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REVIEW ARTICLE

# The multifaceted role of kinases in amyotrophic lateral sclerosis: genetic, pathological and therapeutic implications

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Amyotrophic lateral sclerosis is the most common degenerative disorder of motor neurons in adults. As there is no cure, thousands of individuals who are alive at present will succumb to the disease. In recent years, numerous causative genes and risk factors for amyotrophic lateral sclerosis have been identified. Several of the recently identified genes encode kinases. In addition, the hypothesis that (de)phosphorylation processes drive the disease process resulting in selective motor neuron degeneration in different disease variants has been postulated. We re-evaluate the evidence for this hypothesis based on recent findings and discuss the multiple roles of kinases in amyotrophic lateral sclerosis pathogenesis. We propose that kinases could represent promising therapeutic targets. Mainly due to the comprehensive regulation of kinases, however, a better understanding of the disturbances in the kinome network in amyotrophic lateral sclerosis is needed to properly target specific kinases in the clinic.

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**Keywords:** kinase; amyotrophic lateral sclerosis; motor neuron; phosphorylation

**Abbreviations:** ALS = amyotrophic lateral sclerosis; AMPK = AMP activated kinase; DNA-PK = DNA-dependent protein kinase; ER = endoplasmic reticulum; GSK3β = glycogen synthase kinase 3β; iPSC = induced pluripotent stem cell; JNK = c-Jun N-terminal kinases; Miro1 = mitochondrial Rho GTPase 1; MOK = MAPK/MAK/MRK overlapping kinase; NF-M/H = neurofilament medium/heavy chain; p38 MAPK = p38 mitogen-activated protein kinases; PERK = protein kinase R-like endoplasmic reticulum kinase; PtdIns(3,5)P2 = phosphatidylinositol (3,5)-bisphosphate; TDP-43 = TAR DNA-binding protein 43

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# Introduction

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease characterized by the selective and progressive degeneration of motor neurons in the brain, brainstem and spinal cord. Motor neuron deterioration leads to muscle weakness and results in death of the patient due to respiratory failure typically within 3 to 5 years after diagnosis. The life time risk of developing ALS is estimated to be ~1 in 400 (Alonso *et al.*, 2009).

In most patients, ALS begins at ~50–60 years of age with asymmetric, painless weakness in one limb. Initially, abnormalities can be focal, with the disease spreading relentlessly over time. Nevertheless, ALS is a heterogeneous disorder. Time of disease onset varies between the first and the seventh decade of life and there are clear differences in location of symptom onset, rate of disease progression, and degree of cognitive impairments (reviewed in Swinnen and Robberecht, 2014). Besides variations in clinical presentation, there is also considerable variation in disease aetiology. Approximately 10% of patients report a family member also diagnosed with ALS (familial ALS), strongly supporting a direct genetic cause of the disease. However, 90% of patients suffer from the sporadic form of the disease (sporadic ALS), as no family member was ever diagnosed with ALS. Therefore, considerable efforts to identify environmental factors causing ALS were made, but these were unsuccessful until now. This supports the idea that for most ALS cases, including sporadic ALS, there might be an important genetic contribution (Simpson and Al-Chalabi, 2006).

A cascade of different processes could result in motor neuron death, independent of the exact cause of each ALS case. One of the processes that could drive selective motor neuron degeneration is the aberrant regulation of kinases (Hu *et al.*, 2003; Krieger *et al.*, 2003). The beneficial effects of inhibiting the Src/c-Abl pathway in multiple models of ALS suggests that kinases can closely regulate shared downstream processes in ALS pathogenesis (Katsumata *et al.*, 2012; Wenqiang *et al.*, 2014; Imamura *et al.*, 2017). A compound screening based on a survival assay of induced pluripotent stem cell (iPSC) derived motor neurons from ALS patients (Imamura *et al.*, 2017) also identified the Src/c-Abl as a major pathway related to motor neuron survival (Imamura *et al.*, 2017). In addition, genetic knockdown and pharmacological inhibition of Src/c-Abl rescued the degeneration of iPSC-derived motor neurons from patients with familial ALS mutations in *SOD1*, *C9orf72*, *TARDBP*, and from sporadic ALS patients (Imamura *et al.*, 2017). In line with these *in vitro* observations, Src/c-Abl inhibitors also attenuated ALS phenotypes both in mutant SOD1 and in TDP-43 transgenic mice (Katsumata *et al.*, 2012; Wenqiang *et al.*, 2014). Moreover, phosphorylation of Src/c-Abl was increased in post-mortem spinal cord tissue from ALS patients (Katsumata *et al.*, 2012) indicating that this pathway could also play a role in motor neurons from ALS patients. Taken together, the beneficial effects obtained by inhibiting the Src/c-Abl pathway indicate that a shared downstream kinome pathway could be involved in the selective death of motor neurons in ALS.

Aberrant phosphorylation of various ALS-related proteins (e.g. SOD1 TDP-43 and FUS) by kinases could affect the localization and function of these proteins. Furthermore, kinases play a pivotal role in biochemical reactions involved in protein, lipid and nucleotide metabolism (Box 1). However, the exact role these alterations play in motor neuron degeneration remains elusive. In addition, multiple studies showed that mutations in genes encoding different kinases can cause or confer susceptibility to ALS, suggesting that alterations in the function of specific kinases and/or their downstream targets are vital to (motor) neuron survival (Freischmidt *et al.*, 2015; Higelin *et al.*, 2018). In this review, we provide a focused update on the potential role of kinases in ALS genetics and pathophysiology in view of some recent data.

# ALS genetics: the emerging role of kinases

Since the discovery that mutations in the superoxide dismutase 1 (*SOD1*) gene cause ALS (Rosen *et al.*, 1993), a multitude of genes have been linked to ALS (reviewed in Volk *et al.*, 2018) Remarkably, the gene products can be grouped into a few biological processes: proteostasis (*UBQLN2*, *VCP*, *OPTN*, *TBK1*, *C9orf72*, *VAPB*), RNA metabolism (*FUS*, *TARDBP*, *HNRNPA1*, *MATR3*), and cytoskeletal dynamics (*PFN1*, *TUBA4A*, *KIF5A*, *DCTN1*). This indicates the potential importance of these processes in ALS pathobiology, especially when considering loss-of-function mutations in these genes (Taylor *et al.*, 2016). While mutations in several genes are associated with ALS, point mutations in *SOD1*, *TARDBP*, *FUS*, or a hexanucleotide repeat expansion in *C9orf72* explain more than half of the familial ALS cases (Fig. 1 and Box 2) (Taylor *et al.*, 2016). Recently developed sequencing techniques resulted in multiple new discoveries, and suggest also polygenetic modes of inheritance (Oskarsson *et al.*, 2018). Several of these newly discovered ALS-causing or ALS risk genes encode protein kinases.

## TBK1

*TBK1* encodes TANK-binding kinase, a serine/threonine kinase interacting with proteins involved in the innate immune response and autophagy (Pottier *et al.*, 2015). Genetic variants in *TBK1* are associated with glaucoma (Traynis *et al.*, 2014) and herpes (Herman *et al.*, 2012). In addition, two independent whole exosome and whole genome sequencing studies linked different mutations in *TBK1* to ALS (Fig. 1) (Cirulli *et al.*, 2015; Freischmidt *et al.*, 2015). Mutations in *TBK1*, as identified in ALS, caused a loss of kinase function (de Majo *et al.*, 2018) and TBK1 knockout mice showed dendritic swellings, abnormally shaped astrocytes, and p62-

## Box 1 Classification of kinases

- Kinases are transferases catalysing the addition of a phosphate group ($PO_4^{3-}$) to hydroxyl groups of various substrates including lipids, nucleic acids, and amino acids. The phosphate normally originates from adenosine triphosphate (ATP). Phosphorylation is involved in nearly all signal transduction processes, and thus kinases play a pivotal role in regulating cellular metabolism, cell cycle, transport, secretory processes, and many other pathways (for a review see Rask-Andersen *et al.*, 2014).
- The total number of kinases is very large; more than 900 genes in the human genome encode kinases. Kinases are classified based on their substrates and functions. Based on the substrate, kinases can be classified as protein kinases, lipid kinases and nucleotide kinases (Rask-Andersen *et al.*, 2014).
- Protein kinases are the largest category of kinases with more than 500 encoding genes identified. These kinases are responsible for phosphorylating amino acids. Based on its specificity, protein kinases can be categorized into four classes: (i) protein-histidine kinases, which phosphorylate histidine residue; (ii) protein-tyrosine kinases, which phosphorylate tyrosine residue; (iii) protein-serine/threonine kinases, which phosphorylate serine and/or threonine residues; and (iv) dual-specificity kinases, which phosphorylate both tyrosine and serine/threonine residues. Protein tyrosine kinases can be classified into two additional subtypes: the receptor-type and the non-receptor-type based on the function of the substrates (Rask-Andersen *et al.*, 2014).
- Lipid kinases are a group of kinases that are responsible for phosphorylating lipid molecules. These lipids comprise membrane structures including the plasma membrane, as well as the membranes of the organelles. Inositol is phosphorylated by lipid kinases to generate phosphoinositol and phosphoinositide lipids. The lipid phosphorylation process is involved in the membrane signal transmission throughout the endomembrane system.
- Nucleotide kinases are responsible for the phosphorylation of nucleic acids that are the basic units of RNA and DNA. In RNA and DNA polymers, the backbone is composed of repeating phospho-ribose units. Kinases transfer the phosphate to the nucleoside, creating a nucleotide monophosphate. This process is also involved in regulating the synthesis of nucleotides.

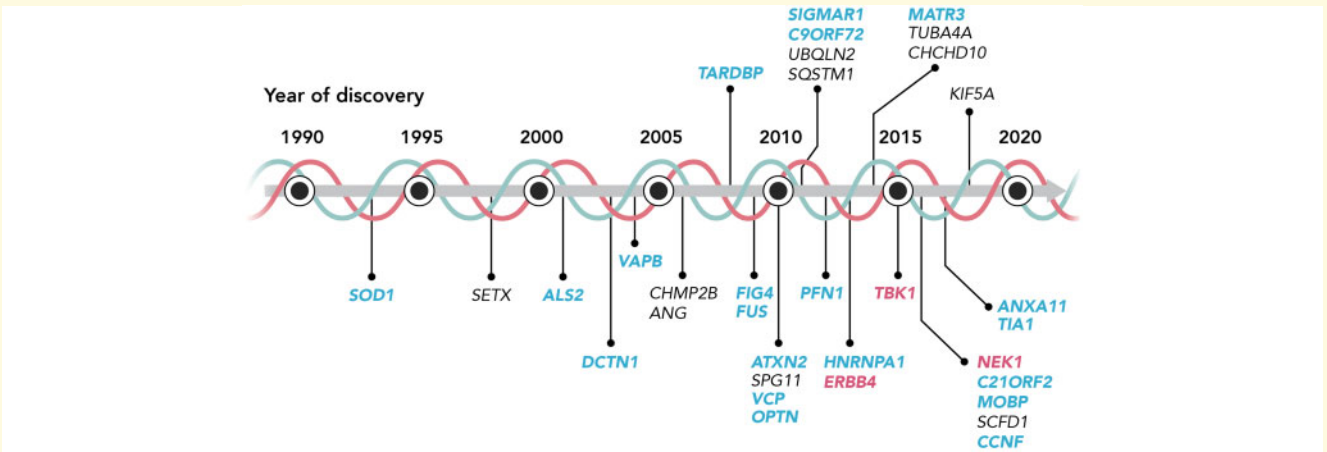


**Figure 1 ALS genetics, the emerging role of kinases.** Chronological overview of the discovery of gene mutations involved in ALS. Genes in blue are ALS genes of which the gene product could interact with kinases pathologically. Genes in pink are encoding kinases. Each of these gene mutations is reported in more than one ALS-affected family, or in multiple, unrelated cases of sporadic ALS.

and ubiquitin-positive aggregates in the cerebellum (Duan *et al.*, 2019). Interestingly, families with *TBK1* mutations showed an increased risk to develop cognitive defects in addition to their motor symptoms (Oakes *et al.*, 2017), and ALS patients with *TBK1* mutations displayed a bulbar onset more frequently (van der Zee *et al.*, 2017). Post-mortem neuropathological analysis of *TBK1* mutation carriers showed massive TDP-43-positive perinuclear inclusions in temporal lobe neurons, but not in the spinal cord, and showed p62/sequestosome 1 (SQSTM1)-positive perinuclear inclusion in the right para-hippocampal gyrus (van der Zee *et al.*, 2017). p62/SQSTM1 is encoded by the ALS gene *SQSTM1* (Fecto *et al.*, 2011), and acts as a major autophagy receptor (Aparicio *et al.*, 2019). Interestingly, TBK1 phosphorylates p62/SQSTM1 to ensure its binding to polyubiquitinated proteins and to efficiently target these proteins for degradation in autophagosomes (Matsumoto *et al.*, 2015). As most patients with ALS displayed p62/SQSTM1 positive cytoplasmic inclusions (Teyssou *et al.*, 2013), and as TBK1 was an inducer of type-1 interferons and affected autophagy

### Box 2 Main ALS genes and pathology

- At present, mutations in four genes are linked to classical ALS and explain up to 80% of familial ALS cases. More than 25 years ago, linkage analysis identified dominant missense mutations in the *SOD1* gene as the first genetic cause for ALS (Rosen *et al.*, 1993). This Cu-Zn superoxide dismutase is an abundant, ubiquitously expressed, cytoplasmic enzyme that catalyses the conversion of highly reactive superoxide into hydrogen peroxide. More than 170 *SOD1* mutations were reported occurring in ~12% of familial ALS and ~2% of sporadic ALS cases (for a review see Renton *et al.*, 2014). The most commonly used mouse model for ALS is the mutant $SOD1^{G93A}$ mouse (Gurney *et al.*, 1994). This transgenic model develops adult-onset neurodegeneration of spinal motor neurons and progressive motor deficits leading to paralysis (Gurney *et al.*, 1994).
- An important discovery in the context of ALS was the identification of the TDP-43 protein as the major constituent of ubiquitin-positive neuronal inclusions (Neumann *et al.*, 2006). This was followed by the discovery of mutations in the *TARDBP* gene encoding TDP-43 (Gitcho *et al.*, 2008; Sreedharan *et al.*, 2008). *TARDBP* mutations are relatively rare and it is estimated that ~4% of familial ALS patients and only a small percentage of sporadic ALS cases is caused by these mutations (Renton *et al.*, 2014). As TDP-43 plays an important role in DNA/RNA metabolism, the discovery of TDP-43 in ALS pathogenesis highlighted the importance of RNA processing in ALS (for a review see Ling *et al.*, 2013).
- Soon after the discovery of the *TARDBP* mutations, mutations in *FUS*, another gene encoding a DNA/RNA-binding protein, were discovered (Kwiatkowski *et al.*, 2009; Vance *et al.*, 2009). *FUS* mutations are also responsible for a small subset of ALS cases. It is estimated that they account in the Western world for 4% and 1% of familial ALS and sporadic ALS, respectively (Kwiatkowski *et al.*, 2009; Vance *et al.*, 2009; Van Damme *et al.*, 2010). ALS mutations in *FUS* are mainly in the N-terminal low-complexity domain and in the highly-conserved C-terminal nuclear localization signal (NLS) (Ling *et al.*, 2013). They lead to the mislocalization of FUS to the cytoplasm and this results in the formation of cytoplasmic FUS inclusions (Dormann *et al.*, 2010).
- More recently, an intronic hexanucleotide (GGGGCC) tandem repeat expansion was identified in the *C9orf72* gene (DeJesus-Hernandez *et al.*, 2011; Renton *et al.*, 2011), which is the most common cause of ALS explaining ~40% of familial ALS and ~7% of sporadic ALS cases in the Western world (for a review see Renton *et al.*, 2014). While control individuals usually harbour two to eight repeats, patients have more than 30, and up to hundreds or even thousands (Renton *et al.*, 2014). The exact underlying pathological mechanism remains elusive, although a combination of loss-of-function and gain-of-function was suggested (Shi *et al.*, 2018).

and mitophagy (de Majo *et al.*, 2018), it is possible that reduced TBK1-mediated p62/SQSTM1 phosphorylation disrupts cellular proteostasis (Vinet and Zhedanov, 2011). As a consequence, disturbed autophagy could (partially) explain the *TBK1*-related pathophysiology in ALS. An additional ALS-related protein that is also a target of TBK1 is optineurin. This protein is encoded by the *OPTN* gene, is highly abundant, and is involved in the inflammatory response, autophagy, Golgi maintenance, and vesicular transport. Recessive mutations in *OPTN* are considered as a rare genetic cause of ALS (Richter *et al.*, 2016). In view of the loss of kinase function from *TBK1* mutations identified in ALS (de Majo *et al.*, 2018), it is likely that this also affects the ubiquitin-directed breakdown of aggregates through decreased optineurin targeting (Li *et al.*, 2018).

Based on the targets of TBK1 (e.g. p62/SQSTM1 and optineurin) and because ALS-causing *TBK1* mutations result in a loss of kinase function, we hypothesize that the impaired kinase function of TBK1 induces impairments in the clearance of proteins by autophagy or by the ubiquitin proteasome system, thereby contributing to the motor neuron degeneration. These mechanisms may act alone or in combination with other affected processes. Therapeutically stimulating the kinase function of TBK1 may be beneficial. However, more studies are needed to find out the exact therapeutic potential of TBK1 modulation in ALS, eventually also in those ALS patients without *TBK1* mutations.

## NEK1

Another kinase associated with ALS is NIMA related kinase 1 (*NEK1*) (Brenner *et al.*, 2016; Kenna *et al.*, 2016). Recently, different *NEK1* variants have been identified in both familial and sporadic ALS (Kenna *et al.*, 2016; Gratten *et al.*, 2017; Nguyen *et al.*, 2018; Shu *et al.*, 2018; Tripolszki *et al.*, 2019). *NEK1* risk variants occur in ~3 to 5% of ALS cases, although no ALS pedigrees have been identified with a clear segregation of *NEK1* mutations with the disease (Nguyen *et al.*, 2018). While most of the variants are missense variants, a large proportion of *NEK1* variants lead to a loss-of-function (Nguyen *et al.*, 2018). *NEK1* variants are either heterozygous or homozygous in ALS patients (Shu *et al.*, 2018; Goldstein *et al.*, 2019), and often occur in ALS patients with a mutation in another ALS gene, such as *SOD1*, *C9orf72*, *TUBA4A*, or *TARDBP* (Nguyen *et al.*, 2018; Shu *et al.*, 2018).

The NEK1 protein contains an N-terminal kinase domain and an extended C-terminal domain with several predicted coiled-coil regions interacting with other proteins (Melo-Hanchuk *et al.*, 2017). Although NEK1 contains two

classical nuclear localization signals (NLS), the full length protein exclusively localizes to the cytoplasm, as the cytoplasmic localization signal originates from the extended C-terminal domain (Feige *et al.*, 2006). Interestingly, a short protein fragments with its C-terminal ending at the first NLS has also been identified and this truncated version of NEK1 enters the nucleus (Feige *et al.*, 2006). Overexpression of these nuclear NEK1 isoform caused abnormal chromatin condensation and dispersal of the nuclear pore complex (Feige *et al.*, 2006). Therefore, a (genetic) modification of NEK1 might affect chromatin modifications and DNA stability, as well as several other cellular functions, including cilia formation, DNA-damage response, microtubule stability, neuronal morphology and axonal polarity (reviewed in Nguyen *et al.*, 2018). This is supported by the phenotype of the NEK1 null mice suffering from developmental abnormalities including pleiotropic malfunctions, including facial dysmorphism, male sterility, dwarfism and anaemia, although no neurodegeneration was reported (Upadhya *et al.*, 2000). Human fibroblasts from ALS patients with homozygous NEK1 truncations showed abnormalities in cilia number, cilia structure and microtubule stability (Kenna *et al.*, 2016). Moreover, *in vitro* silencing of *NEK1* led to distorted neuronal morphology with disturbed polarity and deacetylation of microtubules via histone deacetylase 6 (HDAC6) and to disrupted microtubule stability and growth (Chang *et al.*, 2009; Cohen *et al.*, 2013). Besides neuronal morphology and axonal polarity, NEK1 also regulates cellular viability and the permeability of the mitochondrial membrane through phosphorylation of the voltage-dependent anion channel 1 (VDAC1) (Chen *et al.*, 2009). In addition, compromised NEK1 expression in patient-derived cells showed increased DNA damage which was accompanied by the deregulation of the cell cycle (Higelin *et al.*, 2018). Interestingly, NEK1 can also interact with multiple ALS-related gene products, including alsin, VAPB and C21ORF2 (Nguyen, *et al.*, 2018). One example is the interaction of NEK1 with C21ORF2, which is needed for efficient DNA damage repair responses (Fang *et al.*, 2015).

While the above information highlights potential mechanisms by which variants in *NEK1* might affect motor neuron viability in ALS, it is currently unclear which of these processes is involved in motor neuron degeneration and/or whether these are viable therapeutic targets. The generation of NEK1-ALS patient-derived iPSCs and subsequent motor neuron studies could aid in gaining a better understanding of this.

## ERBB4

Mutations in *ERBB4* have been identified in ALS patients (Takahashi *et al.*, 2013), although these findings have not (yet) been replicated. However, the modifying role of ERBB4 and neuregulin 1 in ALS has been extensively investigated (Takahashi *et al.*, 2013; Mancuso *et al.*, 2016; Mòdol-Caballero *et al.*, 2017). Erb-B2 receptor tyrosine kinase 4 (ERBB4) is a tyrosine kinase receptor that is able to activate multiple signal transduction cascades including the mitogen-activated protein kinase (MAPK), Agrin/MuSK, mTORC1, and STAT pathways (Trinidad *et al.*, 2000; Eto *et al.*, 2010; Sundvall *et al.*, 2012; Nie *et al.*, 2018). ERBB4 plays a role in various biological processes, including neurodevelopment. It belongs to the epidermal growth factor (EGF) subfamily of receptor tyrosine kinases (RTKs) and can be activated upon binding of neuregulins (NRGs) to the extracellular ligand-binding domain (Takahashi *et al.*, 2013). In primate brain, expression of both full length and shorter fragments of ERBB4 was widely found in neuronal soma and nucleus throughout the brain of juvenile and adult primates, which could indicate a regulatory role for the ERBB4/NRG pathway in the CNS (Thompson *et al.*, 2007). *ERBB4* mutations identified in ALS patients decreased the auto-phosphorylation of ERBB4 upon neuregulin 1 stimulation *in vitro* (Takahashi *et al.*, 2013). As a transmembrane receptor tyrosine kinase, ERBB4 binds to neuregulin 1 activating its signalling, and an impaired neuregulin ERBB4 pathway is involved in the pathogenesis of ALS (Takahashi *et al.*, 2013; Mancuso *et al.*, 2016; Mòdol-Caballero *et al.*, 2017). The shortest ectodomain fragments of ERBB4 are generated in the presence of neuregulin 1 (Lopez-Font *et al.*, 2019). Interestingly, ERBB4 ectodomain fragments were decreased in the CSF from ALS patients, as well as in the plasma of $SOD1^{G93A}$ and $TDP\text{-}43^{A315T}$ mice, indicating an involvement of ERBB4 in different ALS subtypes (Lopez-Font *et al.*, 2019).

ALS is considered as a 'dying back' or 'distal axonopathy', in which the first pathological changes occur at the neuromuscular junction (NMJ) prior to motor neuron degeneration and the onset of clinical symptoms (Campanari *et al.*, 2016). NRG1 is mainly produced by neurons and muscles and it mediates the crosstalk between terminal Schwann cells and the peripheral motor axon at the endplate. This process is tightly regulated by the ERBB receptor family including ERBB3, ERBB4 and their co-receptors ERBB1 and ERBB2 (Morano *et al.*, 2018). ERBB4 is especially enriched in the neuromuscular junctions and inhibition of ERBB4 impaired neuromuscular development in zebrafish embryos (Paatero *et al.*, 2019). This is in line with results showing that loss-of-function mutations in *ERBB4* could likely be the cause of autosomal-dominant ALS (Takahashi *et al.*, 2013). Furthermore, the expression of NRG1 type III isoform was reduced in both ALS patients and $SOD1^{G93A}$ mice in parallel with motor neuron loss (Lasiene *et al.*, 2016). In addition, the expression of NRG1 type I isoform was increased and associated with neuroinflammation and glial activation in spinal cord of ALS patients and $SOD1^{G93A}$ mice (Song *et al.*, 2012). Moreover, overexpression of NRG1 in skeletal muscle promoted NMJ maintenance in the $SOD1^{G93A}$ mouse model of ALS (Mancuso *et al.*, 2016). This suggests a potential cell type specific effect, which should be taken into consideration in the development of potential therapeutic strategies targeting this kinase.

As abnormal expression and activation of ERBBs is associated with many human cancers (Hynes and Lane, 2005), modulation of the activity of these kinases has already been studied (Qiu *et al.*, 2008), and could facilitate the development of ERBB4 modulation as a potential ALS therapeutic strategy. However, more work is needed to evaluate how exclusive the interaction between ERBB4 and neuregulin 1 is, and whether other ERBB4 interactors play a pathogenic role in ALS.

# The multiple roles of kinases in ALS pathophysiology

While some kinases are associated with ALS as they are encoded by (potential) ALS-related genes, other kinases are involved in processes linked to selective motor neuron death (Fig. 2). p38 mitogen-activated protein kinase (p38 MAPK), c-Jun N-terminal kinase (JNK), TBK1, and DNA-dependent protein kinase (DNA-PK) are kinases that have been associated with different ALS-related pathophysiological changes (Deng *et al.*, 2014; Oakes *et al.*, 2017; Naumann *et al.*, 2018).

p38 MAPK belongs to the class of mitogen-activated protein kinases (MAPKs), which participate in signalling responses to cytokines and stress. Increased levels of active p38 MAPK were detected in post-mortem spinal cord and brain tissue of both familial and sporadic ALS patients (Bendotti *et al.*, 2004). Moreover, we and others observed an upregulation of activated p38 MAPK in the $SOD1^{G93A}$ mice during disease progression (Tortarolo *et al.*, 2003; Dewil *et al.*, 2007), and inhibition of p38 MAP kinase had a moderate positive effect on the survival of the $SOD1^{G93A}$ mice (Dewil *et al.*, 2007). More recently, it was shown that p38 MAPK regulates axonal transport and neuroinflammation in different ALS models (Sama *et al.*, 2017; Gibbs *et al.*, 2018).

JNKs, another subgroup of the MAPK family, function as stress-activated protein kinases. Activation of JNK/c-jun signalling occurs in motor neurons of $SOD1^{G93A}$ mice and is involved in the phosphorylation of TDP-43. Phosphorylated TDP-43 is a major component of the inclusions observed in neurons and glial cells in many cases of frontotemporal dementia (FTD) and/or ALS (Arai *et al.*, 2006; Neumann *et al.*, 2006; Hasegawa *et al.*, 2008; Sreedharan *et al.*, 2008; Mackenzie *et al.*, 2010; Gitler and Shorter, 2011; Suzuki and Matsuoka, 2013; Ratti and Buratti, 2016). Multiple phosphorylation epitopes (pS379, pS403/404, pS409, pS410 and pS409/410) were identified in aggregated TDP-43 via phosphorylation-specific anti-TDP-43 antibodies. This abnormal phosphorylation and accumulation of TDP-43 is regulated by casein kinase-1 (CK1) (Hasegawa *et al.*, 2008). Besides JNK and CK1, glycogen synthase kinase 3β (GSK3β) was shown to be a suppressor of ALS and FTD pathogenesis (Sreedharan *et al.*, 2008; Suzuki and Matsuoka, 2013).

As described above, TBK1 is a serine/threonine-protein kinase implicated in both neuroinflammation and autophagy in ALS (Oakes *et al.*, 2017). DNA-PKs are nuclear protein serine/threonine kinases, which sense DNA damage and participate in the ligation step of the non-homologous end joining (NHEJ) pathway of DNA double strand break repair. Combined with cytoplasmic FUS accumulations, DNA-PKs are activated upon DNA damage in ALS (Naumann *et al.*, 2018). Below, we summarize the regulation of some of the main kinases in the pathophysiology of ALS (Fig. 2).

## Kinases and axonal transport

Intracellular transport of cargoes is essential to maintain the structure and function of motor neurons because of their extreme morphology and polarization. Axonal transport mediates the distribution of cargoes, such as mRNAs, protein and organelles, mainly synthesized in the cell body across the cell (Box 3). In addition, axonal transport maintains the essential long-distance communication between the cell body and the synaptic terminals which allows neurons to react to their surroundings via trafficking of signalling endosomes (for a review see De Vos and Hafezparast, 2017). Axonal transport defects were observed in many different ALS models, and mutations in components of the axonal transport machinery were linked to ALS (López *et al.*, 2015; Wloga *et al.*, 2017). Moreover, axonal transport defects are an early, presymptomatic phenotype, which has also been observed in iPSC-derived motor neurons carrying a wide variety of ALS-causing mutations (Sivadasan *et al.*, 2016; Guo *et al.*, 2017; Kreiter *et al.*, 2018; Naumann *et al.*, 2018; Pal *et al.*, 2018). There is increasing support for the hypothesis that (de)phosphorylation processes are key regulators of axonal transport (Box 3) (reviewed in Brady and Morfini, 2017). Kinases regulate axonal transport by directly phosphorylating molecular motors, adaptors, cargoes and/or the microtubular network, and this affects the interaction between motor proteins and their cargoes or between motor proteins and microtubules, hence affecting axonal transport (Brady and Morfini, 2017; De Vos and Hafezparast, 2017). In addition, modifying the microtubule network affects axonal transport by altering the microtubule stability. Below, we discuss the major kinases described to impact these interactions (Fig. 2A).

### p38 MAPK

Similar to patients, $SOD1^{G93A}$ transgenic mice and transgenic squid axoplasm carrying ALS-linked FUS mutations (G230C, R521G and R495X) showed activation of p38 MAPK and impaired axonal transport (Tortarolo *et al.*, 2003; Bendotti *et al.*, 2004; Sama *et al.*, 2017). The mechanism underlying the toxic function of p38 MAPK overactivation is the phosphorylation of kinesin-1 on its serines 175 and 176 by p38 MAPK. This inhibits translocation of

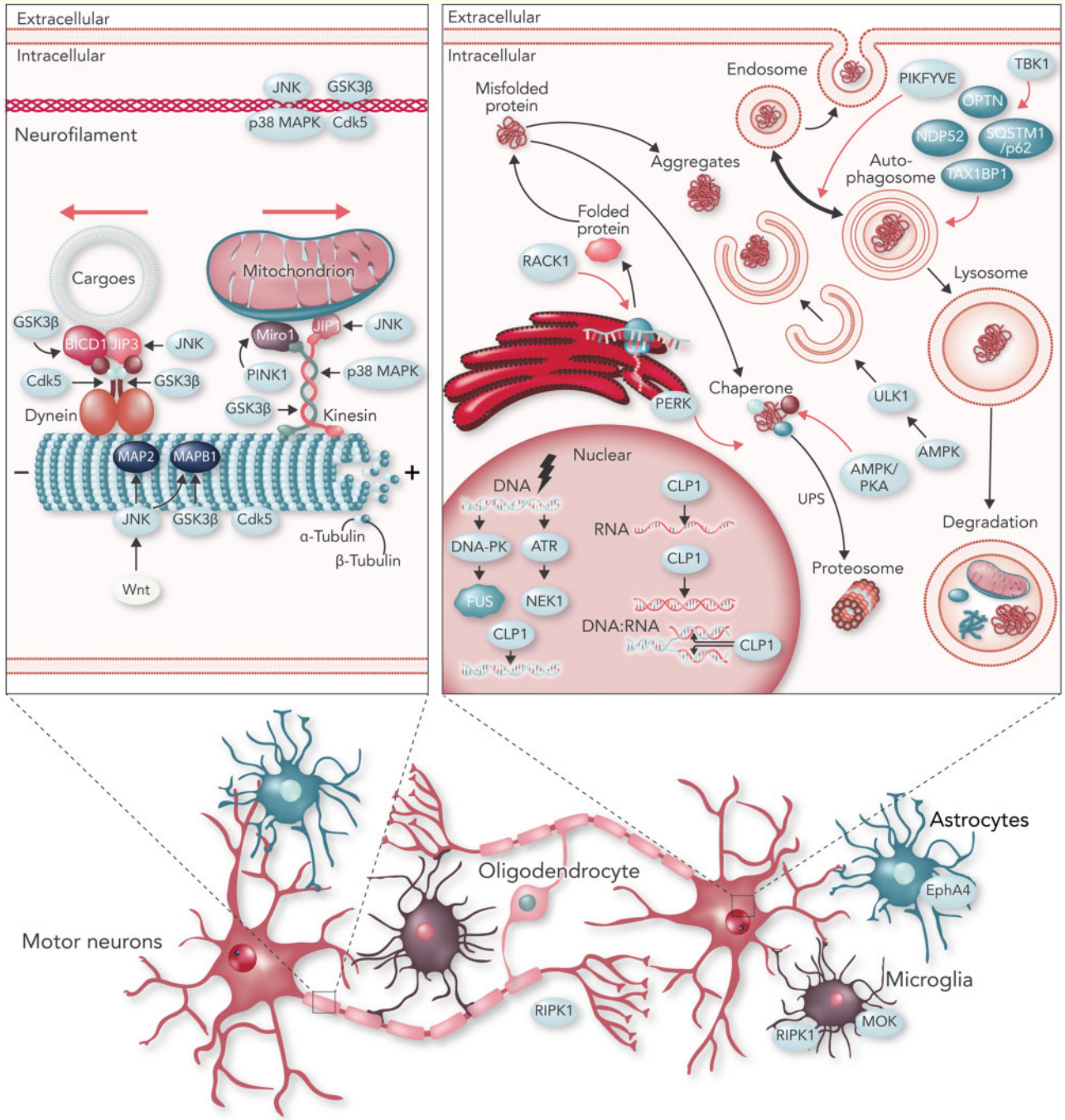


**Figure 2 Kinases in neurodegenerative processes involved in ALS.** Schematic overview of kinases involved in different ALS pathophysiological processes. Names identified in light blue ovals indicate kinases. Names in dark blue ovals indicate adaptors that interact with kinases. *Top left*: Examples of kinases regulating axonal transport by interacting with subunits of the transport process including dynein (retrograde transport), kinesin (anterograde transport) and their adaptors, microtubules, neurofilaments, and cargo specific-adaptors. *Bottom*: Examples of kinases activated in non-neuronal cells including astrocytes, microglia and oligodendrocytes. *Top right*: Examples of kinases regulating proteostasis including protein synthesis through ribosomes on the endoplasmic reticulum (ER), the ubiquitin-proteasome system (UPS) and autophagy by direct or indirect phosphorylation. Examples of kinases activated by DNA damage and involved in RNA-related processes are also shown.

kinesin-1 along axonal microtubules and will cause transport defects (Morfini *et al.*, 2013). Inhibition of p38 MAPK showed multiple beneficial effects in rodent ALS models, including protection of mutant SOD1-induced motor neuron degeneration, prolongation of survival of $SOD1^{G93A}$ mice, restoration of axonal transport defects in both $SOD1^{G93A}$ mice and in murine motor neurons transfected with mutant FUS (Dewil *et al.*, 2007; Sama *et al.*, 2017; Gibbs *et al.*,

### Box 3 Axonal transport and the 'dying back' hypothesis in ALS

- The most particular morphological feature of neuronal cells in comparison to other cell types is their extreme polarity and very long axons. Axons allow for the efficient communication between soma and axonal terminals. Axonal communication is especially important for motor neurons, as they not only need to connect with each other but also reach over a long distance to muscles to properly control muscle contraction (reviewed in De Vos and Hafezparast, 2017). Axonal transport maintains the efficient supply of cargoes including proteins, RNAs, lipids and organelles and it is also responsible to clear or recycle misfolded proteins or aggregates during cellular stress (reviewed in Prior *et al.*, 2017). Axonal transport uses microtubules as tracks and α-tubulin and β-tubulin are the building blocks of these microtubules. The growing 'plus' end of the microtubules is at the axonal distal part, and the 'minus' end is located at the side of the soma. Two types of ATP-dependent motors, including kinesins and dyneins, are responsible for moving cargoes (including organelles, proteins, RNAs etc.) along the microtubules (reviewed in Guo *et al.*, 2019). Kinesins are a superfamily of proteins that are mainly responsible for anterograde axonal transport (from 'minus' to 'plus' end of the microtubules) (Guo *et al.*, 2019), while some kinesins move towards the 'minus' end. This enables the kinesins to transport cargo in both directions (Endow, 1999). Dyneins are also a family of proteins that can be further divided into two isoforms including cytoplasmic dynein and axonemal dynein. Cytoplasmic dynein is responsible for retrograde axonal transport (from 'plus' to 'minus' end of the microtubules) (for a review see Reck-Peterson *et al.*, 2018). Adaptor proteins connect motor proteins to their cargo (Guo *et al.*, 2019).
- The 'dying-back' hypothesis explains the sequence of events during motor neuron degeneration in ALS (Dadon-Nachum *et al.*, 2011; Baker 2014). The underlying idea is that motor neurons lose their connection with muscle fibres and that the axon retracts towards the soma. This ultimately results in motor neuron death. This hypothesis is supported by the observation that motor neuron pathology begins at the terminal part of the axon and proceeds in a 'dying back' pattern (Dadon-Nachum *et al.*, 2011). In addition, the longest and largest neurites with the highest metabolic demand seem to be the most susceptible to this "dying back" phenomenon (Dadon-Nachum *et al.*, 2011).

2018) (Table 1). p38 MAPK can also phosphorylate neurofilament medium and heavy chain (NF-M and NF-H) side-arms, which hampered axonal transport (Ackerley *et al.*, 2004). This is in line with the observation that increased co-localization of p38 MAPK with phosphorylated neurofilaments was present in neurons of $SOD1^{G93A}$ mice (Bendotti *et al.*, 2004). This implies that inhibition of p38 MAPK could be therapeutically relevant in ALS, as we have previously shown by using semapimod in $SOD1^{G93A}$ mice, although the beneficial therapeutic effect was moderate (Dewil *et al.*, 2007). We assume that this moderate beneficial effect *in vivo* was because of the fact that semapimod could not restore the function of the neuromuscular junctions (Dewil *et al.*, 2007). Therefore, we propose a combined treatment regimen targeting the entire motor unit *in vivo*.

## JNK

Increased phosphorylation of c-Jun occurred in motor neurons of $SOD1^{G93A}$ mice and JNK signalling also seemed to be involved in TDP-43-related toxicity (Vlug *et al.*, 2005; Suzuki and Matsuoka, 2013). Limited TDP-43 overexpression in NSC34 motor neuronal cells and primary cortical neurons induced neuronal cell death through the upregulation of Bim and CHOP expression and downregulation of Bcl-xL expression (Suzuki and Matsuoka, 2013). Furthermore, TDP-43 overexpression increased the phosphorylation of JNK and inhibition or downregulation of JNK inhibited TDP-43-induced cell death, suggesting a link between the JNK/c-Jun signalling and TDP-43 induced cell death (Suzuki and Matsuoka, 2013). JNK directly phosphorylated the motor domain of kinesin-1 (DeBerg *et al.*, 2013). Moreover, JNK could disrupt binding between kinesin-1 and JIP1, which is a cargo adaptor of kinesin, via activation of MAP kinase kinase kinase (MAPKKK), Wallenda (a homologue of dual leucine zipper-bearing kinase) and MAPKK Hemipterous (a homologue of MKK7) in *Drosophila* (Horiuchi *et al.*, 2007). Furthermore, JNK regulated retrograde axonal transport by affecting the binding of JIP3 to p150 Glued and dynein light chain (DLIC) (Horiuchi *et al.*, 2007). Similar to p38 MAPK, Jun N-terminal kinase-1 and -3 (JNK1 and JNK3) could also phosphorylate NF-M and NF-H side-arm domains, interrupting axonal transport (Ackerley *et al.*, 2004). JNK signalling can also be activated by dual leucine zipper kinase (DLK). Genetic deletion and pharmacological inhibition of DLK protected against axon degeneration, neuronal loss, and functional decline in $SOD1^{G93A}$ mice by reducing phosphorylated c-Jun (Le Pichon *et al.*, 2017). In addition, JNK can be activated by the Wnt pathway to phosphorylate microtubule-associated proteins, such as microtubule-associated protein 2 (MAP2) and microtubule-associated protein 1B (MAP1B). This results in changes in the microtubular dynamics and eventually disrupts neuronal axonal transport in cooperation with GSK3β (Ciani and Salinas, 2007). Dramatic upregulation of the expression of several members of the Wnt family in astrocytes as a function of disease progression has been shown in spinal cord of $SOD1^{G93A}$ mice, as well as in post-mortem tissue of ALS patients (Chen *et al.*, 2012; Yu *et al.*, 2013;

**Table 1 Compounds targeting kinases in ALS**

| Kinase | Possible target/mechanism | Inhibitor | Preclinical model or clinical trial phase | Effect | BBB permeability | FDA approval (ALS/other diseases) | Ref /clinical trial registration number[a] |
|---|---|---|---|---|---|---|---|
| AMPK | A2A adenosine receptor (A2AR) | JMF1907 | NSC34 cells<br>TDP-43 transgenic mouse | Normalized the mislocalization of TDP-43 *in vitro*<br>Improved the motor function (rotarod performance, forelimb grip strength) *in vivo* | NE | NE | Liu *et al.*, 2015 |
| ASK1 | Stress-responsive | K811, K812<br>NQDI-1 | SOD1$^{G93A}$ transgenic mouse<br>SOD1$^{G85R}$ transgenic squid axoplasm | Increased survival of motor neurons<br>Inhibited the activation of glial cells<br>Extended survival of SOD1$^{G93A}$ transgenic mouse<br>Rescued anterograde axonal transport in SOD1$^{G85R}$ transgenic squid axoplasm | NE | NE | Song *et al.*, 2013; Fujisawa *et al.*, 2016 |
| CK1 | Phosphorylate TDP-43 directly | Inhibitor20 | TDP-43 transgenic fly | Extended fly lifespan | Yes | NE | Salado *et al.*, 2014 |
| DLK | JNK pathway | GNE-3511 | SOD1$^{G93A}$ transgenic mouse | Delayed neuromuscular junction denervation | Yes | NE | Le Pichon *et al.*, 2017 |
| ERK | EGFR pathway signalling | Erlotinib | SOD1$^{G93A}$ transgenic mouse | Delays disease progression; no extend survival | Yes | Yes | Le Pichon *et al.*, 2013 |
| EphA4 | EphA4-LBD | 123C4 | SOD1$^{G93A}$ transgenic mouse | Extended survival | NE | NE | Wu *et al.*, 2017 |
| EphA4 | *EPHA4* | EphA4-ASO | SOD1$^{G93A}$ and PFN1$^{G118V}$ transgenic mouse | No improvement of motor function or survival | NE | NE | Ling *et al.*, 2018 |
| GSK3β | COX-2 | GSK-3 inhibitor VIII | SOD1$^{G93A}$ transgenic mouse | Increased motor neuron survival; delayed disease onset and extended survival | NE | NE | Koh *et al.*, 2007 |
| GSK3β | Changes of transcription factors | Lithium plus valproate | SOD1$^{G93A}$ transgenic mouse | Delayed the onset of motor dysfunction<br>Extended survival and reducing neurological deficits | Yes | Yes | Feng *et al.*, 2008 |
| GSK3β | NE | JGK-263 | SOD1$^{G93A}$ transgenic mouse | Increased motor neuron survival<br>Improved motor function and delayed the onset of motor dysfunction, rotarod failure, and survival | Yes | NE | Ahn *et al.*, 2014 |
| JAK3 | NE | WHI-P131 | SOD1$^{G93A}$ transgenic mouse | Increased survival | NE | NE | Trieu *et al.*, 2000 |
| p38 MAPK | Kinesin1 | SB239063 | SOD1$^{G93A}$ transgenic mouse | Restored the rate of axonal retrograde transport *in vivo* | Yes | NE | Gibbs *et al.*, 2018 |
| p38 MAPK | TNFs | Semapimod | SOD1$^{G93A}$ transgenic mouse | Increased motor neuron survival<br>Delayed disease onset and extended survival | NE | NE | Dewil *et al.*, 2007 |
| p38 MAPK | Stress response | MW069 | SOD1$^{G85R}$ transgenic squid axoplasm | Rescue anterograde axonal transport | NE | NE | Song *et al.*, 2013 |
| PERK | eIF2α | GSK2606414 | Primary rat cortical neurons<br>TDP-43 transgenic fly | Increased survival of neurons; mitigation of TDP-43-induced climbing dysfunction in fly | Yes | NE | Kim *et al.*, 2014 |
| PIKFYVE | RAB5 | YM201636<br>Apilimod | C9orf72 patient iMNs | Increased EEA1 + endosome size<br>Increased patient iMN survival | NE | NE | Shi *et al.*, 2018 |
| RIPK1 | Inflammation | DNL747 | Phase I in ALS | Not yet available | Yes | NE | NCT03757351 |
| ROCK | Actin cytoskeleton and neuronal survival | Fasudil | Phase II in ALS | Not yet available | Yes | NE | Lingor *et al.*, 2019; NCT03792490 |
| Src/c-Abl | Autophagy | Bosutinib | iPSC-MNs (sporadic, TDP-43, C9orf72, SOD1)<br>SOD1$^{G93A}$ transgenic mouse | Increased survival of iPSC-derived MNs<br>Delayed disease onset and extended survival | Yes | Yes | Imamura *et al.*, 2017 |
| Tyrosine kinase (pan) | Inflammation (add-on treatment with riluzole) | Masitinib | Phase II / III in ALS | Phase II: improvement of life quality, respiratory function and delay of death | Yes | NE | Mora *et al.*, 2019; NCT02588677 |

[a]As registered on clinicaltrials.gov.
BBB = blood–brain barrier; iMN = induced motor neuron; NE = no evidence.

González-Fernández *et al.*, 2019). This suggests that glial proliferation-related neurodegeneration activates the Wnt signalling pathway. Subsequently, JNKs are activated by Wnt and eventually disturb the stability of the microtubules. The interaction between different kinases indicates that a series of kinome events could be involved in ALS pathogenesis. As a consequence, targeting a single kinase may also influence the function of other related kinases. On the other hand, targeting only one single kinase could be insufficient to result in positive therapeutic effects.

## GSK3β

GSK3β is a multifunctional serine/threonine kinase that was originally identified as a regulator of glycogen synthase (Du *et al.*, 2010). GSK3β modulated transport of α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate (AMPA)-containing vesicles by mediating phosphorylation of kinesin light chain 2 (KLC2) in kinesin-dependent axonal transport (Du *et al.*, 2010). In addition, GSK3β can also regulate dynein-dependent axonal transport. Two conserved residues from two different dynein intermediate chain (IC) isoforms, S87/T88 in IC-1B and S88/T89 in IC-2C, are targeted by GSK3β. Although these isoforms are fairly ubiquitous, the IC-1B isoform is described to play a prominent role in axonal transport in neurons (Gao *et al.*, 2015). The phosphorylated residues are within an Ndel1-binding domain, which is responsible for the interaction between ICs and Ndel1 (Gao *et al.*, 2015). Moreover, the phosphorylation of a dynein adaptor called BICD1 was GSK3β dependent (Fumoto *et al.*, 2006). Pharmacological and genetic inhibition of GSK3β could increase dynein motility (Gao *et al.*, 2015) (Table 1). GSK3β can also mediate semaphorin3A-induced bidirectional axonal transport through phosphorylation of the axis inhibitor-1 (Hida *et al.*, 2015). Interestingly, semaphorin 3A signalling through neuropilin-1 was an early trigger for the distal axonopathy in $SOD1^{G93A}$ mice (Venkova *et al.*, 2014), implying that GSK3β signalling plays a pivotal role in distal axonal degeneration in ALS. The activation of GSK3β was also observed in ALS-associated defects in endoplasmic reticulum (ER)/mitochondrial communication (Stoica *et al.*, 2016). Therefore, GSK3β may also influence axonal transport indirectly by affecting ER–mitochondria interactions (Stoica *et al.*, 2014, 2016; Guo *et al.*, 2017). Moreover, GSK3β could influence axonal transport by fostering NF-NF associations that compete with transport by phosphorylating the C-terminal tail of the NF-H (Vohnoutka *et al.*, 2017), and GSK3β regulated the release of tau from microtubules by phosphorylation which influenced microtubule stability (Rankin *et al.*, 2007). GSK3β is also the main kinase responsible for TDP-43 phosphorylation and deletion of the GSK3β gene protected against TDP-43-induced toxicity (Sreedharan *et al.*, 2008). Although extensive research identified a direct and indirect involvement of GSK3β in ALS pathology, the real therapeutic potential in ALS patients is not yet clear. Until now, different GSK3β inhibitors with blood–brain barrier (BBB) permeability have been developed and investigated in $SOD1^{G93A}$ mice (Table 1) showing that lithium had a neuroprotective effects in $SOD1^{G93A}$ mice (Fornai *et al.*, 2008). As most of these inhibitors showed beneficial effects including delayed disease onset, increased motor neuron survival, and improved motor function (Feng *et al.*, 2008; Ahn *et al.*, 2014), clinical trials targeting GSK3β have been conducted. A completed Phase III trial administering lithium to ALS patients did not show a benefit on survival (Al-Chalabi *et al.*, 2013), although a meta-analysis identified a beneficial effect on mutant *UNC13A* carriers but not on mutant *C9orf72* carriers (van Eijk *et al.*, 2017). Further research using other GSK3β inhibitors in different ALS model systems should be performed to validate the potential of GSK3β as a therapeutic target.

## CDK5

CDK5 is a serine/threonine kinase that belongs to the mitotic cyclin-dependent kinases family, and activation of CDK5 occurred in the spinal cord of $SOD1^{G37R}$ mice (Nguyen *et al.*, 2001). Similar to GSK3β, CDK5 phosphorylates Ndel1, inducing the formation of the Lis1 and Ndel1 complex. Binding of this complex to dynein inhibited the dynein-mediated transport through blockage of the ATP-dependent release of dynein from microtubules (Klinman and Holzbaur, 2015). This process was validated by monitoring the transport of lysosomes, autophagosomes, mitochondria, and endosomes (Pandey and Smith, 2011; Klinman and Holzbaur, 2015). This was further confirmed by a restoration of axonal transport defects through inhibition of CDK5 in dorsal root ganglion (DRG) neurons from $SOD1^{G93A}$ mice (Klinman and Holzbaur, 2015). However, it is unclear whether this also affected motor neuron survival. Furthermore, activation of CDK5 was also required to maintain initial segment integrity of the axon, which was responsible for both microtubular organization and polarized dynein-dependent sorting of axodendritic cargos in neurons (Klinman *et al.*, 2017). In addition, CDK5 could regulate axonal transport in neuronal cells by phosphorylating the NF-H subunit (Shea *et al.*, 2004). Through sharing both up and downstream pathways with GSK3β (Engmann and Giese, 2009), CDK5 might also participate in a kinase network that regulates axonal transport, which might contribute to motor neuron survival.

## PINK1

PTEN-induced kinase 1 (PINK1) is a mitochondrial serine/threonine-protein kinase that localizes at the outer membrane of mitochondria, but can also be found throughout the cytosol. Mitochondria are one of the most important cargoes transported along the axon to maintain proper energy supply and normal synaptic function of motor neurons (Saxton and Hollenbeck, 2012; Vandoorne *et al.*, 2018, 2019). The efficient clearance of damaged mitochondria largely relies on proper axonal transport (Saxton and Hollenbeck, 2012). PINK1 regulates axonal transport by phosphorylating

mitochondrial Rho GTPase 1 (Miro1) (Wang *et al.*, 2011). Miro1 serves as a component of the primary motor/adaptor complex that anchors kinesin to the mitochondrial surface (MacAskill *et al.*, 2009; López-Doménech *et al.*, 2018). It plays a pivotal role in regulating mitochondrial transport in response to $Ca^{2+}$ levels within and outside of mitochondria by interacting with kinesin (MacAskill *et al.*, 2009; López-Doménech *et al.*, 2018) (Fig. 2A). When mitochondria are damaged, the depolarized mitochondria stabilize PINK1 on their surface, promoting the interaction between PINK1 and Miro, and causing PINK1 to phosphorylate Ser156 of Miro. Subsequently, the interaction of parkin with Miro removes Miro from the mitochondrial membrane and it is subsequently degraded by the proteasome. Kinesin is released from the mitochondria and this eventually stops mitochondrial transport (Wang *et al.*, 2011). The pivotal role of PINK1, often through Miro1, in ALS pathogenesis has been suggested in different models as well as in ALS patients. In spinal cord tissue of ALS patients as well as in the $SOD1^{G93A}$ and $TDP\text{-}43^{M337V}$ mouse models, it was shown that Miro1 expression was reduced (Zhang *et al.*, 2015). In addition, mutations in Miro genes caused anterograde mitochondrial transport defects in distal synaptic terminals of flies (Guo *et al.*, 2005; Russo *et al.*, 2009). Moreover, PINK1 can be stimulated by mitochondrial damage, can induce Miro degradation and can halt mitochondrial transport (Wang *et al.*, 2011). Interestingly, downregulation of PINK1 suppressed the TDP-43-induced degenerative phenotypes in a *Drosophila* model, and PINK1 also functioned as a genetic modifier of FUS-induced neurodegeneration (Chen *et al.*, 2016; Sun *et al.*, 2018). This implies that PINK1 might serve as a potential therapeutic target for ALS through regulating mitochondrial transport.

## Kinases and neuroinflammation

Neuroinflammation is the inflammatory response within the brain or spinal cord. It is mediated by the production of cytokines, chemokines and reactive oxygen species produced by resident microglia, astrocytes and endothelial cells, and/or by peripherally derived immune cells (reviewed in DiSabato *et al.*, 2016). It is a common denominator in neurodegeneration (Hammond *et al.*, 2019). In the CNS of ALS patients and of ALS animal models, a chronic activation of microglia, astrocytes and T lymphocytes was a prominent pathological observation (Liu and Wang, 2017). A genome-wide transcriptional study on motor cortex samples from 31 patients with sporadic ALS and 10 healthy control subjects identified aberrant expression of 1573 of 2637 inflammatory-related genes in ALS patient tissues (Morello *et al.*, 2017). Several critical mediators of neuroinflammation, including GSK3β, Cdk, JNK, MAPKs, and ERK, are kinases (Wang *et al.*, 2012; Chen *et al.*, 2016). However, it remains to be determined whether neuroinflammation is driving the ALS disease process or represents a mere consequence of the neurodegeneration. This is especially difficult to assess in ALS patients as diagnostic certainty is mostly reached in an advanced stage of the disease. However, the discovery of TBK1 as an ALS-causing gene suggests a direct link between neuroinflammation and kinases in ALS (Cirulli *et al.*, 2015; Freischmidt *et al.*, 2015). In addition, decreasing microgliosis by administration of masitinib, a tyrosine kinase inhibitor, in symptomatic $SOD1^{G93A}$ rats prolonged survival by 40% and offered motor neuron protection in the spinal cord (Trias *et al.*, 2016). Despite this and many other findings that suggest that inflammation is important in ALS, one should also keep in mind that many therapeutic strategies counteracting neuroinflammation failed in clinical trials despite showing promising therapeutic effects in animal models (reviewed in McCauley and Baloh, 2019). Below, we discuss the role of various neuroinflammation-related kinases in the context of ALS (Fig. 2B).

### TBK1

As described above, different genetic studies identified *TBK1* as an ALS-causing gene and/or a disease modifier (Cirulli *et al.*, 2015; Freischmidt *et al.*, 2015). TBK1 belongs to the IKK-kinase family, which is involved in immune signalling pathways by regulating the production of interferon (IFN)α and IFNβ, and activation of TBK1 was observed in microglia (Moore and Holzbaur, 2016; Oakes *et al.*, 2017). TBK1 also functions as an important regulator of dendritic cells, which are crucial for mediating immune responses. Dendritic cell-specific deletion of TBK1 caused T-lymphocyte activation and autoimmune symptoms (Xiao *et al.*, 2017). In T cell-specific TBK1 knockout mice, the migration of T lymphocytes from the lymph nodes was impaired and resulted in reduction of the number of T lymphocytes in the CNS, eventually causing TBK1-dependent neurotoxicity (Yu *et al.*, 2015). Studies in $SOD1^{G93A}$ mice showed that neuroprotective anti-inflammatory mediators were present during the early stage of the disease, and these changed into cytotoxic pro-inflammatory mediators at later stages of the disease (Beers *et al.*, 2011). As a recent phase I clinic trial showed that administration of T lymphocytes to ALS patients was safe and well tolerated (Thonhoff *et al.*, 2018), it will be interesting to investigate whether this strategy could rescue ALS-related phenotypes in TBK1 conditional knockout mice. In addition, further experiments evaluating the effect of TBK1 expression on neuroinflammation would be helpful to pinpoint which of the canonical functions of TBK1 are essential for its link to ALS.

### RIPK1

In post-mortem tissue from ALS patients, multiple biochemical hallmarks of necroptosis—a form of programmed necrosis caused by inflammation—were observed (Ito *et al.*, 2016). Although there is no causal evidence linking receptor-interacting kinase 1 (*RIPK1*) mutations to ALS, this gene is considered to be associated with ALS by its close association to the ALS gene *OPTN* and it is a critical regulator of cell death and inflammation (Humphries *et al.*, 2015; Ito *et al.*, 2016). In $SOD1^{G93A}$ mice, loss-of-function mutations in the *OPTN* gene resulted in progressive demyelination and

axonal degeneration through engagement of the necroptotic machinery in the CNS (Ito *et al.*, 2016). RIPK1 regulates necroptosis via the sequential activation of two downstream targets: RIPK3 and mixed lineage kinase domain-like protein (MLKL) (Wang *et al.*, 2019). In ALS, OPTN significantly suppressed RIPK1-dependent signalling by regulating its turnover (Ito *et al.*, 2016). Moreover, elevated levels of RIPK1, RIPK3 and MLKL were observed in the $SOD1^{G93A}$ mouse model, which could contribute to the axonal pathology and motor dysfunction in these mice (Ito *et al.*, 2016). As the inhibition of RIPK1 prevented progressive axonal degeneration, RIPK1 could also play an important role in mediating axonal degeneration (Ito *et al.*, 2016). In addition, through interaction with TBK1, RIPK1 promoted ageing-related inflammation that has been suggested to contribute to ALS disease progression (Xu *et al.*, 2018). Recently, a phase I clinical ALS trial, which will test the safety, tolerability, pharmacokinetics, and pharmacodynamics of the RIPK1 inhibitor, DNL747, was announced (NCT03757351) (Table 1). Overall, RIPK1 is an interesting target to provide axonal protection in ALS and eventually in other human degenerative disorders characterized by axonal degeneration.

### MOK

MAPK/MAK/MRK overlapping kinase (MOK) belongs to the MAP kinase superfamily. It is localized in the cytoplasm and phosphorylates several exogenous substrates or undergoes autophosphorylation. Cytoplasmic TDP-43 aggregation is a pathological hallmark in both familial and sporadic ALS patients (reviewed in Hergesheimer *et al.*, 2019). TDP-43 aggregates purified from the brains of ALS patients were hyperphosphorylated and/or ubiquitinated (Hergesheimer *et al.*, 2019). These intracellular aggregates were cytotoxic and caused extracellular TDP-43 accumulation that could be internalized and modified by microglia (Leal-Lasarte *et al.*, 2017). MOK seems to play a role as an important mediator in TDP-43 aggregates exposed to microglia. MOK co-localized with TDP-43 aggregates and reduced phosphorylation after exposure of microglia to TDP-43 aggregates (Leal-Lasarte *et al.*, 2017). By targeting MOK, extracellular TDP-43 aggregates stimulated inflammasome dependent IL-1β and IL-18 secretion, and neuroinflammation-linked caspase 3 activation that eventually could cause neurodegeneration (Leal-Lasarte *et al.*, 2017). This may be a direct consequence of abnormal engagement of MOK into TDP-43 aggregates, and, as a consequence, modulation of MOK activation status may affect cellular responses that are modulated by downstream signalling pathways. In addition, TDP-43 also triggered inflammasome activation of the NF-κB pathway through p38 MAPK, which led to secretion of IL-1β and IL-18 (Huang *et al.*, 2019). As both MOK and p38 MAPK belong to the MAPK superfamily, the interaction between MOK and p38 MAPK still needs to be investigated in more detail to obtain a better picture of the kinase network in TDP-43-related neuroinflammation.

### EPHA4

EPHA4 receptor tyrosine kinase belongs to the Ephrin receptor subfamily of the protein-tyrosine kinase family and was initially identified as an ALS modifier in a morpholino-based zebrafish screen (Van Hoecke *et al.*, 2012). EPHA4 receptor tyrosine kinase regulates axonal guidance in the corticospinal tract, but also functions as a mediator of inflammation in spinal cord injury (Goldshmit *et al.*, 2004; Zhao *et al.*, 2018). In mice, EPHA4 expression was also upregulated in activated astrocytes after spinal cord injury (Goldshmit *et al.*, 2004). In our previous study, we showed that EPHA4 modulated motor neuron degeneration and disease progression in ALS (Van Hoecke *et al.*, 2012). Another recent study showed that decreased signalling of EPHA4 improved functional performance and motor neuron survival in the $SOD1^{G93A}$ mice (Zhao *et al.*, 2018). In addition, very rare loss-of-function variants in the *EPHA4* gene were associated with prolonged survival in ALS patients (Van Hoecke *et al.*, 2012). We obtained EPHA4-specific nanobodies (Schoonaert *et al.*, 2017) and selective EPHA4 agonists were also developed to treat ALS (Wu *et al.*, 2017) with promising results in delaying the progression of disease in the $SOD1^{G93A}$ mouse model (Wu *et al.*, 2017) (Table 1). Despite the fact that these results indicate that EPHA4 receptor tyrosine kinase may serve as a therapeutic target for ALS, downregulation of EPHA4 using antisense oligonucleotides had no protective effect (Ling *et al.*, 2018). In addition, it becomes clearer that the ephrin system is complicated and that interfering with this signalling system in the context of ALS does not always result in the expected outcome (Rué *et al.*, 2019).

### RACK1

Receptor of activated protein C kinase 1 (RACK1) functions as an intracellular protein receptor for protein kinase C, and has been associated to ALS as it mislocalized in spinal cord sections of ALS patients (Russo *et al.*, 2017). RACK1 modulated microglial resistance against LPS-induced inflammatory injury (Yin *et al.*, 2017). Moreover, TDP-43 inhibited the inflammatory response by modulating expression of RACK1 in human osteoarthritis (Huang *et al.*, 2017). Intriguingly, mislocalization of RACK1 to TDP-43-positive cytoplasmic inclusions in motor neurons was observed in ALS patients (Russo *et al.*, 2017). Although there is no clear mechanism underlying the role of RACK1 in ALS yet, the current findings suggest that RACK1 might mediate ALS pathogenesis through its effects on neuroinflammation.

## Kinases and disrupted proteostasis

Proteostasis is dependent on a complex regulatory network that maintains protein homeostasis (Box 4). It is vital for cell health and survival and consists of several pathways controlling protein biosynthesis, folding, trafficking, and degradation (reviewed in Webster *et al.*, 2017). It also includes specific protein stress pathways such as the unfolded protein response (UPR) in the ER, the mitochondrial UPR and the cytosolic heat shock

### Box 4 Proteostasis in ALS

- Proteostasis is the concept that there are cellular mechanisms that control the synthesis, folding, trafficking and degradation of proteins inside and outside the cell (Powers *et al.*, 2009). In humans, approximately one-third of all proteins are synthesized in the ER and then transit to membrane compartments (Hetz and Mollereau, 2014). The quality control of protein folding happens in the ER (Hetz and Mollereau, 2014). In pathological conditions, as well as during ageing, the efficient protein folding process is hampered with misfolded proteins accumulating and clumping together as aggregates (for a review see Boeynaems and Gitler, 2018). Under normal physiological conditions, protein aggregation could play a protective and beneficial role to minimize the toxic effects of misfolded proteins. Some proteins can switch between liquid and solid phases, a process called phase transition (Boeynaems *et al.*, 2018; Wang and Zhang, 2019). If this process is impaired, toxicity can result in disease-relevant pathological changes (Boeynaems *et al.*, 2018). It has been shown that the phosphorylation of the prion-like domain can play a pivotal role in phase transition of proteins (Boeynaems and Gitler, 2018). In addition, disrupted proteostasis can cause ER stress (Hetz and Saxena, 2017). ER stress induces the ubiquitin proteasome system (UPS), a signalling network that transduces information about the protein folding status from the ER to the cytosol and nucleus to conjugate ubiquitin to substrates. This results in the degradation of these proteins by the proteasome (Hetz and Saxena, 2017). Besides the UPS, autophagy is another mechanism that contributes to protein degradation, a process in which autophagosomes and lysosome are involved (reviewed in Kwon and Ciechanover, 2017).
- Abnormal protein aggregation is one of the earliest pathological features observed in spinal cord and brain tissue of ALS patients (Blokhuis *et al.*, 2013). Different ALS-related gene mutations in *SOD1*, *TARDBP* or *FUS* lead to protein misfolding and aggregation (Blokhuis *et al.*, 2013). Activation of autophagy delays disease onset, reduces neurological deficits and prolongs survival in $SOD1^{G93A}$ mice (Kim *et al.*, 2013; Staats *et al.*, 2013). As a consequence, proper synthesis, protein folding, and degradation are important processes to maintain efficient proteostasis in motor neurons.

response (Webster *et al.*, 2017). Ubiquitin-positive inclusions are a hallmark of ALS. Hyperphosphorylated, ubiquitinated and/or cleaved TDP-43 are major constituent of these inclusions (Neumann *et al.*, 2006). This, in combination with preclinical studies showing altered autophagy and proteasomal pathways (reviewed in Ramesh and Pandey, 2017), and genetic studies linking mutations in genes encoding proteins involved in these pathways to ALS (Webster *et al.*, 2017; Alexander *et al.*, 2018), suggests that proteostasis could be a key player in ALS pathogenesis. More recently, defects in protein synthesis have also been implicated in ALS (López-Erauskin *et al.*, 2018; Koskimäki *et al.*, 2019). Kinases actively participate in several steps of protein synthesis and degradation (Box 4). Therefore, we discuss the main kinases impacting proteostasis that could be involved in ALS (Fig. 2C).

## AMPK

AMP activated kinase (AMPK) is a serine/threonine kinase that can be activated in response to energetic stress (Garcia and Shaw, 2017). The activity of AMPK is induced by a high ratio of intracellular AMP to ATP levels. It is a member of the protein kinase A (PKA) family, which comprises enzymes that are dependent for their activity on cellular levels of cyclic AMP (cAMP). Levels of cAMP are affected by the ubiquitin-proteasome system (UPS) to further modulate phosphorylation-dependent downstream signalling. The AMPK/PKA pathway regulates multiple aspects of cell survival (Rinaldi *et al.*, 2015). Motor neurons of ALS patients exhibiting cytoplasmic TDP-43 mislocalization showed an increased level of AMPK activation (Liu *et al.*, 2015). Moreover, AMPK-mediated phosphorylation was significantly upregulated in spinal cords of $SOD1^{G93A}$ mice upon disease onset (Liu *et al.*, 2015). C9ORF72 interacts with Rab1a and the Unc-51-like kinase 1 (ULK1) autophagy initiation complex, which might undergo a close regulation by AMPK (Yang *et al.*, 2016; Nwadike *et al.*, 2018). In contrast to the data obtained in $SOD1^{G93A}$ mice, AMPK activity was drastically diminished in spinal cords and brains of presymptomatic and symptomatic $TDP\text{-}43^{A315T}$ mice (Perera *et al.*, 2014). Eventually, this could be explained by the milder motor neuron loss in the spinal cord of these mutant $TDP\text{-}43^{A315T}$ mice (Perera *et al.*, 2014). In the brain cortex of the $TDP\text{-}43^{A315T}$ mice, pronounced neuronal loss and ubiquitin pathology was observed, which could be consistent with a lower AMPK activity in the brain (Wegorzewska *et al.*, 2009; Perera *et al.*, 2014). Furthermore, the $TDP\text{-}43^{A315T}$ mice showed a progressive weight gain, increased body fat and adipocyte hypertrophy leading to AMPK inactivation (Stallings *et al.*, 2013; Perera *et al.*, 2014). Although the presence of activated AMPK in $SOD1^{G93A}$ mice at symptom onset argues against a role for AMPK signalling in disease initiation, it could be a determinant of disease progression (Perera *et al.*, 2014). However, the role of elevated AMPK signalling in $SOD1^{G93A}$ mice should be further investigated, as the AMPK-mediated transcription of its downstream targets was unaltered (Perera *et al.*, 2014). Altogether, these data suggest a different effect of AMPK activation in mutant SOD1 compared to mutant TDP-43 transgenic mice (Liu *et al.*, 2015). In addition, the activation of AMPK in mouse spinal cords induced the mislocalization of TDP-43, recapitulating a key neuropathological characteristic of ALS.

Inhibition of AMPK activity rescued the mislocalization of TDP-43 and delayed disease progression in TDP-43 transgenic mice (Liu *et al.*, 2015) (Table 1). In addition, cAMP-induced phosphorylation by PKA of the 26S proteasome on Rpn6/PSMD11 at Ser14 enhanced its activity to hydrolyse ubiquitinated proteins, ATP and small peptides (Lokireddy *et al.*, 2015). It also stimulated the degradation of protein aggregates, including SOD1, TDP-43, and FUS in motor neurons, as well as in cultured cortical neurons (Lokireddy *et al.*, 2015).

## PERK

Protein kinase R-like endoplasmic reticulum kinase (PERK), also known as eukaryotic translation initiation factor 2-alpha kinase 3, is a transmembrane protein kinase of the pancreatic eukaryotic initiation factor-2alpha kinase (PEK) family located in the ER membrane. ER stress evokes the activation of the UPS which is followed by activation of three major ER transmembrane proteins known as inositol-requiring enzyme 1 (IRE1), PERK, and activating transcription factor 6 (ATF6) (Kametaka *et al.*, 2007). The PERK pathway is the only one modulating protein synthesis as an adaptive response (Bell *et al.*, 2016). Typical ER stress markers were observed in soluble extracts of spinal cord tissue of sporadic ALS patients (Medinas *et al.*, 2017). Modulation of the eIF2α phosphorylation, involved in the PERK signalling, was protective against TDP-43 toxicity in flies and mammalian neurons (Kim *et al.*, 2014). Mutant TDP-43 transgenic flies showed increased eIF2α phosphorylation and an impaired motor function (Kim *et al.*, 2014). In line with this, knockdown of the fly homologue of PERK improved the motor function in these flies (Casci and Pandey, 2015). In addition, treatment of rat neurons and flies with a PERK inhibitor rescued the toxicity induced by TDP-43 overexpression (Kim *et al.*, 2014) (Table 1). Phosphorylation of eIF2α by PERK led to increased expression of ATF4 particularly in spinal cord of $SOD1^{G93A}$ mice (Kikuchi *et al.*, 2006). In addition, $SOD1^{G93A}$ mice showed more ATF4 expression at symptomatic stage compared to earlier stages, which suggests that PERK is activated during the disease progression (Kikuchi *et al.*, 2006). Furthermore, the assembly of stress granules induced by poly-PR encoded from the hexanucleotide repeat sequence in the *C9orf72* gene by repeat-associated non-ATG (RAN) translation was dependent on eIF2α phosphorylation (Boeynaems *et al.*, 2017). This is indicative for a potential toxic function of eIF2α phosphorylation by PERK in C9ORF72-related ALS. Considering the fact that TDP-43, FUS, as well as the arginine-containing dipeptide repeat proteins (DPRs) derived from the hexanucleotide repeats in C9ORF72, are all involved in stress granule dynamics, it could be that there is a more general role for PERK signalling in ALS (Smith and Mallucci, 2016). Phosphorylation of eIF2α and subsequent reduction of protein translation increased stress granules formation (Aulas *et al.*, 2017). Moreover, PERK-mediated phosphorylation of eIF2α initiated stress granule formation (Walker *et al.*, 2013) and a disturbance in stress granule dynamics might eventually cause neuronal dysfunction (Chitiprolu *et al.*, 2018; Fernandes *et al.*, 2018). Whether inhibition of PERK could have a beneficial effect for different ALS subtypes needs to be investigated further.

## TBK1

Besides its role in inflammation, TBK1 also plays an important role in autophagy through phosphorylation of different autophagy adaptors including p62/SQSTM1, OPTN, nuclear dot protein 52 kDa (NDP52) and Tax1 binding protein 1 (TAX1BP1) (Richter *et al.*, 2016). Disrupted autophagy of mutant SOD1 and TDP-43 seems to be involved in ALS patients carrying mutations in p62/*SQSTM1* (Teyssou *et al.*, 2013; Hadano *et al.*, 2016). In addition, TBK1 expression was reduced in $SOD1^{G93A}$ mice and deletion of TBK1 disrupted autophagy reproducing behavioural and locomotor symptoms in mice (Duan *et al.*, 2019). Moreover, ALS-associated mutations in *OPTN* downregulated autophagy and protein clearance (Markovinovic *et al.*, 2017). The assembly of ubiquitin chains triggered autophagy adaptor recruitment resulting in the activation of TBK1. In this process, TBK1 interacted with autophagy-relevant sites including ubiquitin- and LC3-binding domains of OPTN and p62/SQSTM1, as well as with the SKICH domains of NDP52 and TAX1BP1 (Richter *et al.*, 2016). Impairment of autophagy could be the result of defective recognition of LC3 binding domain by mutant p62/SQSTM1, and could lead to neurodegeneration *in vivo* (Goode *et al.*, 2016). TBK1 phosphorylates OPTN to promote mitophagy and it phosphorylates p62/SQSTM1 to promote autophagosome maturation (Vinet and Zhedanov, 2011). Mutations in *TBK1* decreased the clearance of dysfunctional mitochondria by reducing the binding of TBK1 to OPTN (Weil *et al.*, 2016), suggesting that TBK1 could indeed play a role in ALS pathogenesis. As different mutations in the *TBK1* gene cause different diseases, it was proposed that gain-of-function mutations in *TBK1* are associated with normal tension glaucoma, while loss-of-function mutations result in ALS/FTD or in herpes encephalitis (Ahmad *et al.*, 2016). So far, there are at least six different small molecules that are known to inhibit TBK1 that showed beneficial results improving diet-induced metabolic dysfunctions in mice (Cruz and Brekken, 2018). Considering the role of metabolic alterations in ALS patients and models (for a review see Vandoorne *et al.*, 2018), targeting TBK1 could also be considered as a therapeutic approach for ALS, although the effect of TBK1 inhibition on autophagy and mitophagy should be first clarified in more detail.

## PIKFYVE

FYVE finger-containing phosphoinositide kinase (PIKFYVE) belongs to a large family of evolutionarily-conserved lipid kinases, and inhibiting its activity increased C9orf72 motor neuron survival (Shi *et al.*, 2018). PIKFYVE phosphorylates

phosphatidylinositol 3-phosphate (PtdIns3P) to form phosphatidylinositol (3,5)-bisphosphate [PtdIns(3,5)P2]. The FYVE finger domain of PIKFYVE plays a vital role in localizing the protein to the cytosolic leaflet of endosomes through directly binding to membrane PtdIns3P, and is thereby involved in multiple processes of endosome dynamics (reviewed in Hasegawa *et al.*, 2017). Ubiquitous knockout of this protein was embryonic lethal in mice, while one active allele was sufficient for normal embryonic development and survival (Ikonomov *et al.*, 2011). C9ORF72 co-localized with Rab proteins, which are implicated in autophagy and endocytic transport in human spinal motor neurons, indicating a role in Rab-mediated endosomal trafficking in ALS (Farg *et al.*, 2014). In a recent study, induced motor neurons were generated from human iPSCs derived from patients carrying the hexanucleotide repeat expansion in *C9orf72*. The patient-derived induced motor neurons showed a lower survival after stress compared to control induced motor neurons (Shi *et al.*, 2018). In a subsequent small molecule screen, a PIKFYVE inhibitor, YM201636, significantly increased the survival of C9ORF72 patient-derived motor neurons by converting PtdIns3P into PtdIns(3,5)P2, which enhanced the fusion of lysosomes with both endosomes and autophagosomes under cell stress (Shi *et al.*, 2018) (Table 1). These results were confirmed using a different PIKFYVE inhibitor, apilimod (Shi *et al.*, 2018). In addition, brief treatment of apilimod directly into the hippocampal area rescued neurons from excitotoxic injury *in vivo* (Shi *et al.*, 2018), and dose-dependently reduced *N*-methyl D-aspartate (NMDA)-induced neurodegeneration in the hippocampus (Staats *et al.*, 2019). These results suggest that inhibition of PIKFYVE might be a viable therapeutic target in C9-ALS.

## Kinases and DNA/RNA metabolism

DNA/RNA metabolism is the process in which nucleic acids are synthesized and degraded (for a review see: Voet *et al.*, 2016). The discovery that mutations in the genes encoding the DNA/RNA binding proteins FUS and TDP-43 can cause ALS highlights the fact that DNA/RNA metabolism is an important player in ALS (Sreedharan *et al.*, 2008; Kwiatkowski *et al.*, 2009; Vance *et al.*, 2009). Different *in vitro* and *in vivo* experiments concerning FUS and TDP-43 confirmed that both proteins play a key role in various aspects of DNA/RNA metabolism (reviewed in Lagier-Tourenne *et al.*, 2010). Mutations in the angiogenin gene (*ANG*) have also been linked to familial and sporadic ALS (Wu *et al.*, 2007). Mechanistically, ANG-induced tRNA fragmentation was proposed to play a key role in the stress granule formation and neuronal loss (Emara *et al.*, 2010; Hanada *et al.*, 2013). Moreover, interfering with DNA damage represents another therapeutic avenue given the post-mitotic nature of motor neurons. In addition to familial ALS caused by mutations in *SOD1*, where protein misfolding is proposed to lead to a reduced protection against oxidative DNA damage (Barbosa *et al.*, 2010), two recent studies using FUS-ALS patient iPSC-derived motor neurons showed increased levels of DNA damage and an impaired DNA repair response (Naumann *et al.*, 2018; Wang *et al.*, 2018). Moreover, recent studies showed that different kinases are actively involved in DNA repair and tRNA processing in the context of ALS (Hanada *et al.*, 2013; Deng *et al.*, 2014; Kenna *et al.*, 2016; Havali-Shahriari *et al.*, 2017). Below, we focus on the main kinases currently described to play a role in these different processes (Fig. 2C).

### DNA-PK

DNA-PK is a serine/threonine protein kinase that belongs to the phosphatidylinositol 3-kinase-related kinase protein family. It is required for the NHEJ pathway of DNA repair, which repairs DNA double-strand breaks (Yin *et al.*, 2017). This kinase could play a role in ALS as one of its substrates is FUS (Rhoads *et al.*, 2018). Upon DNA damage, DNA-PK phosphorylates the N-terminal serine/threonine residues of FUS, resulting in the cytoplasmic translocation of FUS (Deng *et al.*, 2014). This implies that the proper function of FUS relies on phosphorylation. Recent studies showed that the prion-like low complexity domain (PrLD) of FUS in combination with an RGG domain are responsible for condensing FUS from a soluble condition into a gel-like phase, which could be the stepping stone for macroscopic aggregate formation (Patel *et al.*, 2015; Shorter, 2017; Bogaert *et al.*, 2018). Phosphorylation of the PrLD by DNA-PK inhibited the transition of FUS from a liquid to a solid phase and influenced the formation of FUS aggregates (Deng *et al.*, 2014; Patel *et al.*, 2015; Shang and Huang, 2016). Similar to FUS, TDP-43 also contains a prion-like domain and showed a similar ability to phase separate (Li *et al.*, 2018). It is not a stretch to predict that DNA-PK might also regulates TDP-43 by a similar mechanism.

### NEK1

The NEK kinases are a family of serine/threonine kinases that play an important role in the regulation of the disjunction of the centrosome, the assembly of the mitotic spindle and the DNA damage response (Fry *et al.*, 2012). NEK1 is the only member of the NEK kinase family required for the activation of the DNA damage response through ataxia telangiectasia and Rad3-related (ATR) kinase (Melo-Hanchuk *et al.*, 2017). As described above, variants in *NEK1* are associated with ALS (Brenner *et al.*, 2016; Kenna *et al.*, 2016), and the C21ORF2 protein can interact with NEK1 during DNA repair (Fang *et al.*, 2015). IPSC-derived motor neurons carrying a heterozygous nonsense mutation in *NEK1* showed increased DNA damage and impaired DNA damage response after induction of DNA damage (Higelin *et al.*, 2018). This indicates that loss-of-function of NEK1 can induce deficits in DNA damage repair that might contribute to neurodegeneration in ALS. Therefore, promoting the DNA damage response could be a potential approach to compensate for the loss-of-function of NEK1. As described above, expression of nuclear NEK1 isoform affected chromatin stability and induced nuclear pore complex dispersal (Feige

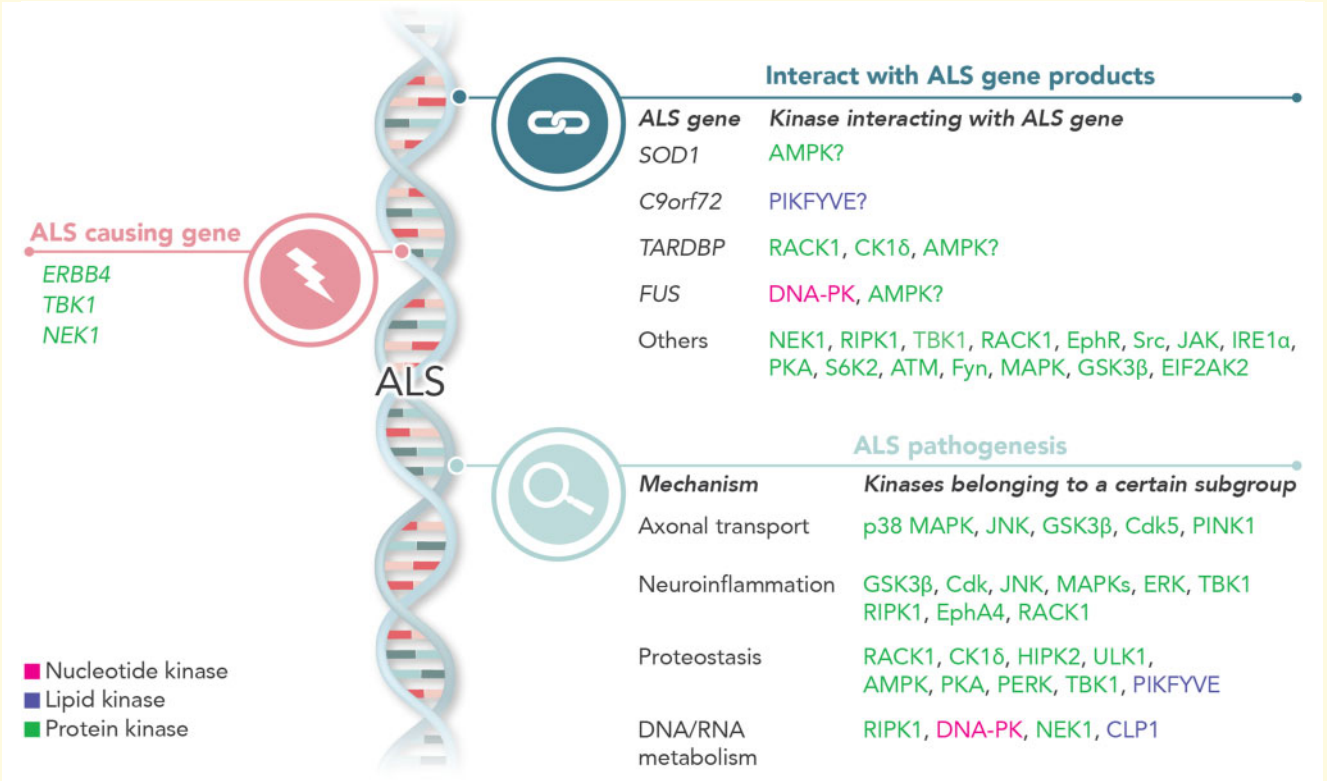


**Figure 3 The multifaceted role of kinases in ALS.** Overview of the different types of kinases involved in ALS genetics and pathophysiology. These include protein kinases (green), lipid kinases (purple) and nucleotide kinases (pink).

*et al.*, 2006). Recently, different studies suggested that defects in nucleocytoplasmic transport serve as a shared downstream consequence in different ALS subtypes (reviewed in Kim and Taylor, 2017). These include the identification of cytoplasmic protein aggregates of TDP-43, FUS, OPTN, UBQLN2 and recognition of impaired nucleocytoplasmic transport of C9ORF72 (Kim and Taylor, 2017). In line with the fact that NEK1 mutants co-occur with different other ALS genes and as it might play a role as an ALS disease modifier, mutations in NEK1 could eventually affect the nuclear pore complex interfering with nucleocytoplasmic transport of proteins and RNAs (White and Sreedharan, 2016; Nguyen *et al.*, 2018; Shu *et al.*, 2018). Targeting NEK1 might contribute to reverse nucleocytoplasmic defects (Walker and El-Khamisy, 2018). Therefore, clarification of the nuclear function and substrates of NEK1 is crucial to understand its role in ALS.

### CLP1

Polyribonucleotide 5′-hydroxyl-kinase Clp1 (CLP1) is a polynucleotide kinase and a component of the tRNA splicing endonuclease (TSEN) complex (Schaffer *et al.*, 2014). As the first discovered mammalian RNA kinase, it phosphorylates the 5′-hydroxyl groups of double-stranded RNA (dsRNA), single-stranded RNA (ssRNA), double-stranded DNA (dsDNA) and double-stranded DNA/RNA hybrids (Hanada *et al.*, 2013). CLP1 knockout mice showed progressive loss of spinal motor neurons, axonal degeneration in peripheral nerves, denervation of neuromuscular junctions resulting in impaired motor function, muscle weakness, paralysis and eventually respiratory failure leading to a reduced life span (Hanada *et al.*, 2013). This indicates that there is a link between CLP1 and the tRNA process which can lead to motor neuron degeneration in mice. Despite being indicative for a link between CLP1 and motor neuron degeneration, more data are required to propose CLP1 as a potential target for treating motor neuron diseases, including ALS.

## Modulating kinase activity: a potential therapeutic avenue for ALS?

As receptor tyrosine kinase signalling pathways have been successfully targeted to inhibit proliferation and angiogenesis in the context of cancer therapies (Bhullar *et al.*, 2018) and as kinase deregulation has been shown to play a role in ALS, kinases could potentially play a pivotal role in novel drug developments for ALS. Interestingly, kinase inhibitor drug discovery programs have recently broadened their

focus, including an expanded range of kinase targets (Ferguson and Gray, 2018).

The discoveries related to the potential role of different kinases in ALS resulted in testing different compounds, mainly kinase inhibitors, in different preclinical ALS models and in clinical trials (Table 1). Except some major kinases mentioned above, inhibiting some other individual kinases (e.g. apoptosis signal-regulating kinase 1 (ASK1), casein kinase 1δ (CK1δ), Janus kinase 3 (JAK3), extracellular signal-regulated kinase (ERK), Rho-associated, coiled-coil containing protein kinase (ROCK) also showed beneficial results in both ALS mouse models and clinical trials (Trieu *et al.*, 2000; Le Pichon *et al.*, 2013; Song *et al.*, 2013; Salado *et al.*, 2014; Fujisawa *et al.*, 2016; Lingor *et al.*, 2019) (Table 1). As a loss-of-function of different kinases could be involved in ALS, it is also possible that activating specific kinases could be a new approach to treat ALS. Interestingly, the most effective FDA-approved ALS drug, riluzole, was reported to function as an antagonist of protein kinase C (PKC) (Krieger *et al.*, 2003), despite the fact that the therapeutic effect of riluzole is mainly connected to an effect on excitotoxicity (Cheah *et al.*, 2010). Based on the significant results in $SOD1^{G93A}$ rats (Trias *et al.*, 2016), a recent phase II clinical trial (NCT02588677) in ALS patients showed a benefit of masitinib as an add-on therapy to riluzole resulting in a 25% delay in disease progression (Mora *et al.*, 2019). It is important that this positive effect will be confirmed in a phase III study (NCT02588677). Masitinib seems to modulate inflammatory processes by targeting a limited number of kinases at a safe dosage (Mora *et al.*, 2019). This is in line with the idea that a combined kinase-based treatment might be necessary to be effective. Given the widespread involvement of different kinases in various biological processes, a 'kinase targeting cocktail' treatment could be an even better option to achieve optimal clinical benefits for ALS patients. Yet, given the widespread physiological roles of kinases in various cell types extensive preclinical testing will be needed to minimize the potential side effects before kinase modulating drugs can be used in clinical practice. Whether this will be possible, given the pleiotropic role of most kinases in different pathways, is an open question and it might be more complicated than anticipated to answer this question.

# Conclusions and future perspectives

Some time ago, alterations of several protein kinases and their pathways were reported in $SOD1^{G93A}$ mice by Krieger *et al.* (2003). Since then, research findings expanded the scope beyond mutant SOD1 and illustrated that there could be a multi-faceted role for kinases in ALS pathogenesis (Fig. 3). Evidence supporting the importance of kinases in ALS stems from many different sources. First, recent genetic studies showed that mutations in kinase-encoding genes can be a risk factor or can even be a cause of ALS. Second, kinases interact with various ALS-related genes/gene products, and affect disease progression in many cases. Even without being a causal gene, different kinases directly regulate ALS-causing gene products and influence their function. Last but not least, kinases actively participate in regulating major pathological processes in ALS. However, it remains unclear how and to what extent these observations are interconnected. Therefore, investigating ALS-related alterations in kinase pathways, as well as their functional consequences is crucial. With the rapidly developing genetic and compound screening platforms, it is expected to be only a matter of time to obtain a clear kinome-wide picture of ALS. This, in combination with the availability of efficient kinase-targeting therapeutics, offers the intriguing opportunity to target multiple ALS-related processes simultaneously, which hopefully could lead to a new treatment strategy for ALS.

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# Competing interests

The authors report no competing interests.

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