Abstract: Early detection of amyotrophic lateral sclerosis (ALS) is critical for better therapeutic outcomes. The median time from symptom onset to diagnosis of ALS is 11 months, with a range of 6-21 months. Given that the median life expectancy is three years, it is important to shorten the diagnostic journey, initiate therapies promptly, and facilitate clinical research participation. Biomarkers may be the key to enhancing early diagnosis, tracking disease progression, and testing target engagement of promising therapeutics. Clinically valid biomarkers for ALS are currently lacking, and research has been ongoing to identify appropriate biomarkers. Ideal biomarkers should be minimally invasive, such as blood. In this chapter, we review our current understanding of blood-based biomarker research in ALS and discuss future directions.

Keywords: amyotrophic lateral sclerosis; biomarker; blood; mitochondria; TDP-43
INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is mostly a sporadic disease that leads to progressive degeneration of the cortical, bulbar, and spinal motor neurons (1–3). The median age of onset of sporadic ALS is 55, with a male predominance (1.5:1) (2). Diagnosis is based on upper motor neuron signs (spasticity, increased tendon reflexes) and lower motor neuron dysfunction, which may be supported by electrophysiological findings (1). Weakness and atrophy begin either in the bulbar region or in the limb muscles in about a third of cases and spread to the contralateral limb. Respiration is usually affected late in the disease and up to 50% may have evidence of frontotemporal dementia (FTD). Patients with older onset age, bulbar dysfunction, greater clinical disability, and low respiratory function have the poorest prognosis (1, 2). The median life expectancy from symptom onset is approximately three years, with a five-year survival rate of 20–25% and a 20-year survival rate of 5% (2). Most cases are sporadic, but 10–15% are of autosomal dominant inheritance.

Biomarkers can serve as tools for early diagnosis, predictors of prognosis, indicators of target engagement or therapeutic response, and enablers of discovery of future therapeutics for ALS. Biomarker development efforts for ALS have been hampered by a number of issues including small sample size, methodological variation, and lack of standardized techniques. On average, time from symptom onset to clinical diagnosis spans 11 months and this time is critical for life-saving interventions and therapies (4). Biomarkers could hasten diagnosis to allow for earlier introduction of therapies. Prognostic biomarkers are critical due to the heterogeneous nature of ALS and could facilitate prediction of how a subgroup of ALS subjects might progress or respond to a therapy. The low prevalence of ALS is an important issue that negatively affects clinical trials and biomarker development (5–7). In general, recruitment to clinical trials in rare diseases like ALS is a challenge. In ALS, several factors reduce the likelihood of participation in clinical trials including delay or uncertainty in diagnosis, slow progression, respiratory compromise, short life expectancy, and in some cases, dislike of being assigned to the placebo group. Discovery of diagnostic, prognostic, and target-engagement biomarkers are essential for accelerating the research and development of ALS therapeutics. In this chapter, we provide an overview of our current understanding of blood-based biomarkers for ALS.

POTENTIAL BIOMARKERS FOR ALS

The body of knowledge on biomarkers of ALS is limited. Ideally, a biomarker for ALS should be easy to quantify, minimally invasive, specific, reliable with an uncomplicated measurement process, and reproducible across multiple laboratories (8). Figure 1 summarizes the main areas of biomarker research in ALS, all of which target pathological findings in the disease. These aim to measure neurodegeneration, neuroinflammation/systemic inflammation, oxidative stress, excitotoxicity, mitochondrial function, and protein aggregation/proteostasis. Tables 1 and 2 summarize the overall findings of blood-based measures (9–30).
Figure 1. **Biomarker Focus in ALS.** Areas of biomarker development are focused on pathological findings in ALS. These include neuroinflammation/systemic inflammation, mitochondrial dysfunction, neurodegeneration, and protein aggregation/proteostasis. Created with BioRender.com

### TABLE 1 Blood Based Biomarker Studies

| Target                | Source           | Sensitivity/Specificity       | n     | Source |
|-----------------------|------------------|-------------------------------|-------|--------|
| TDP-43                | Plasma           | NA                            | 319   | (9)    |
| Exosome miRNA         | Plasma           | NA                            | 40    | (10)   |
| Exosome proteomics    | Plasma           | NA                            | 22    | (11)   |
| Proteomics            | Plasma           | 58% and 90%                   | 295   | (12)   |
| Glutamate Uptake      | Platelet         | NA                            | 82    | (13)   |
| Mito-Respiration      | Platelet         | NA                            | 15    | (14)   |
| Serotonin             | Platelet         | NA                            | 114   | (15)   |
| NfL                   | Serum/Plasma     | 84–100% and 76–97%            | 248   | (16)   |
| NfH                   | Serum/Plasma     | 61–80%, 72.1–83.7%            | 157–331| (16–18)|
| Cytokines             | Serum/Plasma     | NA                            | 87–183| (19–21)|
| Ferritin              | Serum/Plasma     | NA                            | 104–694| (22–24)|
| Creatine Kinase       | Serum/Plasma     | 63.8% and 54.3%               | 216–834| (22, 25)|
| Non-coding RNA        | Whole Blood      | 73.9–93.7%                    | 88    | (26)   |
| Chromosomal Confirmation| Whole Blood     | 83.33–87.5%                   | 58    | (27)   |
| Microarray analysis   | Whole Blood      | 87%                           | 1,116 | (28)   |
| Immune Cell Profiling| Whole Blood      | NA                            | 80    | (29)   |
| T-regs                | Whole Blood      | 73.9–76.9%, 69.6–73.1%        | 217   | (30)   |
In this section, our current knowledge on biomarkers for both familial and sporadic ALS are discussed.

**C9ORF72 protein**

The most common genetic abnormality in frontotemporal lobar degeneration (FTLD) and ALS is the expansion of GGGGCC \((G\_C\_G\_C)_n\) repeat in an intron of chromosome 9 open reading frame72, depicted as C9ORF72 (31, 32). GGGGCC repeat expansions are translated through a repeat associated non-ATG (RAN) mechanism that does not require the AUG start codon (33). This non-canonical type of protein translation takes place without frame shifting or RNA editing, resulting in production of dipeptide repeat (DPR) proteins. There are five known DPR proteins, Poly-GA, Poly-GP, Poly-GR, Poly-PA, and Poly-PR (34, 35). These DPR proteins display different profiles across neurodegenerative diseases and could be potential biomarkers. Poly-GA proteins are associated with inclusion bodies when TDP-43 aggregation is lacking (TDP-43-negative inclusions) (35). In the neurons of post-mortem brain, Poly-GA protein aggregates are surrounded by TDP-43 aggregates (36). Poly-GR and Poly-PR DPR proteins cause neurodegeneration in drosophila without TDP-43 aggregation (37). Some studies suggest Poly-GA aggregation can induce TDP-43 phosphorylation and aggregation (35). Thus, the precise role of DPR proteins in TDP-43 aggregation has not yet been resolved. The \(G\_C\_G\_C\) repeats can be measured in blood (38) and could serve as a blood-based biomarker for ALS. For familial ALS cases, peripheral blood lymphocyte levels of mutated SOD1 and mutated C9ORF72 were used to measure target engagement in a clinical trial. Although primary outcomes of clinical trials are focused on cerebrospinal fluid (CSF)-based biomarkers, blood cell profiles appear to be changing as well. For example, SOD1 levels were reduced in peripheral blood lymphocytes in a pyrimethamine clinical trial (39). Poly-GP repeats in C9ORF72-positive ALS cases are detected in peripheral blood mononuclear cells.
Biomarkers for Amyotrophic Lateral Sclerosis

Neurofilaments

Neurofilaments function to maintain axon structure and transport (40). Neurofilaments exist in three isoforms: high-molecular-weight subunit (180–200 kDa [NfH]), middle-molecular-weight subunit (130–170 kDa [NfM]), and low-molecular-weight subunit (60–70 kDa [NfL])—all are exclusively expressed in neurons (41). Neurofilaments are considered surrogate biomarkers of neuronal degeneration (42). Aberrant NfL accumulation is observed in both familial and sporadic ALS patients (43–47). CSF levels are considered better than blood levels for the diagnostic confirmation of ALS (48). NfL levels increase during early stages of ALS (18). Further studies show NfL increases as early as 12 months prior to symptom onset in ALS and could be a predictive biomarker (49). Single molecule array technology or SIMOA has enabled the quantification of NfL in serum and plasma at picogram/mL sensitivity (50, 51). NfL is widely used as a biomarker of ALS. NfL levels in serum are higher in ALS subjects and correlate well with CSF measurements (52). Overall, NfL strongly correlates with survival, but levels are largely steady over time and show no correlation with functional diagnostic scores such as the El Escorial Criteria (7, 16, 53). Using the SIMOA assay, serum NfL may be not only a clinically validated prognostic biomarker for ALS but may also be a biomarker of treatment effect (54). Plasma neurofilament heavy subunit (pNfH) has shown variable results across studies (7, 53). One study showed elevated pNfH levels predict faster progression at 4 months while another study showed it was associated with higher mortality at 12 months (18). Other studies show pNfH levels are neither steady nor reliable longitudinally and are not correlated with disease progression. Overall, the rate of change in blood pNfH is not reliable to predict disease progression and its utility as a diagnostic marker remains to be realized (16–18).

TDP-43

Transactive response (TAR) DNA binding protein 43 (TDP-43) regulates gene transcription, mRNA splicing, stability, and translation (55). Mutations in TDP-43 cause familial forms of ALS and TDP-43 aggregates are found in most ALS subjects on autopsy (56–58). TDP-43 and its post-translational modifications can be measured across numerous biofluid and could serve as a biomarker for ALS (59–65). Within the ALS field, CSF TDP-43 measurements are preferred over blood-based samples. However, lumbar punctures are invasive, and patients are less likely to agree to this procedure for CSF sampling. Mass spectrometry analysis of post-mortem brain tissue from ALS subjects revealed a number of TDP-43 post-translational modifications including hyperphosphorylation, acetylation, ubiquitination, deamidation, and oxidation (66). Hyperphosphorylation (67, 68) and lysine acetylation increase TDP-43 aggregation (69). Phosphorylation of TDP-43 between amino acids 220-414 is suspected to prevent TDP-43 degradation and increase its expression levels (70). Plasma TDP-43 is higher but is unchanged in serum (9). TDP-43 is mis-localized in cytoplasmic fractions of PBMCs while
overall expression of TDP-43 is not changed. TDP-43 levels in PBMCs correlate with disease burden over time (62, 71, 72). Longitudinal studies showed that TDP-43 plasma levels are highly variable over time, and between individuals (7). These variable findings could be a consequence of blood handling, hemolysis, and coagulation. Classification of TDP-43 expression and post-translation modifications in the blood of ALS subjects could be used as a biomarker for detection/diagnosis and therapeutic outcomes.

**Extracellular RNAs, exosomes and stress granules**

Extracellular RNAs are found outside the cells in extracellular vesicles (EVs) such as exosomes, micro vesicles and apoptotic bodies, or RNA-binding proteins. Their association with lipids and proteins protect them from degradation and allows for their measurement. Extracellular RNAs are found in many forms, such as tRNA, mRNA, microRNA (miRNA), and circular RNA (circRNA) within EVs. tRNA fragments may be disease-specific and should be considered for biomarker development (73, 74). Next generation sequencing of neural enriched exosomes from plasma of ALS patients identified eight miRNAs that could discriminate ALS from healthy subjects (10). circRNA can be detected in extracellular fluid (75–78). The function of circRNA is largely unknown but regulation of gene expression is a likely function (79). High levels of extracellular circRNA in CSF suggest that the central nervous system (CNS) may secrete them (80–82). The potential of circRNA as a biomarker in ALS was recently reviewed (83).

Exosomes are 50-100 nm extracellular vesicles released from cells. In blood, exosomes are released by erythrocytes, platelets, endothelial cells, and lymphocytes (Table 3). Proteomic analysis of exosomes from ALS and Parkinson’s disease (PD) subjects was able to discriminate between these two diseases (11). Exosomes derived from blood, serum, or plasma show high contamination of blood proteins, which decreases the specificity of proteomic analysis (84).

Stress granules are cytoplasmic RNA complexes that form in response to environmental stress. Several ALS-associated proteins, such as FUS (85), TDP-43(86), Ataxin2 (87), and SOD1 variants (88) have been identified as integral components of stress granules. Currently, measurements of stress granules are limited to cell-based assays.

| Exosome Donor Cell     | Marker                          |
|------------------------|--------------------------------|
| Platelets              | CD31, CD41, CD61, CD42b, GPIIb-IIIa |
| Endothelial cells      | CD31, CD42B, CD51, CD105         |
| Monocytes              | CCR2, CD14, CD41a                |
| Neutrophils            | CD43, CD16                       |
| Lymphocytes            | CD4, CD8                         |
| Erythrocytes           | CD235a                          |
Progranulin

Progranulin (PGRN) is a cysteine-rich secretory protein involved in cell proliferation, inflammation, and tumorigenesis (89). Brain progranulin is implicated in neuronal survival as well as pathogenesis of neurodegenerative diseases (90, 91). Progranulin levels can be measured in both CSF and serum of FTD, ALS, and Alzheimer’s disease patients (92). Although no comprehensive study is available to compare progranulin levels in brain with CSF and serum values (92), blood levels are 35 times higher than CSF in ALS subjects with FTD (93). This suggests blood measures of progranulin could serve as a biomarker in ALS.

RNAseq and proteomics

Microarray analysis of blood cells has allowed for machine learning and identification of ALS subjects from the healthy (28) with an accuracy of 87%. Gene expression changes observed in ALS blood cells include increased neutrophil related genes with decreased erythroid lineage-specific genes. The expression of copper chaperone of superoxide dismutase (CCS) and other mitochondrial respiration-linked genes were significantly associated with survival in ALS subjects (28). Further, circulating non-coding RNAs have shown a 73.9-93.7% accuracy in discriminating the healthy from ALS populations (26). Proteomic analysis of ALS blood samples shows changes in proteins involved in the regulation of metabolism and mitochondrial function, particularly carbohydrate, creatine, and lipid metabolism (12). Nitric oxide and reactive oxygen species production are upregulated in macrophages of ALS patients (94). Protein expression of TDP-43, cyclophilin A, and ERp57 in PBMCs were found to associate with disease progression in ALS subjects. A multiprotein expression profile in PBMCs could discriminate ALS from healthy controls with 98% power, and discriminate ALS from other neurologic disease with 91% power. The multiprotein expression profile was further validated in the G93A SOD1 ALS mouse model using both PBMCs and spinal cord tissue (62). Chromosomal conformation in blood samples can also discriminate between ALS and healthy subjects with a sensitivity of 83.33–87.5% and specificity of 75.0–76.92% (27).

Inflammatory markers

Cytokine expression in blood is altered in ALS subjects but do not change over time. Tumor necrosis factor α (TNF-α) and downstream effector interleukins are increased in ALS subjects (19–21). Data from 25 independent studies examining serum and plasma levels of cytokines show that TNFα, IL-1β, IL-6, IL-8, TNF receptor 1, and vascular endothelial growth factor (VEGF) are elevated in ALS (7). Other inflammatory markers such as complement components, C reactive protein, and chitotriosidase have shown equivocal association with ALS (7). Immune cell profiling has shown that higher levels of lymphocytes, monocytes, and T cell subtypes are associated with longer survival times (29). CD4+CD25High T-regs are lower in ALS patients (30, 95), and is a measure of ALS progression. Overall, inflammatory markers have not shown specificity for ALS diagnosis and no association with disease progression has been established yet.
Metabolites

Serum and plasma creatine kinase are elevated in ALS subjects and correlate with the revised ALS functional rating scale (ALSFRS-R) score and other functional outcomes in ALS (22, 25). Plasma and serum ferritin levels are higher in ALS subjects. In some studies, ferritin levels were associated with survival and in others it did not (22–24). Glutamate uptake is impaired in platelets and astrocytes derived from ALS subjects (13). Furthermore, platelet serotonin levels are reduced in ALS subjects and is associated with an increased risk of death (15).

Mitochondrial biomarkers

Mitochondrial dysfunction is observed across numerous tissues in ALS subjects. Spinal cord mitochondrial DNA shows higher levels of mutation, and reduced citrate synthase, complex I+III, II+III and IV activities (96). Induced pluripotent stem cells (iPSCs) derived from ALS patient fibroblasts show reduced mitochondrial function when differentiated into motor neurons. iPSC-derived ALS motor neurons had reduced ATP production and mitochondrial respiration and increased glycolytic flux (97). Muscle samples from ALS patients show a large number of cytochrome oxidase-negative fibers, and some of these patients had reduced enzyme activity (98, 99). Two separate studies of ALS muscles showed reduced mitochondrial respiration and changes in mitochondrial DNA (99, 100). Tissues outside of the spinal cord and muscle also show changes in mitochondrial function. Fibroblasts from ALS patients show reduced basal, uncoupled, and ATP-linked respiration (101). Hepatic mitochondria from ALS subjects show ultrastructural changes with enlarged mitochondria, inclusions, and disorganized structure (102). Lymphocytes from ALS subjects show increased calcium levels and reduced uncoupled respiration (103). These observations show that mitochondrial abnormalities are a systemic finding in ALS. While most mitochondrial respiration indices were reduced in ALS platelets, non-mitochondrial respiration and complex II activities were increased. Complex II activity reduction over three months correlated with decline in function on the ALSFRS-R scale (14). Two separate clinical trials, testing Rasagiline as a therapeutic for ALS, used lymphocyte apoptosis, mitochondrial superoxide, and mitochondrial membrane potential as secondary outcomes (65, 104). Based on abnormal lymphocyte mitochondrial membrane potentials (101), it would seem reasonable to pursue these as potential biomarkers. Blood cell respiration or enzyme Vmax assays could be used to determine if a drug is engaging its target by altering mitochondrial function.

CONCLUSION

ALS is a rare disease. We estimate the ALS population in the US to be about 17,000 people (13,000–24,000) based on a US population of 329,450,000 (105). This is one of the main reasons affecting biomarker development for ALS. The exact mechanisms underlying motor neurodegeneration and muscle impairment in ALS are unknown. Current hypotheses include neuroinflammation, mitochondrial dysfunction, oxidative stress, excitotoxicity, and protein aggregation (1, 106–112). Lack of understanding of how these mechanisms interact at different stages of the
disease is another issue limiting the progress of biomarker development and subsequent drug development for ALS. The lack of validated biomarkers for ALS has directly affected drug development. There are three FDA approved therapies for ALS: riluzole and edaravone for modulating the course of the disease, and dextromethorphan/quinidine for symptomatic treatment of sialorrhea. The effect of riluzole is modest, extending the lifespan by 2–3 months (113–115). Edaravone appears to slow progression and preserve function in ALS patients (115–117). Like riluzole, edaravone (Radicava) can have some side effects but its intravenous route of administration can be an obstacle at times. Nuedexta targets pseudobulbar symptoms and has no known effect on life span (118, 119). Current clinical trials for ALS are listed on https://clinicaltrials.gov/ [accessed on 17 June 2021]. There are 448 ongoing studies in Unites States, and most of these would benefit from a host of exploratory and confirmatory biomarkers.

Blood-based biomarkers are considered non-invasive and have the potential to be cost-effective. Disagreements exist regarding the utility of blood measures as surrogate for reflecting the status of motor neurons in the spinal cord or muscle. However, as shown in Figure 2, neurodegeneration and reactive gliosis contribute to blood brain barrier (BBB) breakdown. This BBB breakdown can lead to leakage of CNS exosomes/EVs and other molecules into the blood stream. Further studies are required to assess the correlation between blood measures and spinal cord/muscle tissue disease status. Validated biomarker application in people with ALS

**Figure 2. Blood Brain Barrier Breakdown and Circulating Biomarkers.** Neurodegeneration and reactive gliosis can lead to blood brain barrier (BBB) disruption (and vice versa). This BBB disruption could allow for CNS derived circulating biomarkers to be measured. Created with BioRender.com
would derive numerous benefits. In addition to shortening the diagnostic journey, disease biomarkers may generate some cost-savings and enhance enrollment in clinical trials. Timely diagnosis will also reduce the time to starting currently available therapies. Biomarkers have the potential to provide valuable information about disease trajectory and critically important early insight into the effectiveness of experimental therapeutics. There is a great unmet need for cost-effective, reliable, accurate, non-invasive and reproducible biomarkers for ALS.

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REFERENCES

1. Gubbay SS, Kahana E, Zilber N, Cooper G, Pintov S, Leibowitz Y. Amyotrophic lateral sclerosis. A study of its presentation and prognosis. J Neurol. 1985;232(5):295–300. https://doi.org/10.1007/BF00313868
2. Rowland LP, Shneider NA. Amyotrophic lateral sclerosis. N Engl J Med. 2001;344(22):1688–700. https://doi.org/10.1056/NEJM20010531442207
3. Statland JM, Barohn RJ, McVey AL, Katz JS, Dimachkie MM. Patterns of Weakness, Classification of Motor Neuron Disease, and Clinical Diagnosis of Sporadic Amyotrophic Lateral Sclerosis. Neurol Clin. 2015;33(4):735–48. https://doi.org/10.1016/j.ncl.2015.07.006
4. Cellura E, Spataro R, Taiello AC, La Bella V. Factors affecting the diagnostic delay in amyotrophic lateral sclerosis. Clin Neurol Neurosurg. 2012;114(6):550–4. https://doi.org/10.1016/j.clineuro.2011.11.026
5. Benveniste O, Goebel HH, Stenzel W. Biomarkers in Inflammatory Myopathies-An Expanded Definition. Front Neurol. 2019;10:554. https://doi.org/10.3389/fneur.2019.00554
6. Katyal N, Govindarajan R. Shortcomings in the Current Amyotrophic Lateral Sclerosis Trials and Potential Solutions for Improvement. Front Neurol. 2017;8:521. https://doi.org/10.3389/fneur.2017.00521
7. Verber NS, Shepheard SR, Sassani M, McDonough HE, Moore SA, Alix JJP, et al. Biomarkers in Motor Neuron Disease: A State of the Art Review. Front Neurol. 2019;10:291. https://doi.org/10.3389/fneur.2019.00291
8. Henley SM, Bates GP, Tabrizi SJ. Biomarkers for neurodegenerative diseases. Curr Opin Neurol. 2005;18(6):698–705. https://doi.org/10.1097/01.wco.0000186842.51129.cb
9. Verstraete E, Kuiperij HB, van Blitterswijk MM, Veldink JH, Schelhaas HJ, van den Berg LH, et al. TDP-43 plasma levels are higher in amyotrophic lateral sclerosis. Amyotroph Lateral Scler. 2012;13(5):446–51. https://doi.org/10.3109/17482968.2012.703208
10. Banack SA, Dunlop RA, Cox PA. An miRNA fingerprint using neural-enriched extracellular vesicles from blood plasma: towards a biomarker for amyotrophic lateral sclerosis/motor neuron disease. Open Biol. 2020;10(6):200116. https://doi.org/10.1098/rsob.200116
11. Tomlinson PR, Zheng Y, Fischer R, Heidasch R, Gardiner C, Evertts S, et al. Identification of distinct circulating exosomes in Parkinson’s disease. Ann Clin Transl Neurol. 2015;2(4):353–61. https://doi.org/10.1002/acn3.175
12. Lawton KA, Brown MV, Alexander D, Li Z, Wulff JE, Lawson R, et al. Plasma metabolomic biomarker panel to distinguish patients with amyotrophic lateral sclerosis from disease mimics. Amyotroph Lateral Scler Frontotemporal Degener. 2014;15(5–6):362–70. https://doi.org/10.3109/21678421.2014.908311
13. Ferrarese C, Sala G, Riva R, Begni B, Zoia C, Tremolizzo L, et al. Decreased platelet glutamate uptake in patients with amyotrophic lateral sclerosis. Neurology. 2001;56(2):270–2. https://doi.org/10.1212/01.WNL.56.2.270
14. Kazamel M, Chacko B, King P, Lee I, Darley-Usmar V. Mitochondrial Bioenergetic Profile in Platelets as a Biomarker for Amyotrophic Lateral Sclerosis (ALS) (4869). Neurology. 2020;94(15 Supplement):4869.
15. Dupuis L, Spreuks-Varooquax O, Bensimon G, Jullien P, Lacomblez L, Salachas F, et al. Platelet serotonin level predicts survival in amyotrophic lateral sclerosis. PLoS One. 2010;5(10):e13346. https://doi.org/10.1371/journal.pone.0013346
16. Feneberg E, Oeckl P, Steinacker P, Verde F, Barro C, Van Damme P, et al. Multicenter evaluation of neurofilaments in early symptom onset amyotrophic lateral sclerosis. Neurology. 2018;90(1):e22–e30. https://doi.org/10.1212/WNL.0000000000004761
17. De Schaepdryver M, Jeromin A, Gille B, Claeyss KG, Herbst V, Brix B, et al. Comparison of elevated phosphorylated neurofilament heavy chains in serum and cerebrospinal fluid of patients with amyotrophic lateral sclerosis. J Neurol Neurosurg Psychiatry. 2018;89(4):367–73. https://doi.org/10.1136/jnnp-2017-316605
18. McCombe PA, Pfluger C, Singh P, Lim CY, Airey C, Henderson RD. Serial measurements of phosphorylated neurofilament-heavy in the serum of subjects with amyotrophic lateral sclerosis. J Neurol Sci. 2018;385:24–8. https://doi.org/10.1016/j.jns.2018.07.044
19. Lu CH, Allen K, Oei F, Leoni E, Kuhle J, Tree T, et al. Systemic inflammatory response and neuromuscular involvement in amyotrophic lateral sclerosis. Neurol Neuroimmunol Neuroinflamm. 2016;3(4):e244. https://doi.org/10.1212/NXI.0000000000000244
20. Cereda C, Baiocchi C, Bongioanni P, Cova E, Guareschi S, Metelli MR, et al. TNF and sTNFR1/2 plasma levels in ALS patients. J Neuroimmunol. 2008;194(1–2):123–31. https://doi.org/10.1016/j.jneuroim.2007.10.028
21. Poloni M, Facchetti D, Mai R, Micheli A, Agnoletti L, Frangolini G, et al. Circulating levels of tumour necrosis factor-alpha and its soluble receptors are increased in the blood of patients with amyotrophic lateral sclerosis. Neurosci Lett. 2000;287(3):211–4. https://doi.org/10.1016/S0304-3940(00)01177-0
22. Patin F, Corcia P, Madji Hounoum B, Veyrat-Durebex C, Respaud E, Piver E, et al. Biological follow-up in amyotrophic lateral sclerosis: decrease in creatinine levels and increase in ferritin levels predict poor prognosis. Eur J Neurol. 2015;22(10):1385–90. https://doi.org/10.1111/ene.12754
23. Goodall EF, Haque MS, Morrison KE. Increased serum ferritin levels in amyotrophic lateral sclerosis (ALS) patients. J Neurol. 2008;255(11):1652–6. https://doi.org/10.1007/s00415-008-0945-0
24. Nadjar Y, Gordon P, Corcia P, Bensimon G, Pieroni L, Meininger V, et al. Elevated serum ferritin is associated with reduced survival in amyotrophic lateral sclerosis. PLoS One. 2012;7(9):e45034. https://doi.org/10.1371/journal.pone.0045034
25. Chio A, Calvo A, Bovio G, Canosa A, Bertuzzo D, Galmozzi F, et al. Amyotrophic lateral sclerosis outcome measures and the role of albumin and creatinine: a population-based study. JAMA Neurol. 2014;71(9):1134–42. https://doi.org/10.1001/jamaneurol.2014.1129
26. Joilin G, Gray E, Thompson AG, Bobeva Y, Talbot K, Weishaupt J, et al. Identification of a potential non-coding RNA biomarker signature for amyotrophic lateral sclerosis. Brain Commun. 2020;2(1):fca053. https://doi.org/10.1093/braincomms/fca053
27. Salter M, Corfield E, Ramadass A, Grand F, Green J, Westra J, et al. Initial Identification of a Blood-Based Chromosome Conformation Signature for Aiding in the Diagnosis of Amyotrophic Lateral Sclerosis. EBioMedicine. 2018;33:169–84. https://doi.org/10.1016/j.ebiom.2018.06.015
28. Swindell WR, Kruse CPS, List EO, Berryman DE, Kopchick JJ. ALS blood expression profiling identifies new biomarkers, patient subgroups, and evidence for neutrophilia and hypoxia. J Transl Med. 2019;17(1):170. https://doi.org/10.1186/s12967-019-1900-0
29. Gustafson MP, Staff NP, Bornschlegl S, Butler GW, Maas ML, Kazamel M, et al. Comprehensive immune profiling reveals substantial immune system alterations in a subset of patients with amyotrophic lateral sclerosis. PLoS One. 2017;12(7):e0182002. https://doi.org/10.1371/journal.pone.0182002
30. Henkel JS, Beers DR, Wen S, Rivera AL, Toennis KM, Appel JE, et al. Regulatory T-lymphocytes mediate amyotrophic lateral sclerosis progression and survival. EMBO Mol Med. 2013;5(1):64–79. https://doi.org/10.1002/emmm.201201544
31. DeJesus-Hernandez M, Mackenzie IR, Boeve BF, Boxer AL, Baker M, Rutherford NJ, et al. Expanded GGGGCC hexanucleotide repeat in noncoding region of C9ORF72 causes chromosome 9p-linked FTD and ALS. Neuron. 2011;72(2):245–56. https://doi.org/10.1016/j.neuron.2011.09.011
32. Renton AE, Majounie E, Waite A, Simon-Sanchez J, Rollinson S, Gibbs JR, et al. A hexanucleotide repeat expansion in C9ORF72 is the cause of chromosome 9p21-linked ALS-FTD. Neuron. 2011;72(2):257–68. https://doi.org/10.1016/j.neuron.2011.09.010
33. Cleary JD, Ranum LP. Repeat-associated non-ATG (RAN) translation in neurological disease. Hum Mol Genet. 2013;22(R1):R45–51. https://doi.org/10.1093/hmg/ddt371
34. Andrade NS, Ramic M, Esanov R, Liu W, Rybin MJ, Gaidosh G, et al. Dipeptide repeat proteins inhibit homology-directed DNA strand break repair in C9ORF72 ALS/FTD. Mol Neurodegener. 2020;15(1):13. https://doi.org/10.1186/s13024-020-00365-9
35. Nonaka T, Masuda-Suzukake M, Hosokawa M, Shimozawa A, Hirai S, Okado H, et al. C9ORF72 dipeptide repeat poly-GA inclusions promote intracellular aggregation of phosphorylated TDP-43. Hum Mol Genet. 2018;27(15):2658–70. https://doi.org/10.1093/hmg/ddy174
36. Mackenzie IR, Arzberger T, Kremmer E, Troost D, Lorenzl S, Mori K, et al. Dipeptide repeat protein pathology in C9ORF72 mutation cases: clinico-pathological correlations. Acta neuropathologica. 2013;126(6):859–79. https://doi.org/10.1007/s00401-013-1181-y
37. Mizielinska S, Gronke S, Niccoli T, Ridler CE, Clayton EL, Devoy A, et al. C9orf72 repeat expansions cause neurodegeneration in Drosophila through arginine-rich proteins. Science. 2014;345(6201):1192–4. https://doi.org/10.1126/science.1256800
38. Cammack AJ, Atassi N, Hyman T, van den Berg LH, Harms M, Baloh RH, et al. Prospective natural history study of C90F72 ALS clinical characteristics and biomarkers. Neurology. 2019;93(17):e1605–e17. https://doi.org/10.1212/wnl.0000000000008359
39. Gendron TF, Chew J, Stankowski JN, Hayes LR, Zhang YJ, Prudencio M, et al. Poly(GP) proteins are a useful pharmacodynamic marker for C9ORF72-associated amyotrophic lateral sclerosis. Sci Transl Med. 2017;9(383).
40. Raine CS. Neurocellular anatomy. In: Siegel GJ, Albers RW, Brady ST, Price DL, editors. Basic Neurochemistry Molecular, Cellular, and Medical Aspects 7th ed: Elsevier Academic Press; 2006. p. 3–19.
41. Pigino G, Kirtkpatrick LL, Brady ST. The cytoskeleton of neurons and glia. In: Siegel GJ, Albers RW, Brady ST, Price DL, editors. Basic neurochemistry. 7th ed: Elsevier Academic press; 2006. p. 123–37.
42. Petzold A. Neurofilament phosphoforms: surrogate markers for axonal injury, degeneration and loss. J Neurol Sci. 2005;233(1–2):183–98. https://doi.org/10.1016/j.jns.2005.03.015
43. Hirano A. Cytopathology of amyotrophic lateral sclerosis. Adv Neurol. 1991;56:91–101.
44. Hirano A, Donnenfeld H, Sasaki S, Nakano I. Fine structural observations of neurofilamentous changes in amyotrophic lateral sclerosis. J Neuropathol Exp Neurol. 1984;43(5):461–70. https://doi.org/10.1097/00005072-198409000-00001
45. Hirano A, Nakano I, Kurland LT, Mulder DW, Holley PW, Saccomanno G. Fine structural study of neurofibrillary changes in a family with amyotrophic lateral sclerosis. J Neuropathol Exp Neurol. 1984;43(5):471–80. https://doi.org/10.1097/00005072-198409000-00002
46. Julien JP. Amyotrophic lateral sclerosis. unfolding the toxicity of the misfolded. Cell. 2001;104(4):581–91. https://doi.org/10.1016/S0092-8674(01)00244-6
47. Rosengren LE, Karlsson JE, Karlsson JO, Persson LI, Wikkelso C. Patients with amyotrophic lateral sclerosis and other neurodegenerative diseases have increased levels of neurofilament protein in CSF. J Neurochem. 1996;67(5):2013–8. https://doi.org/10.1046/j.1471-4159.1996.67052013.x
48. Poesen K, Van Damme P. Diagnostic and Prognostic Performance of Neurofilaments in ALS. Front Neurol. 2018;9:1167. https://doi.org/10.3389/fneur.2018.01167
49. Benatar M, Wu J, Andersen PM, Lombardi V, Malaspina A. Neurofilament light: A candidate biomarker of presymptomatic amyotrophic lateral sclerosis and phenoconversion. Ann Neurol. 2018;84(1):130–9. https://doi.org/10.1002/ana.25276
50. Steinacker P, Barschke P, Otto M. Biomarkers for diseases with TDP-43 pathology. Mol Cell Neurosci. 2019;77:43–59. https://doi.org/10.1016/j.mcn.2018.10.003
51. Rissin DM, Kan CW, Campbell TG, Howes SC, Fournier DR, Song L, et al. Single-molecule enzyme-linked immunosorbent assay detects serum proteins at subfemtomolar concentrations. Nat Biotechnol. 2010;28(6):595–9. https://doi.org/10.1038/nbt.1641
52. Ganesalingam J, An J, Shaw CE, Shaw G, Lacomis D, Bowser R. Combination of neurofilament heavy chain and complement C3 as CSF biomarkers for ALS. J Neurochem. 2011;117(3):528–37. https://doi.org/10.1111/j.1471-4159.2011.07224.x
53. Lu CH, Macdonald-Wallis C, Gray E, Pearce N, Petzold A, Norgren N, et al. Neurofilament light chain: A prognostic biomarker in amyotrophic lateral sclerosis. Neurology. 2015;84(22):2247–57. https://doi.org/10.1212/WNL.0000000000001642
54. Benatar M, Zhang L, Wang L, Granit V, Statland J, Barohn R, et al. Validation of serum neurofilaments as prognostic and potential pharmacodynamic biomarkers for ALS. Neurology. 2020;95(1):e59–e69. https://doi.org/10.1212/WNL.0000000000009559
55. Lee JD, Woodruff TM. TDP-43 Puts the STING in ALS. Trends Neurosci. 2020. https://doi.org/10.1016/j.tins.2020.12.001
56. Dewey CM, Cenik B, Sephton CF, Johnson BA, Herz J, Yu G. TDP-43 aggregation in neurodegeneration: are stress granules the key? Brain Res. 2012;1462:16–25. https://doi.org/10.1016/j.brainres.2012.02.032
57. Wei Y, Lim L, Wang L, Song J. ALS-causing cleavages of TDP-43 abolish its RRM2 structure and unlock CTD for enhanced aggregation and toxicity. Biochem Biophys Res Commun. 2017;485(4):826–31. https://doi.org/10.1016/j.bbrc.2017.02.139
58. Cascella R, Capitini C, Fani G, Dobson CM, Cecchi C, Chiti F. Quantification of the Relative Contributions of Loss-of-function and Gain-of-function Mechanisms in TAR DNA-binding Protein 43 (TDP-43) Proteinopathies. J Biol Chem. 2016;291(37):19437–48. https://doi.org/10.1074/jbc.M116.737726
59. Wilhite R, Sage JM, Bouzid A, Primavera T, Agbas A. Platelet phosphorylated TDP-43: an exploratory study for a peripheral surrogate biomarker development for Alzheimer's disease. Future Sci OA. 2017;3(4):FSO238. https://doi.org/10.4155/fsa-2017-0009
60. Foulds PG, Davidson Y, Mishra M, Hobson DJ, Humphreys KM, Taylor M, et al. Plasma phosphorylated-TDP-43 protein levels correlate with brain pathology in frontotemporal lobar degeneration. Acta neuropathologica. 2009;118(5):647–58. https://doi.org/10.1007/s00401-009-0594-0
61. Kasai T, Tokuda T, Ishigami N, Sasayama H, Foulds P, Mitchell DJ, et al. Increased TDP-43 protein in cerebrospinal fluid of patients with amyotrophic lateral sclerosis. Acta neuropathologica. 2009;117(1):55–62. https://doi.org/10.1007/s00401-008-0456-1
62. Nardo G, Pozzi S, Pignataro M, Lauranzano E, Sapan G, Garbelli S, et al. Amyotrophic lateral sclerosis multiprotein biomarkers in peripheral blood mononuclear cells. PloS one. 2011;6(10):e25545. https://doi.org/10.1371/journal.pone.0025545
63. Noto Y, Shibuya K, Sato Y, Kanai K, Misawa S, Sawai S, et al. Elevated CSF TDP-43 levels in amyotrophic lateral sclerosis: specificity, sensitivity, and a possible prognostic value. Amyotroph Lateral Scler. 2011;12(2):140–3. https://doi.org/10.3109/17482968.2010.541263
64. Steinacker P, Hendrich C, Sperfeld AD, Jesse S, von Arnim CA, Lehner S, et al. TDP-43 in cerebrospinal fluid of patients with frontotemporal lobar degeneration and amyotrophic lateral sclerosis. Arch Neurol. 2008;65(11):1481–7. https://doi.org/10.1001/archneur.65.11.1481
65. Statland JM, Moore D, Wang Y, Walsh M, Mozaffar T, Elman L, et al. Rasagiline for amyotrophic lateral sclerosis: A randomized, controlled trial. Muscle Nerve. 2019;59(2):201–7. https://doi.org/10.1002/mus.26335
66. Kametani F, Obi T, Shishido T, Akatsu H, Murayama S, Saito Y, et al. Mass spectrometric analysis of accumulated TDP-43 in amyotrophic lateral sclerosis brains. Sci Rep. 2016;6:23281. https://doi.org/10.1038/srep23281
67. Neumann M, Kwong LK, Lee EB, Kremmer E, Flatley A, Xu Y, et al. Phosphorylation of S409/410 of TDP-43 is a consistent feature in all sporadic and familial forms of TDP-43 proteinopathies. Acta neuropathol. 2009;117(2):137–49. https://doi.org/10.1007/s00401-008-0477-9
68. Nonaka T, Arai T, Hasegawa M. [The molecular mechanisms of intracellular TDP-43 aggregates]. Brain and nerve = Shinkei kenkyu no shinpo. 2009;61(11):1292–300.
69. Cohen TJ, Hwang AW, Restrepo CR, Yuan CX, Trojanowski JQ, Lee VM. An acetylation switch controls TDP-43 function and aggregation propensity. Nat Commun. 2019;6:5845. https://doi.org/10.1038/s41467-018-05758-z
70. Zhang YJ, Gendron TF, Xu YF, Ko LW, Yen SH, Petrucelli L. Phosphorylation regulates proteasomal-mediated degradation and solubility of TAR DNA binding protein-43 C-terminal fragments. Mol Neurodegener. 2010;5:33. https://doi.org/10.1186/1750-1326-5-33
71. De Marco G, Lupino E, Calvo A, Moglia C, Bucinnia B, Grifoni S, et al. Cytoplasmic accumulation of TDP-43 in circulating lymphomonocytes of ALS patients with and without TARDBP mutations. Acta Neuropathol. 2011;121(5):611–22. https://doi.org/10.1007/s00401-010-0786-7
72. Cereda C, Leoni E, Milani F, Pansarasa O, Mazzini G, Guareschi S, et al. Altered intracellular localization of SOD1 in leukocytes from patients with sporadic amyotrophic lateral sclerosis. PLoS One. 2013;8(10):e75916. https://doi.org/10.1371/journal.pone.0075916
73. Li S, Xu Z, Sheng J. tRNA-Derived Small RNA: A Novel Regulatory Small Non-Coding RNA. Genes. 2018;9(5). https://doi.org/10.3390/genes9050246
74. Hogg MC, Raoof R, El Naggar H, Monsefi N, Delanty N, O’Brien DF, et al. Elevation in plasma tRNA fragments precede seizures in human epilepsy. J Clin Invest. 2019;129(7):2946–51. https://doi.org/10.1172/JCI126346
75. Li Y, Zheng Q, Bao C, Li S, Guo W, Zhao J, et al. Circular RNA is enriched and stable in exosomes: a promising biomarker for cancer diagnosis. Cell Res. 2015;25(8):981–4. https://doi.org/10.1038/cr.2015.82
76. Sheinerman KS, Umanosky SR. Circulating cell-free microRNA as biomarkers for screening, diagnosis and monitoring of neurodegenerative diseases and other neurologic pathologies. Front Cell Neurosci. 2013;7:150. https://doi.org/10.3389/fncel.2013.00150
77. Burgos K, Malenica I, Metpally R, Courtright A, Rakela B, Beach T, et al. Profiles of extracellular miRNA in cerebrospinal fluid and serum from patients with Alzheimer's and Parkinson's diseases correlate with disease status and features of pathology. PLoS one. 2014;9(5):e94839. https://doi.org/10.1371/journal.pone.0094839
78. Sun XY, Zhang J, Niu W, Guo W, Song HT, Li HY, et al. A preliminary analysis of microRNA as potential clinical biomarker for schizophrenia. Am J Med Genet B Neuropsychiatr Genet. 2015;168B(3):170–8. https://doi.org/10.1002/ajmg.b.32292
79. Wilusz JE. A 360 degrees view of circular RNAs: From biogenesis to functions. Wiley Interdiscip Rev RNA. 2018; 9(4):e1478. https://doi.org/10.1002/wrna.1478
80. Zahn Y, Xue W, Li X, Zhang J, Chen S, Zhang JL, et al. The Biogenesis of Nascent Circular RNAs. Cell Rep. 2016;15(3):611–24. https://doi.org/10.1016/j.celrep.2016.03.058
81. Jeck WR, Sorrentino JA, Wang K, Slevin MK, Burd CE, Liu J, et al. Circular RNAs are abundant, conserved, and associated with ALU repeats. RNA. 2013;19(2):141–57. https://doi.org/10.1261/rna.035667.112
82. Salzman J, Gawad C, Wang PL, Lacayo N, Brown PO. Circular RNAs are the predominant transcript isoform from hundreds of human genes in diverse cell types. PLoS one. 2012;7(2):e30733. https://doi.org/10.1371/journal.pone.0030733
83. Ravnik-Glavec M, Glavic D. Circulating RNAs as Potential Biomarkers in Amyotrophic Lateral Sclerosis. Int J Mol Sci. 2020;21(5). https://doi.org/10.3390/ijms21051714
84. Ferrara D, Pasetto L, Bonetto V, Basso M. Role of Extracellular Vesicles in Amyotrophic Lateral Sclerosis. Front Neurosci. 2018;12:574. https://doi.org/10.3389/fnins.2018.00574
85. Lo Bello M, Di Fini F, Notaro A, Spataro R, Conforti FL, La Bella V. ALS-Related Mutant FUS Protein Is Mislocalized to Cytoplasm and Is Recruited into Stress Granules of Fibroblasts from Asymptomatic FUS P525L Mutation Carriers. Neurodegener Dis. 2017;17(6):292–303. https://doi.org/10.1159/000480085
86. Ratti A, Gumina V, Lenzi P, Bossolasco P, Fulceri F, Volpe C, et al. Chronic stress induces formation of stress granules and pathological TDP-43 aggregates in human ALS fibroblasts and iPSC-motoneurons. Neurobiol Dis. 2020;145:105051. https://doi.org/10.1016/j.nbd.2020.105051

87. Becker LA, Huang B, Bieri G, Ma R, Knowles DA, Jafar-Nejad P, et al. Therapeutic reduction of ataxin-2 extends lifespan and reduces pathology in TDP-43 mice. Nature. 2017;544(7650):367–71. https://doi.org/10.1038/nature22038

88. Mateju D, Franzmann TM, Patel A, Kopach A, Boczek EE, Maharana S, et al. An aberrant phase transition of stress granules triggered by misfolded protein and prevented by chaperone function. EMBO J. 2017;36(12):1669–87. https://doi.org/10.15252/embj.201695957

89. De Muyt Y, Van Damme P. Cellular effects of progranulin in health and disease. J Mol Neurosci. 2011;45(3):549–60. https://doi.org/10.1007/s12031-011-9553-z

90. Petkau TL, Leavitt BR. Progranulin in neurodegenerative disease. Trends Neurosci. 2014;37(7):388–98. https://doi.org/10.1016/j.tins.2014.04.003

91. Van Damme P, Van Hoecke A, Lambrechts D, Vanacker P, Bogaert E, van Swieten J, et al. Progranulin functions as a neurotrophic factor to regulate neurite outgrowth and enhance neuronal survival. J Cell Bio. 2008;181(1):37–41. https://doi.org/10.1083/jcb.200712039

92. Wilke C, Gillardon F, Deuschle C, Dubois E, Hobert MA, Muller vom Hagen J, et al. Serum Levels of Progranulin Do Not Reflect Cerebrospinal Fluid Levels in Neurodegenerative Disease. Curr Alzheimer Res. 2016;13(6):654–62. https://doi.org/10.2174/1567205013666160314151247

93. Feneberg E, Steinacker P, Volk AE, Weishaupt JH, Wollmer MA, Boxer A, et al. Progranulin as a candidate biomarker for therapeutic trial in patients with ALS and FTLD. J Neural Transm (Vienna). 2016;123(3):289–96. https://doi.org/10.1007/s00702-015-1486-1

94. Xu Z, Lee A, Nouwens A, Henderson RD, McCombe PA. Mass spectrometry analysis of plasma from amyotrophic lateral sclerosis and control subjects. Amyotroph Lateral Scler Frontotemporal Degener. 2018;19(5-6):362–76. https://doi.org/10.1080/21678421.2018.1433689

95. Beers DR, Henkel JS, Zhao W, Wang J, Huang A, Wen S, et al. Endogenous regulatory T lymphocytes ameliorate amyotrophic lateral sclerosis in mice and correlate with disease progression in patients with amyotrophic lateral sclerosis. Brain. 2011;134(Pt 5):1293–314. https://doi.org/10.1093/brain/awr074

96. Wiedemann FR, Manfredi G, Mawrin C, Beal MF, Schon EA. Mitochondrial DNA and respiratory chain function in spinal cords of ALS patients. J Neurochem. 2002;80(4):616–25. https://doi.org/10.1046/j.0022-3042.2001.00731.x

97. Hor JH, Santosa MM, Lim VJH, Ho BX, Taylor A, Khong ZJ, et al. ALS motor neurons exhibit hallmark metabolic defects that are rescued by SIRT3 activation. Cell Death Differ. 2020. https://doi.org/10.1101/713651

98. Crugnola V, Lamperti C, Lucchini V, Ronchi D, Peverelli L, Prelle A, et al. Mitochondrial respiratory chain dysfunction in muscle from patients with amyotrophic lateral sclerosis. Arch Neurol. 2010;67(7):849–54. https://doi.org/10.1001/archneur.2010.128

99. Vielhaber S, Kunz D, Winkler K, Wiedemann FR, Kirches E, Feistner H, et al. Mitochondrial DNA abnormalities in skeletal muscle of patients with sporadic amyotrophic lateral sclerosis. Brain. 2000;123 (Pt 7):1339–48. https://doi.org/10.1093/brain/123.7.1339

100. Vielhaber S, Winkler K, Kirches E, Kunz D, Buchner M, Feistner H, et al. Visualization of defective mitochondrial function in skeletal muscle fibers of patients with sporadic amyotrophic lateral sclerosis. J Neurol Sci. 1999;169(1–2):133–9. https://doi.org/10.1016/S0022-510X(99)00236-1

101. Szelechowski M, Amoedo N, Obre E, Leger C, Allard L, Bonnae M, et al. Metabolic Reprogramming in Amyotrophic Lateral Sclerosis. Sci Rep. 2018;8(1):3953. https://doi.org/10.1038/s41598-018-22318-5

102. Nakano Y, Hitayama K, Terao K. Hepatic ultrastructural changes and liver dysfunction in amyotrophic lateral sclerosis. Arch Neurol. 1987;44(1):103–6. https://doi.org/10.1001/archneur.1987.00520130079022

103. Curti D, Malaspina A, Facchetti G, Camana C, Mazzini L, Tosca P, et al. Amyotrophic lateral sclerosis: oxidative energy metabolism and calcium homeostasis in peripheral blood lymphocytes. Neurology. 1996;47(4):1060–4. https://doi.org/10.1212/WNL.47.4.1060

104. Macchi Z, Wang Y, Moore D, Katz J, Saperstein D, Wolk D, et al. A multi-center screening trial of rasagiline in patients with amyotrophic lateral sclerosis: Possible mitochondrial biomarker target engagement.
105. Mehta P, Kaye W, Raymond J, Punjani R, Larson T, Cohen J, et al. Prevalence of Amyotrophic Lateral Sclerosis - United States, 2015. MMWR Morb Mortal Wkly Rep. 2018;67(46):1285–9. https://doi.org/10.15585/mmwr.mm6746a1

106. Blokhuis AM, Groen EJ, Koppers M, van den Berg LH, Pasterkamp RJ. Protein aggregation in amyotrophic lateral sclerosis. Acta neuropathologica. 2013;125(6):777–94. https://doi.org/10.1007/s00401-013-1125-6

107. Duffy LM, Chapman AL, Shaw PJ, Grierson AJ. Review: The role of mitochondria in the pathogenesis of amyotrophic lateral sclerosis. Neuropathol Appl Neurobiol. 2011;37(4):336–52. https://doi.org/10.1111/j.1365-2990.2011.01166.x

108. Rothstein JD. Current hypotheses for the underlying biology of amyotrophic lateral sclerosis. Ann Neurol. 2009; 65 Suppl 1:S3–9. https://doi.org/10.1002/ana.21543

109. Smith EF, Shaw PJ, De Vos KJ. The role of mitochondria in amyotrophic lateral sclerosis. Neurosci Lett. 2019;710:132933. https://doi.org/10.1016/j.neulet.2017.06.052

110. Swerdlow RH. The neurodegenerative mitochondrialopathies. J Alzheimers Dis. 2009;17(4):737–51. https://doi.org/10.3233/JAD-2009-1095

111. Swerdlow RH, Parks JK, Cassarino DS, Trimmer PA, Miller SW, Maguire DJ, et al. Mitochondria in sporadic amyotrophic lateral sclerosis. Exp Neurol. 1998;153(1):135–42. https://doi.org/10.1006/exnr.1998.6866

112. Swerdlow RH, Parks JK, Pattee G, Parker WD, Jr. Role of mitochondria in amyotrophic lateral sclerosis. Amyotroph Lateral Scler Other Motor Neuron Disord. 2000;1(3):185–90. https://doi.org/10.1080/14660820050515179

113. Fang T, Al Khleifat A, Meurgey JH, Jones A, Leigh PN, Bensimon G, et al. Stage at which riluzole treatment prolongs survival in patients with amyotrophic lateral sclerosis: a retrospective analysis of data from a dose-ranging study. Lancet Neurol. 2018;17(5):416–22. https://doi.org/10.1016/S1474-4422(18)30054-1

114. Hinchcliffe M, Smith A. Riluzole: real-world evidence supports significant extension of median survival times in patients with amyotrophic lateral sclerosis. Degener Neurol Neuromuscul Dis. 2017;7:61–70. https://doi.org/10.2147/DNND.S135748

115. Schultz J. Disease-modifying treatment of amyotrophic lateral sclerosis. Am J Manag Care. 2018;24(15 Suppl):S327–S35.

116. Abe K, Itoyama Y, Sobue G, Tsuji S, Aoki M, Doyu M, et al. Confirmatory double-blind, parallel-group, placebo-controlled study of efficacy and safety of edaravone (MCI-186) in amyotrophic lateral sclerosis patients. Amyotroph Lateral Scler Frontotemporal Degener. 2014;15(7–8):610–7. https://doi.org/10.3109/21678421.2014.959024

117. Rothstein JD. Edaravone: A new drug approved for ALS. Cell. 2017;171(4):725. https://doi.org/10.1016/j.cell.2017.10.011

118. Smith R, Pioro E, Myers K, Sirdofsky M, Goslin K, Meekins G, et al. Erratum to: Enhanced Bulbar Function in Amyotrophic Lateral Sclerosis: The Nuedexta Treatment Trial. Neurotherapeutics. 2017;14(3):830. https://doi.org/10.1007/s13311-017-0517-z

119. Smith R, Pioro E, Myers K, Sirdofsky M, Goslin K, Meekins G, et al. Enhanced Bulbar Function in Amyotrophic Lateral Sclerosis: The Nuedexta Treatment Trial. Neurotherapeutics. 2017;14(3):762–72. https://doi.org/10.1007/s13311-016-0508-5