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Treatment of Amyotrophic Lateral Sclerosis: Lessons Learned from Many Failures

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ABSTRACT: Amyotrophic lateral sclerosis (ALS) is one of the most complex neurodegenerative diseases, involving both cortical and spinal components of motor neuron circuitry and non-neuronal cells that support the motor neurons. There is no effective therapeutic for ALS, and compounds that have extended the lifespan of ALS mouse models have failed in clinical trials. This viewpoint discusses current information regarding the changing views about ALS and what the failures in clinical trials can teach us in the search for an effective treatment. Previous challenges and roadblocks in drug discovery for ALS are noted, and solutions to current limitations are discussed. Learning from the past and moving forward with a new mindset can translate into successful and effective treatment strategies in ALS and other related diseases.

Amyotrophic lateral sclerosis (ALS), one of the most complex and unique diseases of the central nervous system, is characterized by the progressive degeneration of motor neuron circuitry, which includes neurons and cells that reside in the cortex, spinal cord, and the brainstem. Diminished motor function leads to muscle wasting, paralysis, and death, generally within 3−5 years of diagnosis. There is only one FDA-approved drug for the treatment of ALS, riluzole, which extends lifetime by only 2−3 months, without alleviating disease symptoms. ALS can have a genetic link or may sporadically occur in the patient. Although the symptoms of familial and sporadic ALS are indistinguishable, there are numerous pathways associated with disease etiology and pathology. Therefore, diagnosis and treatment offer many challenges.[1]

The past 10 years have witnessed an unprecedented pace of discoveries in ALS. Since the identification of mutations in the *SOD1* gene, more than 25 genes have been directly linked, and 22 have been closely associated with ALS.[2] Although the function of the *C9orf72* gene is not well understood, detection of its intron expansion in 80% of all ALS patients has been remarkable and has immense clinical implications.[3] Developing insight from pathophysiological studies also began to provide information on how to treat ALS.[4] It has become very clear that even though patients display similar pathologies, the underlying molecular and cellular causes are different, and that, like cancer and heart disease, one drug and one solution to ALS is not the direction medicinal chemists should be heading. This realization sets the stage for future clinical trials. Initially, inclusion criteria for clinical trials were based on gender, age, location of disease onset, and family history. However, we now appreciate the importance of heterogeneity among patients, and selection criteria for clinical trials need to focus on the cellular pathways that go wrong in specific individuals. Therefore, drug discovery should have the mindset that different molecules may be relevant only to select subsets of patient populations, and personalized medicine may be required based on the needs of the patient.[5]

■ CHALLENGES TO SOLUTIONS

We believe that our ability to overcome three principal challenges will determine success in building effective ALS therapeutics: (1) design better compounds that improve the health of motor neurons via distinct cellular pathways; (2) improve preclinical screening approaches and help make educated guesses on compounds' ability to promote motor neuron survival; (3) identify patients that develop the disease from these distinct cellular defects and include them in the most appropriate clinical trials.

Design Better Compounds. There have been numerous advances in methods for drug discovery over the last 20 years, but none has been found to be useful in the search for new ALS therapeutics. Many of the most effective advancements are related to methods for screening compounds and structure-based design. The industry is reticent to use functional assays to screen compounds because the mechanism of action of molecules is deemed important to establish a SAR. However, the newer fragment-based approaches and virtual screening techniques cannot be applied, as there is no one prominent target for ALS, although mutations in certain genes are known to lead to protein aggregation and toxicity that results in the ALS phenotype. What causes the mutations and why those mutations result in protein aggregation are still not known. Structure-based design also cannot be applied, as the targets are not well-defined. This argues for improved approaches toward identifying compounds that affect the root cause of ALS, namely, the degeneration of upper and lower motor neurons; compound screens that target protection of these motor neurons may be the most effective approach.

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Improve Preclinical Screening. Initially, a decision to move a compound into clinical trials in ALS required its ability to extend the lifespan of the hSOD1^{G93A} ALS mouse model, which recapitulates many aspects of human pathology. If a compound did not extend its lifespan, it was not considered further, even if it had excellent pharmacokinetic and toxicological characteristics. Unfortunately, even compounds that extend lifespan of these mice failed in clinical trials,[6] raising doubts about direct translation from mice to humans. The ALS Therapy Development Institute (ALSTDI), among other institutions, suggested guidelines for preclinical studies to reduce false positives, including assessment of the animals' physical and biochemical traits related to ALS, determination of the actual cause of animal death, and development of statistical models related to what animals should be used (gender, littermates, and gene disposition) to minimize experimental noise.

More importantly, failures in clinical trials opened up the field to a new realization and understanding, namely, that it is important to focus on motor neurons, not on the mice.[7] Since both cortical and spinal motor neurons die in ALS, the main focus shifted to the health of motor neurons and motor neuron circuitry in patients, and it became important to identify compounds with that capability.

One approach to investigate motor neuron survival is to dedifferentiate patient-isolated fibroblasts into motor neurons *in vitro*. Production of spinal motor neurons (SMN) from skin biopsies of ALS patients is possible, and they have been instrumental for preclinical screening.[8] Although these neurons express motor neuron markers in culture upon dedifferentiation, their unique properties are under investigation. The advantages of using fibroblast-differentiated human SMN for preclinical screening are their availability in large numbers and presence of assays to determine survival rate under specific conditions. However, because these are genetically manipulated cells, they may not truly manifest *in vivo* characteristics of SMN. In addition, these dedifferentiated cells do *not* turn into corticospinal motor neurons (CSMN), which limits long-term treatment options for ALS.

The second approach is to use model systems in which motor neurons mimic disease pathology at a cellular level; one potential caveat is the choice of the model system. To reveal important insight, motor neurons in the model must closely mimic human motor neurons. Mouse is again most commonly used, not only because mouse motor neurons are almost identical to human motor neurons at a cellular level but also because they offer great versatility resulting from transgenic approaches. Generation and characterization of the UCHL1-eGFP reporter line, which allows visualization of CSMN and SMN, have been illuminating.[9] It is now possible to locate, isolate, purify, and study motor neuron populations. Upon crossing UCHL1-eGFP with model systems of ALS, disease reporter lines in which motor neurons are fluorescently labeled, can be generated. Such models will be invaluable to investigate survival needs of diseased motor neurons.

Today we can both engineer human cells toward SMN lineage and use motor neurons in model systems that recapitulate human conditions at a cellular level. These important developments in two complementary approaches will reveal important information about the efficacy of compounds to improve motor neuron survival.

Since nonneuronal cells (i.e., astrocytes and microglia) are implicated in ALS pathology, long-term treatment will also require information from nonneuronal cells. Riluzole acts primarily on astrocytes and reduces the rate of astrogliosis; identification of compounds that reduce astrogliosis and microgliosis is an active area of research. There are already drug discovery platforms that incorporate reduced astrogliosis as their read-out for success.[10] In sum, extensive knowledge of the cell biology of motor neurons and non-neuronal cells and their requirements for survival is emerging, and this will form a basis to make educated guesses for future clinical trials.

Improve Inclusion Criteria in Clinical Trials. When compounds are identified and classified based on their ability to improve upper versus lower motor neuron survival and/or suppression of astrogliosis and microgliosis via distinct mechanisms using either engineered human cells or model systems that mimic human pathology, we will have a better understanding about which compounds could improve the health of distinct ALS subpopulations. There are numerous genetic developments revealing cellular mechanisms that become affected in motor neurons.[11] For example, patients with mutations in *KIFAP3*, *DCTN1*, and *NEFH* genes would most likely suffer from axonal transport defects, whereas patients with mutations in *VCP*, *VAPB*, *SQSTM1*, and *Ubiquilin2* will have increased ER-stress and protein accumulation defects (Table 1). Therefore, it could be possible to group patients accordingly, to the cellular pathways that are potentially affected and build better patient groups for clinical trials. Another important development will be the identification of biomarkers that suggest the timing and extent of disease pathology and the rate of disease progression, although such biomarkers are not yet available. For the past 30 years, all but one of the clinical trials failed; nonetheless, numerous

Table 1. List of Genes Linked and Associated with ALS, the Pathways That Would Potentially Be Affected in the Presence of Mutations in These Genes, and Compounds That Are Implicated to Have Efficacy on a Particular Pathway[a]

genes linked and associated with ALS	potentially related pathways	compounds with suggested efficacy on the pathway
SOD1, *DAO*	oxidative stress	ederavone, AEOL 10150
KIFAP3, *NEFH*, *DCTN1*	axonal transport defects	
FUS, *TARDP43*, *HNRNPA1*, *SETX*, *TAF15*, *C9orf72*	mRNA and transcription defects	
SQSTM1, *Ubiqulin2*, VCP, VAPB	ER-stress and protein accumulation defects	arimoclomol
	neuroinflammation	celecoxib, thalidomide, lenalidomide, minocycline
SOD1	mitochondrial defects	creatine
SOD1	glutamate excitatoxicity	riluzole, ceftriaxone, ZK 187638
SQSTM1	autophagy	lithium, rapamycin, resveratol, trehalose
PFN1, *Cdh22*, *PRPH*, *PFN1*, *SPAST*	defects in maintaining cell structure	

[a]Previous clinical trials included patients with a wide spectrum of underlying pathology, and it was not possible to group patients based on compounds' efficacy and the potential pathways that are affected in patients. However, future clinical trials will have better inclusion criteria and improved outcomes.

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compounds displayed positive results for a subpopulation of patients. However, their overall numbers were not sufficiently high to present a significant outcome. The individuals that responded to that particular compound may indeed represent the subpopulation of ALS patients that would benefit from the treatment. Moving forward, it is imperative to include the right population of patients and to use compounds with known and proven function on a given cellular pathway that becomes affected in the disease for that particular subpopulation of patients. Unfortunately, setting up meaningful clinical trials is also challenging because of the limited number of patients that can be recruited: relatively few people develop ALS, and those who do have very limited lifespan after diagnosis. A joint effort from multiple different centers and different countries is required, and ALS associations are now coordinating a global effort, such as the Northeast Amyotrophic Lateral Sclerosis (NEALS) consortium.

■ CONCLUSIONS

Although there have been many failures in the past, the future is bright. Today we have a more informed basis to synthesize better compounds, can test the efficacy of compounds on motor neurons, and global networks among ALS centers allow improved clinical trial settings. Our failures have shaped our critical thinking. Improved methodologies in medicinal chemistry and new approaches to understand motor neuron biology will guide us to a better future to find effective treatment options for ALS and other related motor neuron diseases.

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Notes

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