CLINICAL INVESTIGATION

Outcome measures in amyotrophic lateral sclerosis clinical trials

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease with an average survival of 3–5 years. While therapies for ALS remain limited, basic and translational ALS research has been host to numerous influential discoveries in recent years. These discoveries have led to a large pipeline of potential therapies that await testing in clinical trials. Until recently, ALS clinical trials have relied on a limited cadre of 'traditional' outcome measures, including survival and measures of function. These measures have proven useful, although imperfect, in Phase III ALS trials. However, their utility in early-phase ALS trials is limited. For these early trials, outcome measures focused on target engagement or biological pathway analysis might improve trial outcomes and better support the drug development process.

Keywords: biomarker • end point • function • survival • surrogate

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Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease. ALS is characterized by motor neuron loss resulting in weakness, disability, and eventually death from failure of the ventilatory muscles [1]. The median age of onset is 55 years and average survival is 3–5 years [2]. The only US FDA-approved, disease-modifying medication, riluzole, confers a modest survival benefit [3]. While the incidence of ALS is comparable to that of multiple sclerosis (~2/100,000), its prevalence is much lower because of its rapid progression (~5/100,000) [4].

ALS is a diagnosis of exclusion that is confirmed based on history, clinical features, exam findings, and history of progressive spread. Laboratory evaluations and neuroimaging are performed to exclude the presence of competing disorders. Electrodiagnostic findings both exclude potential disease mimics and support the clinical diagnosis. Consensus diagnostic criteria, known as the revised El Escorial Criteria (EEC) provide a standardized framework to classify patients for enrollment into research studies [5]. However, many people with ALS do not fulfill the strict EEC criteria until later in the disease course [6]. To enable earlier diagnosis, the Awaji criteria have been developed and shown to increase diagnostic sensitivity without substantial reduction in specificity [7,8]. Many ALS experts find these criteria useful clinically, but enrollment into clinical trials has thus far continued to employ EEC criteria.

Given the poor prognosis, rapid disease course, and dearth of effective treatments, clinical trials are of primary importance for many people with ALS and their providers [9]. Fortunately, ALS clinical research has been a vibrant area in recent decades. At the same time, the challenges to ALS clinical trials, are substantial: disease rarity, patient heterogeneity, limited understanding of disease pathophysiology, lack of robust biomarkers, and a relatively rapid disease course [10]. In fact, despite a concerted effort to identify new disease-modifying therapies, in the 20 years since riluzole was approved by the FDA, no new disease-modifying therapies have been identified. At the same time, each passing year brings more new drug candidates, and the pipeline of novel therapeutics requiring testing remains full.

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Given the challenges of developing new disease-modifying therapies for ALS, and the long line of candidate agents that await testing, more efficient clinical trials hold the potential to substantially speed the discovery of a new successful ALS therapy. The most influential design feature of ALS clinical trials is the selection of appropriate outcome measures. Selection of such outcome measures can support the trial objectives, design, sample size, duration, and ultimately reduce the cost and complexity of the trial. Thus far, ALS clinical trials in both early and late phases of testing have relied on measures of function and survival. While these measures might serve late-stage trials fairly well, they are poorly suited for smaller, shorter, early-phase trials. In these early, proof-of-concept trials, biomarkers might provide more rapid information about a drug's ability to reach and engage a target, affect the target molecular pathway, and ultimately meet early ALS efficacy benchmarks.

Framework for ALS outcome measures

The WHO International Classification of Functioning, Disability and Health, or ICF model [11], provides a conceptual framework for understanding the range of outcome measures available for use in ALS clinical trials (Table 1). In the ICF model, human function occurs on multiple levels. 'Disease pathology' directly reflects the specific molecular events that cause disease. These events cause deficits within the performance of an organ or body system, such as muscle weakness in ALS, or 'impairments'. Ultimately, disease may result in deficient performance of functional tasks, such as ambulation, or 'activity limitations'.

Direct measures of 'disease pathology' in ALS would include biomarkers that are causally related to motor neuron loss. For example, an imaging marker of glutamate receptor occupancy could demonstrate target engagement in a theoretical trial of a glutamate receptor antagonist in the CNS. In parallel, pharmacokinetic studies in the cerebrospinal fluid (CSF) could provide evidence for the drug's presence at the putative site of action. Both may be important to appropriately interpret results of a study. Measures of motor neuron loss are direct measures of pathology, but are downstream from the aforementioned biomarkers. Any or all of these measures, could be employed in early, small, highly informative proof-of-concept trials to rapidly evaluate the potential of candidate drugs and speed drug discovery. Unfortunately, reliable measures of motor neuron

Table 1. Framework for amyotrophic lateral sclerosis outcome measures.

	ALS-specific consequences	Available outcomes	Outcomes in validation	Outcomes in development
	Neurodegenerative processes			Pharmacodynamic biomarkers
Pathology	Motor neuron loss		• MUNE • EIM	
Impairment	Muscle weakness	• Measure of limb muscle strength (e.g., HHD) • Measures of ventilatory muscle strength (e.g., VC)	ATLIS	
Activity limitation	Changes in function (e.g., gait difficulties, dysphagia, dysarthria and dyspnea)	Functional rating scale (e.g., ALSFRS-R)	CAFS	
	Death	Tracheostomy-free survival	CAFS	

Outcome measures are listed starting from the ones closest to the molecular events leading to ALS to the ones that capture the ultimate functional consequences of disease progression.
ALS: Amyotrophic lateral sclerosis; ALSFRS-R: Amyotrophic Lateral Sclerosis Functional Rating Scale, revised; ATLIS: Accurate test of limb isometric strength; CAFS: Combined assessment of survival and function; EIM: Electrical impedance myography; HHD: Hand-held dynamometry; MUNE: Motor unit number estimation; VC: Vital capacity.

loss and biomarkers of disease pathophysiology and drug target engagement remain elusive in ALS.

'Impairment', or muscle weakness due to ALS, is the direct consequence of motor neuron loss and therefore a relatively direct surrogate marker of neurodegeneration in ALS. This opens the possibility of measuring muscle strength using precise, quantitative tools in small, efficient ALS trials. Muscle strength is also a relevant outcome measure in larger late-stage trials, since muscle weakness is the primary driver of morbidity and mortality in people with ALS.

'Activity limitation' is now typically measured using the revised ALS Functional Rating Scale (ALSFRS-R) [12], although this has not always been the case. In fact, the trials of riluzole employed a measure of function known as the Norris scale, which has fallen out of favor, as it is less sensitive than the ALSFRS-R [13]. In general, measurement of disability is a less-direct assessment of pathology and requires a larger sample size due to inter-subject heterogeneity. Even more heterogeneous is survival. However, these measures are clinically meaningful and, as such, satisfy this key regulatory requirement for Phase III registration trials.

In general, outcome measures of activity limitation are more robust and have been more widely applied than measures of impairment, although a few well-heeled measures of impairment exist. These widely used, or 'traditional', ALS outcome measures constitute the majority of outcome measures in use today. Few outcome measures related to disease pathology have been used, but the future is bright for this type of outcome measure. Measures of disease pathology are likely to become more central to ALS clinical trials as concepts of ALS as a syndrome with multiple underlying etiologies take hold and novel therapeutics targeting specific underlying pathophysiology for various subsets of disease are developed.

Traditional ALS outcome measures: measures of muscle strength, disability & survival

Phase III trials meant to support the marketing of drugs are strictly regulated, and the FDA and analogous European entity, the EMA, require that Phase III trials employ outcome measures with direct clinical relevance. In ALS, measures of function and/or survival are the mainstay for these trials. These metrics include limb muscle strength, respiratory strength, functional disability (usually measured by the ALSFRS, revised [ALSFRS-R]) and tracheostomy-free survival (Table 2).

Measures of muscle strength

Morbidity and mortality from ALS are driven by the progressive paralysis of skeletal muscles, including bulbar muscles, limb muscles, the diaphragm, and other muscles of ventilation. Therefore, the evaluation of muscle strength is an important measure of disease progression.

Vital capacity (VC) is commonly used to measure the strength of the ventilatory muscles. VC is the maximum amount of air a person can expel from the lungs after a maximum inhalation. When measuring VC, the patient is asked to take as deep a breath as possible, make a tight seal around the device mouthpiece, and then exhale. VC can then be measured as either forced VC (FVC; when exhalation is performed rapidly with maximum effort) or slow VC (SVC; when exhalation is performed slowly). FVC declines with ALS progression, is clinically relevant and related to survival [14–16]. Furthermore, the rate of FVC decline was found to be an independent predictor of survival [16]. Portable, inexpensive, user-friendly devices are now available to quickly assess FVC in the clinic and follow it longitudinally [15]. Therefore, measurement of FVC has been commonly employed in ALS clinical trials [17–19]. However, FVC can be insensitive to early disease progression in some people with ALS who experience FVC decline only as a late feature of the disease. In addition, its measurement is sometimes problematic in patients with significant bulbar weakness because the portable devices require the patient to make a tight seal with pursed lips for accurate measurement. In recent years, there has been a trend in ALS trials to measure SVC rather than FVC as FVC may underestimate the true VC if there is concomitant obstructive lung disease [20]. In addition, many subjects have a tendency to cough during forced exhalation required for FVC, making SVC easier to perform [21].

Other measures related to breathing function include measures of the strength of the diaphragm and other inspiratory muscles such as the maximal inspiratory pressure and the maximal sniff nasal inspiratory pressure. These measures are sometimes used clinically to determine the need for noninvasive ventilation as they are more sensitive than FVC to ALS-related weakness of the muscles of inspiration [22,23]. While maximal inspiratory pressure and sniff nasal inspiratory pressure have been shown to correlate with ALS survival [15,24], they have not replaced FVC in most trials, probably due to FVC's relative ease, availability and reliability.

Measures of limb muscle strength are intuitive and relevant. Furthermore, muscle strength has been shown to be a valid measure of disease progression in ALS [25], although its decline over the course of the disease is not completely linear. Deviations from linearity are most notable in early and late stages of the disease [16,26].

Table 2. Commonly used outcome measures in amyotrophic lateral sclerosis clinical trials.

Level of assessment	Outcome measure	Description	Benefits	Drawbacks
Impairment (loss of strength)	FVC	Quantitative means of assessing reduction in the strength of the diaphragm and of the other muscles of ventilation	Declines with ALS progression, clinically relevant, related to survival and easily followed in clinic	Site of onset affects timing of FVC decline, concurrent bulbar weakness causes inaccuracy in measurement
Impairment (loss of strength)	HHD	Quantitative means of assessing reduction in limb muscle strength	Clinically relevant, objectively measured, portable and reproducible	Requires rigorous training, relies upon examiner strength and patient effort
Activity limitation (disability)	ALSFRS-R	Provides a snapshot of functional status by assessing ALS-related disability in the domain of gross and fine motor tasks, bulbar function and respiratory status	Widely accepted, reproducible and easily administered	Subjective, affected by symptomatic treatment, statistical handling can be complicated
Survival	Survival	Percentage of trial participants surviving without the need for tracheostomy or permanent noninvasive ventilation	Clinically relevant, evaluates the true effect of the therapeutic intervention	Increases trial duration and cost, no information about disability or quality of life

ALS: Amyotrophic lateral sclerosis; ALSFRS-R: Amyotrophic Lateral Sclerosis Functional Rating Scale, revised; FVC: Forced vital capacity; HHD: Hand-held dynamometry.

Measures of muscle strength are routinely included as outcome measures in ALS clinical trials through one of several available methods [17–19,27,28]. Manual muscle testing using clinical scales such as the Medical Research Council scale has been employed in multiple trials, including the seminal riluzole study [27]. A limitation of manual muscle testing, however, is that it is poorly sensitive [29]. The Medical Research Council is a five-grade scale, but grades 4 and 5 occupy the majority of the expected strength on the scale. This may lead to underestimation of treatment effect, particularly when analyzing small cohorts of patients over a short time period [29]. In addition, extensive training is required to ensure inter-rater reliability. Maximal voluntary isometric contraction is a quantitative measure of limb strength producing more reliable interval data [18,19]. Multiple devices are available to measure maximal voluntary isometric contraction. Most recent ALS clinical trials have employed hand-held dynamometry (HHD) as it is an objective, reproducible, inexpensive and easily performed measure [30], although it is subject to patient effort, floor and ceiling effects. HHD relies on the ability of the evaluator to overcome the patient's strength to perform a valid measurement. This can limit its sensitivity when strong muscles are tested (ceiling effect) [31,32]. On the other hand, if the patient is too weak to move against gravity, some muscles cannot be tested using HHD (floor effect). Finally, extensive outcome training is required to ensure intra- and inter-rater reliability, particularly when HHD is used in multicenter studies [33]. Regardless of the method used, quantitative muscle strength is limited by its reliance on subject cooperation and effort.

ALSFRS-R

The ALSFRS is the most widely accepted outcome measure of activity limitation for people with ALS. The original ALSFRS was developed and validated in the 1990s by the Ciliary Neurotrophic Factor Study Group and rapidly gained acceptance [34].

In the original ALSFRS, gross motor, fine motor and bulbar abilities were evaluated by three questions each, and breathing ability by one question, for a total number of ten questions. Each of the questions was rated on a 0–4 ordinal scale (0 indicates absence of function or severe impairment and 4 indicates normal function). Thus, the maximum score in the original ALSFRS was 40 [34]. The ALSFRS was revised in 1999 to more fully capture disability from ventilatory impairment. The ALSFRS-R contains a total of 12 questions, has a maximum score of 48, and allows a more complete assessment of breathing function. The ALSFRS-R assesses gross motor tasks (turning in bed, walking and climbing stairs), fine motor tasks (cutting food, handwriting and dressing/hygiene), bulbar function (speech, swallowing and salivation), and breathing function (dyspnea, orthopnea and need for ventilatory support) [12,34].

The ALSFRS-R [12], is now commonly employed in Phase II and III ALS trials [17,28,35–38]. The ALSFRS-R captures many clinically relevant features of disease progression, is reproducible, and is validated for administration in person or by phone [39]. It has high inter-rater and intra-rater reliability [39]. Importantly, it has been shown to predict survival in ALS [16,40,41].

Despite all the benefits of the ALSFRS-R, it remains a subjective score. Some questions focus on symptoms that can respond to symptomatic therapy (e.g., anticholinergic therapy for sialorrhea). In addition, it does not always decline in a linear fashion [26] and can have uncertain clinical meaning. A recent survey suggested that most ALS physicians consider a drop of two points in the ALSFRS-R to be clinically meaningful [42]. However, the generalizability of this observation is unclear as the scale is ordinal and points on the scale are not equidistant. Additionally, if a study participant drops out or dies during a trial, no further ALSFRS-R scores are available. In this situation, statistical methods, such as random effects models, are required to analyze the subject's data, although which statistical techniques are best is unclear. Finally, the predictive value of the ALSFRS-R as an outcome measure in early-phase trials has come into doubt after a number of recent Phase II trials (based on ALSFRS-R) failed in Phase III trials [36,37,43–45].

Tracheostomy-free survival

Survival may be the most intuitive and clinically meaningful outcome measure – prolonging survival is clearly a major goal for ALS therapies. Riluzole, has a modest survival benefit [3,27,46], but no proven functional benefit, underlining the importance of this outcome measure for ALS trials. Tracheostomy-free survival is defined as death, tracheostomy or permanent noninvasive positive pressure ventilation (NIPPV). Permanent NIPPV is often arbitrarily defined within the context of ALS clinical trials as the use of NIPPV for longer than 22 or 23 h a day [27,46]. This peculiar definition of survival reflects the complexities of end-of-life care for people with ALS, some of whom choose to undergo placement of tracheostomy coupled with mechanical ventilation. This type of intervention can prolong survival by many years [47–50], but does not alter the underlying disease pathophysiology.

The use of tracheostomy-free survival as a primary outcome measure in Phase III clinical trials has additional limitations. First and foremost, given disease heterogeneity and variation in expected disease course, large sample sizes with long follow-up are required for adequate statistical power. Furthermore, survival alone does not provide information about activity limitation or quality of life (QOL), important outcomes for patients. The long follow-up required by trials relying on survival as a primary outcome measure may be a barrier to enrollment and retention, since patients may be reluctant to commit to such long-term trials. These drawbacks are generally outweighed by benefits and have not discouraged the use of survival as a primary outcome measure for Phase III ALS trials. Survival, however, is impractical as an outcome measure for early phase trials. The selection of more efficient outcome measures for Phase II trials remains a challenge and priority.

Combined assessment of survival & function

Both physical function and survival are clinically meaningful, but analysis of either alone might underestimate the treatment effect of a therapy. Recently, the Combined Assessment of Survival and Function (CAFS) was developed to assess patient's outcomes based on both survival time and change in the ALSFRS-R score [51]. CAFS is not itself an outcome measure; it is a novel analysis of the ALSFRS-R and survival together. This method allows analysis of functional outcomes that adjust for mortality and has been used in recent Phase II and Phase III clinical trials [43,52]. Each patient's outcome during a trial is compared with every other patient's outcome and assigned a score. The worst outcome is assigned to the individual who dies first and the best outcome is assigned to the one who survives with the least functional decline. The scores are then ranked and the mean rank score for each treatment group is calculated [51]. A higher mean CAFS score indicates a better group outcome. A drawback of CAFS, however, is that it is a nonparametric rank analysis and therefore changes in CAFS scores cannot be directly compared across trials (Table 3).

Additional measures of disability & function

Outcome measures of spasticity, cognitive ability, mood, behavior changes, fatigue and QOL have been used to supplement primary outcome measures in trials of disease-modifying therapies. They have been used as primary outcomes in trials evaluating options for management of disease-related symptoms, an area of critical need for optimizing treatment of people with ALS [53].

The Modified Ashworth Scale (MAS) is the standard clinical measure of spasticity in multiple neurologic diseases [54,55]. Spasticity is an important ALS symptom, yet treatment options are based mostly on research in other patient populations [53,56]. The main limitation of the MAS is that it is subjective and only a few muscles can be reliably tested. Yet, a small study of a daily exercise program suggested a short-term positive effect on spasticity as measured by MAS [57], and other

Table 3. Novel amyotrophic lateral sclerosis outcome measures.

Level of assessment	Outcome measure	Description	Benefits	Drawbacks
Pathology (motor neuron loss)	MUNE	Many techniques available to estimate the number of remaining motor units	Direct marker of motor neuron loss, sensitive measure of disease progression	May be cumbersome and require rigorous training, must choose which muscles to test, may be painful
Pathology (muscle health)	EIM	Measures muscle electrical properties as a reflection of muscle health	Painless, reproducible, requires little training, evaluates the disease final common pathway, shows promise as a proxy for clinically relevant end points	Muscle health can be affected by multiple factors, questions about specificity remain, must decide which muscles to test, summary scores may not reflect individual muscle scores
Impairment (loss of strength)	ATLIS	Quantitative means of assessing reduction in limb muscle strength	Eliminates need for examiner counterforce, is sensitive across a broad range of muscle strength	As other measures of muscle strength, relies on patient effort
Activity limitation (disability) and survival combined	CAFS	Each patient's outcome is compared with every other patient's outcome and assigned a score. Higher mean CAFS score in a group indicates better outcome	Captures both survival and functional status in a single outcome measure, adjusts functional status for mortality	It is a nonparametric rank analysis and therefore changes in CAFS scores cannot be directly compared across trials

ATLIS: Accurate test of limb isometric strength; CAFS: Combined assessment of function and survival; EIM: Electrical impedance myography; MUNE: Motor unit number estimation.

studies have included this scale as a secondary outcome measure. Thus, having a validated scale has led to exploration of therapies for this symptom. Validation of outcome measures for other ALS-related symptoms could lead to the development of symptomatic therapies as they have for affective lability [58]. Thus, the Center for Neurologic Studies-Lability Scale was instrumental in evaluating the effect of dextromethorphan/quinidine for the treatment of pseudobulbar affect [59,60].

Other ALS-specific scales have been developed to measure specific domains of function when the available scales were recognized to be inaccurate due to the complexity of the disease. The ALS Depression Inventory (ADI-12) is a 12-item scale that has been designed to screen for depression in ALS [61]. This scale was developed because conventional questionnaires to measure depression commonly include assessment of somatic and motor-related symptoms leading to the potential for bias in a population where motor function is affected by the underlying neurodegenerative process. QOL is clearly an important dimension when caring for people with ALS. However, instruments that are commonly available to capture QOL in clinical and research settings rely heavily on assessment of strength and physical function [62]. The ALS-Specific Quality of Life instruments was developed to disentangle QOL from physical function in people with ALS. It relies on many important nonhealth-related factors such as support, existential and spiritual issues [63,64]. Lastly, recent emphasis on the importance of cognitive and behavior dysfunction in ALS [10,65] has been accompanied by the development of several scales to measure these domains [66–68]. Clinical use of these scales, especially the ALS Cognitive Behavioral Screen and the Edinburgh Cognitive and Behavioral ALS Screen, in the USA and Europe, respectively, is gaining momentum [65,66,68]. It is reasonable to anticipate that these scales will be used to detect treatment effect in future trials of therapies targeted to the cognitive and behavioral manifestations of ALS. Additional areas of need include pain, cramping, weight loss, fall risk and bulbar dysfunction, to name a few.

Novel outcome measures in development & validation

Currently, ALS clinical trials require large sample sizes to demonstrate treatment effect, in part because traditional ALS outcome measures focus on activity limitation, and thus include the wide phenotypic variation encountered amongst people with ALS. While these traditional ALS outcome measures are clinically

meaningful and work well for late-stage trials, early-phase trials could gain statistical power by employing outcome measures with reduced intersubject variance. These tend to be measures of impairments, or even of disease pathology. A few of these novel outcome measures are currently in development or validation and include neurophysiologic measures and a new strength testing device (Table 3). Insofar as these measures are more proximate to the underlying ALS pathophysiology, they could reduce variability, boost statistical power and reduce study duration.

Motor unit number estimation

Motor unit number estimation (MUNE) is a measure of remaining motor units and therefore a direct measure of motor neuron loss. The technique is attractive because it is directly related to the underlying pathology. MUNE is based on the ratio of the maximal compound muscle action potential (CMAP), representing the sum of all motor units, divided by the average single motor unit. Routine nerve conduction studies are used to elicit the CMAP. There are, however, multiple methods to record single motor units, which differ among MUNE techniques. Importantly, MUNE has been shown to represent a more sensitive marker of disease progression than measures of strength or disability, suggesting that it may be used as an efficient marker of motor neuron degeneration in early ALS trials [69–71]. However, some MUNE techniques have proven cumbersome and problematic due to poor inter-rater reliability when employed in multicenter trials [72,73]. In addition, many MUNE techniques are time-consuming, may be painful for the subject, and requires rigorous training to maintain acceptable inter-rater reliability. Still, under appropriate circumstances, MUNE might be used detect treatment effects in small Phase II clinical trials.

The motor unit number index (MUNIX) is a quick, simple, and reproducible MUNE technique [74–76]. MUNIX can be used to assess multiple muscles and determine a global average index, a desirable characteristic as ALS often has an asymmetric distribution. Early studies of MUNIX showed that the index is a sensitive marker of motor neuron loss and declines over time with disease progression [77,78]. MUNIX improves on prior MUNE techniques and its potential applications as an ALS outcome measure are currently under investigation.

Neurophysiological index

The neurophysiological index (NI) is a multimetric index that is derived from CMAP amplitude, F-wave frequency and distal motor latency of the ulnar nerve-innervated abductor digiti minimi muscle [79]. Importantly, NI is a sensitive parameter in evaluating progression in ALS and is therefore a promising candidate outcome measure [79–82]. The major advantage of NI is that it is calculated from standard neurophysiological measurements and requires no special equipment. Further studies will clarify whether NI can be utilized as an outcome measure in ALS clinical trials.

Electrical impedance myography

Electrical impedance myography (EIM) is a noninvasive electrophysiologic technique that uses transdermal application of high-frequency, low-intensity electrical stimulation to derive muscle impedance. Muscle impedance changes as muscle health declines. The technique is painless, highly reproducible and requires little training to perform. EIM parameters correlate with survival in both preclinical models [83,84] and ALS patients [85,86]. Importantly, its coefficient of variation compares favorably to those of HHD and ALSFRS-R [85]. However, because overall muscle health can be affected by multiple factors, questions remain about the specificity of EIM for motor neuron dysfunction. In addition, only certain muscles can be tested, and summary scores may misrepresent the disease progression. Studies are ongoing to determine whether EIM can be used as an efficient biomarker in early ALS clinical trials.

Accurate test of limb isometric strength

Accurate test of limb isometric strength (ATLIS) is a portable, user-friendly device for quantitative, objective measurement of strength (The US Patent and Trademark Office awarded The Massachusetts General Hospital Corporation, Boston, MA, a patent for this device in 2009). ATLIS tests 12 muscle groups and produces interval data. Its validity and reliability in ALS have been recently established [87,88]. Importantly, ATLIS does not rely on examiner's strength, a feature that compares favorably to the more widely used HHD. In addition, ATLIS is sensitive to small changes in muscle strength at all stages of the disease, suffering from fewer ceiling or floor effects than HHD because for resistance, it relies on a fixed load cell rather than the strength of the evaluator [87]. The characteristics of ATLIS responsiveness longitudinally as the disease progresses are under investigation [89], and its application to Phase II trials looks promising.

Biomarkers as surrogate end points for early clinical trials

The NIH defines a biomarker as "a characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes or pharmacologic responses to a therapeutic

intervention" [90]. Biomarkers hold the potential to dramatically alter early phase ALS clinical trials (Figure 1). Successful markers of early diagnosis could hasten enrollment. Prognostic markers might allow for enrollment enrichment or even comparison of observed rate of progression to predicted rate, reducing the need for placebos in early trials. Biomarkers that reflect disease progression might be used to monitor response to treatment. Finally, pharmacodynamic biomarkers can be utilized in early trials to demonstrate a drug's ability to affect the pathway of interest.

Diagnostic biomarkers

The development of diagnostic biomarkers may facilitate early enrollment in clinical trials and potentially define pathophysiologic subgroups. Because the average diagnostic delay is approximately 1 year [91,92], trial enrollment generally comes after the neurodegeneration is well underway.

Pathophysiology biomarkers

Pathophysiology biomarkers might reflect underlying cellular dysfunction or abnormal biochemical pathways and define pathophysiologically relevant subgroups of people with ALS. Patients within a given pathophysiologic subgroup might be more likely to benefit from a certain drug based on their specific disease mechanisms (cohort enrichment) [93]. As an example, trials of immunomodulatory treatment may enroll only patients that exhibit specific patterns of inflammatory cell activation [94].

Prognostic biomarkers

The phenotypic variability of ALS is remarkable. Rate of progression is one of the most widely varied, and this heterogeneity greatly diminishes statistical power in trials [95]. New prognostic factors such as BMI and uric acid levels have been recently recognized, adding to a long list of possible prognostic markers [95–97]. Prediction algorithms to determine expected disease progression are being developed [98]. Future trials might gain statistical power by comparing observed disease progression to predicted progression for individual trial participants using prognostic biomarker profiling and predictive algorithms.

Biomarkers of disease progression

More sensitive measures of disease progression, such as more precise measures of strength, are being developed and have the potential to be used as surrogate outcome measures. Validation of more sensitive and precise biomarkers of disease progression will lead to reduced sample size and shorter trial duration in Phase II trials, thus accelerating the path to efficacy trials.

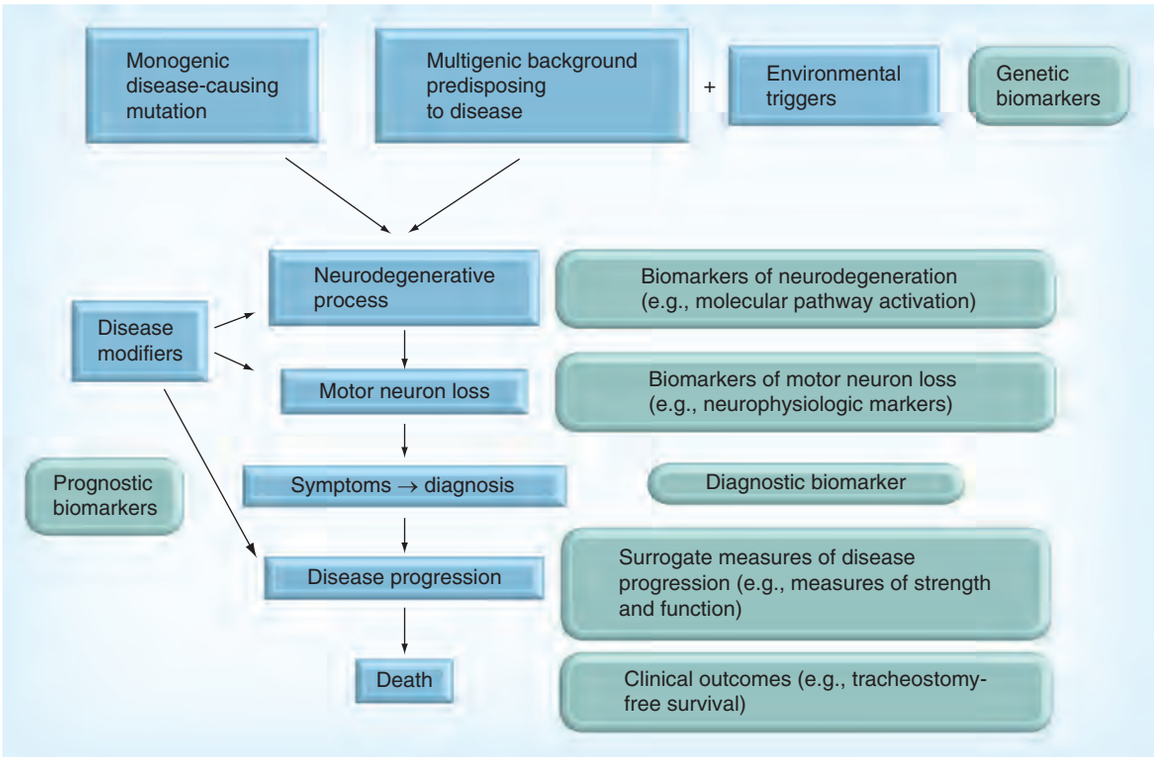

Figure 1. Framework for biomarker development. Biomarkers are urgently needed in amyotrophic lateral sclerosis for early diagnosis, prognostic determination, monitoring of disease progression and response to treatment.

Pharmacodynamic biomarkers

Already in Phase II ALS trials, pharmacodynamic biomarkers are being used to test whether the compound under investigation is biologically active at the motor neurons and may help identify the dosage range required to achieve this effect [99]. Pharmacodynamic markers confirm that drug is engaging its target and thus that the biological question has been appropriately tested in the early trial. Historically, most ALS trials have not included measures of biological activity of the investigational compound. It is possible that prior Phase III trials may have failed because the dosages or routes of administration tested did not allow the compound to reach, and engage, its therapeutic target(s).

Development of biomarkers for use in early-phase ALS trials could speed ALS drug development and increase the likelihood of Phase III trial successes. In the ideal Phase II trial, candidate therapy is designed to modify known pathways involved in ALS pathogenesis, likely in a subgroup of patients at a certain disease stage(s). Such a trial would include pharmacokinetic and pharmacodynamic biomarkers and robust outcomes of disease progression. Recent progress in genetics, preclinical models, and biomarker discovery suggest that these goals are attainable, although further research is urgently needed to make these ideal scenarios a reality.

Among the most promising biomarkers, the development of genetic, imaging and CSF biomarkers deserves special attention.

Recent genetic discoveries have changed our understanding of ALS and have provided novel clues about the underlying pathophysiology (reviewed in [100]). Genetic markers already act as diagnostic biomarkers, facilitating earlier and more certain diagnosis. Disease-modifying genes, such as *EPHA4*, might soon begin to act as predictive biomarkers [101]. Finally, genetically determined subgroups of patients can allow targeting of specific pathophysiologies, likely in the form of gene silencing or modification. A recent Phase I trial of antisense oligonucleotide delivery for patients carrying the *SOD1* mutation is a notable example [102]. Efforts are underway to utilize similar paradigms to target C9orf72 hexanucleotide repeat expansions.

Neuroimaging is another active area of ALS research [103]. Clinically, MRI is used to exclude disease mimics. Newer, advanced MRI techniques, however, are being investigated as a source of biomarkers for ALS. These techniques include diffusion tensor imaging, functional MRI and magnetic resonance spectroscopy (MRS). Additional promising imaging includes nuclear medicine methods such as positron emission tomography. These emerging neuroimaging techniques allow evaluation of alterations in neuronal networks and in the chemistry, metabolism and receptor distribution in the brain [103]. As disease biomarkers, they may allow early diagnosis, phenotypic characterization, monitoring of disease progression, and, potentially, detection of response to treatment.

Diffusion tensor imaging is, perhaps, the best characterized novel MRI technique and has already proven useful in characterizing features of specific ALS genotypes [104], presymptomatic abnormalities in people at risk for developing ALS [105], and spread along functional connections [106,107]. Positron emission tomography studies have begun to provide information about ALS pathophysiology such as inflammatory [108] and metabolic [109,110] abnormalities in well-defined patient subgroups. These promising techniques should be applied in early, proof-of-concept trials targeting specific disease mechanisms. These studies may identify cohorts of responders and pave the way for cohort-enriched efficacy trials.

Finally, the search for ALS-specific biochemical markers in either the blood or the CSF is ongoing [111]. The identification of a reliable diagnostic ALS test in a biofluid could revolutionize ALS diagnosis and trial enrollment [112,113]. At the same time, a biofluid marker that changes with disease progression or predicts progression could be even more useful for ALS trials and therapy development. While no single biochemical marker has been established, many have been proposed. Among the most promising molecules, the phosphorylated neurofilament heavy chain was recently shown to be able to reproducibly differentiate between ALS and control cases [114]. Multicenter, prospective efforts are now being conducted to determine which candidate biomarker can be translated into the clinical setting [111,115]. Because of the potential implications of biomarkers in early stage ALS trials, these studies are of critical importance.

Conclusion & future perspective

The last decade has seen tremendous advances in our understanding of ALS genetics and pathophysiology and, at the same time, has opened the door to novel avenues of research to address the many unanswered questions that surround this disease [10,116]. In parallel, an extremely collaborative research community has developed robust infrastructure resources and shared metrics to optimize ALS clinical research, as well as trial design and implementation [93,111,117–119]. The combination of preclinical breakthroughs, translational advances and solid infrastructure is likely to bring about major developments in the field of ALS over the next few years. Given the critical importance of biomarkers as outcome measures for Phase II trials, the development of such biomarkers should be a major effort in the coming years. A major, coordinated,

international collaboration that includes industry, academia and patient-advocacy organizations holds the potential to develop viable biomarkers for use in Phase II trials. Optimization of outcome measures and routine incorporation of biomarker measurement into future clinical trials will be critical steps forward and will dramatically improve the chances of success in these endeavors.

Author contribution

S Paganoni: study concept/design; analysis/interpretation of data; drafting/revising the manuscript; M Cudkowicz: study concept/design; analysis/interpretation of data; drafting/revising the manuscript; and JD Berry: study concept/design; analysis/interpretation of data; drafting/revising the manuscript.

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Executive summary

- Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease characterized by loss of motor neurons and progressive weakness. Survival averages 3–5 years after symptom onset.
- Riluzole, the only US FDA-approved disease-modifying agent, has a modest effect.

Challenges in ALS clinical trials

- Disease rarity and heterogeneity, rapid course, lack of knowledge of pathogenesis, and absence of biomarkers are challenges for ALS clinical trialists.
- ALS clinical trials have traditionally relied on outcome measures of muscle strength, function and survival. These outcomes are needed to measure clinically meaningful effects in Phase III trials. However, their use in early-phase drug development is problematic due to the need to employ large cohorts and follow them for a prolonged period of time in order to detect small treatment effects.

Recent innovations & new directions in ALS clinical research

- Biomarker discovery efforts have been stepped up in an effort to identify reliable markers of drug target engagement for use in early proof-of-concept clinical trials.
- Novel surrogate measures of neurodegeneration are being developed. The goal is to use these markers in Phase II studies to help select the most promising compounds to bring into Phase III testing.

Future perspective

- Expansion of knowledge about disease genetics and pathophysiology, incorporation of biomarker measurement into the drug development process, and optimization of outcome measures will create opportunities to better investigate potential therapies for ALS.

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