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The pre-clinical discovery of Amyotrophic Lateral Sclerosis Drugs

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Abstract

Introduction—Amyotrophic Lateral Sclerosis, also referred to as Lou Gehrig's disease is characterized by the progressive loss of cells in the brain and spinal cord that leads to debilitation and death in 3–5 years. Only one therapeutic drug, Riluzole, has been approved for ALS and that drug improves survival by 2–3 months. The need for new therapeutics, either that can postpone or slow the progression of the motor deficits and prolong survival, is still a strong unmet medical need.

Areas Covered—Although there are a number of drugs currently in clinical trials for ALS, this review provides an overview of the most promising biological targets and preclinical strategies that are currently being developed and deployed. The list of targets for ALS was compiled from a variety of websites including: individual companies that have ALS programs, and the author's experience.

Expert Opinion—Progress is being made in the identification of possible new therapeutics for ALS with recent efforts in: understanding the genetic causes of the disease, susceptibility factors, and the development of additional preclinical animal models. However, many challenges remain in the identification of new ALS therapeutics including: the use of relevant biomarkers, the need for earlier diagnosis of the disease, and additional animal models. Multiple strategies need to be tested, in the clinic, in order to determine what will be effective in patients.

Keywords

genetic diseases; motoneuron disease; neurodegeneration; stem cells; therapeutic strategies

1. Introduction

Amyotrophic Lateral Sclerosis (ALS) was first described in 1873 and became also referred to as Lou Gehrig's disease after the famous baseball player who was diagnosed with ALS in 1939. ALS is a progressive disease of nerve cells in the brain and spinal cord that control voluntary muscles [1]. The degeneration of these motoneurons to the point where they can no longer signal the muscles causes muscle weakening leading to the inability to move arms and legs and eventually leads to inability to breathe on ones own. ALS most commonly affects people between 40–60 years of age, and men are affected slightly more often than women (1.4 :1) [1]. The incidence of ALS is 1–2 per 100,000, while prevalence is 4–6 per 100,000 of total population [1].

Unfortunately, there is no known cure for ALS. ALS is diagnosed based on the history of the patient and physical examination of the patient. It can be difficult to diagnose ALS because symptoms can vary between individuals but a combination of Electromyography (EMG) and nerve conduction tests is used for diagnosis. EMG records the electrical activity from the brain and/or spinal cord to the arms and legs during contraction and at rest. From

the time to diagnosis to death is usually from 3–5 years. This rapid debilitating disease has a known genetic cause in only about 10% of the cases that follow a familial inheritance (FALS). Most cases have no known inheritance and are called sporadic ALS (SALS). Both FALS and SALS produce similar pathological changes and symptoms [2, 3–5].

There is one approved drug for ALS called Riluzole that was approved in 1995 and has been shown to extend life by 2–3 months but not relieve symptoms of the disease. Riluzole has anti-excitotoxic properties [6–7], by presumably inhibiting the release of glutamate and it also blocks some of the postsynaptic effects of glutamate by inhibiting NMDA and AMPA receptors. Due to the degeneration of the motor neurons, there are a variety of symptoms including spasticity, difficulty swallowing, and degeneration and wasting of muscle. There are drugs available that can help provide symptomatic relief. For example, Baclofen or diazepam may be effective in controlling spasticity. In addition, physical therapy can help alleviate the pain from muscle contractures and related joint pain and the use of braces or a wheelchair, or other orthopedic measures may be needed to maximize muscle function. With only one approved drug that does not provide any symptomatic relief, there is clearly a need for new and better drugs.

This review will focus on the multiple strategies for new therapeutics for ALS. The genes that have been associated with ALS can provide some clues to the mechanism of the disease. $\text{Cu}^{+2}/\text{Zn}^{+2}$ superoxide dismutase 1 (SOD1) was the first causative gene identified in 1993. 15–25% of the FALS cases are linked to a genetic defect in the gene coding the antioxidant enzyme SOD1. The rest of the FALS cases have not been linked to SOD1 indicating other yet unidentified genetic causes. Recently, mutations in the TAR DNA-binding protein gene (TDP43) [8] and FUS/TLS gene have been found to occur in about 4 to 5 percent of the familial ALS cases [9–11].

More recently, additional genes have been linked to ALS including angiogenin, KIFAP3, and UNC13A [2]. The function of these genes and their associated networks is one of the strategies for therapeutics development. It is believed that multiple factors may underlie the disease mechanism [12–13].

In 2011, there are more than 15 clinical trials involving the testing of therapeutics [14] taking place for ALS, so the outlook for the disease is looking more promising. The results of these current clinical trials will address the value of a number of strategies, although most of the drugs in clinical trials for ALS have been originally developed for other indications. This also means that the regulatory path continues to get better defined. However, the biggest concerns are the lack of sensitive biomarkers to effectively monitor progression of disease and the fear that by the time the patients are symptomatic, much damage has already occurred minimizing the chances for impacting the disease process. For the sake of focus, however, this review will provide discussion of multiple therapeutic strategies that are currently in preclinical development. In addition, stem cell therapies will also be reviewed.

Multiple mechanisms have been proposed to account for the progressive motor neuron death in ALS. These include oxidative stress, neurofilament damage, mitochondrial abnormalities, glutamate-mediated excitotoxicity, altered response to hypoxia and gene regulation based defects. A number of cellular pathways have been shown to be dysregulated in both patients and in animal models of ALS. The sequence of events and the causative nature of the events are still far from understood, however, many of the cellular pathways are inter-related suggesting that these could have an impact on the disease progression. The list of targets is quite long and includes targets involved in protein misfolding causing ER stress, proteasome inhibition and autophagy. Possibly related is impaired axonal transport, mitochondrial damage and misregulation of apoptosis, oxidative stress, glutamate induced excitotoxicity,

neuroinflammation, and transcriptional dysregulation. Most of the recent strategies are focused on one or more of these cellular pathologies.

2. Existing drugs

Riluzole is the only approved drug for ALS and this drug has been demonstrated to increase life expectancy for patients with ALS by a few months [15].

3. Drugs Currently in Clinical Trials

Isis Pharmaceuticals is currently running Phase I clinical trials with anti-sense oligonucleotides to SOD1 [16]. Because antisense drugs do not cross the blood-brain barrier the drug is administered directly into the cerebral spinal fluid. KNS-760704 is in Phase II (dexpramipexole; also referred to by others as R-(+) pramipexole, RPPX) is an orally administered small molecule in clinical development by Knopp and Biogen Idec for the treatment of ALS. The drug is the optical enantiomer of pramipexole, a selective, high affinity dopamine agonist for Parkinson's disease and restless legs syndrome as Mirapex® (U.S.) and Sifrol® (Europe). Pramipexole is exclusively the S(-) enantiomer (i.e., not a mixture of both enantiomers) and KNS-760704 is exclusively the R(+) enantiomer. Both KNS-760704 and pramipexole demonstrate neuroprotective properties independent of dopamine receptor interaction, but KNS-760704 exhibits greatly reduced dopamine receptor affinity allowing a broader dose range than pramipexole. NeuroNova's sNN0029 is a novel drug candidate that contains vascular endothelial growth factor (VEGF) protein, a naturally occurring protein scientifically proven to be an important survival factor for motor neurons. The intended therapy involves direct infusion of sNN0029 into the cerebrospinal fluid (CSF) that circulates around the brain and spinal cord by intracerebroventricular (ICV) administration. In Phase I/II, Trophos currently has their drug olesoxime (TRO19622), in ALS Phase III clinical trials. Olesoxime is a mitochondrial pore modulator, a member of a series of cholesterol-oxime compounds that were identified in their neuronal cell screening platform [17]. Preclinical studies have demonstrated that these compounds promote the function and survival of neurons and other cell types under disease relevant stress conditions [18] through interactions with the mitochondrial permeability transition pore (mPTP). Trophos has completed Phase Ib clinical trials. Cytokinetics has their drug CK-2017357 in Phase II clinical trials as a potential treatment for diseases and conditions including ALS, associated with aging, muscle weakness and wasting or neuromuscular dysfunction. CK-2017357 is a fast skeletal muscle troponin activator that results in increased skeletal muscle force and slowing of time to muscle fatigue.

4. Targets Strategies (see also Table 1)

4.1. Protein aggregation, refolding, and clearance

Protein aggregation is a common feature of most of the neurodegenerative diseases but it takes different forms depending on the disease. In both sporadic and familial forms of ALS inclusions are found that contain SOD1, TDP-43, or FUS/TLS proteins. Strikingly, the ALS inclusions don't stain with the same dyes that stain amyloid aggregates found in Alzheimer's disease patients. There is also some debate over whether the inclusions are the same in SOD1 as they are in TDP-43 and FUS/TLS and whether they are toxic to the cells or more of a protective defense [19]. FUS/TLS and TDP-43 both contain a prion domain prone to pathological misfolding [20]. Cambria has a small molecule compound in preclinical development for ALS that inhibits the mutant SOD1 aggregation formed in ALS [21–22]. A slightly different strategy is being taken by Chaperone Therapeutics who has identified small molecules that have been shown to be effective at suppressing protein aggregation by activating the human heat shock transcription factor 1 (HSF1) which activates the entire

chaperone protein network. Chaperone proteins prevent both assembled protein subunits and newly synthesized proteins from aggregating and can be involved in facilitating both refolding and clearance of abnormally folded proteins. Chaperone proteins can work synergistically to combat protein aggregation; therefore, enabling the natural chaperone protein response to be effective at reducing levels of protein aggregation. Many chaperones are heat shock proteins because the tendency for proteins to aggregate is increased in response to proteins that are denatured in response to cellular stress. NexGenix Pharmaceuticals platform is also based on the role of chaperone activity and their target is the heat shock protein, Hsp90. Their lead compounds are chemically modified synthetic derivatives of the natural product radicicol. A number of Hsp90 inhibitors are in development mainly for cancer applications but those that cross the blood brain barrier could be effective in ALS [23]. Reata Pharmaceuticals has identified a novel molecule that binds directly to SOD1 and acts as a molecular chaperone to stabilize misfolded mutant SOD1. It is called RTA 801 and is the first SOD1 folding stabilizer to emerge from their discovery program. This strategy has also been pursued in academics [24]. Debiopharm Group and Curis Inc has a small molecule called Debio 0932 (formerly CUDC-305) that is an Hsp90 inhibitor [25]. It is orally bioavailable, and in preclinical testing, this compound was shown to effectively cross the blood brain barrier. Hsp70 is another member of the family of heat shock proteins and participates in protein folding and cellular stress and participates in the disposal of damaged proteins. ALS Biopharma, LLC is developing both Hsp70 as a protein biologic in addition to small molecule modulators of Hsp70. They are pursuing both ALS and Alzheimer's disease indications. Histone deacetylase (HDAC) inhibitors are an established class of drugs that modulate the structure and function of proteins regulating a number of physiological processes including gene expression, differentiation, migration, mitosis, autophagy and stem cell identity. Inhibition of HDACs control a critical intracellular mechanism for degradation of misfolded proteins called the "aggresome" pathway. A number of HDAC inhibitors have been in clinical use for cancer but are limited by poor tolerability and lack of target specificity among the HDAC family [26]. Repligen Corporation is developing HDAC inhibitors for Friedrich's Ataxia and Huntington's and would also have applications to ALS. They are currently evaluating their compound in preclinical models. The biotech company, Acetylon has licensed technology from Dana Farber Cancer Center and Harvard University and combines their knowledge of acetylated protein networks and powerful chemical synthesis methodology to develop inhibitors of HDAC6. Acetylon is developing highly selective small-molecule HDAC inhibitors for specific diseases within the areas of cancer, inflammation, neurodegeneration, genetic protein deficiencies, and infectious diseases. This drug target is of particular interest in ALS because there is data to suggest that HDAC6 is involved in regulating various proteins involved in axonal transport including tubulin and possibly dynactin [27]. EnVivo Pharmaceuticals is developing a small molecule brain penetrant HDAC inhibitor for Alzheimer's disease and schizophrenia, but could also be effective for ALS. ExonHit Therapeutics and Allergan uses gene profiling technology to identify genes whose splice variants produce abnormal proteins which may trigger or contribute to the development of disease. Their most relevant initiative is a series of small molecule HDAC inhibitors being developed for neurodegenerative indications. FoldRx (now owned by Pfizer) has developed technology to mitigate the toxicity from protein misfolding and protein aggregation diseases. They have so far successfully applied this technology with their drug that stabilizes the native state of transthyretin to treat the disease Transthyretin amyloidosis. They are applying their technology to TDP-43 for ALS and Frontal Temporal dementia. NeuroPhage has developed novel technology for diseases that involve misfolding or protein aggregation using phage technology. The new approach, developed at Tel Aviv University, consists of using phage viruses to physically disaggregate aggregated proteins, such as amyloid and is also being applied to TDP-43 for ALS. Proteostasis is focused on discovering and developing novel small molecule therapeutics designed to control the pathways such as the

ubiquitin-proteasome pathway, that maintain a critical balance among protein synthesis, folding, aggregation, trafficking and degradation. Their therapeutic focus is on emphysema, type II diabetes, and neurodegenerative diseases including ALS. Sirtris (now owned by Glaxo SmithKline) is also developing small molecules that up-regulate sirtuins, a family of seven NAD⁺-dependent, protein deacetylase enzymes associated with the aging process. Preclinical and clinical studies are underway in a variety of models including neurodegenerative diseases [28–29].

4.2. Reactive oxygen species

Strategies to prevent reactive oxygen species are being pursued by Takeda with the small molecule Pioglitazone which is an FDA-approved drug for diabetes. Pioglitazone has also been shown to be protective in brains of Relapse-Remitting Multiple Sclerosis [30] and in a mouse ALS model [31–33]. It reduced production of cytokines & reactive oxygen species in cultured neurons. However, Phase II clinical trials were terminated due to lack of efficacy (clinicaltrials.gov). Vasopharm is developing a small molecule VAS2870, which is an NAD(P)H oxidase (NOX) modulator that confers neuroprotection by preventing the generation of reactive oxygen species. There is support for this in the literature for stroke and neurodegenerative diseases. [34]. The company GenKyoTex is taking a similar strategy with developing novel NOX inhibitors for a number of indications including ALS. The company Stealth Peptides has a small molecule (despite their name) that is an antioxidant that functions by blocking the activity of CD36, thereby providing mitochondrial protection via antioxidant scavenging action. It has very good blood brain barrier penetration and has provided significant reduction in infarct volume in animal studies for stroke. It is being evaluated for full toxicology in preparation for an IND. Their drug could have therapeutic potential for ALS, as well. Retrotope, Inc. has a small molecule novel anti-oxidant approach that isotopically stabilizes polyunsaturated fatty acids to protect against free-radical oxidative damage targeting mitochondria. This is a new approach to controlling ROS-induced cellular damage through oxidative stress. Monounsaturated oleic acid or with one of the deuterated 11,11-D2-linoleic or 11,11,14,14-D4- α -linolenic acid, confer protection in their yeast model. Deuterated polyunsaturated fatty acids (PUFAs) also confer protection to wild-type yeast subjected to heat stress. These results indicate that isotope reinforced PUFAs are stabilized compared to standard PUFAs, and they protect yeast cells against the toxic effects of lipid autoxidation products [35].

4.3. Inflammation

Inflammation is the body's response to tissue damage. There is increasing evidence that inflammation is associated with motoneuron death in ALS, although there is no evidence, so far, that ALS is an autoimmune disease. Glial cells are part of the immune system and when they become over-activated, they may add to the damage [36]. There is evidence that ALS is non-cell-autonomous from the observation that wild type glial cells extend survival of SOD1 mutant motor neurons [37]. There are a number of strategies underway to interrupt the inflammatory cascade in ALS. Transition Therapeutics is developing anti-inflammatory compounds that inhibit glial cell activation and inhibit over-production and release of pro-inflammatory cytokines (IL-1 β , TNF α , IL-6 and MCP-1) from glia. ALS Therapy Development Institute (ALS-TDI) has a number of compounds they are developing with anti-inflammatory properties including TDI-00846 which is a blocking antibody to CD40L in order to modulate the progression of ALS. For ALS-TDI's other strategies, see their website [38]. Cognosci has three lead small synthetic peptides of 10–17 amino acids that are in preclinical development that are based on the ApoE protein that have strong in vitro and in vivo anti-inflammatory activity. Cognosci is initially focused on other neurodegenerative disorders but their peptides could also help in ALS. Daval International Ltd is developing what they call AIMSPRO which is derived from the serum of goats that have been

inoculated with a variety of vaccines so that it contains a broad spectrum of antibodies. AIMSPRO has anti-inflammatory properties, as it contains cytokines that induce a predominately functional cytokine response that has an anti-inflammatory profile. Reportedly, several "open-label" observations in patients with demyelinating diseases of CNS/PNS have suggested improved axonal conduction by the modification of voltage gated ion channels. Some studies suggest a lowering of sodium channel triggering voltage change which is the opposite of how local anesthetics work. AIMSPRO has orphan disease status for treatment of ALS and Krabbe Leukodystrophy (neurodegenerative disease affecting myelin sheath), with potential beneficial effects on axonal conduction. A single ALS patient case study has been documented with AIMSPRO and this study is now being expanded to include additional patients [39–41]. ZZ Biotech is developing new protein biologic therapy based on recombinant Activated Protein C (APC), a protease with anticoagulant that is currently being used for sepsis. It also has cytoprotective properties. They have developed a genetically engineered variant of recombinant APC, named 3K3A-APC that has 90% reduced anticoagulant activity, but preserved cell-protective and anti-inflammatory activities compared to APC. Scientists studied the ability of APC to slow the cell death that occurs in the mouse model of ALS. They were able to extend the lifespan of mice with an aggressive form of the disease significantly, by about 25 percent. The compound also extended the length of time that the mice were able to function well despite showing some symptoms of the disease, and it reduced the pace of muscle wasting that is a hallmark of ALS [42].

4.4. Iron chelation

The company Varinel is developing a small molecule that has been developed by Professor Youdim at the Technion University. The small molecule has multifunctional activity that includes (1) iron chelation, presumably reducing oxidative damage to brain cells caused by iron-catalyzed free radical reactions; (2) selective irreversible inhibition of the important mitochondrial brain enzymes, monoamine oxidases A and B (MAO-A and -B), providing for improved levels of neurotransmitters; (3) properties that support neuronal rescue and growth of new neuronal tissue; (4) anti-apoptotic properties that prevent the initiation of cell suicide programs in damaged brain cells. The lead compound, VAR10300 includes in its chemical structure a propargylamine moiety. Based on work conducted by Professor Youdim [43] and others, propargylamine has anti-apoptotic activity via multiple neuroprotective and neurorestorative pathways that include activation of the tyrosine kinase receptor (Trk A and B), the stimulation of PKC phosphorylation; upregulation of PKC alpha and epsilon mRNA and encoded proteins; induction of Bcl-X(L), Bcl-w, glia derived neurotrophic factor (GDNF) and brain-derived neurotrophic factor (BDNF) mRNAs; and down regulation of PKCdelta and gamma, Bad, and Bax mRNAs and the inhibition of the cleavage and activation of pro-caspase-3 and poly(ADP-ribose) polymerase (PARP) [43].

4.5. Glutamate-mediated excitotoxicity

One potential mechanism for neurodegeneration in ALS is through glutamate-mediated excitotoxicity. Excitotoxicity is defined as excessive exposure to the excitatory neurotransmitter glutamate and is mediated in part by overstimulation of N-methyl-D-aspartate (NMDA) type glutamate receptors, resulting in excessive Ca^{2+} influx and neuronal injury or death. A novel approach to reduce glutamate levels is being developed by the start-up Braintact. Their approach is to develop drugs that remain in the blood circulation and boost a natural mechanism that reduces glutamate levels in the blood and leads to the lowering of glutamate concentrations in the brain. This strategy will be applied to both acute and chronic neurodegenerative disorders including ALS. The scavenging system was developed by Vivian Teichberg from the Weizmann Institute of Science in Rehovot, Israel. Braintact's drug is composed of two molecules: the human enzyme glu-oxaloacetate transaminase (GOT) and one of its substrates oxaloacetate [44–45]. In the presence of

oxaloacetate, GOT plays a role in reducing glutamate levels in the blood by converting glutamate into another molecule. GOT is a natural human enzyme normally present in the blood in moderate concentrations. When injected with oxaloacetate, higher levels of the enzyme are activated and reduce the glutamate concentration in the blood. As a result, excess glutamate from the brain enters the blood circulation and its levels in the brain decrease. Both GOT and oxaloacetate naturally exist in the body. They have validated this approach in animal models including for ALS and no side-effects were shown following intravenous administration. Excessive exposure of neurons to glutamate can also occur through deficiency in expression or activity of Na⁺-dependent excitatory amino acid transporters (EAATs), primarily through the family member EAAT2 which is responsive for termination of glutamate-mediated synaptic transmission by removal of glutamate from the synapse. In ALS, there is reduced EAAT2 expression and activity which is believed to be a contributing factor in excitotoxic cell death in disease. Overexpression of EAAT2 in ALS animal model resulted in a delay in disease onset and prolonged survival of motor neurons [46–50]. Therefore it is expected that increased expression of EAAT2 will prevent glutamate-mediated excitotoxic cell death. Expression of EAAT2 is thought to be regulated at the translational level and has been targeted by small molecules that are currently being tested in ALS mouse model [51–52].

4.6. Growth factors

No genetic defects have been identified to date that interfere with any of the growth factor genes. This may be explained by the fact that dysfunction of growth factors would likely be quite devastating for development and result in embryonic lethality. The idea that neurotrophic factors might promote cell survival and therefore rescue dying neurons is an attractive one for potential disease modifying effects [53]. One strategy is to deliver a peptide derived from a growth factor or the growth factor itself. In this case, an effective delivery system needs to be developed because proteins or peptides are generally not very stable in blood and unlikely to cross the blood brain barrier. An alternative strategy is to develop small molecules that can mimic the effects of growth factors either by acting directly on the receptor or by stimulating the relevant growth factor pathway. A number of products are already in clinical trials. PharmaTrophix has successfully developed small molecule ligands for the growth factor receptor p75 and exploring them for Alzheimer's disease (licensed to Elan Pharmaceuticals) but they will likely also have applications to ALS [54]. Amarantus Therapeutics has their lead drug candidate, AMRS001, also known as MANF (mesencephalic astrocyte derived growth factor) which is a highly potent growth factor exhibiting anti-apoptotic effects [55]. Enkam is developing trophic factor mimetics using peptides that are derived from multifunctional proteins, such as Neural Cell Adhesion Molecule (NCAM), Fibroblast Growth Factors (FGF), the neurotrophins, Glia Derived Neurotrophic Factors (GDNF), and Human Epidermal Growth Factor Receptor family (HER). These peptides are applied to stem cells from cord blood to promote their survival [56]. Their most advanced development candidate is with a Neuronal Cell Adhesion Molecule (NCAM)-derived FGF peptide receptor agonist. Signaling pathways are another way to provide neurotrophic support and prevent cell death. ShanaRx is developing small molecules that are novel activators of the PI3K pathway as disease modifying therapies for ALS. Oxford Biomedica has in preclinical development their gene based treatment called MoNuDin which uses the Company's LentiVector gene delivery technology to deliver a neuroprotective gene, vascular endothelial growth factor (VEGF), to prevent further degeneration of the motor neurons and potentially restore motor function. Oxford Biomedica has in preclinical development for ALS, their lead product, Cogane which is a small-molecule member of the Sapogenin class of compounds. They are orally bioavailable neurotrophic factor inducers that cross the blood brain barrier. Both compounds have demonstrated neuroprotective effects in a variety of preclinical models [57].

4.7. RNA Strategies

microRNAs are short RNA molecules that are posttranslational regulators that bind to complementary sequences of target mRNAs effectively resulting in translational repression and gene silencing. miRNA binding proteins may be causative in motoneuron diseases with causative mutations in genes known to bind to RNA (e.g. TDP, FUS, SMN) although there is no direct evidence for this. miRNA have only been recently recognized as biological regulators and we are in the initial stages of understanding how this novel class of gene regulators is involved in neurological functions. However, a body of evidence is growing to support that miRNAs are important regulators of diverse biological processes including cell differentiation, growth, proliferation, and apoptosis. Moreover, miRNAs have been found to be key modulators of both CNS development and plasticity. Some miRNAs have already been implicated in several traumatic CNS injuries and neurodegenerative diseases [58–60] including ALS [61–62]. The company miRagen Therapeutics is developing potential drugs based on miR-206, a microRNA localized to the neuromuscular junction that has been shown to play a crucial role in the progression of ALS [62]. RXi Pharmaceuticals a biotechnology company focused on discovering, developing and commercializing innovative therapies using RNA-targeted technologies, to develop a new treatment for ALS using RXI's proprietary self-delivering RNAi therapeutic platform (sd-rxRNA™), a novel class of RNAi compounds that do not require a delivery vehicle to enter cells and has improved pharmacology compared to traditional RNAs. The work focuses on delivery to the spinal cord and brain to silence the SOD1 gene in ALS, and completion of the project is expected to generate supportive data for clinical development of anti-SOD1 sd-rxRNA therapy.

4.8. Stem cells

The promise of stem cells as a therapeutic strategy is very high for multiple diseases including ALS [63–64]. Stem cells are undifferentiated cells that have the capacity to differentiate to any number of multiple specialized cell types that can form into muscle, nerve, and blood vessels, for example. When injected into the body, at least a portion of them will migrate to areas of injury and become new cells based on the tissue they contact. However, there are a lot of issues with stem cell treatment that still need to be resolved [65–66]. There can be tissue rejection with the implanted stem cells so that patients have to receive anti-rejection drugs at the same time as receiving the stem cells. It is unclear whether any beneficial effects will be temporary or long term and therefore will repeated treatments be required. If stem cells that begin as healthy cells are placed in an unhealthy environment, only transient benefits will be realized. In the case of ALS, success with stem cell strategies depends on the replacement of cells injured or dead that will be able to make functional contacts to replace lost or weakened motor functions. This functionality needs to include contact with the correct muscle fibers.

There are currently at least thirteen companies that are developing stem cell strategies with applications for ALS. Some of the programs are in preclinical development or Phase I clinical trials and include the following companies: NeuralStem, Pluristem, ReNeuron, SanBio, Saneron, StemCells, and Brainstorm Cell Therapeutics. Other companies are utilizing strategies to upregulate the endogenous, dormant stem cell populations found in the nervous system. This strategy is being applied to ALS by the following companies: Fate Therapeutics and Samaritan Pharmaceuticals. A third strategy involves modifying stem cells genetically in order to augment their natural ability to provide trophic support with growth factors such as brain derived growth factor (BDNF). BioFocus, for example, is developing strategies to use stem cells and iPS cells in assays to understand more about the disease and provide tools for research. RhinoCyte is developing therapies based on autologous olfactory neural progenitor stem cells that they call RhinoCytes that are developed by culturing and

isolating human adult stem cells from the olfactory epithelium. Rhinocyte has received orphan drug status for treatment of ALS. NeuroGeneration is pursuing stem cell-based therapeutics both transplantation and reprogramming approaches for neurodegenerative diseases including ALS. NeuroNascent, Inc is developing small molecule therapeutic candidates that promote proliferation and new neurons from the brain's own neural stem/progenitor cell populations. Preclinical studies of these candidates to measure improved critical function and to diminish loss of neurons due to disease progression are currently under way for a number of diseases including ALS. Q Therapeutics is developing cell-based therapeutic products to restore or preserve normal neuronal function by replacing support cells of the nervous system (astrocytes and oligodendrocytes). Their Q Cells® are glial precursors that provide important support functions necessary for neuron health through generation of astrocytes. Astrocytes provide direct neuroprotection through multiple means including production of growth factors, other trophic support, reduction of toxic metabolites and direct interactions that promote healthy neurons. Diseases which may benefit from such direct neuroprotection include ALS, spinal cord injury, traumatic brain injury, stroke and Alzheimer's disease.

4.9. Vaccine approach

A vaccine approach is being taken by the company Amorfix together with Biogen Idec. Amorfix is developing a vaccine for active immunization against disease-specific epitopes on misfolded or aggregated mutant SOD-1. The vaccine is expected to eliminate the misfolded mutant SOD1 protein, while sparing wild-type SOD-1 and has some support in the literature [67–68]. They have issued patents for protection of three targets of SOD1 misfolded protein called “disease-specific epitopes”. They are applying their technology to therapeutics as well as diagnostics. They have also applied their technology to beta amyloid for Alzheimer's disease. They are taking two approaches, a passive infusion of manufactured monoclonal antibodies and an active immunization approach designed to elicit the production of similar antibodies by the patient's own body. This strategy is based on the assumption that the primary cause of motoneuron death is due to the misfolded and aggregated SOD1. If the misfolded SOD1 can be recognized and its toxic activity neutralized, that ALS progression would be slowed and potentially reversed. Neurimmune Therapeutics AG has been developing human-derived antibodies to TDP-43 that are protective in nervous system diseases. These antibodies have been shown to protect against neurodegeneration in ALS disease mouse models. Biogen-Idec has an agreement in which they will be responsible for the development of the pre-clinical candidates and the commercialization of all products.

5. Conclusion

Multiple strategies are being applied to develop new therapeutics for ALS, a very devastating disease that compromises motor control and progresses in severity until death generally from respiratory failure. The following strategies are actively being applied to ALS therapies: altering protein misfolding, improving clearance of toxic species, reducing inflammation and oxidative damage, reducing excitotoxicity. In addition, newer strategies using stem cells, vaccines, and RNA regulation are also being pursued. With this combination of approaches and the increase in the number of drugs tested in clinical trials, the optimism increases that new and effective strategies for ALS will be realized.

6. Expert Opinion

Progress is being made in the identification of new therapeutics for ALS with recent efforts in understanding the genetic causes of the disease, susceptibility factors, and development of additional preclinical animal models. However challenges remain in the identification of

new ALS therapeutics that include the use of relevant biomarkers. Relevant biomarkers will lead to improved clinical trials. When does the disease begin? Can the disease be diagnosed earlier? For example, could electrical impedance myography be used as a tool for earlier diagnosis [69]. With earlier diagnosis of the disease, it will be more likely to change the course of the disease.

What is remarkable in the field of ALS therapeutics is the broad scope of strategies being undertaken. The multiple strategies are based on the likelihood that there may be multiple causes of ALS. Therefore strategies are based on what is known about neuronal death and the known genetic causes of ALS. Also very evident are the existence of common features with other neurodegenerative diseases. Perhaps many of the same therapeutic strategies being developed for other neurodegenerative diseases can be applied to ALS. Common hallmarks that produce a number of cellular and functional alterations among the neurodegenerative diseases include accumulation of misfolded proteins, so a variety of strategies include blocking the aggregation of the misfolded proteins and facilitating their clearance.

A successful therapeutic strategy for ALS is highly dependent on multiple factors. First is the need for a thorough analysis of the complex pathology and genetics of ALS. Questions related to ALS as a motoneuron disease are still unanswered. Like other neurodegenerative diseases, combinations of approaches are likely to be the most successful. ALS is defined as a disease with selective degeneration of upper and lower motor neurons. Upper motor neurons are the cells in the nervous system that include the neurons that are located in the motor region of the cerebral cortex or the brain stem that carry movement information through a common pathway to the lower motor neurons in the brain stem and spinal cord that connect directly to muscles. However, ALS is likely a disease that is multisystemic with neuronal and non-neuronal cells that are affected. Other systems are found to be involved and it is unknown whether other organ systems are involved as a consequence of the motoneuron disease process or in addition to the motoneuron disease process. Autonomic dysfunction, for example, appears to be an early stage in ALS progression [70]. Future treatments need to consider targeting motoneurons and neighboring cells and networks which contribute to the persistence and diffusion of the disease. A better understanding of underlying cause(s) of sporadic forms of ALS will also lead to improved animal models which have also hampered the development of therapeutic candidates. Multiple organizations provide resources for ALS and are listed in Table 2.

There are three new therapeutic directions that are also actively being pursued. The first is in the area of genetics where key findings include the identification of additional genetic causes for ALS. For many years, the Cu^{2+} Zn^{2+} superoxide dismutase (SOD1) gene had been the only gene known to cause ALS. 5–10% patients have a familial history and now nine additional genes have been identified as additional genetic factors associated with ALS ([2,71]. The recently discovered genes have to be explored and five additional loci without an identified gene have also been identified [2]. These new genetic results offer the potential for new targets and the potential to better understand the biology of the disease. In addition, new animal models are being developed based on the new genes. The second new therapeutic direction is in the area of stem cells. Stem cell models based on embryonic stem cells from ALS animal models and based on the differentiation of patients induced pluripotent stem (iPS) cells into susceptible neurons and glia could serve as an additional tool for the identification of new therapeutics for ALS [72]. There is a great deal of interest in the use of stem cells also for treatment of neurodegenerative diseases [73–74]. This strategy is currently being tested in FDA-approved clinical trials for a variety of conditions other than ALS and a number of companies are developing a strategy for ALS (see below). Current clinical trials for stroke [75] and traumatic brain injury with mesenchymal stem cells

[76] are currently ongoing. The third new therapeutic effort is towards the development of a biomarker strategy. Biomarkers are important to the development of new therapeutics as well as for improved diagnostics. Searches have been made using imaging techniques and analysis of CSF and plasma of ALS patients to identify a biomarker and it has been difficult to identify and validate biomarkers [77–81].

To justify moving compounds into clinical trials, one animal model for ALS that was developed in the 1990's has until very recently, been the only available model. This is the transgenic mouse overexpressing the SOD1^{G93A} mutation. These mice show many of the same defects as in the human ALS disease. However, many compounds that showed good activity in the SOD1 transgenic model have resulted in disappointing lack of efficacy in human clinical trials. More recently, compounds have been held to a higher standard which is to test them in these mice after symptoms appear to more closely mimic the clinical trial. However, the fact remains that only a small subset of patients have mutations in SOD1 and therefore call into question the reliance of efficacy in the SOD1^{G93A} transgenic mice. More recently there are reports of a new ALS mouse model that expresses a mutant form of the TDP-43 gene [82]. Additional animal models are needed as well as a correlation with a drug-targeted mechanism that can be assessed in both animals and clinical trials.

Perhaps the most striking outcome of this review of current strategies for ALS is that the efforts are largely through small biotechnology companies. ALS is classified as a rare disease with fewer than 30,000 patients in the US and therefore represents a very small commercial market potential. This has largely been an unattractive disease for the large pharmaceutical companies. However, a number of companies are focused on mechanisms that would have commercial potential for other indications in addition to ALS. For example, HDACs and chaperones also have commercial applications to the oncology arena.

Article highlights

1. ALS is a debilitating, fatal disease in which there is no existing therapeutic that alters the progression of the disease and therefore therapeutic development for ALS remains a high unmet medical need.
2. There is a broad range of strategies being undertaken to identify new therapeutics for ALS.
3. Newly discovered genes that cause ALS offer new therapeutic strategies for ALS.
4. The Biotech industry is very active in the identification of new therapeutics for ALS and other neurodegenerative diseases.
5. Recent efforts in stem cells, genetics, and biomarkers offer new strategies for ALS therapeutics.

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Declaration of Interest

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Table 1

List of companies with Amyotrophic Lateral Sclerosis therapeutic relevant strategies, their location and targets

Company	Location	Target
Acetylon	US- Massachusetts	Protein aggregation
ALS Biopharma	US-Pennsylvania	Protein aggregation
ALS Therapy Development Institute	US-Massachusetts	Inflammation
Amarantus Therapeutics	US-California	Growth factor
Amorfix	Canada	Vaccine
Brainstorm Cell Therapeutics	US-New York and Israel	Stem cells
Braintact	Israel	Excitotoxicity
Cambria	US-Massachusetts	Protein aggregation
Chaperone Therapeutics	US-North Carolina	Protein aggregation
Cognosci	US-North Carolina	Inflammation
Cytokinetics	US-California	Muscle contractility
Daval International	UK	Inflammation
Debiopharm Group / Curis Inc	Switzerland / US- Massachusetts	Protein aggregation
Enkam	Denmark	Growth factor
EnVivo Pharmaceuticals	US- Massachusetts	Protein aggregation
ExonHit Therapeutics/Allergan	France	Protein aggregation
Fate Therapeutics	US-California	Stem cells
FoldRx	US- Massachusetts	Protein aggregation
Genervon Biopharmaceuticals	US-California	Growth factors
GenKyoTex	Switzerland	Oxidative stress
Isis Pharmaceuticals	US-California	RNA strategies
Knopp Biosciences/Biogen	US-Pennsylvania, Massachusetts	Oxidative stress
miRagen Therapeutics	US-Colorado	RNA strategies
NeuralStem	US-Maryland	Stem cells
Neurimmune Therapeutics	Switzerland	Vaccine
Neurogeneration	US-California	Stem cells
NeuroNascent, Inc	US-Maryland	Stem cells
NeuroNova	Sweden	Growth factor
NeuroPhage	US- Massachusetts and Israel	Protein aggregation
NexGenix Pharmaceuticals	US-New York	Protein aggregation
Oxford Biomedica	UK	Growth factor
PharmaTrophix	US-North Carolina	Growth factor
Phytopharm	UK	Growth factor
Pluristem Therapeutics	Israel	Stem cells
Proteostasis	US- Massachusetts	Protein aggregation
Q Therapeutics	US-Utah	Stem cells
Reata Pharmaceuticals	US-Texas	Protein aggregation

Company	Location	Target
ReNeuron	UK	Stem cells
Repligen Corporation	US- Massachusetts	Protein aggregation
Retrotope, Inc	US-California	Oxidative stress
RhinoCyte	US-Kentucky	Stem cells
RXi Pharmaceuticals	US- Massachusetts	RNA strategies
Samaritan Pharmaceuticals	US-Nevada	Stem cells
SanBio	US-California	Stem cells
Saneron	US-Florida	Stem cells
ShanaRx	US-California	Growth Factors
Sirtris/GSK	US- Massachusetts	Protein aggregation
Stealth Peptides	US-Maryland	Oxidative stress
StemCells	US-California and UK	Stem cells
Takeda	Japan	Oxidative stress
Transition Therapeutics	Canada	Inflammation
Trophos	France	Oxidative stress
Varinel	Israel	Iron chelation
Vasopharm	Germany	Oxidative stress
ZZ Biotech	US-New York	Inflammation

Table 2

List of useful resources for more information on Amyotrophic Lateral Sclerosis

Foundation	website
ALS Association	http://www.alsa.org/
ALS Forum	http://www.researchals.org/
ALS Therapy Development Institute	http://www.als.net/
ALS Therapy Alliance	http://alstherapyalliance.org/
Prize4Life	http://www.prize4life.org/
Project ALS	http://www.projectals.org/
NINDS Fact Sheet on ALS	http://www.ninds.nih.gov/disorders/amyotrophiclateralsclerosis/detail_ALS.htm
MDA division ALS	http://www.als-mda.org/