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Roots to start research in amyotrophic lateral sclerosis: molecular pathways and novel therapeutics for future

Abstract: Amyotrophic lateral sclerosis (ALS) is a devastating neurological disease that rapidly progresses from mild motor symptoms to severe motor paralysis and premature death. There is currently no cure for this devastating disease; most ALS patients die of respiratory failure generally within 3–5 years from the onset of signs and symptoms. Approximately 90% of ALS cases are sporadic in nature, with no clear associated risk factors. It is reported that ALS is a complex and multifaceted neurodegenerative disease. Less is known about the key factors involved in the sporadic form of the disease. The intricate pathogenic mechanisms that target motor neurons in ALS includes oxidative stress, glutamate excitotoxicity, mitochondrial damage, protein aggregation, glia and neuroinflammation pathology, defective axonal transport, and aberrant RNA metabolism. Despite aggressive research, no therapy has been yet proven to completely reverse the core symptoms of the disease. Riluzole is the only drug approved by the Food and Drug Administration and recommended by the National Institute for Clinical Excellence so far proven to be successful against ALS and may prevent progression and extend life for a few months or so. This article provides a novel understanding in key findings of pathogenesis and interventions currently under investigation to slow disease progression in ALS.

Keywords: amyotrophic lateral sclerosis; motor neuron disease; pathogenesis; therapy.

Introduction

Amyotrophic lateral sclerosis (ALS), also known as Lou Gehrig's disease or Charcot's disease, is a rapidly progressive, fatal neurological disease that attacks motor neurons of the brain (called upper motor neurons), brain stem, and spinal cord (called lower motor neurons), including anterior horn cells, consequently causing progressive atrophy of associated muscle tissues responsible for controlling voluntary movements (Rowland and Shneider, 2001).

The earliest symptoms of ALS are muscle twitching (fasciculation), cramping, stiffness of muscle and muscle weakness affecting an arm or a leg, slurred speech, and difficulty chewing or swallowing (dysphagia). In the final stages of the disease, patients have trouble breathing as the respiratory muscles become weak (Table 1, primary and secondary symptoms). There is no cure for this devastating disease; most ALS patients die of respiratory failure normally within 3–5 years from the onset of signs and symptoms (Gil et al., 2008; Spataro et al., 2010). However, disease progression varies widely among affected individuals; about 10% of ALS patients survive up to 10 years (Turner et al., 2003).

Approximately 90% of ALS cases exist with no clear associated risk factors, known as sporadic ALS (SALS). About 10% of cases are thought to be inherited, known as familial ALS (FALS) (Ticozzi et al., 2011). Of these, 20% of all FALS cases result from mutation of copper-zinc superoxide dismutase 1 (SOD1); the *C9orf72* gene accounts for 30%–40%; and *TARDBP*, *FUS*, *ANG*, *ALS2*, *SETX*, and VAPB account for the remaining cases. The clinical presentation (symptoms) of SALS and FALS is almost very similar. Mutations in two genes, *ALS2* and *SETX*, have been reported in juvenile ALS (JALS) families (Al-Saif et al., 2011).

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Table 1 Symptoms attributable to amyotrophic lateral sclerosis.

Direct impact/primary symptoms (owing to motor neuronal degeneration)	Indirect/secondary symptoms (as a result of primary symptoms)
Weakness and atrophy of muscle	Psychological disturbances
Fasciculations and muscle cramps	Sleep disturbances
Spasticity	Constipation
Dysarthria	Drooling
Dysphagia/choking	Chronic hypoventilation
Dyspnea	Thick mucus secretions
Emotional lability	Pain

The incidence of SALS is approximately 2 per 100,000 people worldwide every year (Logroscino et al., 2008). The exception to this number was seen in the Western Pacific island of Guam and the Kii peninsula of Japan, where the incidence has been 50–100 times more than elsewhere in the world. This is probably due to an environmental toxin β-methylamino-L-alanine (Cox et al., 2003). The incidence was slightly higher in males than females, with a M:F ratio of about 1.5:1. The reason for this male excess has been the possible protective hormonal factors present in women. Mostly, ALS strikes people between 55 and 75 years of age (Byrne et al., 2013). Rarely, ALS will attack before 25 years of age, called as JALS (Gouveia and De Carvalho, 2007).

Early and accurate diagnosis of ALS has become a major challenge to neurologists. There are many different kinds of motor neuron diseases (like Kennedy's disease, or X-linked spinobulbar muscular atrophy, cervical spondylotic myelopathy, and myasthenia gravis) that can cause symptoms similar to ALS, and thus, a complete history of both general and neurological examination is an important first step in diagnosing ALS and ruling out other disorders.

Incorrect diagnosis, not surprisingly, is very common, ranging from 5% to 10% of the cases (Davenport et al., 1996). It is not often realized that there is no single test that absolutely proves the diagnosis of ALS. However, the combination of history, clinical examination, nerve tests, and blood tests often establishes the diagnosis beyond reasonable doubt. The World Federation of Neurology Research Group on Motor Neuron Diseases have developed the 1994 El Escorial diagnostic criteria (Brooks, 1994) and the revised 2000 Airlie House criteria (Brooks et al., 2000; El Escorial Criteria for the Diagnosis of Amyotrophic Lateral Sclerosis: www.medicalcriteria.com/site/index.php?option=com_content&view=article&id=54%3Aneuroals&catid=64%3Aneurology&Itemid=73&lang=en) to aid in diagnosing and classifying patients for research studies and clinical trials.

Current pathogenesis of ALS for future medication development

Based on the previous studies from ALS patients, transgenic animal models, and *in vitro* studies, it is believed that ALS is likely to be a complex, multifactorial, and multisystem disease. Several mechanisms have been implicated in the pathologenesis of ALS, such as oxidative stress, glutamate excitotoxicity, mitochondrial damage, protein aggregation, glia and neuroinflammatory pathology, defective axonal transport, and aberrant RNA metabolism (Figure 1).

Each of these pathophysiological processes has a distinct time frame of occurrence, some occurring instantaneously within minutes, whereas others take hours and days. These processes share overlapping and redundant features and cause injury to neuron, glia, and astrocyte cells, thus causing paralysis and loss of function of a particular part of the body supplied by the offended motor neurons. Targeting these pathways should facilitate the discovery of better clinical biomarkers and improved medication for the diagnosis, monitoring, and treatment of patients with ALS.

Oxidative stress

Oxidative stress is one of the major hallmarks of tissue damage and plays an important role in neurodegenerative diseases. It arises when there is an elevated level of ROS/RNS than is required for normal redox signaling in the body. This is caused primarily by insufficient levels of antioxidants or by inhibition of the antioxidant enzymes. Reactive oxygen intermediates, including hydrogen peroxide (H_2O_2), superoxide anions (O_2^-), hypochlorous acid, and hydroxyl radicals (OH^-), are formed as by-products of normal cellular metabolism in all eukaryotic cells. Generally, within the mitochondria, 1%–3% of molecular oxygen escapes out from electron
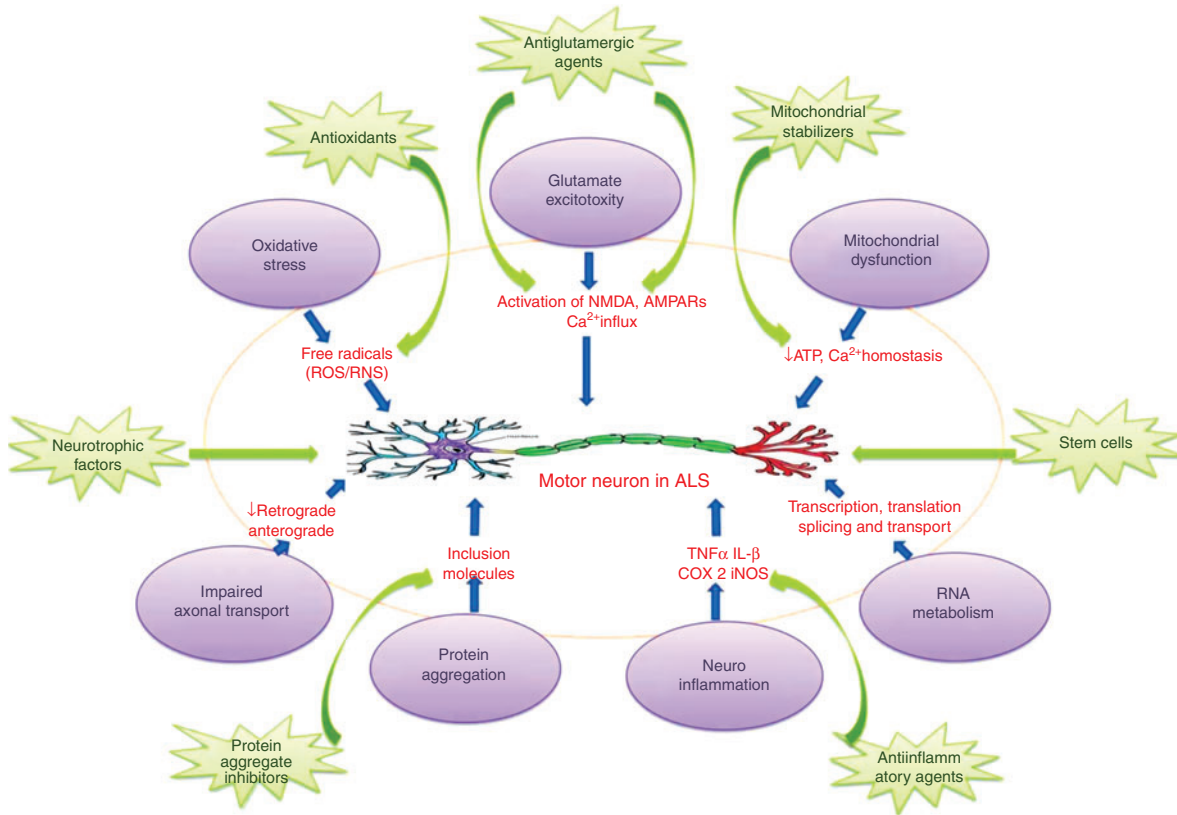
Figure 1 Molecular pathways and therapeutic strategies of motor neuron injury in ALS.

The figure denotes the pathogenic pathways as well as the therapeutic strategies for ALS. Excessive glutamate will stimulate NMDA and AMPA receptors, which leads to influx of excessive calcium and, ultimately, glutamate excitotoxicity. Oxidative stress produced by reactive oxygen species (ROS)/reactive nitrogen species (RNS) leads to membrane, protein, and DNA damage. Mitochondrial dysfunction leads to loss of Ca^{+2} homostasis, signaling, and decreased ATP production. Glia activates inflammatory cascade via secretion of TNF-α, ILβ, COX2, and iNOS. Aberrant proteins can form aggregates/inclusion molecules. Impaired axonal transport may contribute to an energy deficit in the distal axon and loss of retrograde and anterograde transport. Motor neurons might also undergo dysregulation of transcription, translation, splicing, transport, and abnormal RNA processing, which contribute to aberrant protein folding. Neurotrophic factors and stem cells directly help in protecting motor neurons. Antioxidants, mitochondrial stabilizers, and antiglutamergic agents reduce the burden of oxidative stress, mitochondrial dysfunction, and glutamate excitotoxicity, respectively. Anti-inflammatory agents inhibit and reduce the level of proinflammatory agents. Protein aggregate inhibitors hamper the formation of inclusion molecules. In this figure, oval shaped cartoons indicate pathogenic pathways, and star shape, therapeutic strategies in ALS.

transport chain and forms the superoxide anion O_2^-. This superoxide anion is converted by copper zinc SOD1 into H_2O_2. With the help of glutathione peroxidase and catalase, the H_2O_2 is converted into water and is extinguished from the cell. Besides these enzymatic detoxification processes, the cell contains nonenzymatic antioxidants such as α-tocopherol (vitamin E), retinol (vitamin A), and ascorbic acid (vitamin C). Further, activation of nitric oxide synthase (NOS) generates nitric oxide (NO), which merges with superoxide to form peroxynitrite (ONOO$^-$), a potent oxidant (Beckman and Koppenol, 1996; Drechsel et al., 2012). In the presence of ferrous ion (Fe^{+2}), peroxynitrite and H_2O_2 are converted into reactive hydroxyl radicals, which causes damage to various

cellular components such as DNA, proteins, and lipids (Figure 2). Besides this, a DNA repair enzyme, poly (ADP-ribose) polymerase-1 (PARP1), is also linked with the production of NO, which is implicated in oxidative stress. If there is any breakdown in DNA strands, this enzyme is activated and catalyzes the conversion of β-nicotinamide adenine dinucleotide into nicotinamide (NA) and long polymers of poly (ADP-ribose).

Even though the brain comprises 2% of the body weight, it consumes 20% of total oxygen utilized by the body, thus resulting in the generation of a large amount of ROS. The neuronal cells are highly susceptible to degeneration by ROS generated by redox dysregulation (Halliwell, 2006). In pathological conditions like ALS, the same
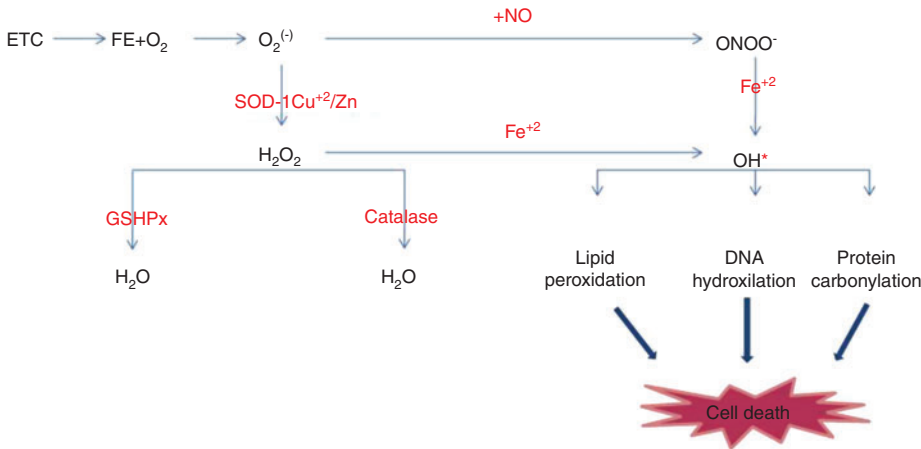
Figure 2 Scheme of the generation and role of free radicals in ALS.

radicals and other species are produced in enormous quantities, and some of them may be transformed into more toxic molecules (Said Ahmed et al., 2000). Increased oxidative stress damage to proteins, lipids, and DNA has been found in the central nervous system (CNS) tissue of the transgenic mouse model of FALS expressing mSOD1 (Andrus et al., 1998; Liu et al., 1998, 1999). This is further supported by the presence of increased amounts of oxidative damage markers like lipid peroxidation (Siciliano et al., 2002), oxidized DNA (Fitzmaurice et al., 1996; Ferrante et al., 1997), 3-nitrotyrosine levels (Abe et al., 1995; Beal et al., 1997), and protein carbonylation (Ferrante et al., 1997) in postmortem CNS tissue samples (mainly spinal cord) of both SALS and FALS patients.

Glutamate excitotoxicity

Glutamate is the major excitatory neurotransmitter in the CNS of mammals and consists of around 5–10 mmol/kg. The synaptic nerve impulse transmission in motor neurons is dependent upon the magnitude of glutamate available (Butcher and Hamberger, 1987). Rothstein first proposed glutamate-mediated excitotoxicity as an imperative mechanism of motor neuron degeneration when elevated levels of glutamate were observed in the cerebrospinal fluid of ALS patients (Rothstein et al., 1990). Excessive glutamate stimulates the postsynaptic glutamate receptors such as NMDA and AMPA receptors (Shaw, 2005), which leads to excitotoxicity by increasing the influx of calcium ions (Ca^{2+}) to enter the cell (Sen et al., 2005). Excessive Ca^{2+} entered into cell activates a myriad of enzymes, including phospholipases, endonucleases, and proteases such as calpain, which will lead to neuronal injury and cell death.

The excitatory activity of glutamate is usually terminated by reuptake of the neurotransmitter by excitatory amino acid transporters (EAATs) (Shaw, 2005), primarily by the EAAT1 and EAAT2 proteins on perisynaptic astrocytes. This is supported by preclinical and clinical investigations. Diminution of EAAT2 directly roots neuronal death in transgenic mice (Rothstein et al., 1996). At the final stage of the disease, the level of EAAT2 protein is approximately reduced to 50% of normal in the spinal cord of *SOD1*G85R mutant mice (Bruijn et al., 1997). Rothstein proposed the selective loss of EAAT2 glutamate transport in patients with SALS (Rothstein, 1994). This loss of EAAT2 was attributed to aberrant splicing of EAAT2 mRNA in affected areas of the CNS, which leads to increased levels of glutamate in synapses (Lin et al., 1998). Ameliorating glutamate excitotoxicity by inhibiting the glutamate release from motor neurons is a promising approach to prevent neuronal death in ALS, as riluzole, the only drug approved by the Food and Drug Administration (FDA) for ALS treatment, acts by inhibiting glutamate release from presynaptic nerve ends.

Mitochondrial dysfunction

The mitochondrion is a highly dynamic cell organelle within the cell that plays a pivotal role in ATP production, maintains calcium homeostasis, regulates cellular metabolism, and participates in calcium signaling (Manfredi and Xu, 2005; Santa-Cruz et al., 2012) and thus maintains membrane potential. However, in response to changes in the intracellular environment, mitochondria can rapidly change into death promoting organelle by producing ROS, release of prodeath proteins (BCl-2 family proteins),
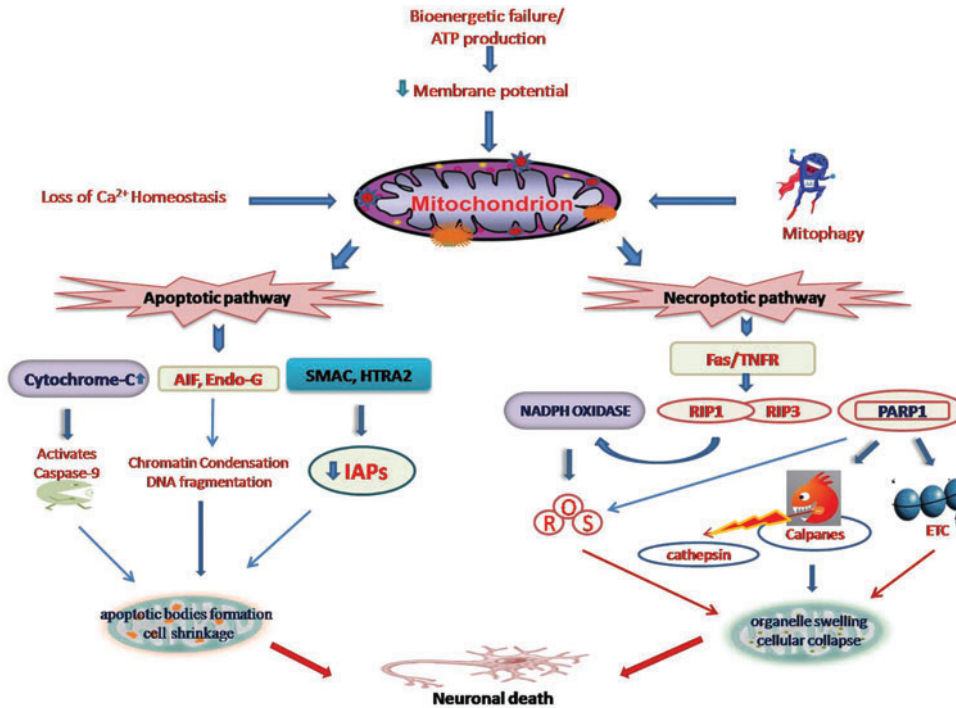
Figure 3 Role of mitochondria in neuronal death.
Mitochondria dysfunction is mediated by a plethora of intracellular mechanisms, such as loss of Ca^{2+} homeostasis, membrane potential, bioenergetic failure, and mitophagy, which ultimately leads to neuronal death by inducing a myriad of intracellular signals. Release of cytochrome C activates the caspase-9; AIF, endo-G, breaks the nuclear material. A rise in caspase level or a drop in inhibitor of apoptosis proteins (IAPs) causes neuronal death by forming apoptotic bodies and cell shrinkage. Activation of TNFR and Fas death receptors induces RIP complex, which will further act on NADPH oxidases to generate reactive oxygen species (ROS). Activated calpaines, cathepsins, and loss of ETC functioning causes cellular collapse and neuronal death.

and activation of many cell death pathways involved in neuronal death like necrosis, apoptosis, and autophagy (Figure 3).

Even though the majority of the mitochondrial polypeptides are encoded by nuclear genome, some of the mitochondrial polypeptides are encoded by its own DNA. Human mitochondrial DNA (mtDNA) is a closed circular molecule that consists of 16,569 DNA base pairs, and each cell, on average, consists of approximately five such mtDNA molecules. The mitochondrial genome encodes 13 polypeptides components of ETC as well as mitochondrial rRNA and tRNA that support the intramitochondrial protein synthesis.

Apoptosis

Apoptosis is also known as programmed cell death, in which cells induce to commit suicide. Several mitochondrial intermembrane space proteins become proapoptotic if they are released into the cytosol. Cytochrome c release causes the activation of apaf-1 (apoptosome),

which cleaves the proenzyme of caspase-9 into its active form, which targets several cellular components. Apoptosis inhibitor factor (AIF), a protein initiating a caspase-independent pathway of apoptosis and endonuclease G (endo-G), is a mitochondrial enzyme that cleaves DNA at GC tracts. Both AIF and endo-G exit through a mitochondrial membrane into cytoplasm and from there translocates into the nucleus in order to prepare the cell for death by damaging the DNA by triggering chromatin condensation and DNA fragmentation. Bcl-2 (antiapoptotic) and BAX (proapoptotic) proteins regulate the release of AIF and endo-G into the cell. Second mitochondria-derived activator of caspase (SMAC), also referred to as DIABLO, is a mitochondrial protein that in humans is encoded by the *DIABLO* gene. HTRA2, a serine protease, is a mitochondrial enzyme that in humans is encoded by the *HTRA2* gene. If present in the mitochondria, both help in removing denatured proteins within the mitochondria. Once these two proteins (SMAC and HTRA2) are released from mitochondria, they will inhibit or degrade the cytosolic inhibitor of apoptosis proteins. All these processes will mingle together, causing plasma membrane bebbling,

apoptotic body formation, cell shrinkage, and finally, cell death by a process called apoptosis (Lin and Beal, 2006).

Necroptosis

Necrosis is a random, uncontrolled, and accidental premature cell death characterized by the loss of cell membrane integrity, organelle swelling, mitochondrial dysfunction, release of cell death products into intracellular space, and cellular collapse. Regulated necrosis is termed as *necroptosis* or *programmed necrosis*, a complex phenomenon, and mitochondria play a pivotal role in this pathway. Recently, this new concept of necroptosis came into existence with the discovery of some key signaling components like receptor interacting protein kinases (RIP1 and RIP3), PARP1, and NADPH oxidases in the cell death process. RIP1 and RIP3 are serine/threonine-protein kinases, key signaling molecules that are activated by the activation of the death receptors like Fas and tumor necrotic factor receptor (TNFR). These proteins are dissociated from TNFR and form a complex in cytosol, which subsequently acts on the mitochondria. In addition, NADPH oxidase is activated by the RIP1/RIP3 complex, which generates ROS. PARP1 has multiple roles; it activates RIP complex and calpains. The activated calpains cause the rupture of lysosomes and release of cathepsins, which will lyse the mitochondria. Excessive activation of PARP1 also stimulates the AIF-mediated DNA degradation, which will further activate the production of PARP1. The intermediate molecules produced in all these pathways, such as ROS, RIP complex, and cathepsins, act on mitochondria and lead to necroptotic neuronal cell death (Galluzzi and Kroemer, 2008; Baines, 2010).

Mitochondrial dysfunction is found in many neurodegenerative disorders; previous evidence suggests that mitochondrial dysfunction as a major event is involved in the pathogenesis of ALS (Cozzolino and Carri, 2012). Pathological studies have explored the existence of morphologically abnormal or damaged mitochondria in motor neurons from ALS patients (SZi and Iwata, 1996; Wiedemann et al., 2002), mouse models (Kong and Xu, 1998), and cultured neuronal cellular models, implicating mitochondrial dysfunction in the disease pathogenesis.

Many structural and functional defects, including flawed respiratory function, diminished oxygen consumption, lack of respiratory control, and reduced membrane potential, were seen in mitochondria obtained from astrocytes of genetically mutated SOD1-G93A rats (Cassina et al., 2008). Consistent deficits in the subunits of ETC and activity of mitochondrial complex I and IV of the ETC activities have been noticed in the spinal cord and skeletal muscle of ALS patients (Borthwick et al., 1999; Vielhaber et al., 2000; Sasika and Iwata, 2007; Crugnola et al., 2010). Mutant *SOD1* is localized in mitochondria and accumulates on the outer membrane and inside the intermembrane space (Kawamata and Manfredi, 2008). Increased Ca^{+2} concentration and mitochondrial dysfunction were found in ALS animal models (Kruman et al., 1999). Thus, mitochondrial dysfunction plays a critical role in ALS pathogenesis.

Protein aggregation

Cytoplasmic inclusions or aggregates have been found in all neurodegenerative diseases including ALS and are formed by abnormal accumulation of proteins (Ross and Poirier, 2004). A variety of protein aggregates have been identified as implicated in the pathogenesis of the disease, including SOD1 (Sheng et al., 2012), TDP-43, FUS (Vance et al., 2009; Corrado et al., 2010), OPTN (Ying and Yue, 2012), UBQLN2 (Synofzik et al., 2012), and C9ORF72 (Mori et al., 2013). Of all these, SOD1 was the first protein to be identified that has a key role in causing cellular toxicity in FALS cases. The ubiquitin proteasome system, molecular chaperones, and the autophagy-lysosome system help to monitor protein structure and defend cells from dysfunctional, mal-folded, denatured, and damaged proteins. The existence of ubiquitin, p62, and molecular chaperones in ALS aggregates implicates the role for all these systems in the pathogenesis of ALS. Molecular chaperones, mainly heat shock proteins, are upregulated in ALS spinal cord (Anagnostou et al., 2010). These findings therefore highlight the importance of protein aggregation in ALS, and preventing the aggregation of one or all of the proteins will have potential benefit in the treatment of neuronal injury in ALS.

Glia and neuroinflammation

Glia are the resident innate immune cells found in brain and are implicated as active contributors of neuronal damage in ALS, in which the overactivation and dysregulation of microglia might result in disastrous and progressive neurotoxic consequences (Sargsyan et al., 2005; Block et al., 2007). Histological investigations of tissue from ALS patients confirmed the presence of abundant activated microglia in brain, brain stem, corticospinal tract, and spinal cord (Ince et al., 1996). Experimental studies with transgenic murine models of ALS implicate

the correlation of microglia activation and proliferation in the pathogenesis of the disease. Disease course examination and genetic manipulations have particularly reveled this fact. Studies with asymptomatic transgenic mice models of ALS revealed the fact that activation of microglia starts well before the appearance of motor defects (Hall et al., 1998; Alexianu et al., 2001). Further positron emission tomography (PET) imaging reveals the activation of microglial in patients with neurodegenerative diseases and in animal models (Turner et al., 2004).

NF-κB was activated in microglia and astrocytes of murine models of autoimmune encephalomyelitis and thermal ablation injury of brain (Kaltschmidt et al., 1994). Activated NF-κB initiates the transcription of a wide variety of inflammatory-associated genes, which consist of inducible NOS (iNOS), cyclooxygenase-2 (COX2), matrix metalloproteinase-9 (MMP-9), Bcl-xL, interleukins (IL-2, IL-6, IL-8, IL-12), p40, intracellular adhesion molecule-1 and -2 (ICAM-1, ICAM-2), vascular cell adhesion molecule-1, TNF-α, and interferon-γ (Baldwin, 2001). This results in the secretion of a wide variety of inflammatory molecules such as NO, prostaglandins (PGE_2, PGI_2, PGD_2, $PGF_{2\alpha}$), cytokines (IL-2, IL-6, TNF-α, and TGF-β), adhesion molecules that includes selectins (P-, E-, L-selectin), integrins (β2-integrin), and immunoglobulins. Several experimental animal models of ALS confirmed that expression of proinflammatory molecules is an early event that occurs before the development of clinical symptoms (Xie et al., 2004; Lewis et al., 2012). Down streaming these mediators will be helpful in suppressing neuroinflammation. However, recent reports indicate that microglia has not only neurotoxic consequences but also neuroprotective properties (Block et al., 2007; Henkel et al., 2009) (Table 2). Further studies are mandatory to resolve these issues.

Impaired axonal transport

Axonal transport is a cellular process responsible for the transport of mitochondria, lipids, proteins, and other cell organelles to and from a neuron's cell body through the cytoplasm of its axon. Axonal and cell body accumulations of organelles and other proteins are hallmark pathologies for many human neurodegenerative diseases, mainly in the case of ALS. Axonal transport can be divided into anterograde (movement of molecules/organelles towards the axon tip) and retrograde (movement of molecules/organelles towards the cell body). The motor proteins

Table 2 Neurotoxic and neuroprotective effects of inflammatory mediators.

Inflammatory mediator	Neurotoxic effect	Neuroprotective effect
TNF-α	Increase in neurotoxic mediators	Increase in neutrophilic factors
	Inhibition of glutamate uptake	Control of extracellular Ca^{2+}
	Increase in Ca^{2+} signaling in neurons	Induction of antiapoptotic factors
	Stimulation of apoptosis of endothelial cells	Induction of antioxidants
	Edema formation	
	BBB breakdown	
IL-1β	Endorse gliosis	Increase in survival promoting factors
	Increase in Ca^{2+} in neurons	Induction of IL-1ra
	Edema formation	
	BBB breakdown	
	Endogenous pyrogen	
iNOS	Stimulation of inflammatory response	Vasodilatation and increase in energy supply to neurons
	Lipid peroxidation	
	Inhibition of enzymes for DNA replication	
	Stimulation of inflammatory mediators	
MMP-9	Stimulation of leukocyte adherence and transmigration	Clearance of necrotic cell debris
	BBB breakdown	Stimulation of neuroplasticity, recovery, and repair
	Edema formation	
ICAM-1	Attachment of leukocytes to endothelium	
	Stimulation of diapedesis	
MCP-1	Regulation and migration of leukocyte trafficking	Stimulation of neuroplasticity, recovery, and repair
	Stimulation of phagocytes	
IL-6, IL-8	Endogenous pyrogen	Induction of IL-1ra
	Attraction of T-lymphocytes	

kinesin and dynein are mechanochemical enzymes that move cargoes in the anterograde and retrograde directions, respectively (Schwartz, 1979). Neuronal expression of point mutation in the p150^{Glued} subunit of the dynactin gene was observed by Puls in a family with motor neuron disease (Puls et al., 2003).

Neurofilaments (NFs) are the most abundant cytoskeletal proteins in motor neurons that facilitate in providing structural support for the axon, initiating axonal growth, and determining the axonal diameter. Abnormal accumulation of NF in cytoplasm will leads to degradation of motor neurons. NF side-arm phosphorylation results in increased NF pausing (Ackerley et al., 2003). Codon deletions and insertions in the phosphorylation repeat domain of *NEFH* (which encodes NFH) have been identified in some cases of SALS (Tomkins et al., 1998; Al-Chalabi et al., 1999). Overexpression of wild-type NFL, NFH, peripherin, or mutant NFL in mice causes motor neuron dysfunction, axonal atrophy, and perikaryal accumulations of neurofilaments (Collard et al., 1995). Alterations in the stoichiometry of NF subunits and microtubule disorganization can cause defective axonal transport and ALS. SOD1 transgenic mouse models of ALS illustrate the evidence of slow axonal transport in motor neurons (Williamson and Cleveland, 1999). A considerable amount of research work has suggested the importance of impaired axonal transport in the neuronal death of ALS cases.

C9orf72 mutation

Recently, mutation in *C9orf72* (chromosome 9 open reading frame 72) was reported as a chief genetic cause of ALS. Hexanucleotide consists of four guanines, and two cytosines repeat expansions (GGGGCC)n in a noncoding region of *C9orf72* were identified as an initiatory molecular cascade of disease pathology and defects (Renton et al., 2011; Williams et al., 2013). When this sequence of hexanucleotides is repeated too many times, it can lead to ALS. There is no exact numerical value for the number of repeats needed to cause the disease. But from previous studies, researchers believed that a minimum of 30 repeats will initiate the pathogenesis, while a maximum of 1600 repeats was found in diseased individuals (DeJesus-Hernandez et al., 2011; Renton et al., 2011). Mutations in this gene were found not only in ALS but also in frontotemporal dementia (FTD), a neurodegenerative disorder that affects personality, memory, behavior, and language, and ALS-FTD. This expanded repeat of hexanucleotide was the main genetic cause of the disease and accounts for 33% FALS and 8% of SALS cases (Farg et al., 2014).

The typical function of protein synthesized by the *C9orf72* gene is unknown. Three isoforms of the protein are synthesized by the *C9orf72* gene. The hexanucleotide repeat diminishes the amount of protein produced by the *C9orf72* gene. The protein is found in many parts of the nervous system, including the cerebellum, cortex, and spinal cord. Within the cytoplasm of the neuron, the protein is presented mainly around the nucleus. In addition to this, vesicles present at synaptic bulbs also contain a large amount of this protein. Manal suggested the possible role of *C9orf72* in endosomal trafficking and autophagy by depletion of *C9orf72* using siRNA dsyregulation (Manal et al., 2014). Canadian researchers developed the first animal model to study the function of *C9orf72* mutation in ALS.

RNA processing (TDP-43 and FUS/TLS)

Lately, RNA processing has become implicated in the pathogenesis of ALS with the identification of mutations in two genes with RNA/DNA-binding functions, TDP-43 (transactive response DNA-binding protein) (Kabashi et al., 2008; Rutherford et al., 2008; Sreedharan et al., 2008; Daoud et al., 2009) and FUS/TLS (fused in sarcoma/translocated in liposarcoma) (Kwiatkowski et al., 2009; Vance et al., 2009; Hewitt et al., 2010). TDP-43 is composed of 414 amino acids that consist of two RNA-recognition motifs: a nuclear localization signal and a nuclear export signal (Da Cruz and Cleveland, 2011). This protein is normally concentrated in the nucleus but also shuttles back and forth between the nucleus and the cytoplasm (Ayala et al., 2008). Increased transport into the cytoplasm leads to the formation of aggregates in the cytoplasm. This was evidenced by experimental studies as aggregates were seen in the cytoplasm of the brain and spinal cord of ALS patients (Arai et al., 2006; Neumann et al., 2006; Giordana et al., 2010), in murine models (Tatom et al., 2009; Wils et al., 2010), and in *Drosophila* (Li et al., 2010).

The discovery of mutations of TDP-43 in ALS was followed by the invention of mutations in another RNA/DNA-binding protein, that is, FUS/TLS. Mutations of this protein primarily cause the accumulation of aggregates in the nucleus, with low levels detected in cytoplasm (Andersson et al., 2008). Abnormal accumulation of FUS/TLS inclusions was observed in the neurons and glial cells of postmortem brain and spinal cord of ALS patients with FUS/TLS mutations (Kwiatkowski et al., 2009; Tateishi et al., 2009; Vance et al., 2009). The distinct role of these two proteins is not fully explored. Although these two proteins have structural and functional similarities and belong to

a family of heterogeneous ribonucleoproteins, there is no evidence that these proteins acts together. The involvement of these two proteins in ALS pathogenesis is shifting the research towards the understanding of RNA processing for the treatment of neurodegenerative disorders.

Cognitive impairment associated with ALS

Initially, it was believed that ALS is devoid of cognitive functions, but it is now proven to involve a number of cognitive impairments. Mostly patients who have ALS have mild cognitive impairment, and 5% have a clinical subtype of frontotemporal lobar degeneration (FTLD) called FTD (Lomen-Hoerth et al., 2003).

FTLD was initially described as Pick's disease, which is the second most common cause of progressive cognitive impairment after Alzheimer's disease. The three forms of FTLD were defined by consensus criteria in 1998. The most common form that is described in patients with ALS is frontal variant FTD; the other two forms are nonfluent progressive aphasia, which is characterized by language impairment, and semantic dementia, which is characterized by loss of conceptual knowledge. Clinically cognitive decline in ALS has been visualized by personality change, irritability, obsessions, poor insight, and pervasive deficits on frontal executive tests. Some studies have reported cognitive decline in well-characterized clinical cohorts of patients with ALS, which includes neuronal loss in frontal and temporal lobar atrophy and ubiquitinated tau-negative and synuclein-negative intraneuronal inclusions (Gallassi et al., 1985; Strong, 2001).

Some studies (David and Gillham, 1986; Kew et al., 1993) have reported that free picture recall is affected in patients with ALS. Immediate recall, as judged by the registration component of the Mini-Mental State Examination, might not be affected (Rakowicz and Hodges, 1998), although this examination is not a very sensitive test of memory. Language seems to be impaired in MRI and PET studies of ALS patients (Neary et al., 1998), which supports the previously mentioned findings that extramotor pathways are affected in ALS. Language deficits have included mainly reduced verbal output (Strong et al., 1999), deficits in naming of objects (Bak and Hodges, 2004), perseverations, echolalia (repetition of words said by other people), stereotypic expressions, and semantic paraphasias (substitution of words that relate closely to one another, e.g., sock for glove).

There is no agreement for behavioral impairment in ALS, but clinically, it is thought to represent abnormalities that do not meet the Neary criteria for FTD. Behavioral impairment in ALS can be classified on the basis of presentation of frontal-lobe-type behavioral impairment in two or more areas, as measured from a standardized caregiver interview (Murphy et al., 2007). However, it is still under debate whether to classify patients with ALS who have cognitive impairment together with or separate from those who have behavioral impairment.

Genetically, both ALS and FTD seem to be same but are clinically different. There might be a clinical pathological spectrum of neurodegeneration in only a subset of both conditions. For example, there is emerging pathological evidence that motor neuron degeneration associated with depletion of *C9orf72* and deposition of TARDBP is linked to a different pathogenic mechanism from that associated with mutations in SOD1 (Mackenzie et al., 2007), particularly in mice.

Pharmacotherapy of ALS

Despite aggressive research, no therapy has been yet proven to completely reverse the core symptoms of this devastating disease, and thus, the development of new therapeutics is imperative. The recent approaches to ALS treatment included pharmacotherapy, stem cell therapy, ventilation therapy, and psychotherapy.

Pharmacotherapy focuses mainly on relieving symptoms associated with the pathogenesis and maintaining an optimal quality of life. Treatment is based on individual therapy with a gold standard drug and or continual adaptation with substituent medication. Riluzole is the only drug approved by the FDA and recommended by the National Institute for Clinical Excellence (NICE) so far proven to be effective against ALS and may prevent progression and prolong life for a few months or so. Riluzole acts by decreasing the release of glutamate. Oxidative stress and excitotoxicity are early events in ALS pathogenesis, occurring before any cytopathology changes can be identified. Therefore, antioxidants and antiexcitotoxic agents are ALS prevention strategies appropriate for investigation. More recently, there has been some epidemiological evidence that long-term antioxidant supplementation with vitamin E may reduce the risk of ALS. In addition, clinicopathologic and neuroradiologic studies show that inflammation via activation of microglia will follow the process of neuron destruction in ALS. Nonsteroidal anti-inflammatory drug (NSAID) use has been associated

with decreased risk for ALS. Therefore, despite the negative clinical trials with NSAIDs for the treatment of ALS, these may still have a role in reducing the risk for ALS. Other neurodegenerative processes, such as mitochondrial dysfunction, impaired axonal transport, and protein aggregation, may then chase the pathological process and aggregate the disease condition. Use of drugs like creatine, olesoxime, ariclomol, and scriptaid is helpful as an add-on therapy to slow down the progression of the disease. New treatments under investigation are aimed at slowing the progression and treating the symptoms of ALS (Table 3).

Pharmacological treatments currently under investigation

Gilenya/Fingolimod

Fingolimod (Gilenya, Novartis, Dorval, Canada) is an oral sphingosine-1-phosphate-receptor modulator that is currently being evaluated for the treatment of ALS. It acts on G-protein couple receptors, regulating proliferation, adhesion, migration, differentiation, and adhesion. There is also evidence that fingolimod acts by preventing lymphocyte egress from lymph nodes (Matloubian et al., 2004). This leads to a reduced infiltration of potentially autoaggressive lymphocytes into the CNS (Bartholomãus et al., 2009). *In vitro* models of ALS have proved that fingolimod has neuroprotective properties (Aiden and Ralf, 2010). Recently, a randomized, double-blind, phase II study was designed to determine the acute safety and tolerability of oral administration of 0.5 mg fingolimod versus the matching placebo. This study is ongoing and recruitment of participants has been initiated (clinicaltrials.gov: Gilenya in Amyotrophic Lateral Sclerosis, https://clinicaltrials.gov/ct2/show/NCT01786174?term=NCT01786174&rank=1).

Ariclomol/BRX-220

Ariclomol (CytRx Corporation, Los Angeles, CA, USA), an analog of bimoclomol, induces heat shock protein during cell stress by amplyfing heat shock protein gene expression. Arimoclomol has been shown to extend life in an animal model of ALS (Kalmar et al., 2008). A randomized, double-blind, and placebo-controlled phase 2/3 trial was designed to determine the safety and efficacy of

arimoclomol 200 mg in patients with SOD1-positive FALS. Rate of decline of ALS Functional Rating Scale-Revised (ALSFRS-R) over a period of 12 months, disease progression as measured by the rate of decline in forced expiratory volume in 6 s, and motor unit number estimation are the primary and secondary outcome measures designed, respectively. This study is currently recruiting participants (clinicaltrials.gov: Phase II/III Randomized, Placebo-controlled Trial of Arimoclomol in SOD1 Positive Familial Amyotrophic Lateral Sclerosis, https://clinicaltrials.gov/ct2/results?term=NCT00706147&Search=Search).

Rasagiline

Rasagiline (Teva Pharmaceutical Industries Ltd, Petah Tikva, Israel) is a MAO-B inhibitor used in Parkinson's disease (Lecht et al., 2007). The drug had a significant dose-dependent therapeutic effect on preclinical motor functions and survival of animals (Waibel et al., 2004). A phase II multicenter, randomized, double-blind trial is ongoing to compare the effect of rasagiline as an add-on therapy to riluzole in the treatment of ALS. The primary aim of the trial is to examine the survival time, and secondary outcome measures are change in ALSFRS and quality of life. This study is currently ongoing (clinicaltrials.gov: Study of Rasagiline in Patients With Amyotrophic Lateral Sclerosis, https://clinicaltrials.gov/ct2/results?term=NCT01879241&Search=Search).

Ozanezumab

Ozanezumab (GlaxoSmithKline, Brentford, London) is a monoclonal antibody that acts by inhibiting the neurite outgrowth inhibitor-A (NOGO-A). Preclinical studies revealed that a high expression of NOGO-A affects neuromuscular junction integrity, and destabilization of nerve terminals and suppression of the same increased the health of motor neurons in mice, indicating the effective role of NOGO-A in ALS (Jokic et al., 2006). A phase II, multicenter, randomized, double-blind trial was designed to investigate the efficacy and safety of intravenous administration of ozanezumab as compared to matching placebo. The study is currently ongoing to assess the effect of intervention on the function and survival time in ALS patients (clinicaltrials.gov: Study of Ozanezumab (GSK1223249) Versus Placebo in the Treatment of Amyotrophic Lateral Sclerosis, https://clinicaltrials.gov/ct2/results?term=NCT01753076&Search=Search).

Table 3 Pharmacotherapeutic agents for ALS.

Target	Agent/Compound	Mechanism of action	Route of administration	Phase/Trial	Remarks/Study outcome	References
Glutamate excitotoxicity	Riluzole[a]	Reduces influx of calcium ions by blocking NMDA receptors	PO	FDA approved	Slowed the progression of disease and improved survival in patients	Bensimon et al., 1994
	Lamotrigine	Blocks calcium channels and inhibits release of glutamate	PO	DB RCT	No significant value in altering the disease progression	Ryberg et al., 2003
	Dextrometorphan	Blocking NMDA receptors	PO	DB RCT	No improvement in survival of ALS patients	Blin et al., 1996; Gredal et al., 1997
	Gabapentin	Interaction with voltage-gated calcium channels	PO	Phase 3	No evidence of a beneficial effect on disease progression or symptoms in patients with ALS	Miller et al., 2001; Kalra et al., 2003
	Topiramate	Antagonist of AMPA/kainate subtype of the glutamate receptors	PO	DB RCT	Faster rate of decline in muscle strength, lack of efficacy, and fabricate adverse effects	Cudkowicz et al., 2003
	Memantine	Blocking NMDA-type glutamate receptors	PO	Phase 2/3	Well tolerated, safe, and positive outcome on survival time	De Carvalho et al., 2010
	Ceftriaxone	Decreases glutamate levels near nerves	IV/IM	Phase 3	No improvement in survival in ALS	NCT00349622
	LY300164	AMPA antagonist	PO	Phase 2	Change in ALS functional rating score	NCT00696332
	L-arginine	Antiglutamatergic	PO	Preclinical	Significantly slowed the progression of neuropathology in lumbar spinal cord, delayed onset of motor dysfunction, and prolonged life span in mice	Lee et al., 2009
Oxidative stress	CoQ10	Antioxidant	PO	Phase 2	No significant differences in survival and safety concern	Levy et al., 2006; Kaufmann et al., 2009
	Vitamin E	Free-radical scavenger	PO	Phase 3	No effect observed on primary outcome measured by Norris limb scale, but patients are less prone to develop the disease state from mild to more sever state	Desnuelle et al., 2001; Wang et al., 2011
	N-acetylcysteine	Antioxidant	SC	Phase 2DB RCT	Did not result in survival or reduction in disease progression	Louwerse et al., 1995; NCT00539513
	Edavarone (MCI-186)	Free-radical scavenge	IV	Phase 3 DB RCT	Safe and well tolerated and there was a suggestion of reducing the progression of disease	NCT00330681
	Zinc and copper	Antioxidant	PO	Phase 1/2	High doses of zinc and copper are safe in patients with ALS	NCT01259050
	Apocynine	Inhibitor of NADPH oxidase	PO	Pre-clinical	Extend the survival of the $SOD1^{G93A}$ ALS mice	Harraz et al., 2008
	Epigallocatechin-3-gallate (EGCG)	Antioxidant	PO	Preclinical	Delayed the onset of disease and extended life span	Xu et al., 2006b

(Table 3   Continued)

Target	Agent/Compound	Mechanism of action	Route of administration	Phase/Trial	Remarks/Study outcome	References
Mitochondrial dysfunction	Creatine	Stabilizes the mitochondrial transition pore and supports mitochondrial ATP production presumptively abating dysfunction occurring early in the course of ALS	PO	DB RCT	No benefit in improving motor, respiratory, or functional capacity in ALS patient	Shefner et al., 2004; Rosenfeld et al., 2008
	Olesoxime (TRO19622)	Prevention of mitochondrial dysfunction and improved microtubule dynamics	PO	Phase 2/3	Safe and well tolerated, slowed the progression of disease	NCT01285583
	Dexpramipexole	Increases the efficiency of mitochondria	PO	Phase 2	Dose-dependent benefit of 300 mg dexpramipexole on functional decline and survival	Rudnicki et al., 2013
	Melatonin	Antagonism of both caspase-mediated and caspase-independent mitochondria-induced cell death	PR	Phase 1/2	High-dose melatonin suitable for normalizing serum protein carbonyls an oxidative stress marker	Weishaupt et al., 2006
Neuroinflammation	Celecoxib	Inhibition of prostaglandin synthesis	PO	Phase 2 DB RCT	No beneficial effect of celecoxib in improvement of motor functions in ALS	Cudkowicz et al., 2006
	Gilenya[b] (fingolimod)	Sphingosine 1-phosphate receptor modulator	PO	Phase 2	Study currently recruiting participants	NCT01786174
	Glatiramer acetate	Shifts proinflammatory Th1 cells to regulatory Th2 cells that suppress the inflammatory response in ALS pathogenesis	SC	Phase 2	Enhanced lymphocyte proliferation observed in patients	Gordon et al., 2006
	Thalidomide	Potent anti-inflammatory effects through the modulation of key cytokines including TNF-α	PO	Phase 2	Does not appear to effectively modulate disease progression and can cause adverse effects	Stommel et al., 2009
	ONO-2506	Modulator of astrocyte activation	SC	Phase 2	Rate of decline of respiratory function determined as vital capacity	NCT00403104
	Anakinra	IL-1 receptor antagonist	SC	Phase 2	No effect of anakinra on the progression of symptoms of ALS	NCT01277315
	Celastrol	TNF-α ↓ iNOS, CD40	PO	Preclinical	Significantly improved weight loss and motor performance	Kiaei et al., 2006
Protein aggregation	Ariclomol[b]	Inhibit protein aggregation by heat shock protein induction	PO	Phase 2/3	Study currently recruiting participants	NCT00706147
	Sodium phenylbutyrate	Histone deacetylase inhibition	PO	Phase 2	Safe and effective, therapeutically efficient in improving histone acetylation levels	Cudkowicz et al., 2009
	Sodium Valproate	Histone deacetylase inhibition	PO	Phase 3	Safe but not effective in ameliorating the disease progression	NCT00136110
	Scriptaid	Histone deacetylase inhibition	NA	Preclinical	Prevented aggresome formation	Corcoran et al., 2004

(Table 3 Continued)

Target	Agent/Compound	Mechanism of action	Route of administration	Phase/Trial	Remarks/Study outcome	References
Neurotrophic factors	BDNF	BDNF is agonist of TrkB and LNGFR receptors	SC	Phase 3	Significant benefit in ALS patients with early respiratory impairment by preventing the progression of the disease	Kasarskis et al., 1999
	G-CSF	Induce neurogenesis and counteract apoptosis	SC	Phase 2, 3	Study currently recruiting participants	NCT01825551
	Xaliproden	Secretion of several endogenous motor neuron growth factors and serotonin 5-HT1A receptor agonist	PO	Phase 3	No statistical significance on functional parameters, especially vital capacity	Meininger et al., 2004
	r-IGF-1	Support neuronal regeneration and antiapoptotic	SC	Phase 3 DB RCT	No beneficial effect in patients with ALS	Sorenson et al., 2008
	Ciliary neurotrophic factor	Promotes neurotransmitter synthesis and neurite outgrowth and reducing tissue destruction during inflammatory attacks	IT	Phase 2/3 DB RCT	No statistically significant in treating ALS symptoms and there were increased adverse events and deaths with 5 µg/kg treatment	Miller et al., 1996
	VEGF	Stimulate cellular responses by binding to tyrosine kinase receptors	IT/IV	Phase 1/2	Safe and tolerable through assessments of adverse events, vital signs, clinical laboratory tests, and MRI of brain	NCT01384162
	Mechano-growth factor	Increases in muscle strength	SC	Preclinical	Significant improvement in hindlimb muscle strength and an increase in motor unit and motoneuron survival	Riddoch-Contreras et al., 2009
Miscellaneous	Rasagiline[b]	MAO-B inhibitor	PO	Phase 2	Study currently recruiting participants	NCT01879241
	Ozanezumab[b]	Neurite outgrowth protein (NOGO-A) inhibitor	IV	Phase 2	Study currently recruiting participants	NCT01753076
	Fasudil[b]	Selective RhoA/Rho kinase (ROCK) inhibitor	IV	Phase 2	Study currently recruiting participants	NCT01935518
	CK-2017357[b]	Selectively activates fast skeletal muscle troponin complex	PO	Phase 2	Study currently recruiting participants	NCT01709149
	Cannabis sativa extract[b]	Antispasticity effect	Oromucosal spray	Phase 2/3	Study currently recruiting participants	NCT01776970

[a]FDA-approved drug.
[b]Agents being currently investigated in clinical trials for ALS.PO, per orally; SC, subcutaneous; IV, intravenous; PR, rectally; IM, intramuscular; IT, intrathecal; NA, not available.

Fasudil

Fasudil (Tocris A Bio-Techne Brand, Bristol, UK) is a potent Rho-kinase inhibitor and vasodilator approved in Japan for the treatment of cerebral vasospasm. Recently, various *in vitro* and *in vivo* studies have proved that fasudil prevents motor neuron cell death, slows down disease progression, and increases survival time (Takata et al., 2013; Tönges et al., 2014). A phase II open-label study was designed by Peking University Third Hospital. The study is currently recruiting participants to test the safety and efficacy of fasudil on the lives of ALS patients (clinicaltrials.gov: A Clinical Trial of Safety and Efficacy of Fasudil in Subjects With Amyotrophic Lateral Sclerosis (ALS), https://clinicaltrials.gov/ct2/results?term=NCT01935518&Search=Search).

CK-2017357

CK-2017357 (Cytokinetics Inc., South San Francisco, CA, USA) (Tirasemtiv) is novel small molecule that selectively activates fast skeletal muscle troponin complex and thereby enhances sensitivity to weak electrical impulse generated by worsening motor nerves. Preclinical studies conducted on mice have found that a high dose of tirasemtiv significantly increased the cross-sectional area of muscle fiber as well as respiratory response (Miciak et al., 2013). A phase II randomized, double-blind 20-week study was designed to evaluate the efficacy and safety of CK-2017357 when taken alone or in combination with riluzole. The primary outcome measure is ALSFRS-R score, and secondary outcome measures are maximum voluntary ventilation and sniff nasal inspiratory pressure measurement (clinicaltrials.gov: Study of Safety, Tolerability & Efficacy of CK-2017357 in Amyotrophic Lateral Sclerosis (ALS), https://clinicaltrials.gov/ct2/results?term=NCT01709149&Search=Search.).

Cannabis sativa

Cannabis sativa is an herbaceous plant that yields around 60 cannabinoids; tetrahydrocannabinol (THC) is the main constituent having an antispasticity effect. Cannabinoids delay the onset of disease symptoms and prolong the survival rate significantly in SOD1 (G93A) ALS mouse (Weydt et al., 2005; Shoemaker et al., 2007). A multicenter, randomized, double-blind, phase II/III trial was designed to study the effect of the intervention on the safety and efficacy of decreasing the spasticity symptoms in ALS patients. The primary outcome measure is the 5-point modified Ashworth Scale score, and secondary measures are spasticity, spasm frequency, and sleep disruption numeric rating scale score. This study is currently recruiting participants (clinicaltrials.gov: Safety and Efficacy on Spasticity Symptoms of a *Cannabis Sativa* Extract in Motor Neuron Disease, https://clinicaltrials.gov/ct2/results?term=NCT01776970&Search=Search).

Stem cell therapy in ALS

The recent breakthroughs in stem cell research might nevertheless provide possibilities for neural implantation and cell replacement therapy for patients with ALS. The attraction of cell implantation or transplantation is that it might help to overcome the inability of the CNS to replace lost neurons. Stem cell studies have yielded positive results in various *in vivo* ALS models using a variety of different stem cell types. Mesenchymal stem cells (bone marrow derived) and neural progenitor cells (spinal cord derived) are the two cell types with the most evidence for use in ALS. Both mesenchymal stem cells and neural progenitor cells showed positive results on an SOD1 animal model, demonstrating improved survival, when compared with control animals (Xu et al., 2006a; Uccelli et al., 2012). There have only been a few human studies that have produced encouraging results, most of them having small, nonrandomized samples (Table 4) (Mazzini et al., 2008). Much work remains to be done before stem cell treatment can ever be regarded as even an experimental therapeutic modality in ALS.

Recently, in September 2013, two Harvard Stem Cell Institute scientists, Drs. Eggan and Rubin, collaborated with the German biotech company Evotec to screen potential drugs in their ALS stem cell models in the hopes of quickly identifying molecules that can be developed into ALS treatments (Evotec and Harvard Stem Cell Institute Form CureMN Collaboration to Advance ALS Research, http://www.menafn.com/27c0eb16-8e5b-47e7-8e92-fb2dfa29dd9d/Evotec-and-Harvard-Stem-Cell-Institute-Form-CureMN-Collaboration-to-Advance-ALS-Research?src=main).

Human neural stem cells

Human neural stem cells (HNSCs) have the ability to divide and differentiate continuously to produce different types of neurons, astrocytes, and oligodendrocytes. HNSCs are widely tested in various neurodegenerative diseases like

Table 4 Stem cell therapy for ALS.

Target/Agent	Mechanism of action	Route of administration	Phase/Trial	Remarks/Study outcome	References
Autologous mesenchymal stromal cells	Can differentiate into functional motor neurons	IT	Phase 1/2	Clinically feasible and relatively safe and induces immediate immunomodulatory effects in patients	Karussis et al., 2010
HNSCs[a]	Primarily differentiate into neurons, astrocytes, and oligodendrocytes	IT	Phase 1/2	This study is currently recruiting participants	NCT01640067
BM mononuclear cells	Have the capacity to differentiate into neural lineages	Intraspinal	Phase 1/2	Stabilized the disease progression as measured by forced vital capacity and the neurologic scale measure	Blanquer et al., 2010
HSSCs[a]	Replacing the nerve cells that have died and generating new supporting cells	IT	Phase 1	Study ongoing	NCT01348451

[a]Agents being currently investigated in clinical trials.
IT, intrathecal.

ALS, Parkinson's disease, Alzheimer's disease, and traumatic brain injury like cerebral stroke. HNSCs can have the ability to migrate and replace the dying neurons in CNS (Imitola et al., 2004). Various preclinical studies with HNSCs showed promising results by slowing muscle degeneration (Yan et al., 2007). A phase I trial was conducted in 18 ALS patients to test the safety of the intervention by giving 1/10 of cell dose that is intended for use for therapeutic dose. The results of the phase I trial were very promising, as quality of life was significantly increased and the intervention is safe to use. The FDA approved the start of phase II dose escalation and safety studies. This study is currently recruiting participants (Human Neural Stem Cell Transplantation in Amyotrophic Lateral Sclerosis (ALS), https://clinicaltrials.gov/ct2/results?term=NCT01640067+&Search=Search.).

Human spinal stem cells

Human spinal stem cells (HSSCs) are derived from spinal cord and are known to express amino acid transporters and thereby reduce glutamate excitotoxicity caused by accumulation of glutamate. They also have the ability to secrete neurotropic factors that are helpful in growth and survival of budding neurons. Preclinical studies conducted on rodent models reveal the survival of HSSCs in various neurodegenerative diseases like spinal ischemia and ALS. This effect was significantly increased with the use of combined delivery of various immunosuppression regimens after intraspinal transplantation in SOD1 (G93A) rats (Hefferan et al., 2011). A phase I study is ongoing to determine the safety of HSSC in ALS (Human Spinal Cord Derived Neural Stem Cell Transplantation for the

Treatment of Amyotrophic Lateral Sclerosis, https://clinicaltrials.gov/ct2/results?term=NCT01348451&Search=Search).

Ventilation therapy

The majority of ALS patients experience difficulty in breathing due to weakening of respiratory muscles. Unlike patients with other respiratory problems, ALS patients have difficulty in pulling oxygen in and releasing carbon dioxide out into the air. This results in depletion of oxygen and buildup of carbon dioxide in the blood. Therefore, the use of supplemental oxygen is normally not suitable. The solution to this problem in ALS patients is to use either noninvasive mechanical ventilation called intermittent positive pressure ventilation or bilevel positive air pressure or invasive ventilation called a ventilator. Use of mechanical ventilators improved quality of life and survival in ALS patients (Radunovic et al., 2009; Dreyer et al., 2014).

Psychotherapy

Depression is very common among ALS patients (Lou et al., 2003; Atassi et al., 2011); most of the patients undergo a state of depression after identification of the disease. Treatment with antidepressants and patient counseling will help the patients and their family members to cope. Sometimes, it is necessary to give psychological therapy, which is usually best if given by family members, in addition to prescribing the antidepressants. Substances with low anticholinergic activity, such as fluoxetine, are

preferable. If chronic pain, excessive salivation, sweating, or sleep disturbances are present, one of the tricyclic antidepressants will give more benefit (Atassi et al., 2011).

Discussion and conclusion

ALS is still a fatal neuromuscular disease. Only the future will decide which among the described molecular pathways will best serve as the new targets for the therapeutic manipulation of motor neuronal death in ALS. Early and accurate diagnosis of ALS has become a major hurdle to neurologists to treat the disease properly in time. Despite aggressive research, riluzole remains the only FDA-approved and NICE-recommended pharmacological therapy proven to be effective against ALS that may prevent progression of the disease. Thus, new treatments that will treat the underlying disease are desperately needed. Several promising randomized clinical trials are ongoing, and an increased collaboration between pharmaceutical companies, basic researchers, and clinical researchers, neurologists, and psychologists has the potential to bring closer to developing an optimum treatment for ALS. The greatest therapeutic success will probably be derived by the use of a single medication that targets more than one pathogenic pathway or combining agents with different mechanisms of actions illustrated. It is very soon to predict the precise compound that will be the effective treatment option for ALS.

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