Thinking About

ALS Exploration

Literature Reading Group

ALS Volunteer Research Progress and Reflections

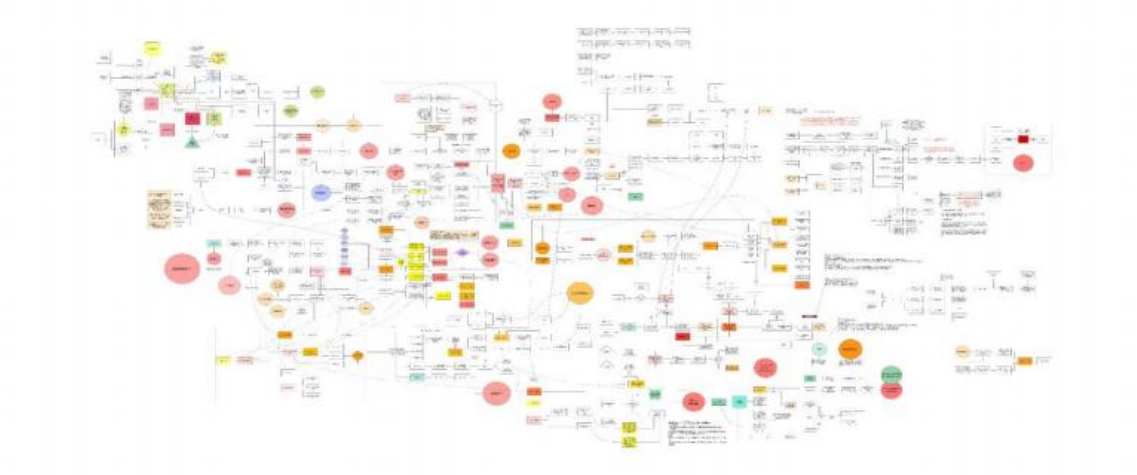
(Phase I: 2024-08 — 2025-03; Phase II: 2025-04 — 2025-09)

1. Exploration of ALS Mechanisms and Therapeutic Strategies

Abstract: *This article briefly describes the potential targets, therapeutic strategies, evaluation scales and biomarkers, research paradigms, and inspirational reflections on Amyotrophic Lateral Sclerosis (ALS).*

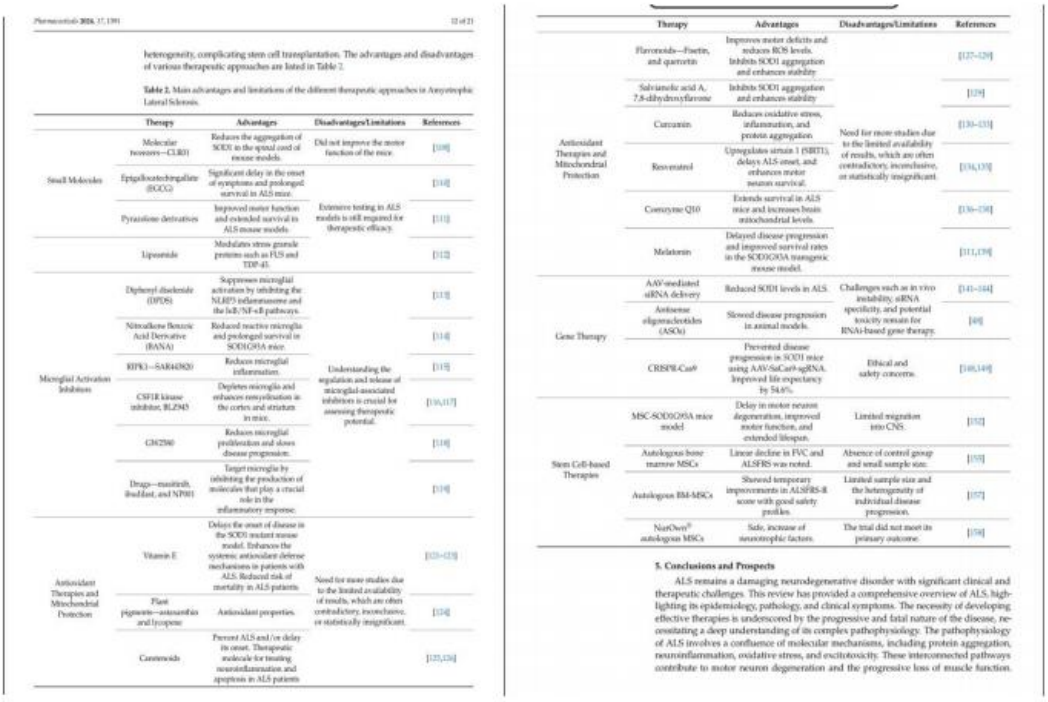
1.1 Potential Targets: ALS Mechanism Knowledge Graph and Core Event Screening

Cai's research team has compiled over **60,000 "entity events"** (i.e., biological processes, such as "TDP-43 phosphorylation" and "chronic inflammation") and their causal relationships, constructing a comprehensive knowledge graph covering ALS mechanisms. Based on this foundation, the team further screened out **700 "core events"**. These events are biological processes that occur at high frequency or play key driving roles in ALS pathology (such as TDP-43 abnormality, SOD1 mutation, oxidative stress, etc.). However, identifying potential targets based on this graph still faces major challenges. (Source: Can't understand the ALS mechanism graph? Use the "factory story" to help you understand ALS)



1.2 Therapeutic Strategies: Development and Limitations of ALS Therapeutic Strategies

Therapeutic strategies for ALS are in a stage of rapid development. Current main therapeutic strategies include: using small molecule drugs to inhibit abnormal protein aggregation, regulating neuroinflammatory pathways to reduce neuronal damage, protecting mitochondrial function through antioxidant therapy, and adopting emerging approaches such as gene therapy and stem cell therapy. However, aforementioned strategies and their corresponding drugs and therapies all have their own advantages and limitations in clinical applications. (Source: Amyotrophic Lateral Sclerosis: Insights and New Prospects in Disease Pathophysiology, Biomarkers and Therapies (2024)).



heterogeneity, complicating stem cell transplantation. The advantages and disadvantages of various therapeutic approaches are listed in Table 2.

Table 2. Main advantages and limitations of the different therapeutic approaches in Amyotrophic Lateral Sclerosis.

Therapy		Advantages	Disadvantages/Limitations	References
Small Molecules	Molecular tweezers—CLR01	Reduces the aggregation of SOD1 in the spinal cord of mouse models.	Did not improve the motor function of the mice.	[108]
	Epigallocatechingallate (EGCG)	Significant delay in the onset of symptoms and prolonged survival in ALS mice.	Extensive testing in ALS models is still required for therapeutic efficacy.	[110]
	Pyrazolone derivatives	Improved motor function and extended survival in ALS mouse models.		[111]
	Lipoamide	Modulates stress granule proteins such as FUS and TDP-43.		[112]
Microglial Activation Inhibitors	Diphenyl diselenide (DPDS)	Suppresses microglial activation by inhibiting the NLRP3 inflammasome and the IκB/NF-κB pathways.	Understanding the regulation and release of microglial-associated inhibitors is crucial for assessing therapeutic potential.	[113]
	Nitrosalkene Benzoic Acid Derivative (BANA)	Reduced reactive microglia and prolonged survival in SOD1G93A mice.		[114]
	RIPK1—SAR443820	Reduces microglial inflammation.		[115]
	CSF1R kinase inhibitor, BLZ945	Depletes microglia and enhances remyelination in the cortex and striatum in mice.		[116,117]
	GW2580	Reduces microglial proliferation and slows disease progression.		[118]
	Drugs—masitinib, ibudilast, and NP001	Target microglia by inhibiting the production of molecules that play a crucial role in the inflammatory response.		[119]
Antioxidant Therapies and Mitochondrial Protection	Vitamin E	Delays the onset of disease in the SOD1 mutant mouse model. Enhances the systemic antioxidant defense mechanisms in patients with ALS. Reduced risk of mortality in ALS patients.	Need for more studies due to the limited availability of results, which are often contradictory, inconclusive, or statistically insignificant.	[121–123]
	Plant pigments—astaxanthin and lycopene	Antioxidant properties.		[124]
	Carotenoids	Prevent ALS and/or delay its onset. Therapeutic molecule for treating neuroinflammation and apoptosis in ALS patients		[125,126]

Therapy		Advantages	Disadvantages/Limitations	References
Antioxidant Therapies and Mitochondrial Protection	Flavonoids—Fisetin, and quercetin	Improves motor deficits and reduces ROS levels. Inhibits SOD1 aggregation and enhances stability	Need for more studies due to the limited availability of results, which are often contradictory, inconclusive, or statistically insignificant.	[127–129]
	Salvianolic acid A, 7,8-dihydroxyflavone	Inhibits SOD1 aggregation and enhances stability		[129]
	Curcumin	Reduces oxidative stress, inflammation, and protein aggregation		[130–133]
	Resveratrol	Upregulates sirtuin 1 (SIRT1), delays ALS onset, and enhances motor neuron survival.		[134,135]
	Coenzyme Q10	Extends survival in ALS mice and increases brain mitochondrial levels.		[136–138]
	Melatonin	Delayed disease progression and improved survival rates in the SOD1G93A transgenic mouse model.		[111,139]
Gene Therapy	AAV-mediated siRNA delivery	Reduced SOD1 levels in ALS.	Challenges such as in vivo instability, siRNA specificity, and potential toxicity remain for RNAi-based gene therapy.	[141–144]
	Antisense oligonucleotides (ASOs)	Slowed disease progression in animal models.		[40]
	CRISPR-Cas9	Prevented disease progression in SOD1 mice using AAV-SaCas9-sgRNA. Improved life expectancy by 54.6%.	Ethical and safety concerns.	[140,145]
Stem Cell-based Therapies	MSC-SOD1G93A mice model	Delay in motor neuron degeneration, improved motor function, and extended lifespan.	Limited migration into CNS.	[152]
	Autologous bone marrow MSCs	Linear decline in FVC and ALSFRS was noted.	Absence of control group and small sample size.	[153]
	Autologous BM-MSCs	Showed temporary improvements in ALSFRS-R score with good safety profiles.	Limited sample size and the heterogeneity of individual disease progression.	[157]
	NurOwn® autologous MSCs	Safe, increase of neurotrophic factors.	The trial did not meet its primary outcome.	[158]

5. Conclusions and Prospects

ALS remains a damaging neurodegenerative disorder with significant clinical and therapeutic challenges. This review has provided a comprehensive overview of ALS, highlighting its epidemiology, pathology, and clinical symptoms. The necessity of developing effective therapies is underscored by the progressive and fatal nature of the disease, necessitating a deep understanding of its complex pathophysiology. The pathophysiology of ALS involves a confluence of molecular mechanisms, including protein aggregation, neuroinflammation, oxidative stress, and excitotoxicity. These interconnected pathways contribute to motor neuron degeneration and the progressive loss of muscle function.

1.3 Evaluation Scales and Biomarkers: ALSFRS-R & Biomarker NfL

1.3.1 ALSFRS-R:

This scale was originally developed and promoted in **1996** by a neuroscience research team from institutions including the **Massachusetts General Hospital (MGH)** in the United States. It was subsequently refined, and gained recognition from organizations such as the World Federation of Neurology (WFN) and the International Alliance of ALS/MND Associations. It is widely used in clinical research, guideline development, and educational materials. Currently, **there is no single organization, association, or individual fully responsible for its updates and copyright**

management, and its maintenance relies more on consensus and collaboration within the academic community.

Although the ALSFRS-R scale is widely adopted, some limitations have gradually emerged in its application. For example, the ROADS (Rasch-Built Overall Amyotrophic Lateral Sclerosis Disability Scale) proposed at the 2024 ALS Summit shows that ROADS may be more sensitive to changes in ALS conditions compared to ALSFRS-R (Source: The ALSFRS-R Summit: a global call to action on the use of the ALSFRS-R in ALS clinical trial). Furthermore, Dr. Fan Dongsheng pointed out as early as 2021 that ALSFRS-R scores have insufficient sensitivity—the actual changes in condition felt by patients often cannot be fully reflected in the scores (Source: https://mp.weixin.qq.com/s/kxPe7HvzQWM9-JADG9_VLA).

Nevertheless, as of now, **ALSFRS-R remains the primary clinical endpoint indicator used by regulatory agencies to evaluate the effectiveness of ALS treatments**.

1.3.2 NfL:

As early as the 1980s, researchers successfully identified multiple subunits of neurofilament proteins, including light chain (NfL), medium chain (NfM), and heavy chain (NfH). In the early stages, due to technical limitations, NfL detection mainly relied on cerebrospinal fluid (CSF) samples. **It was not until around 2010, with the advent of ultra-sensitive detection technologies such as Single Molecule Array (Simoa), that accurate measurement of extremely low concentrations of NfL in blood was achieved**. In April 2023, the U.S. Food and Drug Administration (FDA) made a landmark decision: they accelerated the approval process of the ALS therapeutic drug Tofersen, which lowers blood NfL levels as a surrogate endpoint, forthe treatment of ALS caused by SOD1 gene mutations.
This marks the first time NfL has been used as a biomarker to support approval of innovative drugs, and this indicator has now been incorporated into the efficacy evaluation systems of multiple investigational drugs and therapies.

Currently, ALS biomarkers under development mainly include "wet" biomarkers based on blood and cerebrospinal fluid (such as NfL, NfH, IL-6, etc.), imaging biomarkers (such as MRI and PET), and neurophysiological measurement indicators. However, **due to the highly complex mechanism of ALS, there is currently no single biomarker that can fully meet the needs of diagnosis, classification, prognosis, and drug efficacy evaluation, to fully support the successful development of new therapies**. This also explains why some patients' conditions continue to progress even when their NfL levels are low or even in single digits. Therefore, in the future, it may be necessary to integrate multiple types of biomarkers based on specific clinical application scenarios to form a more comprehensive and accurate evaluation system. (Source: Paper published in Nature "《Biomarkers in amyotrophic lateral sclerosis: current status and future prospects》").

1.4 Research Paradigm: Limitations of Neurodegenerative Disease Research and New Pathway Exploration

Currently, an increasing number of scholars, experts, and enterprises worldwide are actively engaged in research and development work to tackle neurological diseases represented by ALS. In particular, Academician Pu Muming (Academician of the Chinese Academy of Sciences, Foreign Associate of the U.S. National Academy of Sciences, Director of the Institute of Neuroscience), who leads China's strategy to delay the onset age of Alzheimer's disease, pointed out that according to his statistics, **from 1970 to 2024, approximately one-quarter of the Nobel Prizes in Physiology or Medicine were awarded to Western neuroscientists. However, even so, neurological/psychiatric diseases that are more common than neurodegenerative diseases still lack effective treatment options**. This reality reflects that the current research paradigm has limitations and urgently needs to explore new research pathways to achieve breakthroughs. (Source: https://www.toutiao.com/video/7543445466621411883/).

1.4.1 Inspirational Reflections:

ALS not only has complex pathological mechanisms, but its industrial chain also involves multiple stakeholders such as patients, hospitals, industry associations, and research organizations. There are practical challenges such as small sample sizes and huge research funding requirements, which cannot be solved by any single individual or institution alone. However, **what may be more fundamental than research funding and samples is our understanding of this disease: Have we raised that fundamental "right question" within ALS therapeutic research? Have directly truly progressed the ideal of "curing ALS"—rather than just delaying the disease—as a goal itself**?

We all understand that the prerequisite for effectively solving a problem is to confront the critical question. In the ALS community, many patients have "become doctors through chronic illness" and have accumulated professional knowledge that may supplement the expertise of general practitioners. Here, we propose some ideas and raise the following questions, hoping to inspire more in-depth discussions and systematic thinking:

1.4.1.1 Treatment Standard Questions:

How do we systematically measure the "curing" of ALS? How should the delay of disease progression be measured? Should we still rely entirely on the currently flawed ALSFRS-R scale and biomarkers such as NfL? Do we need to establish, or how can we establish, a more sensitive, comprehensive, and predictive new evaluation system?

1.4.1.2 Pathological Target Questions:

Is the key to curing ALS a single target that has been discovered, or a combination of multiple targets? Or does it stem from a completely new mechanism that we have not yet discovered? Furthermore, does the "target" approach itself limit our understanding and breakthroughs to some extent?

1.4.1.3 Therapeutic Drug Questions:

Which molecular mechanisms do existing drugs target? What potential influencing factors are overlooked? Is drug use necessary in the process of curing ALS?

1.4.1.4 Clinical Trial Questions:

Are current clinical observation projects comprehensive enough? Are patients participating in trials receiving other interventions during the process, and are these factors fully recorded and analyzed? Is the observation period reasonable? Are longitudinal investigations of patient conditions conducted after the trial ends? Before producing statistical clinical reports, can we strengthen comprehensive and in-depth analysis of individual treatment efficacy differences?

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2. Inspirational Reflections and Future Directions for ALS Research

Abstract: *This article outlines the diagnosis, healing, and reversal standards for ALS, the current application status of biomarkers, and the diverse therapeutic strategies and future directions of investigational drugs.*

2.1 Patient Diagnosis Standards: Based on Diagnosis by Regular Hospitals and Authoritative Experts, Referring to the "2022 Chinese Expert Consensus" to Establish Western Medicine Standards

Due to the complexity of ALS, regular hospitals currently mainly use the process of elimination to arrive at a diagnosis, and many patients still cannot be diagnosed when their condition is severe, resulting in some patients dying within a few months after diagnosis. Therefore, we hope that patients can be diagnosed through certified and licensed hospitals, especially by experts, to reduce unnecessary disputes. "ALS" referred to in Western medicine and differs from "ALS" referred to in Traditional Chinese Medicine, and this divergence stems from different definitions. From an academic research perspective, the diagnostic criteria should refer to the "Chinese Expert Consensus on Diagnosis and Treatment of Amyotrophic Lateral Sclerosis 2022" (Link: Click to view), after all, the current definition of ALS is mainly based on Western medicine standards.

2.2 Patient Healing Standards: Mainly Based on ALSFRS-R and Survival Time, Combined with CAFS, ROADS, and Patient Perception

Academic Traditional Chinese Medicine defines the diagnostic basis and therapeutic efficacy of "wilting disease" in the "Standards for Diagnosis and Treatment of Traditional Chinese Medicine Diseases and Syndromes (2024 Edition)", while the evaluation standards of major Western pharmaceutical companies also vary. For ALS, commonly used outcome measurement indicators include survival time, Functional Rating Scale (ALSFRS-R), Comprehensive Assessment of Function and Survival (CAFS), and the Rasch-built Overall ALS Disability Scale (ROADS), etc. Currently, **survival time and Functional Rating Scale (ALSFRS-R) are the most commonly used measurement indicators** (see the paper led by the U.S. government "Considerations for Amyotrophic Lateral Sclerosis (ALS) Clinical Trial Design", Link: Click to view). The effectiveness of drugs or therapies is usually evaluated by observing changes in primary endpoint indicators over an evaluation period (generally 3, 6, 9, or 12 months). Current biomarker assessments (such as neurofilament light chain protein NfL, etc.) are still secondary endpoint indicators in most drug experiments, and the focus on NfL has mainly increased with the emergence of the Sod1 gene drug Toferson. Therefore, **it is recommended to use the Functional Rating Scale (ALSFRS-R) as a basis for efficacy quantification** (which can be viewed in group files, this scale provides a comprehensive evaluation based on multiple aspects of the patient), while also considering other patient reports.

2.3 Patient Reversal Standards: Following International Definitions, Requiring Sustained Functional Improvement Without Accompanying Deterioration

The international definition of ALS symptom reversal is when a patient's disease progression meets any of the following conditions: the ALS Functional Rating Scale (ALSFRS-R) increases by at least 4 points and is sustained for at least 6 months; manual muscle testing shows significant objective improvement in strength; significant improvement in gait (for example, from using a wheelchair to being able to walk long distances); or improvement in other activities of daily living and resolution of EMG denervation. Although patients only need to improve on one measure, there should be no deterioration on other measures. If the patient's improvement is sudden (i.e., inconsistent with gradual reinnervation), or if there is no sustained improvement during the follow-up period, it will be excluded (see the literature "Genetic Associations With an Amyotrophic Lateral Sclerosis Reversal Phenotype", Link: Click to view). Therefore, to avoid controversy, it is recommended to temporarily approve this definition.

2.4 Biomarker Standards: Integrating Blood/CSF, Imaging, and Neurophysiological Indicators for Multimodal Combined Application

Biomarkers are biological indicators that can be used to diagnose, measure disease progression, and evaluate drug performance. Potential reliable markers may reveal the targeting and efficacy of drugs. Currently, ALS biomarkers under development include "wet" biomarkers based on blood and cerebrospinal fluid (CSF) (such as NfL, NfH, and IL-2, etc.), imaging (MRI and PET) biomarkers, and measurements using neurophysiology. Given the complexity of ALS, there is currently no single biomarker modality that can fully meet all diagnostic, classification, prognostic, and pharmacodynamic requirements to support the successful development of new therapies. This also explains why some patients have very low NfL values, even single digits, but their condition continues to deteriorate. Therefore, in the future, it may be necessary to integrate multiple biomarker modalities based on specific clinical requirements (see the paper published in Nature "Biomarkers in amyotrophic lateral sclerosis: current status and future prospects", Link: Click to view).

2.5 Therapeutic Strategy Standards for Investigational Drugs: Covering Small Molecule Inhibition, Inflammation Regulation, Antioxidant Protection, Gene and Stem Cell Therapy

The key therapeutic strategies adopted by marketed or investigational Western drugs and therapies each have their advantages and disadvantages, including: **Small molecule inhibition of protein aggregation**: such as InFlectis BioScience's IFB-088 and Guangzhou Magpie Pharmaceuticals' nitrone; **Regulating neuroinflammation to reduce neuronal damage**: such as masitinib, MN-166 (ibudilast), and NP001; **Antioxidant therapy and mitochondrial protection**: such as Relyvrio (Amx0035, withdrawn) and Pridopidine; **Gene therapy**: such as Toferson using antisense oligonucleotides (ASO) to treat SOD1 genes. **Stem cell-based neuroprotection and regenerative therapy**: such as lenzumestrocel (NurOwn); Industry insiders are relatively clear about the advantages and disadvantages of various treatment methods, and drug developers

are also comprehensively addressing issues while seeking benefits and avoiding harm. Patients should avoid being misled by exaggerated information about certain drugs and pay attention to the progress of drug experimental results (see link: Click to view).

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