Harris, Berger et al., 2017) and the peripheral drug candidate GSK2982772 has completed a phase II clinical trial for psoriasis (Weisel, Berger et al., 2020). More recently, evidence from human genetic analysis has linked the dysregulation of RIPK1 to the pathogenesis of ALS. Mutations in TANK-binding kinase 1 (TBK1) leads to a partial loss of function being one of the causes of familial ALS (Cui, Tuo et al., 2018). It has been shown that TBK1 acts as an endogenous RIPK1 inhibitor being the embryonic lethality of knock-out *TBK1* mice dependent on RIPK1 kinase activity. Furthermore, TGF-β-activated kinase 1 (TAK1), with a marked decreased expression on human aging brains, acts also as RIPK1 inhibitor, reducing its myeloid expression in *TBK1-*heterozygotesknock-out mice. In that transgenic animal the observed phenotype is compatible with ALS main hallmarks such as neuroinflammation, TDP-43 aggregation, axonal degeneration and neuronal loss among others (Xu, Jin et al., 2018). These findings may explain that age-dependent reduction of RIPK1 inhibition in brain predisposes to neuroinflammation and neurodegeneration in carriers of TBK1 mutations. Furthermore, axonal degeneration is linked to an increased function of RIPK1, which enhances the potential of RIPK1 inhibitors for the treatment of ALS and neurodegenerative disorders (Ito, Ofengeim et al., 2016).

DNL104, a brain penetrant RIPK1 inhibitor, was tested in 68 healthy volunteers being safe and well-tolerated. DNL104 leads to RIPK1 kinase inhibition measured in peripheral blood mononuclear cells, and this is not associated with central nervous system (CNS) toxicities, supporting the future development of CNS penetrant RIPK1 inhibitors (Grievink, Heuberger et al., 2020). However, Denali discontinued the Phase I study of DNL104, because of liver toxicity and they have moved to the design of new selective and brain high-permeable compounds being the clinical development of DNL747 (structure not disclosed) currently ongoing (ClinicalTrials.gov Identifier: NCT03757351) with results expected by the spring of 2021.

The increase in the knowledge of RIPK1 structure and regulation mechanisms enables the possibility to develop highly specific new RIPK1 inhibitors (Degterev, Ofengeim et al., 2019). If the physicochemical properties of these compounds are also optimized to obtain high brain permeable compounds, a good opportunity for RIPK1 inhibitors will be open for the treatment of ALS and other diseases of the central nervous system.



**Figure 1.** Chemical structures of protein kinase inhibitors currently in clinical trials for ALS. The structures of GDC-0134 and DNL747 (DLK and RIPK1 inhibitors, respectively) are undisclosed.

**Protein kinase inhibitors in preclinical development for ALS**

In this section, those kinase inhibitors that have been advanced to *in vivo* pharmacology for future ALS therapy have been included.

*Casein kinase 1 (CK-1) inhibitors*

CK-1 became involved in ALS in 2008 when it was discovered to be responsible for direct TDP-43 phosphorylation (Hasegawa, Arai et al., 2008). CK-1 is a Ser/Thr kinase ubiquitously expressed in humans. There have been described 7 different isoforms (α, β, γ1 − 3, δ, and ε), that show a high homology, especially at the kinase domain and with more variation at the C-terminal, which regulates kinase activity and is key in substrate recognition (Schittek & Sinnberg, 2014). In 2009 it was shown that CK-1δ phosphorylated TDP-43 in various sites (Kametani, Nonaka et al., 2009). This isoform together with CK-1ε had previously been associated with the regulation of circadian rhythm in eukaryotic cells (Vielhaber, Eide et al., 2000). Since its involvement with TDP-43 phosphorylation, there has been an effort to validate CK-1 inhibitors for the treatment of ALS. Benzothiazole-based small molecules are potent and selective CK-1 inhibitors, having found promising results in reduction of TDP-43 phosphorylation in human neuroblastoma cell line and in hTDP-43 transgenic *Drosophila* (Salado, Redondo et al., 2014). More recently, compounds IGS2.7 and IGS2.37 (Figure 2) have shown their ability to decrease TDP-43 phosphorylation in human cellular models derived from frontotemporal lobar degeneration (FTLD) patients (Alquezar, Salado et al., 2016) and ALS patients (Posa, Martinez-Gonzalez et al., 2019). Furthermore, the good pharmacokinetic profile of compound IGS2.7 motivated its study in the transgenic TDP-43 mice, showing motoneuron preservation, decrease of microglia activation and TDP-43 phosphorylation in spinal cord (Martinez-Gonzalez, Rodriguez-Cueto et al., 2020). Up to date these inhibitors are still in preclinical trials for ALS, however Pfizer completed two clinical phase I studies with a CK-1δ/ε inhibitor, PF-05251749 (ClinicalTrials.gov Identifier: NCT02443740, NCT02691702) that is being developed for irregular sleep-awake disorders in Parkinson’s patients, showing good safety and tolerability, validating the safety of these therapeutic targets at a clinical level. Lastly, it was shown in 2018 that riluzole, one of the approved ALS therapeutics, inhibits CK-1δ with an IC50 of 16.1 µM (Bissaro, Federico et al., 2018). However, it still remains to be demonstrated if this *in vitro* inhibitory activity is enough to exert CK-1 engagement *in vivo* since last year it was published that riluzole was unable to modify the disease in the TDP-43 mouse model of ALS (Chen, Liao et al., 2020).

*Cell division cycle 7 (CDC7)* *inhibitors*

CDC7 is a Ser/Thr kinase highly conserved among eukaryotic cells, from yeast to human (Jiang & Hunter, 1997). It is mainly located in the nucleus of the cell and plays an essential role in the initiation of DNA replication and cell cycle progression (Kim, Yamada et al., 2003). Due to its pivotal function in the cell division cycle, it has been associated with many types of cancer, becoming an attractive target for the treatment of these pathologies (Montagnoli, Moll et al., 2010). In 2013, an homolog of the CDC7 protein in *C. elegans* was identified being implicated in the pathological phosphorylation of TDP-43 *in vivo* (Neumann, Kwong et al., 2009). Furthermore, they showed that the inhibition of this kinase with a previously described ATP-competitive inhibitor known as PHA-767491 (Figure 2) prevents the neurodegeneration driven by TDP-43 hyperphosphorylation (Liachko, McMillan et al., 2013). Among the different CDC7 inhibitors analysed for the treatment of primary or solid tumors found in the literature, only PHA-767491 has shown good results in preventing pathological TDP-43 phosphorylation and neurodegeneration. However, this CDC7 inhibitor presents low blood-brain barrier permeability, so it cannot be employed for the treatment of neurodegenerative diseases (Vanotti, Amici et al., 2008). Recently, a new family of CDC7 kinase inhibitors based in the purine scaffold have been designed and synthesized as potential candidates for the treatment of ALS (Martinez, Perez et al., 2018). These inhibitors are ATP-competitive, selective among different kinases and predicted to be permeable to the CNS according to the *in vitro* PAMPA assay. Moreover, the best compounds of this purine family were selected for further studies *in vitro* and showed that they were able to recover TDP-43 homeostasis (decreasing TDP-43 phosphorylation and recovering nuclear localization) in lymphoblasts from ALS and FTLD patients (Martinez, Rojas et al., 2017). Finally, these inhibitors, including ERP2.37 (Figure 2) were tested in different *in vivo* models, such *C. elegans* and TDP-43 transgenic mice, confirming the same behavior (Martinez-Gonzalez et al., 2019). For these reasons, the inhibition of CDC7 by small molecules could be a novel strategy for the treatment of ALS.

*Apoptosis signal-regulating kinase 1 (ASK1, MAP3K5) inhibitors*

ASK1 is a mitogen-activated protein kinase kinase kinase (MAP3K) that plays a central role in the cellular stress response by modulating inflammation and apoptosis. Experimental evidences that link ASK1 signaling with the pathogenesis of several neurodegenerative diseases have been reviewed elsewhere (Guo, Namekata et al., 2017). With regard to ALS, it was reported that ASK1 activation induced motoneuron death caused by mutant SOD1 protein. This fact was demonstrated on the basis of the observation that *ASK1* deficiency mitigates motoneuron loss and extends the life span in SOD1G93A transgenic mice (Nishitoh, Kadowaki et al., 2008). Due to these results, two specific ASK1 inhibitors, K811 and K812 (Figure 2) (Hidenori Ichijo, Nakagawa et al., 2012), were tested in the SOD1G93A mouse model of ALS. Both compounds were administered orally starting at disease onset (week 28) and during 100 days. The selected doses, 100 mg/kg/day for K811 and 30 mg/kg/day for K812, were chosen based on previous *in vivo* pharmacokinetic studies with both inhibitors. As a result of this long-term treatment, the survival of the animals increased in both cases and the motoneuron death in the spinal cord of the animals was decreased (Fujisawa, Takahashi et al., 2016).

Despite this data, the hypothesis that modulation of the ASK1 pathway might be beneficial for the treatment of neurological diseases has not yet been tested in the clinic, and the main focus has been centered on peripheral human diseases. Specifically, the ASK1 inhibitor Selonsertib (GS-4997), developed by Gilead Sciences, is being tested in phase II clinical trials for the treatment of liver fibrosis.

*p38 kinase inhibitors*

The p38 family of kinases comprises several homologues: p38α (MAPK14), p38β (MAPK11), p38γ [SAPK (stress-activated protein kinase) 3, ERK (extracellular-signal-regulated kinase) 6 or MAPK12] and p38δ (SAPK4 or MAPK13). They possess a 60% of identity in their sequences and different distribution in the body: p38α (ubiquitous), p38β (brain), p38γ (skeletal muscle), p38δ (endocrine glands) (Cuadrado & Nebreda, 2010). Their physiological role is highly complex as they are implicated in a vast number of cellular processes.

In ALS, p38 is closely related with neuroinflammation, secretion of neurotoxic factors and FUS, one of the proteins that may produce familial ALS when mutated. The toxic effects of FUS and SOD1 in motoneurons are mediated by p38 (Sama, Fallini et al., 2017), when translocated to the cytoplasm. Accumulation of p38 in the spinal motoneurons of transgenic SOD1 mice produced neurotoxic effects due to the appearance of reactive glial cells, increasing the production of nitric oxide and formation of peroxynitrite. Moreover, activation of p38 in brain has other deleterious effects such as the hyperphosphorylation of neurofillaments in mice (Ackerley, Grierson et al., 2004) and the activation of the apoptotic cell death cascade by caspases (Torcia, De Chiara et al., 2001).

Treatment of SODG39A animal model with the small molecule SB-239063 an inhibitor of p38α (Figure 2) reverts the axonal transport deficits associated with this model (Gibbs, Kalmar et al., 2018). The vast number of clinical trials involving p38 kinase inhibition in other different pathologies gives a hope to modulation of this protein kinase in this devastating disease.

*Protein tyrosine kinase 2 (PTK2) inhibitors*

PTK2, also known as focal adhesion kinase (FAK), is a well-known kinase involved in multiple advanced-stage solid cancers (Sulzmaier, Jean et al., 2014) but its role in ALS has recently been unveiled. Overexpression of TDP-43 in mouse cellular models impairs the activity of the ubiquitin-proteasome system triggering the accumulation of poly-ubiquitinated toxic proteins (Lee, Jeon et al., 2019). This effect can be reverted by inhibiting PTK2 either with a small molecule, PF573228 (Figure 2) or with siRNA. PTK2 inhibition reduced cytoplasmic TDP-43 levels and eliminated as well the TDP-43-induced cellular death in N2a cells. The pharmacological effect was further confirmed in a *Drosophila* model that expresses human *TARDBP* and *ATXN2-32Q* in the nervous system (Hart & Gitler, 2012). When PTK2 activity was reduced after the treatment with the inhibitor PF573228 a decrease of polyubiquitinated aggregates in their brains and a moderate reversion of phenotypic deficits were observed.

When PTK2 is activated, the kinase TBK1 phosphorylates SQSTM1 at Ser403. This event reduces the ability of SQSTM1 to eliminate the poly-ubiquitinated proteins by autophagy clearance creating a toxic microenvironment for the neurons and thus cell death (Lee et al., 2019). This demonstration of the toxic gain of function of TBK1 is in contrast with other genetic studies that link haploinsufficiency of TBK1 with the appearance of ALS.

However, it has been recently unveiled that accumulation of TDP-43 compromises the dual role of TBK1 in ALS indicating a more complex mechanism for this PTK2-TBK1-SQSTM1 axis.

According to its close involvement in advanced-stage solid cancers, multiple PTK2 inhibitors are known and several clinical trials have been developed, indicating moderate activity and minimal toxic effects. Once the role of PTK2 in ALS is clearly defined, all these clinical studies may enhance the use of PTK2 inhibitors for the treatment of this neurodegenerative disorder.

*Protein kinases C (PKC) inhibitors*

PKC are a family of Ser/Thr kinases involved in many signaling pathways that regulate cellular growth, proliferation, differentiation and cell death (Black & Black, 2012). There are at least 11 different isoforms of PKC classified in three subfamilies based on their cofactor dependence: conventional PKC (cPKC), novel PKC (nPKC) and atypical PKC (aPKC) families (Steinberg, 2008). As they play a key role in the cell cycle regulation, they have been associated with the pathogenesis of several diseases such as cancer, diabetes and cardiovascular, pulmonary, immune and infectious diseases, becoming attractive targets for therapeutic development (Isakov, 2018; Mackay & Twelves, 2007). In the CNS, PKC isoforms have been involved in memory impairment and neurodegeneration as they are associated with the control of short and long term brain functions like synapsis, ion channel regulation and neurotransmission (Battaini, 2001). Regarding their implications in ALS, it was described that PKC activity was increased in ALS patients, suggesting that alterations in this kinase have effects on neuronal viability through the regulation of voltage-dependent Ca2+ channels (Krieger, Lanius et al., 1996). Later, it was demonstrated that several PKC isoforms are regulated and localized in the presynaptic and postsynaptic components in the neuromuscular junctions (NMJ) (Lanuza, Santafe et al., 2014), and they are affected in ALS muscles (Camerino, Fonzino et al., 2019). Finally, as there is a link between PKC activation and NMJ disintegration, a study showed that the inhibition of PKCθ by C20 (Figure 2) was able to rescue the morphology of NMJ in SOD1G93A mouse model (Dobrowolny, Martini et al., 2018).

*EpH4 receptor tyrosine kinase inhibitors*

EphA4 belongs to the EpH receptor tyrosine kinase family, that interacts with A-type and B-type Ephrins. It plays an important role in the developing nervous system and in adults it regulates synaptic plasticity, memory and synapse formation (Klein, 2009). Its relation with ALS pathology was demonstrated in 2012 when it was found that Epha4 regulates the degeneration vulnerability of motoneurons (Van Hoecke, Schoonaert et al., 2012). Moreover, it was shown that in ALS patients, EphA4 loss of function mutations are related with longer survival and its expression negatively correlates with disease onset and survival. In animal models, EphA4 inhibition, both genetically and pharmacologically, rescued pathological phenotypes. These results suggest the potential of EphA4 inhibitors as a therapeutic strategy for ALS. In 2017 an EphA4 ligand with nanomoloar activity, 123C4 (Figure 2) was able to delay disease progression in the SOD1 ALS mouse model (Wu, De et al., 2017). Currently this inhibitor is undergoing preclinical trials for ALS with Iron Horse therapeutics.

In 2018, a virtual screening of FDA-approved drugs identified 5 new EphA4 inhibitors, including ergoloid, cyproheptadine, nilotinib, abiraterine acetate and retapamulin, that inhibited EphA4 clustering by 30% at 50µM (Gu, Fu et al., 2018). Nilotinib was assayed in neurons and showed to block growth cone collapse induced by Ephrin. This finding provides novel scaffolds that could be further optimized for the inhibition of EphA4 and treatment of ALS.

*Tyrosine receptor kinase B (TrKB) inhibitors*

TrKB is a receptor for several neurotrophins, including brain-derived neurotrophic factor (BDNF). This receptor contains three main regions, an extracellular one, the transmembrane domain and the tyrosine kinase domain located in the cytoplasm (Tejeda & Diaz-Guerra, 2017). While the BDNF/TrKB pathway is related to neuronal survival and neuroprotective effect against toxic insults, the complete role of this cascade is not well understood (Pradhan, Noakes et al., 2019), since it has also been shown that TrKB activation through BDNF results in glutamate-induced signaling in rat neuroblastoma cells, and that BDNF toxicity in neural populations could be rescued through TrKB inhibition. In regard to ALS, BDNF has shown to be elevated in muscle of ALS patients (Kust, Copray et al., 2002), and an increase of TrkB mRNA was also identified in the spinal cord (Mutoh, Sobue et al., 2000). Activation of the TrkB receptor finally leads to initiation of extracellular signal regulated kinase (ERK) activation (Wheaton, Salamone et al., 2007). Furthermore, TrkB deletion in SOD1 mice demonstrated to delay the onset of the disease and improved clinical scores of mice compared to controls (Yanpallewar, Barrick et al., 2012), and indirect TrkB inhibition showed to be neuroprotective in motoneurons derived from embryonic cells with toxic insults related to ALS (Mojsilovic-Petrovic, Jeong et al., 2006). Finally, the modulation of TrkB via small molecule inhibition with the potent and selective 7,8-dihydroxyflavone (7,8-DHF) (Figure 2), a TrkB inhibitor that mimics the effect of BDNF, improved motor function and motoneuron loss in the SOD1G93A ALS model mice (Korkmaz, Aytan et al., 2014). These data suggest that inhibitors of TrKB such as 7,8-DHF should be considered as a future potential therapy for ALS.



**Figure 2**. Protein kinase inhibitors assayed in different animal models of ALS.

**Protein kinase inhibitors in early phases of drug discovery for ALS**

Recent years have witnessed a great advance in ALS molecular pathology and several protein kinases are being under active research to confirm their relevance in the pathology. At the same time, specific protein kinase inhibitors are being explored as potential drug candidates. The main results are described below.

*Tau tubulin kinase 1 and 2 (TTBK1/TTBK2) inhibitors*

These Ser/Thr and Tyr kinases are linked to ALS due to the role they play in the phosphorylation of TDP-43: TTBK1 and 2 are two of the four kinases described so far related to abnormal TDP-43 hyperphosphorylation (Liachko, McMillan et al., 2014). Besides, both isoforms co-localize with TDP-43 inclusions in spinal cords of ALS patients and have been shown to phosphorylate key residues Ser409 and Ser410 in the TDP-43 deposition process. TTBK2 plays other fundamental roles in multiple cellular mechanisms such as ciliogenesis, microtubule dynamics and neurotransmitter trafficking, and therefore its inhibition could trigger deleterious effects for the patients (Jackson, 2012; Nieding, Matschke et al., 2016; Watanabe, Kakeno et al., 2015). Furthermore, while TTBK2 is expressed ubiquitously, TTBK1 isoform is specifically expressed in the CNS (Sato, Cerny et al., 2006) and its biological role is usually linked to pathological conditions, making it an ideal target for the reduction of TDP-43 phosphorylation in a selective manner (Nozal & Martinez, 2019).

Only three TTBK1/2 inhibitors have been reported so far and all the compounds showed affinity for both isoforms (Kiefer, Chang et al., 2014; Xue, Wan et al., 2013). Liachko *et al*. showed that siRNA targeting TTBK1 mammalian cells presented reduced TDP-43 phosphorylation upon ethacrynic acid treatment and co-expression of TTBK1/TDP-43 transgenes exacerbated the phosphorylation and the behavioral defects in *C. elegans* animal model (Taylor, McMillan et al., 2018). In cellular human neuroblastoma models, TTBK1/2 inhibitors, referred as AZ-1 and AZ-2 (Figure 3), were assayed showing a neuroprotective profile from the cell death induced by phosphorylated TDP-43 (Nozal, Benítez-Fernández et al., 2018).

All these data suggest that the development of new and TTBK1 selective inhibitors would be an effective and promising therapeutic strategy to treat ALS.

*Mitogen-activated protein kinase kinase kinase kinase 4 (MAPK4K) inhibitors*

MAP4PK4 Ser/Thr kinase is linked to environmental stress response and it acts upstream in the c-Jun N-terminal kinase (JNK) pathway. Among its therapeutic effects it has been related to cancer (Gao, Gao et al., 2016), immune disorders and inflammation (Chuang, Wang et al., 2016). It was first related to ALS disease in 2013, through the analysis of a dual inhibitor of GSK-3 and MAP4K4 kinases, named kenpaullone, in primary motoneurons from mutant SOD1 and wild-type mouse embryonic stem cells (Yang, Gupta et al., 2013).

Later, several groups have tested the druggability of this kinase as it was shown that MAP4K4 is upregulated in stressed motoneurons (Crawford, Ndubaku et al., 2014). The MAP4K4 inhibitor (Figure 3) (MAP4K4, IC50 = 17nM) was tested in different ALS cellular models using motoneurons derived from human iPSC cells (SOD1L144F, TDP-43G298S and TDP-43M377V (Wu, Watts et al., 2019)) showing regulation in motoneuron survival in a dose dependent manner stimulating autophagy and reducing the JNK3-Junc apoptotic pathway (Watts, Wu et al., 2019). More studies are necessary to confirm the relevance of MAP4K4 inhibitors in the future treatment of motoneuron diseases, specially ALS.

*Homeodomain interacting protein kinase 2 (HIPK2) inhibitors*

HIPK2 has usually been linked to cancer, however, unlike other kinases, it has a protective role as its activation produces anti-tumorigenic mechanisms such as apoptosis (Feng, Zhou et al., 2017). Recently, it has been demonstrated that HIPK2 might be a mediator in the endoplasmic reticulum (ER) stress driving neurodegeneration since this kinase has a central role in the ASK1-JNK pro-apoptotic pathway. Under continued ER stress conditions, ASK1 gets phosphorylated and thus activated. This enzyme interacts with HIPK2, phosphorylating Thr360 and Ser359 which drives activation of JNK and therefore cell death. Spinal motoneurons of SOD1G93A mice showed increased HIPK2 expression, and phosphorylation of HIPK2 and all its pathway related proteins (IRE, ASK1 and JNK) is increased in this animal model. Activation of this pathway was also found in fALS spinal cords compared to healthy controls.

Tunicamycin driven toxicity was reversed when HIPK2 was genetically silenced in neurons. Treatment with the HIPK2 inhibitor known as A64 (Miduturu, Deng et al., 2011) (Figure 3) has the same neuroprotective effect. Interestingly, the authors also related HIPK2 with TDP-43 toxicity as they found increased pHIPK2 levels correlating with pTDP-43 and pJNK in a great number of sporadic ALS (sALS) and C9ORF72 cases, suggesting that the toxicity of TDP-43 might be ER mediated.

*Cyclin dependent kinases* *(CDKs) inhibitors*

CDKs are the first family of protein Ser/Thr kinases linked to the regulation of the cell division cycle (Malumbres, 2014). There are 20 different CDKs divided in 8 subfamilies, but only CDK1, 2, 4 and 6 play an important role in the cell cycle. Up to date, there are many CDKs inhibitors in clinical and pre-clinical trials for the treatment of different types of cancer. Some of them have been recently approved by the FDA for the treatment of breast cancer like palbociclib, abemaciclib and ribociclib (Roskoski, 2019). In contrast, the relation between CDKs and ALS is not yet fully understood. It has been reported that the inhibition of CDK2 kinase reduces heterogeneous ribonucleoprotein (hnRNP) K phosphorylation, preventing its incorporation into TDP-43 positive stress granules *in vitro* (Moujalled, James et al., 2015)*.* However, the role of hnRNP K in TDP-43 proteinopathies is not well known. CDK4 protein has been also related to ALS, since an increased expression of this protein has been found in sALS patients (Wang, Bu et al., 2009). In addition, the treatment of SOD1 mice with minocycline, which primary target is not clear, suppressed microglia activation via indirect p38 kinase inhibition (Kim & Suh, 2009), prevented microglia activation and motoneuron degeneration *in vivo* together with a expression decrease of CDK4 and CDK5 (Nguyen, Boudreau et al., 2003). Upregulated levels of CDK5 have also been found in sALS patients, which points to CDK5 as a new protein kinase target for ALS (Bajaj, 2000). CDK5 is unique in the CDK family as it is not involved in the cell cycle but in neuronal migration and differentiation, axon elongation and synaptogenesis as well as in memory and pain perception in adult neurons (Dhariwala & Rajadhyaksha, 2008). Recent studies have demonstrated that treatment with CDK5 inhibitors, olomoucine, roscovitine and flavopiridol, increased motoneuron survival in cell culture, reversed defective axonal transport and reduced microglial neurotoxicity (Appert-Collin, Hugel et al., 2006).

*Mitogen-activated kinase kinase (MAP2K) inhibitors*

MAP2K, also known as MEK kinase, catalyzes the phosphorylation of Thr and Tyr residues of mitogen-activated protein kinases (MAPKs) including extracellular signal-regulated kinase(ERK), p38 and Jun N-Terminal Kinase (JNK) (Morrison, 2012). There are seven subtypes of MEK kinases which are implicated in cell proliferation, survival, differentiation and response to different growth signals. Among them, MEK1/2 and MEK5 have been related to different pathologies such as cancer and neurodegeneration (Neuzillet, Tijeras-Raballand et al., 2014). MEK1/2 kinases mediate the phosphorylation of ERK1/2, which is involved in many neurological disorders as AD, Parkinson’s disease and ALS (Sun & Nan, 2017). Increased levels of phosphorylated ERK1/2 have been reported in the SOD1G93A transgenic mouse model and microglia model, showing the participation of these kinases in the pathogenesis of ALS associated with oxidative stress (Apolloni, Parisi et al., 2013; Chung, Joo et al., 2005).Different studies have also pointed to the relationship between ERK1/2 and TDP-43 as abnormal phosphorylated ERK1/2 aggregates are present in phosphorylated TDP-43 positive inclusions in ALS (Ayala, Granado-Serrano et al., 2011). Furthermore, the activation of ERK1/2 pathway leads to neuronal death in TDP-43 depleted microglia (Xia, Hu et al., 2015). Furthermore, in ALS, it has been reported that the inhibition of MEK1/2 by the specific inhibitor PD98059 (Figure 3) is able to reduce ERK1/2 activity and the phosphorylation of neurofilaments-M (NF-M), neurofilaments-H (NF-H), tau and proteins associated with microtubules (Strong, Kesavapany et al., 2005). However, this study did not show any additional results. On the other hand, MEK5 is able to phosphorylate ERK5 but the MEK5/ERK5 pathway is not well understood. A recent study showed that the inhibition of MEK5 by the small molecule MEK5 inhibitor known as BIX02189 (Figure 3), reduces TDP-43 neurotoxicity and mislocalization by the activation of the autophagy-lysosome pathway (Jo, Lee et al., 2019). Finally, MEK was found to phosphorylate TDP-43 in different human cell lines and that the inhibition of this kinase with PD184352 (Figure 3) and PD98059 was able to block TDP-43 phosphorylation (Li, Reeb et al., 2017).

This kind of kinase inhibitors together with p38 and JNK inhibitors may open new avenues for the potential disease modifying treatment of ALS.

*c-Jun N-terminal kinases (JNKs) inhibitors*

JNKs belong to the mitogen-activated protein kinase family and consist of ten isoforms derived from three genes: *JNK1, JNK2* and *JNK3*.While JNK1 and JNK2 are ubiquitously expressed in the organism, JNK3 is mainly found in the brain, being a therapeutic target for neurological disorders (Yarza, Vela et al., 2015). JNKs modify the activity of numerous proteins that reside at the mitochondria or act in the nucleus by direct phosphorylation and are in relation with inflammatory response.

Many JNK inhibitors based on different molecular scaffolds have been discovered in the past decade, although only a few have advanced to clinical trials being JNK-isoforms selectivity the major obstacle (Li, Qi et al., 2020). Due to the great therapeutic potential of JNK inhibitors, several strategies, including allosteric modulation have been explored (Lombard, Davis et al., 2018). Regarding ALS, JNKs signaling pathway is relevant in the control of TDP-43-induced cell death (Suzuki & Matsuoka, 2013). Treatment of NSC34 motoneuron cells with the JNK inhibitor AS601245 (Figure 3), inhibited the TDP-43-induced cell death. Furthermore JNK also controls the accumulation of TDP-43 in the stress granules after chronic oxidative stress, and treatment with the JNK inhibitors SP600125 and BI-78D3 (Figure 3), resulted in almost complete inhibition of TDP-43-positive stress granules (Meyerowitz, Parker et al., 2011). JNK inhibitors may be a good approach to prevent motoneuron death from TDP-43 toxicity.

*Protein kinase RNA-activated (PKR) inhibitors*

PKR is an apoptotic inducer belonging to the Ser/Thr kinases family. It was first identified to play an important role in the innate immune response to viral infections (Balachandran, Roberts et al., 2000) and its enzymatic activity is regulated by its interaction with double-stranded RNA (dsRNA) (Lemaire, Anderson et al., 2008). Additionally to its antiviral function, PKR is involved in many intracellular regulatory pathways related to different human pathologies including inflammation, cancer, diabetes, metabolic disorders and neurodegeneration (Gal-Ben-Ari, Barrera et al., 2018). PKR interacts with the pro-inflammatory NF-kappaB pathway (NF-ĸB) being linked to neuronal cell death (Mattson & Camandola, 2001). Although the relation between PKR and ALS is not well known, levels of activated PKR were shown to be increased in spinal cord of ALS patients (Peel, 2004). Recently, it has been demonstrated that TDP-43 knockdown results in dsRNA accumulation and astrocyte activation in primary rat astrocytes, and that PKR inhibition by C16 (Figure 3) reduces pro-inflammatory effects of TDP-43 knockdown (LaRocca, Mariani et al., 2019). Thus, PKR inhibition could be a novel strategy to protect motoneuron from the loss of TDP-43 function preventing reactive astrocytosis in ALS.

*Ribosomal s6 kinase 1 (S6K1) inhibitors*

S6K1 is one of the two known mammalian isoforms of p70 ribosomal s6 kinase (RSK), a Ser/Thre kinase activated by the MAP/ERK pathway. It is involved in signal transduction and phosphorylates ribosomal protein S6 a part of the translational machinery (Magnuson, Ekim et al., 2012). Elevated expression of activated S6K1 has been found in thoracic spinal cord tissue from sALS patients that may be related to the neuronal death evident in the pathology (Hu et al., 2003). Recently, it has been shown a reduction of mutant SOD1G93A protein aggregates in NSC34 cells after treatment with S6K1 inhibitors (Sun, Mu et al., 2018). Two small molecules, known as PF-4708671 and A77 1726 were tested, being the last one the active metabolite of leflunomide, an anti-inflammatory drug used in clinic (Doscas, Williamson et al., 2014). This study provides evidences linking the S6K1 inhibition with an increase in autophagy machinery mediated by AMPK activation, and provides new clues to support the use of inhibitors of S6K1 and specific repurposing of leflunomide a new strategy for ALS pharmacotherapy.

**Figure 3**. Protein kinase inhibitors in early research stages as potential pharmacotherapy for ALS.

**CONCLUSIONS AND FUTURE PERSPECTIVES**

ALS is a fatal and devastating neurodegenerative disease characterized by the progressive loss of motoneurons. Patients suffer from increasing muscle paralysis and die within 2-5 years after diagnosis. The lack of validated cellular and animal models together with the unknown etiology, assumed as a combination of genetic and environmental factors, may explain the lack of effective treatments. Finding a cure for ALS is one of the greatest challenges for those working in CNS drug discovery.

Protein kinases, the major drug target family exploited in the last decades, have been linked to multiple pathological mechanisms present in ALS. Their modulation by small molecules offers an exceptional opportunity to discover an effective therapy for this pathology. In fact, protein kinase inhibitors have gained stage in ALS pharmacology being different drug candidates in clinical trials (Figure 4).



Figure 4. Protein kinase inhibitors landscape for ALS treatment. Colour refers to the primary molecular ALS hallamark pursued to be corrected in the preclinical development. Intensity of colour refers to development stage (more intense the colour, more advanced the drug is).

The more advanced protein kinase inhibitors started their ALS development in clinical trials phase II using a repurposing strategy, such masitinib, fasudil, bosutinib or lithium. In all these cases, the specific preclinical model used was the transgenic SODG93A mice which mimics only a reduced percentage of ALS patients. This strategy has been the main one used until this moment and, despite having a lot of failures, has enabled market approval for the only two palliative drugs available for ALS, riluzole and edaravone. The competitive advantage of the current kinase inhibitors in clinical trials is their neuroprotective profile against different motoneurons insults such as oxidative stress or pro-inflammatory cytokines. More interesting is the repurposing of rapamycin, an oncological treatment that increase autophagy trough mTOR inhibition. This cellular process is essential for the clearance of protein aggregates, being rapamycin able to decrease TPD-43 aggregates, the main hallmark of ALS that was discovered only a decade ago. This trial is very relevant to decipher the real therapeutic value of TDP-43 homeostasis recovery in patients. Finally, two new drug candidates for ALS have recently started clinical trials targeting DLK and RIPK1, respectively. Both compounds are first-in-man, and human pharmacokinetic studies under phase I clinical trials are needed prior the ALS proof of concept in phase II. In the next future, some other advanced drugs such as tideglusib, a GSK-3 inhibitor, or leflunomide, in clinical use for reumathoid arthritis with its main metabolite being inhibitor of S6R kinase, may be repurposed for ALS treatment. Both compounds have shown their ability to modify TDP-43 pathology in different cellular and animal models.

The future is very interesting in this field. New protein kinase inhibitors in developmentare selective and brain penetrant, being their primary mechanism of action focused in the modulation of the toxicity caused by TDP-43 dysfunction. This is a pathological feature present in more than 97% of ALS patients but until now the human proof of concept has not been achieved. Only with the advance of all the molecules in the current landscape and intensive research in the pathobiology of the disease, a good treatment to stop this devastating disease will be achieved.

**ACKNOWLEDGEMENTS**

Funding from Comunidad de Madrid (B2017/BMD3813 ELA-Madrid), EU structural funds (FSE and FEDER), MINECO (SAF2016-76693-R), ISCiii (CIBERNED, CB18/05/00040), and MECD (FPU14-00204 to E.R-P. and FPU16/04466 to V.N.) is acknowledged. V.P. has received financial support through the Postdoctoral Junior Leader Fellowship Programme (LCF/BQ/PR18/11640007) from “la Caixa” Banking Foundation.

**CONFLICTS OF INTEREST STATEMENT**

The authors declare that there is no conflict of interest

**References**

Ackerley S, Grierson AJ, Banner S, Perkinton MS, Brownlees J, Byers HL, . . . Miller CC (2004). p38alpha stress-activated protein kinase phosphorylates neurofilaments and is associated with neurofilament pathology in amyotrophic lateral sclerosis. Mol Cell Neurosci 26**:** 354-364.

Aggarwal SP, Zinman L, Simpson E, McKinley J, Jackson KE, Pinto H, . . . Canadian Amyotrophic Lateral Sclerosis c (2010). Safety and efficacy of lithium in combination with riluzole for treatment of amyotrophic lateral sclerosis: a randomised, double-blind, placebo-controlled trial. Lancet Neurol 9**:** 481-488.

Ahn SW, Kim JE, Park KS, Choi WJ, Hong YH, Kim SM, . . . Sung JJ (2012). The neuroprotective effect of the GSK-3beta inhibitor and influence on the extrinsic apoptosis in the ALS transgenic mice. J Neurol Sci 320**:** 1-5.

Alquezar C, Salado IG, de la Encarnacion A, Perez DI, Moreno F, Gil C, . . . Martin-Requero A (2016). Targeting TDP-43 phosphorylation by Casein Kinase-1delta inhibitors: a novel strategy for the treatment of frontotemporal dementia. Mol Neurodegener 11**:** 36.

Apolloni S, Parisi C, Pesaresi MG, Rossi S, Carri MT, Cozzolino M, . . . D'Ambrosi N (2013). The NADPH oxidase pathway is dysregulated by the P2X7 receptor in the SOD1-G93A microglia model of amyotrophic lateral sclerosis. J Immunol 190**:** 5187-5195.

Appert-Collin A, Hugel B, Levy R, Niederhoffer N, Coupin G, Lombard Y, . . . Gies JP (2006). Cyclin dependent kinase inhibitors prevent apoptosis of postmitotic mouse motoneurons. Life Sci 79**:** 484-490.

Ayala V, Granado-Serrano AB, Cacabelos D, Naudi A, Ilieva EV, Boada J, . . . Portero-Otin M (2011). Cell stress induces TDP-43 pathological changes associated with ERK1/2 dysfunction: implications in ALS. Acta Neuropathol 122**:** 259-270.

Bajaj NP (2000). Cyclin-dependent kinase-5 (CDK5) and amyotrophic lateral sclerosis. Amyotroph Lateral Scler Other Motor Neuron Disord 1**:** 319-327.

Balachandran S, Roberts PC, Brown LE, Truong H, Pattnaik AK, Archer DR, & Barber GN (2000). Essential role for the dsRNA-dependent protein kinase PKR in innate immunity to viral infection. Immunity 13**:** 129-141.

Battaini F (2001). Protein kinase C isoforms as therapeutic targets in nervous system disease states. Pharmacol Res 44**:** 353-361.

Bissaro M, Federico S, Salmaso V, Sturlese M, Spalluto G, & Moro S (2018). Targeting protein kinase CK1delta with riluzole: Could it be one of the possible missing bricks to interpret its effect in the treatment of ALS from a molecular point of view? ChemMedChem 13**:** 2601-2605.

Black AR, & Black JD (2012). Protein kinase C signaling and cell cycle regulation. Front Immunol 3**:** 423.

Bowerman M, Murray LM, Boyer JG, Anderson CL, & Kothary R (2012). Fasudil improves survival and promotes skeletal muscle development in a mouse model of spinal muscular atrophy. BMC Med 10**:** 24.

Caccamo A, Majumder S, Deng JJ, Bai Y, Thornton FB, & Oddo S (2009). Rapamycin rescues TDP-43 mislocalization and the associated low molecular mass neurofilament instability. J Biol Chem 284**:** 27416-27424.

Camerino GM, Fonzino A, Conte E, De Bellis M, Mele A, Liantonio A, . . . Pierno S (2019). Elucidating the contribution of skeletal muscle ion channels to amyotrophic lateral sclerosis in search of new therapeutic options. Sci Rep 9**:** 3185.

Chen S, Liao Q, Lu K, Zhou J, Huang C, & Bi F (2020). Riluzole exhibits no therapeutic efficacy on a transgenic rat model of amyotrophic lateral sclerosis. Curr Neurovasc Res**:** doi:10.2174/1567202617666200409125227.

Cheng CW, Lin MJ, & Shen CK (2015). Rapamycin alleviates pathogenesis of a new Drosophila model of ALS-TDP. J Neurogenet 29**:** 59-68.

Chico LK, Van Eldik LJ, & Watterson DM (2009). Targeting protein kinases in central nervous system disorders. Nat Rev Drug Discov 8**:** 892-909.

Chuang HC, Wang X, & Tan TH (2016). MAP4K family kinases in immunity and inflammation. Adv Immunol 129**:** 277-314.

Chung YH, Joo KM, Lim HC, Cho MH, Kim D, Lee WB, & Cha CI (2005). Immunohistochemical study on the distribution of phosphorylated extracellular signal-regulated kinase (ERK) in the central nervous system of SOD1G93A transgenic mice. Brain Res 1050**:** 203-209.

Cohen P (2002). Protein kinases--the major drug targets of the twenty-first century? Nat Rev Drug Discov 1**:** 309-315.

Cohen P, & Alessi DR (2013). Kinase drug discovery--what's next in the field? ACS Chem Biol 8**:** 96-104.

Cortes JE, Apperley JF, DeAngelo DJ, Deininger MW, Kota VK, Rousselot P, & Gambacorti-Passerini C (2018). Management of adverse events associated with bosutinib treatment of chronic-phase chronic myeloid leukemia: expert panel review. J Hematol Oncol 11**:** 143.

Crawford TD, Ndubaku CO, Chen H, Boggs JW, Bravo BJ, DeLaTorre K, . . . Ye W (2014). Discovery of selective 4-amino-pyridopyrimidine Inhibitors of MAP4K4 using fragment-based lead identification and optimization. J Med Chem 57**:** 3484-3493.

Cuadrado A, & Nebreda AR (2010). Mechanisms and functions of p38 MAPK signalling. Biochem J 429**:** 403-417.

Cui R, Tuo M, Li P, & Zhou C (2018). Association between TBK1 mutations and risk of amyotrophic lateral sclerosis/frontotemporal dementia spectrum: a meta-analysis. Neurol Sci 39**:** 811-820.

de Munck E, Palomo V, Munoz-Saez E, Perez DI, Gomez-Miguel B, Solas MT, . . . Arahuetes RM (2016). Small GSK-3 inhibitor shows efficacy in a motor neuron disease murine model modulating autophagy. PLoS One 11**:** e0162723.

Degterev A, Ofengeim D, & Yuan J (2019). Targeting RIPK1 for the treatment of human diseases. Proc Natl Acad Sci U S A 116**:** 9714-9722.

Dhariwala FA, & Rajadhyaksha MS (2008). An unusual member of the Cdk family: Cdk5. Cell Mol Neurobiol 28**:** 351-369.

Dobrowolny G, Martini M, Scicchitano BM, Romanello V, Boncompagni S, Nicoletti C, . . . Musaro A (2018). Muscle expression of SOD1(G93A) triggers the dismantlement of neuromuscular junction via PKC-Theta. Antioxid Redox Signal 28**:** 1105-1119.

Doscas ME, Williamson AJ, Usha L, Bogachkov Y, Rao GS, Xiao F, . . . Xu X (2014). Inhibition of p70 S6 kinase (S6K1) activity by A77 1726 and its effect on cell proliferation and cell cycle progress. Neoplasia 16**:** 824-834.

Dubreuil P, Letard S, Ciufolini M, Gros L, Humbert M, Casteran N, . . . Hermine O (2009). Masitinib (AB1010), a potent and selective tyrosine kinase inhibitor targeting KIT. PLoS One 4**:** e7258.

Feng HL, Leng Y, Ma CH, Zhang J, Ren M, & Chuang DM (2008). Combined lithium and valproate treatment delays disease onset, reduces neurological deficits and prolongs survival in an amyotrophic lateral sclerosis mouse model. Neuroscience 155**:** 567-572.

Feng Y, LoGrasso PV, Defert O, & Li R (2016). Rho kinase (ROCK) inhibitors and their therapeutic potential. J Med Chem 59**:** 2269-2300.

Feng Y, Zhou L, Sun X, & Li Q (2017). Homeodomain-interacting protein kinase 2 (HIPK2): a promising target for anti-cancer therapies. Oncotarget 8**:** 20452-20461.

Fornai F, Longone P, Cafaro L, Kastsiuchenka O, Ferrucci M, Manca ML, . . . Paparelli A (2008). Lithium delays progression of amyotrophic lateral sclerosis. Proc Natl Acad Sci U S A 105**:** 2052-2057.

Freland L, & Beaulieu JM (2012). Inhibition of GSK3 by lithium, from single molecules to signaling networks. Front Mol Neurosci 5**:** 14.

Fujisawa T, Takahashi M, Tsukamoto Y, Yamaguchi N, Nakoji M, Endo M, . . . Ichijo H (2016). The ASK1-specific inhibitors K811 and K812 prolong survival in a mouse model of amyotrophic lateral sclerosis. Hum Mol Genet 25**:** 245-253.

Gagalo I, Rusiecka I, & Kocic I (2015). Tyrosine kinase inhibitor as a new therapy for ischemic stroke and other neurologic diseases: is there any hope for a better outcome? Curr Neuropharmacol 13**:** 836-844.

Gal-Ben-Ari S, Barrera I, Ehrlich M, & Rosenblum K (2018). PKR: A kinase to remember. Front Mol Neurosci 11**:** 480.

Gao X, Gao C, Liu G, & Hu J (2016). MAP4K4: an emerging therapeutic target in cancer. Cell Biosci 6**:** 56.

Gibbs KL, Kalmar B, Rhymes ER, Fellows AD, Ahmed M, Whiting P, . . . Schiavo G (2018). Inhibiting p38 MAPK alpha rescues axonal retrograde transport defects in a mouse model of ALS. Cell Death Dis 9**:** 596.

González-Muñoz M, I. Rodríguez-Mahillo A, Gil C, Morán Y, Moneo I, Martínez A, & S. Mora J (2013). Glycogen synthase kinase-3β expression and phosphorylation in peripheral blood mononuclear cells of patients with amyotrophic lateral sclerosis. Br J Med Med Res 4**:** 263-271.

Grievink HW, Heuberger J, Huang F, Chaudhary R, Birkhoff WAJ, Tonn GR, . . . Groeneveld GJ (2020). DNL104, a centrally penetrant RIPK1 inhibitor, inhibits RIP1 kinase phosphorylation in a randomized phase I ascending dose study in healthy volunteers. Clin Pharmacol Ther 107**:** 406-414.

Gu S, Fu WY, Fu AKY, Tong EPS, Ip FCF, Huang X, & Ip NY (2018). Identification of new EphA4 inhibitors by virtual screening of FDA-approved drugs. Sci Rep 8**:** 7377.

Günther R, Balck A, Koch JC, Nientiedt T, Sereda M, Bahr M, . . . Tönges L (2017). Rho kinase inhibition with fasudil in the SOD1(G93A) mouse model of amyotrophic lateral sclerosis-symptomatic treatment potential after disease onset. Front Pharmacol 8**:** 17.

Guo W, Vandoorne T, Steyaert J, Staats KA, & Van Den Bosch L (2020). The multifaceted role of kinases in amyotrophic lateral sclerosis: genetic, pathological and therapeutic implications. Brain**:** doi:10.1093/brain/awaa1022.

Guo X, Namekata K, Kimura A, Harada C, & Harada T (2017). ASK1 in neurodegeneration. Adv Biol Regul 66**:** 63-71.

Hahn KA, Ogilvie G, Rusk T, Devauchelle P, Leblanc A, Legendre A, . . . Hermine O (2008). Masitinib is safe and effective for the treatment of canine mast cell tumors. J Vet Intern Med 22**:** 1301-1309.

Hall AC, Brennan A, Goold RG, Cleverley K, Lucas FR, Gordon-Weeks PR, & Salinas PC (2002). Valproate regulates GSK-3-mediated axonal remodeling and synapsin I clustering in developing neurons. Mol Cell Neurosci 20**:** 257-270.

Hardiman O, Al-Chalabi A, Chio A, Corr EM, Logroscino G, Robberecht W, . . . van den Berg LH (2017). Amyotrophic lateral sclerosis. Nat Rev Dis Primers 3**:** 17071.

Harris PA, Berger SB, Jeong JU, Nagilla R, Bandyopadhyay D, Campobasso N, . . . Bertin J (2017). Discovery of a first-in-class receptor interacting protein 1 (RIP1) kinase specific clinical candidate (GSK2982772) for the treatment of inflammatory diseases. J Med Chem 60**:** 1247-1261.

Hart MP, & Gitler AD (2012). ALS-associated ataxin 2 polyQ expansions enhance stress-induced caspase 3 activation and increase TDP-43 pathological modifications. J Neurosci 32**:** 9133-9142.

Hasegawa M, Arai T, Nonaka T, Kametani F, Yoshida M, Hashizume Y, . . . Akiyama H (2008). Phosphorylated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. Ann Neurol 64**:** 60-70.

Hidenori Ichijo SM, Nakagawa H, Nakoji M, Osawa T, Shimizu T, & Takahashi M (2012). Patent WO2012011548A1.

Honjo M, & Tanihara H (2018). Impact of the clinical use of ROCK inhibitor on the pathogenesis and treatment of glaucoma. Jpn J Ophthalmol 62**:** 109-126.

Hu JH, Zhang H, Wagey R, Krieger C, & Pelech SL (2003). Protein kinase and protein phosphatase expression in amyotrophic lateral sclerosis spinal cord. J Neurochem 85**:** 432-442.

Imamura K, Izumi Y, Banno H, Uozumi R, Morita S, Egawa N, . . . Inoue H (2019). Induced pluripotent stem cell-based Drug Repurposing for Amyotrophic lateral sclerosis Medicine (iDReAM) study: protocol for a phase I dose escalation study of bosutinib for amyotrophic lateral sclerosis patients. BMJ Open 9**:** e033131.

Imamura K, Izumi Y, Watanabe A, Tsukita K, Woltjen K, Yamamoto T, . . . Inoue H (2017). The Src/c-Abl pathway is a potential therapeutic target in amyotrophic lateral sclerosis. Sci Transl Med 9.

Isakov N (2018). Protein kinase C (PKC) isoforms in cancer, tumor promotion and tumor suppression. Semin Cancer Biol 48**:** 36-52.

Ito Y, Ofengeim D, Najafov A, Das S, Saberi S, Li Y, . . . Yuan J (2016). RIPK1 mediates axonal degeneration by promoting inflammation and necroptosis in ALS. Science 353**:** 603-608.

Jackson PK (2012). TTBK2 kinase: linking primary cilia and cerebellar ataxias. Cell 151**:** 697-699.

Jiang W, & Hunter T (1997). Identification and characterization of a human protein kinase related to budding yeast Cdc7p. Proc Natl Acad Sci U S A 94**:** 14320-14325.

Jin Y, & Zheng B (2019). Multitasking: Dual leucine zipper-bearing kinases in neuronal development and stress management. Annu Rev Cell Dev Biol 35**:** 501-521.

Jo M, Lee S, Kim K, Lee S, Kim SR, & Kim HJ (2019). Inhibition of MEK5 suppresses TDP-43 toxicity via the mTOR-independent activation of the autophagy-lysosome pathway. Biochem Biophys Res Commun 513**:** 925-932.

Kametani F, Nonaka T, Suzuki T, Arai T, Dohmae N, Akiyama H, & Hasegawa M (2009). Identification of casein kinase-1 phosphorylation sites on TDP-43. Biochem Biophys Res Commun 382**:** 405-409.

Kannaiyan R, & Mahadevan D (2018). A comprehensive review of protein kinase inhibitors for cancer therapy. Expert Rev Anticancer Ther 18**:** 1249-1270.

Kiefer SE, Chang CJ, Kimura SR, Gao M, Xie D, Zhang Y, . . . Sheriff S (2014). The structure of human tau-tubulin kinase 1 both in the apo form and in complex with an inhibitor. Acta Crystallogr F Struct Biol Commun 70**:** 173-181.

Kim HS, & Suh YH (2009). Minocycline and neurodegenerative diseases. Behav Brain Res 196**:** 168-179.

Kim JM, Yamada M, & Masai H (2003). Functions of mammalian Cdc7 kinase in initiation/monitoring of DNA replication and development. Mutat Res 532**:** 29-40.

Klein R (2009). Bidirectional modulation of synaptic functions by Eph/ephrin signaling. Nat Neurosci 12**:** 15-20.

Koch JC, Tatenhorst L, Roser AE, Saal KA, Tonges L, & Lingor P (2018). ROCK inhibition in models of neurodegeneration and its potential for clinical translation. Pharmacol Ther 189**:** 1-21.

Koh SH, Kim Y, Kim HY, Hwang S, Lee CH, & Kim SH (2007). Inhibition of glycogen synthase kinase-3 suppresses the onset of symptoms and disease progression of G93A-SOD1 mouse model of ALS. Exp Neurol 205**:** 336-346.

Korkmaz OT, Aytan N, Carreras I, Choi JK, Kowall NW, Jenkins BG, & Dedeoglu A (2014). 7,8-Dihydroxyflavone improves motor performance and enhances lower motor neuronal survival in a mouse model of amyotrophic lateral sclerosis. Neurosci Lett 566**:** 286-291.

Krieger C, Lanius RA, Pelech SL, & Shaw CA (1996). Amyotrophic lateral sclerosis: the involvement of intracellular Ca2+ and protein kinase C. Trends Pharmacol Sci 17**:** 114-120.

Kust BM, Copray JC, Brouwer N, Troost D, & Boddeke HW (2002). Elevated levels of neurotrophins in human biceps brachii tissue of amyotrophic lateral sclerosis. Exp Neurol 177**:** 419-427.

Langeswaran K, Jeyaraman J, R JB, Biswas A, & Dhurgadevi KR (2019). Identifying dual leucine zipper kinase (DLK) inhibitors using e-pharamacophore screening and molecular docking. J Recept Signal Transduct Res 39**:** 99-105.

Lanuza MA, Santafe MM, Garcia N, Besalduch N, Tomas M, Obis T, . . . Tomas J (2014). Protein kinase C isoforms at the neuromuscular junction: localization and specific roles in neurotransmission and development. J Anat 224**:** 61-73.

LaRocca TJ, Mariani A, Watkins LR, & Link CD (2019). TDP-43 knockdown causes innate immune activation via protein kinase R in astrocytes. Neurobiol Dis 132**:** 104514.

Le Pichon CE, Meilandt WJ, Dominguez S, Solanoy H, Lin H, Ngu H, . . . Lewcock JW (2017). Loss of dual leucine zipper kinase signaling is protective in animal models of neurodegenerative disease. Sci Transl Med 9.

Lee S, Jeon YM, Cha SJ, Kim S, Kwon Y, Jo M, . . . Kim HJ (2019). PTK2/FAK regulates UPS impairment via SQSTM1/p62 phosphorylation in TARDBP/TDP-43 proteinopathies. Autophagy**:** 1-17.

Lemaire PA, Anderson E, Lary J, & Cole JL (2008). Mechanism of PKR Activation by dsRNA. J Mol Biol 381**:** 351-360.

Li G, Qi W, Li X, Zhao J, Luo M, & Chen J (2020). Recent advances in c-Jun N-terminal kinase (JNK) inhibitors. Curr Med Chem**:** doi:10.2174/0929867327666200210144114.

Li M, Yasumura D, Ma AA, Matthes MT, Yang H, Nielson G, . . . Diamond MI (2013). Intravitreal administration of HA-1077, a ROCK inhibitor, improves retinal function in a mouse model of huntington disease. PLoS One 8**:** e56026.

Li W, Reeb AN, Lin B, Subramanian P, Fey EE, Knoverek CR, . . . Ayala YM (2017). Heat shock-induced phosphorylation of TAR DNA-binding protein 43 (TDP-43) by MAPK/ERK kinase regulates TDP-43 function. J Biol Chem 292**:** 5089-5100.

Liachko NF, McMillan PJ, Guthrie CR, Bird TD, Leverenz JB, & Kraemer BC (2013). CDC7 inhibition blocks pathological TDP-43 phosphorylation and neurodegeneration. Ann Neurol 74**:** 39-52.

Liachko NF, McMillan PJ, Strovas TJ, Loomis E, Greenup L, Murrell JR, . . . Kraemer BC (2014). The tau tubulin kinases TTBK1/2 promote accumulation of pathological TDP-43. PLoS Genet 10**:** e1004803.

Lingor P, Weber M, Camu W, Friede T, Hilgers R, Leha A, . . . Investigators R-A (2019). ROCK-ALS: Protocol for a randomized, placebo-controlled, double-blind phase IIa trial of safety, tolerability and efficacy of the Rho kinase (ROCK) inhibitor Fasudil in amyotrophic lateral sclerosis. Front Neurol 10**:** 293.

Lipton JO, & Sahin M (2014). The neurology of mTOR. Neuron 84**:** 275-291.

Liscic RM (2017). Als and Ftd: Insights into the disease mechanisms and therapeutic targets. Eur J Pharmacol 817**:** 2-6.

Lombard CK, Davis AL, Inukai T, & Maly DJ (2018). Allosteric modulation of JNK docking site interactions with ATP-competitive inhibitors. Biochemistry 57**:** 5897-5909.

Lovestone S, Boada M, Dubois B, Hull M, Rinne JO, Huppertz HJ, . . . investigators A (2015). A phase II trial of tideglusib in Alzheimer's disease. J Alzheimers Dis 45**:** 75-88.

Mackay HJ, & Twelves CJ (2007). Targeting the protein kinase C family: are we there yet? Nat Rev Cancer 7**:** 554-562.

Mackenzie IR, Bigio EH, Ince PG, Geser F, Neumann M, Cairns NJ, . . . Trojanowski JQ (2007). Pathological TDP-43 distinguishes sporadic amyotrophic lateral sclerosis from amyotrophic lateral sclerosis with SOD1 mutations. Ann Neurol 61**:** 427-434.

Madill M, McDonagh K, Ma J, Vajda A, McLoughlin P, O'Brien T, . . . Shen S (2017). Amyotrophic lateral sclerosis patient iPSC-derived astrocytes impair autophagy via non-cell autonomous mechanisms. Mol Brain 10**:** 22.

Magnuson B, Ekim B, & Fingar DC (2012). Regulation and function of ribosomal protein S6 kinase (S6K) within mTOR signalling networks. Biochem J 441**:** 1-21.

Malumbres M (2014). Cyclin-dependent kinases. Genome Biol 15**:** 122.

Mandrioli J, D'Amico R, Zucchi E, Gessani A, Fini N, Fasano A, . . . group R-Ai (2018). Rapamycin treatment for amyotrophic lateral sclerosis: Protocol for a phase II randomized, double-blind, placebo-controlled, multicenter, clinical trial (RAP-ALS trial). Medicine (Baltimore) 97**:** e11119.

Marech I, Patruno R, Zizzo N, Gadaleta C, Introna M, Zito AF, . . . Ranieri G (2014). Masitinib (AB1010), from canine tumor model to human clinical development: where we are? Crit Rev Oncol Hematol 91**:** 98-111.

Martinez A, Palomo Ruiz MD, Perez DI, & Gil C (2017). Drugs in clinical development for the treatment of amyotrophic lateral sclerosis. Expert Opin Investig Drugs 26**:** 403-414.

Martinez A, Perez DI, Gil C, Martin-Requero A, Rojas Prats E, Martinez-Gonzalez L, & Perez C (2018). Patent WO2018172587A1.

Martinez A, Rojas E, Alquezar C, Perez DI, Gil C, & Martin-Requero A (2017). Regulation of TDP-43 pathology by CDC-7 inhibitors treatment. AD/PD2017 meeting.

Martinez-Gonzalez L, Porras G, Gil C, Palomo V, De Lago E, Gonzalo Consuegra C, . . . Martinez A (2019). Tideglusib, a drug candidate for the treatment of ALS. European Network to Cure ALS (ENCALS) meeting.

Martinez-Gonzalez L, Rodriguez-Cueto C, Cabezudo D, Bartolome F, Andres-Benito P, Ferrer I, . . . de Lago E (2020). Motor neuron preservation and decrease of in vivo TDP-43 phosphorylation by protein CK-1delta kinase inhibitor treatment. Sci Rep 10**:** 4449.

Mathis S, Goizet C, Soulages A, Vallat JM, & Masson GL (2019). Genetics of amyotrophic lateral sclerosis: A review. J Neurol Sci 399**:** 217-226.

Mattson MP, & Camandola S (2001). NF-kappaB in neuronal plasticity and neurodegenerative disorders. J Clin Invest 107**:** 247-254.

Meyerowitz J, Parker SJ, Vella LJ, Ng D, Price KA, Liddell JR, . . . White AR (2011). C-Jun N-terminal kinase controls TDP-43 accumulation in stress granules induced by oxidative stress. Mol Neurodegener 6**:** 57.

Miduturu CV, Deng X, Kwiatkowski N, Yang W, Brault L, Filippakopoulos P, . . . Gray NS (2011). High-throughput kinase profiling: a more efficient approach toward the discovery of new kinase inhibitors. Chem Biol 18**:** 868-879.

Mojsilovic-Petrovic J, Jeong GB, Crocker A, Arneja A, David S, Russell DS, & Kalb RG (2006). Protecting motor neurons from toxic insult by antagonism of adenosine A2a and Trk receptors. J Neurosci 26**:** 9250-9263.

Montagnoli A, Moll J, & Colotta F (2010). Targeting cell division cycle 7 kinase: a new approach for cancer therapy. Clin Cancer Res 16**:** 4503-4508.

Mora JS, Genge A, Chio A, Estol CJ, Chaverri D, Hernandez M, . . . Ab10015 Study G (2020). Masitinib as an add-on therapy to riluzole in patients with amyotrophic lateral sclerosis: a randomized clinical trial. Amyotroph Lateral Scler Frontotemporal Degener 21**:** 5-14.

Morrison DK (2012). MAP kinase pathways. Cold Spring Harb Perspect Biol 4**:** doi:10.1101/cshperspect.a011254.

Moujalled D, James JL, Yang S, Zhang K, Duncan C, Moujalled DM, . . . White AR (2015). Phosphorylation of hnRNP K by cyclin-dependent kinase 2 controls cytosolic accumulation of TDP-43. Hum Mol Genet 24**:** 1655-1669.

Moura RP, Pacheco C, Pego AP, des Rieux A, & Sarmento B (2020). Lipid nanocapsules to enhance drug bioavailability to the central nervous system. J Control Release 322**:** 390-400.

Mueller BK, Mack H, & Teusch N (2005). Rho kinase, a promising drug target for neurological disorders. Nat Rev Drug Discov 4**:** 387-398.

Mukhopadhyay S, Frias MA, Chatterjee A, Yellen P, & Foster DA (2016). The enigma of papamycin dosage. Mol Cancer Ther 15**:** 347-353.

Mutoh T, Sobue G, Hamano T, Kuriyama M, Hirayama M, Yamamoto M, & Mitsuma T (2000). Decreased phosphorylation levels of TrkB neurotrophin receptor in the spinal cords from patients with amyotrophic lateral sclerosis. Neurochem Res 25**:** 239-245.

Neumann M, Kwong LK, Lee EB, Kremmer E, Flatley A, Xu Y, . . . Lee VM (2009). Phosphorylation of S409/410 of TDP-43 is a consistent feature in all sporadic and familial forms of TDP-43 proteinopathies. Acta Neuropathol 117**:** 137-149.

Neumann M, Sampathu DM, Kwong LK, Truax AC, Micsenyi MC, Chou TT, . . . Lee VM (2006). Ubiquitinated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. Science 314**:** 130-133.

Neuzillet C, Tijeras-Raballand A, de Mestier L, Cros J, Faivre S, & Raymond E (2014). MEK in cancer and cancer therapy. Pharmacol Ther 141**:** 160-171.

Nguyen MD, Boudreau M, Kriz J, Couillard-Despres S, Kaplan DR, & Julien JP (2003). Cell cycle regulators in the neuronal death pathway of amyotrophic lateral sclerosis caused by mutant superoxide dismutase 1. J Neurosci 23**:** 2131-2140.

Nieding K, Matschke V, Meuth SG, Lang F, Seebohm G, & Strutz-Seebohm N (2016). Tau Tubulin Kinase TTBK2 sensitivity of glutamate receptor GluK2. Cell Physiol Biochem 39**:** 1444-1452.

Nishitoh H, Kadowaki H, Nagai A, Maruyama T, Yokota T, Fukutomi H, . . . Ichijo H (2008). ALS-linked mutant SOD1 induces ER stress- and ASK1-dependent motor neuron death by targeting Derlin-1. Genes Dev 22**:** 1451-1464.

Nozal V, Benítez-Fernández R, Martínez-González L, Pérez DI, Gil C, & Martínez A (2018). TTBK1 inhibitors: A novel multifactorial approach for the treatment of AD and ALS. 10th Summer School on Medicines São Paulo School of Advanced Science.

Nozal V, & Martinez A (2019). Tau Tubulin Kinase 1 (TTBK1), a new player in the fight against neurodegenerative diseases. Eur J Med Chem 161**:** 39-47.

Oetjen E, & Lemcke T (2016). Dual leucine zipper kinase (MAP3K12) modulators: a patent review (2010-2015). Expert Opin Ther Pat 26**:** 607-616.

Palomo V, Perez DI, Gil C, & Martinez A (2011). The potential role of glycogen synthase kinase 3 inhibitors as amyotrophic lateral sclerosis pharmacological therapy. Curr Med Chem 18**:** 3028-3034.

Palomo V, Tosat-Bitrian C, Nozal V, Nagaraj S, Martin-Requero A, & Martinez A (2019). TDP-43: A key therapeutic target beyond amyotrophic lateral sclerosis. ACS Chem Neurosci 10**:** 1183-1196.

Patel S, Cohen F, Dean BJ, De La Torre K, Deshmukh G, Estrada AA, . . . Siu M (2015). Discovery of dual leucine zipper kinase (DLK, MAP3K12) inhibitors with activity in neurodegeneration models. J Med Chem 58**:** 401-418.

Patel S, Harris SF, Gibbons P, Deshmukh G, Gustafson A, Kellar T, . . . Lewcock JW (2015). Scaffold-hopping and structure-based discovery of potent, selective, and brain penetrant N-(1H-pyrazol-3-yl)pyridin-2-amine inhibitors of dual leucine Zzpper kinase (DLK, MAP3K12). J Med Chem 58**:** 8182-8199.

Peel AL (2004). PKR activation in neurodegenerative disease. J Neuropathol Exp Neurol 63**:** 97-105.

Piepers S, Veldink JH, de Jong SW, van der Tweel I, van der Pol WL, Uijtendaal EV, . . . van den Berg LH (2009). Randomized sequential trial of valproic acid in amyotrophic lateral sclerosis. Ann Neurol 66**:** 227-234.

Piette F, Belmin J, Vincent H, Schmidt N, Pariel S, Verny M, . . . Hermine O (2011). Masitinib as an adjunct therapy for mild-to-moderate Alzheimer's disease: a randomised, placebo-controlled phase 2 trial. Alzheimers Res Ther 3**:** 16.

Pignataro G, Capone D, Polichetti G, Vinciguerra A, Gentile A, Di Renzo G, & Annunziato L (2011). Neuroprotective, immunosuppressant and antineoplastic properties of mTOR inhibitors: current and emerging therapeutic options. Curr Opin Pharmacol 11**:** 378-394.

Pizzasegola C, Caron I, Daleno C, Ronchi A, Minoia C, Carri MT, & Bendotti C (2009). Treatment with lithium carbonate does not improve disease progression in two different strains of SOD1 mutant mice. Amyotroph Lateral Scler 10**:** 221-228.

Posa D, Martinez-Gonzalez L, Bartolome F, Nagaraj S, Porras G, Martinez A, & Martin-Requero A (2019). Recapitulation of pathological TDP-43 features in immortalized lymphocytes from sporadic ALS patients. Mol Neurobiol 56**:** 2424-2432.

Pradhan J, Noakes PG, & Bellingham MC (2019). The role of altered BDNF/TrkB signaling in amyotrophic lateral sclerosis. Front Cell Neurosci 13**:** 368.

Reber L, Da Silva CA, & Frossard N (2006). Stem cell factor and its receptor c-Kit as targets for inflammatory diseases. Eur J Pharmacol 533**:** 327-340.

Remsing Rix LL, Rix U, Colinge J, Hantschel O, Bennett KL, Stranzl T, . . . Superti-Furga G (2009). Global target profile of the kinase inhibitor bosutinib in primary chronic myeloid leukemia cells. Leukemia 23**:** 477-485.

Riancho J, Gil-Bea FJ, Castanedo-Vazquez D, Sedano MJ, Zufiria M, de Eulate GFG, . . . de Munain AL (2018). Clinical evidences supporting the Src/c-Abl pathway as potential therapeutic target in amyotrophic lateral sclerosis. J Neurol Sci 393**:** 80-82.

Roskoski R, Jr. (2019). Properties of FDA-approved small molecule protein kinase inhibitors. Pharmacol Res 144**:** 19-50.

Roskoski R, Jr. (2020). Properties of FDA-approved small molecule protein kinase inhibitors: A 2020 update. Pharmacol Res 152**:** 104609.

Ryu HH, Jun MH, Min KJ, Jang DJ, Lee YS, Kim HK, & Lee JA (2014). Autophagy regulates amyotrophic lateral sclerosis-linked fused in sarcoma-positive stress granules in neurons. Neurobiol Aging 35**:** 2822-2831.

Salado IG, Redondo M, Bello ML, Perez C, Liachko NF, Kraemer BC, . . . Perez DI (2014). Protein kinase CK-1 inhibitors as new potential drugs for amyotrophic lateral sclerosis. J Med Chem 57**:** 2755-2772.

Sama RR, Fallini C, Gatto R, McKeon JE, Song Y, Rotunno MS, . . . Bosco DA (2017). ALS-linked FUS exerts a gain of toxic function involving aberrant p38 MAPK activation. Sci Rep 7**:** 115.

Sato S, Cerny RL, Buescher JL, & Ikezu T (2006). Tau-tubulin kinase 1 (TTBK1), a neuron-specific tau kinase candidate, is involved in tau phosphorylation and aggregation. J Neurochem 98**:** 1573-1584.

Schittek B, & Sinnberg T (2014). Biological functions of casein kinase 1 isoforms and putative roles in tumorigenesis. Mol Cancer 13**:** 231.

Shi Y, & Mader M (2018). Brain penetrant kinase inhibitors: Learning from kinase neuroscience discovery. Bioorg Med Chem Lett 28**:** 1981-1991.

Simmons DL (2013). Targeting kinases: a new approach to treating inflammatory rheumatic diseases. Curr Opin Pharmacol 13**:** 426-434.

Siu M, Sengupta Ghosh A, & Lewcock JW (2018). Dual leucine zipper kinase inhibitors for the treatment of neurodegeneration. J Med Chem 61**:** 8078-8087.

Sreedharan J, Neukomm LJ, Brown RH, Jr., & Freeman MR (2015). Age-dependent TDP-43-mediated motor neuron degeneration requires GSK3, hat-trick, and xmas-2. Curr Biol 25**:** 2130-2136.

Stankov K, Popovic S, & Mikov M (2014). C-KIT signaling in cancer treatment. Curr Pharm Des 20**:** 2849-2880.

Steinberg SF (2008). Structural basis of protein kinase C isoform function. Physiol Rev 88**:** 1341-1378.

Strong MJ, Kesavapany S, & Pant HC (2005). The pathobiology of amyotrophic lateral sclerosis: a proteinopathy? J Neuropathol Exp Neurol 64**:** 649-664.

Sugai F, Yamamoto Y, Miyaguchi K, Zhou Z, Sumi H, Hamasaki T, . . . Sakoda S (2004). Benefit of valproic acid in suppressing disease progression of ALS model mice. Eur J Neurosci 20**:** 3179-3183.

Sulzmaier FJ, Jean C, & Schlaepfer DD (2014). FAK in cancer: mechanistic findings and clinical applications. Nat Rev Cancer 14**:** 598-610.

Sun J, Mu Y, Jiang Y, Song R, Yi J, Zhou J, . . . Xu X (2018). Inhibition of p70 S6 kinase activity by A77 1726 induces autophagy and enhances the degradation of superoxide dismutase 1 (SOD1) protein aggregates. Cell Death Dis 9**:** 407.

Sun J, & Nan G (2017). The extracellular signal-regulated kinase 1/2 pathway in neurological diseases: A potential therapeutic target (Review). Int J Mol Med 39**:** 1338-1346.

Suzuki H, & Matsuoka M (2013). The JNK/c-Jun signaling axis contributes to the TDP-43-induced cell death. Mol Cell Biochem 372**:** 241-248.

Takata M, Tanaka H, Kimura M, Nagahara Y, Tanaka K, Kawasaki K, . . . Hara H (2013). Fasudil, a rho kinase inhibitor, limits motor neuron loss in experimental models of amyotrophic lateral sclerosis. Br J Pharmacol 170**:** 341-351.

Taniguchi J, Seki C, Takuwa H, Kawaguchi H, Ikoma Y, Fujinaga M, . . . Ito H (2014). Evaluation of Rho-kinase activity in mice brain using N-[11C]methyl-hydroxyfasudil with positron emission tomography. Mol Imaging Biol 16**:** 395-402.

Tatenhorst L, Eckermann K, Dambeck V, Fonseca-Ornelas L, Walle H, Lopes da Fonseca T, . . . Lingor P (2016). Fasudil attenuates aggregation of alpha-synuclein in models of Parkinson's disease. Acta Neuropathol Commun 4**:** 39.

Taylor JP, Brown RH, Jr., & Cleveland DW (2016). Decoding ALS: from genes to mechanism. Nature 539**:** 197-206.

Taylor LM, McMillan PJ, Liachko NF, Strovas TJ, Ghetti B, Bird TD, . . . Kraemer BC (2018). Pathological phosphorylation of tau and TDP-43 by TTBK1 and TTBK2 drives neurodegeneration. Mol Neurodegener 13**:** 7.

Tejeda GS, & Diaz-Guerra M (2017). Integral characterization of defective BDNF/TrkB signalling in neurological and psychiatric disorders leads the way to new therapies. Int J Mol Sci 18.

Tönges L, Frank T, Tatenhorst L, Saal KA, Koch JC, Szego EM, . . . Lingor P (2012). Inhibition of rho kinase enhances survival of dopaminergic neurons and attenuates axonal loss in a mouse model of Parkinson's disease. Brain 135**:** 3355-3370.

Tönges L, Günther R, Suhr M, Jansen J, Balck A, Saal KA, . . . Lingor P (2014). Rho kinase inhibition modulates microglia activation and improves survival in a model of amyotrophic lateral sclerosis. Glia 62**:** 217-232.

Torcia M, De Chiara G, Nencioni L, Ammendola S, Labardi D, Lucibello M, . . . Cozzolino F (2001). Nerve growth factor inhibits apoptosis in memory B lymphocytes via inactivation of p38 MAPK, prevention of Bcl-2 phosphorylation, and cytochrome c release. J Biol Chem 276**:** 39027-39036.

Trias E, Ibarburu S, Barreto-Nunez R, Babdor J, Maciel TT, Guillo M, . . . Barbeito L (2016). Post-paralysis tyrosine kinase inhibition with masitinib abrogates neuroinflammation and slows disease progression in inherited amyotrophic lateral sclerosis. J Neuroinflammation 13**:** 177.

Trias E, Ibarburu S, Barreto-Nunez R, Varela V, Moura IC, Dubreuil P, . . . Barbeito L (2017). Evidence for mast cells contributing to neuromuscular pathology in an inherited model of ALS. JCI Insight 2.

Trias E, King PH, Si Y, Kwon Y, Varela V, Ibarburu S, . . . Barbeito L (2018). Mast cells and neutrophils mediate peripheral motor pathway degeneration in ALS. JCI Insight 3.

U KMND-LiCALS Study Group, Morrison KE, Dhariwal S, Hornabrook R, Savage L, Burn DJ, . . . Majid T (2013). Lithium in patients with amyotrophic lateral sclerosis (LiCALS): a phase 3 multicentre, randomised, double-blind, placebo-controlled trial. Lancet Neurol 12**:** 339-345.

Van Hoecke A, Schoonaert L, Lemmens R, Timmers M, Staats KA, Laird AS, . . . Robberecht W (2012). EPHA4 is a disease modifier of amyotrophic lateral sclerosis in animal models and in humans. Nat Med 18**:** 1418-1422.

Vanacore N, & Galeotti F (2008). A clinical specification for a randomized clinical trial on lithium in amyotrophic lateral sclerosis. Proc Natl Acad Sci U S A 105**:** E35.

Vanotti E, Amici R, Bargiotti A, Berthelsen J, Bosotti R, Ciavolella A, . . . Santocanale C (2008). Cdc7 kinase inhibitors: pyrrolopyridinones as potential antitumor agents. 1. Synthesis and structure-activity relationships. J Med Chem 51**:** 487-501.

Vermersch P, Benrabah R, Schmidt N, Zephir H, Clavelou P, Vongsouthi C, . . . Hermine O (2012). Masitinib treatment in patients with progressive multiple sclerosis: a randomized pilot study. BMC Neurol 12**:** 36.

Verstraete E, Veldink JH, Huisman MH, Draak T, Uijtendaal EV, van der Kooi AJ, . . . van den Berg LH (2012). Lithium lacks effect on survival in amyotrophic lateral sclerosis: a phase IIb randomised sequential trial. J Neurol Neurosurg Psychiatry 83**:** 557-564.

Vielhaber E, Eide E, Rivers A, Gao ZH, & Virshup DM (2000). Nuclear entry of the circadian regulator mPER1 is controlled by mammalian casein kinase I epsilon. Mol Cell Biol 20**:** 4888-4899.

Villanueva MT (2017). Neurodegenerative disease: DLK zips across neurodegeneration. Nat Rev Drug Discov 16**:** 678-679.

Wang IF, Guo BS, Liu YC, Wu CC, Yang CH, Tsai KJ, & Shen CK (2012). Autophagy activators rescue and alleviate pathogenesis of a mouse model with proteinopathies of the TAR DNA-binding protein 43. Proc Natl Acad Sci U S A 109**:** 15024-15029.

Wang W, Bu B, Xie M, Zhang M, Yu Z, & Tao D (2009). Neural cell cycle dysregulation and central nervous system diseases. Prog Neurobiol 89**:** 1-17.

Watanabe T, Kakeno M, Matsui T, Sugiyama I, Arimura N, Matsuzawa K, . . . Kaibuchi K (2015). TTBK2 with EB1/3 regulates microtubule dynamics in migrating cells through KIF2A phosphorylation. J Cell Biol 210**:** 737-751.

Watts ME, Wu C, & Rubin LL (2019). Suppression of MAP4K4 signaling ameliorates motor neuron degeneration in amyotrophic lateral sclerosis-molecular studies toward new therapeutics. J Exp Neurosci 13**:** 1179069519862798.

Weisel K, Berger S, Papp K, Maari C, Krueger JG, Scott N, . . . Tak PP (2020). Response to inhibition of receptor-interacting protein kinase 1 (RIPK1) in active plaque psoriasis: a randomized placebo-controlled study. Clin Pharmacol Ther.

Weisner J, Gontla R, van der Westhuizen L, Oeck S, Ketzer J, Janning P, . . . Rauh D (2015). Covalent-allosteric kinase inhibitors. Angew Chem Int Ed Engl 54**:** 10313-10316.

Wheaton MW, Salamone AR, Mosnik DM, McDonald RO, Appel SH, Schmolck HI, . . . Schulz PE (2007). Cognitive impairment in familial ALS. Neurology 69**:** 1411-1417.

Wu B, De SK, Kulinich A, Salem AF, Koeppen J, Wang R, . . . Pellecchia M (2017). Potent and selective EphA4 agonists for the treatment of ALS. Cell Chem Biol 24**:** 293-305.

Wu C, Watts ME, & Rubin LL (2019). MAP4K4 activation mediates motor neuron degeneration in amyotrophic lateral sclerosis. Cell Rep 26**:** 1143-1156.

Wu P, Clausen MH, & Nielsen TE (2015). Allosteric small-molecule kinase inhibitors. Pharmacol Ther 156**:** 59-68.

Xia Q, Hu Q, Wang H, Yang H, Gao F, Ren H, . . . Wang G (2015). Induction of COX-2-PGE2 synthesis by activation of the MAPK/ERK pathway contributes to neuronal death triggered by TDP-43-depleted microglia. Cell Death Dis 6**:** e1702.

Xu D, Jin T, Zhu H, Chen H, Ofengeim D, Zou C, . . . Yuan J (2018). TBK1 suppresses RIPK1-driven apoptosis and inflammation during development and in aging. Cell 174**:** 1477-1491 e1419.

Xue Y, Wan PT, Hillertz P, Schweikart F, Zhao Y, Wissler L, & Dekker N (2013). X-ray structural analysis of tau-tubulin kinase 1 and its interactions with small molecular inhibitors. ChemMedChem 8**:** 1846-1854.

Yang H, Rudge DG, Koos JD, Vaidialingam B, Yang HJ, & Pavletich NP (2013). mTOR kinase structure, mechanism and regulation. Nature 497**:** 217-223.

Yang W, Leystra-Lantz C, & Strong MJ (2008). Upregulation of GSK3beta expression in frontal and temporal cortex in ALS with cognitive impairment (ALSci). Brain Res 1196**:** 131-139.

Yang YM, Gupta SK, Kim KJ, Powers BE, Cerqueira A, Wainger BJ, . . . Rubin LL (2013). A small molecule screen in stem-cell-derived motor neurons identifies a kinase inhibitor as a candidate therapeutic for ALS. Cell Stem Cell 12**:** 713-726.

Yanpallewar SU, Barrick CA, Buckley H, Becker J, & Tessarollo L (2012). Deletion of the BDNF truncated receptor TrkB.T1 delays disease onset in a mouse model of amyotrophic lateral sclerosis. PLoS One 7**:** e39946.

Yarza R, Vela S, Solas M, & Ramirez MJ (2015). c-Jun N-terminal kinase (JNK) signaling as a therapeutic target for Alzheimer's disease. Front Pharmacol 6**:** 321.

Yuan J, Amin P, & Ofengeim D (2019). Necroptosis and RIPK1-mediated neuroinflammation in CNS diseases. Nat Rev Neurosci 20**:** 19-33.

Zhang X, Li L, Chen S, Yang D, Wang Y, Zhang X, . . . Le W (2011). Rapamycin treatment augments motor neuron degeneration in SOD1(G93A) mouse model of amyotrophic lateral sclerosis. Autophagy 7**:** 412-425.