Review

Translating preclinical insights into effective human trials in ALS

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Abstract

Amyotrophic lateral sclerosis (ALS) is a rapidly progressive, adult-onset neurodegenerative disease characterized by selective dysfunction and death of motor neurons in the brain and spinal cord. The disease is typically fatal within 3–5 years of symptom onset. There is no known cure and only riluzole, which was approved by the FDA in 1996 for treatment of ALS, has shown some efficacy in humans. Preclinical insights from model systems continue to furnish ample therapeutic targets, however, translation into effective therapies for humans remains challenging. We present an overview of clinical trial methodology for ALS, including a summary rationale for target selection and challenges to ALS clinical research. © 2006 Elsevier B.V. All rights reserved.

Keywords: Amyotrophic lateral sclerosis; Clinical trial; Preclinical model; Drug evaluation

1. Introduction

Because ALS is a uniformly fatal disease for which no adequate treatment exists, there is a pressing need to develop effective therapies. Preclinical research aimed at elucidating mechanisms of disease in large part specifies the kind of therapies that may be effective against motor neuron demise, and increasingly identifies specific candidate compounds for testing. Taking a drug from “bench-to-bedside” is an enormously costly and complex process. Here, we present an overview of important features in the discovery, development, and validation of disease-modifying therapies and interventions for ALS.

2. Rationale for target selection

2.1. Disease pathogenesis

The pathologic hallmark of ALS is the selective loss of motor neurons in the cortex, brainstem, and spinal cord. Determining molecular and cellular mechanisms that contextually favor motor neuron death in ALS is the starting point in identifying neuroprotective strategies. The pathogenesis of ALS is not completely understood and while much controversy remains about its molecular and biochemical biology, there is increasing consensus that multiple mechanisms of injury converge as the disease progresses and that caspase activation is a final pathway of cell death [1–3].

Important early insights into ALS pathogenesis resulted from a Paracelsian approach, in a phrase, that used the “patient as textbook” to learn about the disease. Studies in serum, cerebrospinal fluid (CSF), and post-mortem tissue implicated four principle mechanisms of injury: oxidative stress, excitotoxicity, axonal transport defects and protein mishandling. These pathways have been extensively explored in the context of cellular and/or animal systems used both to model disease and as major platforms for drug discovery. These model systems have provided further insights about potential contributors to disease including mitochondrial dysfunction, apoptosis, and inflammation.

2.2. Rationale for oxidative stress hypothesis

The hypothesis that oxidative damage is central to ALS was prompted by the discovery that mutations in a key cellular antioxidant, superoxide dismutase (SOD1), can cause ALS. While the majority of ALS cases are sporadic, about 10% of cases are inherited as an autosomal dominant trait and of these, 25% arise due to mutations in the gene encoding SOD1 [4].

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More than 100 mutations have been described to date [5] (for an updated list see the online database at www.alsod.org). Transgenic mice bearing point mutations found in humans, including the first and most widely studied SOD1G93A mutant [6], develop progressive motor neuron disease with clinical and pathologic features of the human disease [6–9]. The primary biochemical function of SOD1 is to convert superoxide, a toxic oxygen species generated by mitochondrial respiration, into hydrogen peroxide and water. While oxidative damage is an attractive proposal for the toxicity of mutant SOD1, it has been difficult to establish in experimental paradigms. SOD1 null mutant mice do not develop disease [10], and the level of enzyme activity is unchanged or elevated in mutant models that do develop disease [9], suggesting that diminished SOD1 activity does not cause motor neuron disease. Toxic copper-mediated chemistries involving aberrant substrates for mutant SOD1, including peroxynitrate [11,12] and hydrogen peroxide [13], have been proposed. Altering copper-loading onto SOD1 [14,15] or peroxynitrate precursors levels by manipulating nitric-oxide synthetase [16,17], however, had no effect on toxicity, suggesting that these aberrant reactions are not likely to have a major role in SOD-mediated disease.

Whether the primary toxicity of SOD1 mutants is oxidative remains uncertain. Some studies suggest that the toxicity of mutant SOD1 may relate to secondary effects of protein aggregates, including disruption of apoptotic regulatory mechanisms within cells (see below for discussion). Certain evidence, though, implicates oxidative injury in the pathogenesis of ALS. Studies of serum, CSF, and human post-mortem tissue have found increased markers of oxidative damage [18–21]. 8-hydroxydeoxyguanosine (8-OHDG), a marker of oxidative damage to DNA, is present in the blood and CSF of ALS patients and increases significantly with disease progression [22]. Clinical efforts to address oxidative damage currently include an early phase trial of the manganese porphyrin AEOL10150, which acts as a free-radical scavenger [103] (Table 1 lists all ongoing human trials).

The timing of oxidative stress in the disease process and the relevant cell type(s) responsible for elaborating reactive species are not known. There is biochemical and pathologic evidence, however, favoring involvement of this pathway in motor neuron dysfunction, and a variety of anti-oxidant compounds already exist. Thus, modulating oxidative stress remains a reasonably promising approach to neuroprotection and development of drugs to slow the progression of ALS.

### Table 1

Current Clinical Trials in ALS

<table>
<thead>
<tr>
<th>Compound</th>
<th>Mechanism</th>
<th>Clinical stage</th>
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<tbody>
<tr>
<td>Ceftriaxone</td>
<td>Anti-excitotoxic</td>
<td>Phase I-III</td>
</tr>
<tr>
<td>Minocycline</td>
<td>Anti-inflammatory, anti-apoptotic</td>
<td>Phase III</td>
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<tr>
<td>AEOL10150</td>
<td>Anti-oxidant</td>
<td>Phase I</td>
</tr>
<tr>
<td>Arimoclomol</td>
<td>Protein misfolding</td>
<td>Phase II</td>
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<td>High-Dose Coenzyme Q</td>
<td>Mitochondrial dysfunction</td>
<td>Phase II</td>
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<tr>
<td>Sodium phenylbutyrate</td>
<td>Histone deacetylase inhibitor</td>
<td>Phase II</td>
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<td>Ritonavir and hydroxyurea</td>
<td>Anti-apoptotic</td>
<td>Phase II</td>
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<tr>
<td>Thalidomide</td>
<td>Anti-apoptotic</td>
<td>Phase II</td>
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</table>

2.3. Rationale for glutamate hypothesis

The neurotransmitter glutamate can induce excitotoxic injury by accumulating at the synapse and causing repetitive depolarizations, cellular edema from sodium influx, and loss of calcium homeostasis in the post-synaptic neuron [23,24]. Compelling evidence for glutamate-mediated injury to motor neurons in ALS is the ability of riluzole to prolong survival in human trials [25,26]. Riluzole is thought to inhibit release of glutamic acid and act at the calcium-permeable NMDA receptor and voltage-gated sodium channels to attenuate cellular responses to glutamate. Defective glutamate handling in ALS was initially suspected based on markedly elevated levels of glutamate in the CSF of affected individuals [27–29] and the finding of impaired glutamate transport associated with loss of the EAAT2 transporter [30]. Five subtypes of glutamate transporters have been identified, but the astrocytic EAAT2 transporter buffers ~90% of the extracellular glutamate surrounding motor neurons [31,32]. Loss of astrocytic EAAT2 protein has been found in 60% of patients with sporadic ALS [31]. A mutation in the EAAT2 coding region detected in one patient with sporadic ALS was found to alter membrane targeting for the protein and impair glutamate uptake [33]. Interestingly, SOD1 mutants are associated with functional loss of EAAT2 in mice [34]. Because motor neurons possess a relative lack of calcium buffering proteins [35], and the configuration of the AMPA receptor on these cells confers increased permeability to calcium [36], motor neurons may be especially vulnerable to excitotoxic injury. Thus, targeting glutamate-mediated injury is a uniquely promising approach to drug development for ALS. Recently, Rothstein and colleagues performed an elegant screen of FDA-approved drugs in vitro to identify compounds that could up-regulate EAAT2 expression [37]. Many β-lactam antibiotics turned out to be potent inducers of EAAT2 expression, however, ceftriaxone showed the added benefit of increasing brain expression and activity of EAAT2 in animals and demonstrated a clear neuroprotective effect in both in vitro and vivo models of motor neuron disease. Whether ceftriaxone confers the same benefit in humans will be determined in an upcoming clinical trial in patients with ALS. Another approach would be to concentrate efforts on the sole drug that has proven efficacy against ALS. A better understanding of the drug–receptor interactions that mediate riluzole’s beneficial effect may bring to light rational modifications to its structure that could improve efficacy. In all events, since a mechanism of injury can be described from a ligand–receptor interaction at the cell surface through multiple well-described downstream signaling cascades, the glutamate injury pathway offers a promising array of potential targets for therapeutic intervention.

2.4. Rationale for axonal dysfunction hypothesis

A pathologic hallmark of ALS is the accumulation of neurofilaments, abundant cytoskeletal components, in the soma and proximal axons of motor neurons [38]. In both human ALS and SOD1 transgenic animals, the largest caliber, most
neurofilament-rich motor neurons are lost [39]. Approximately 1% of sporadic ALS cases have small deletions or insertions in the repetitive tail domain of the neurofilament heavy gene [40]. Moreover, transgenic mice bearing mutant neurofilament light gene display axonal disorganization and selective motor neuron death [41–43]. Manipulating expression levels of neurofilament subunits has a robust effect on disease in the SOD1 mouse [44–46], suggesting that neurofilament organization could be a factor in the selective vulnerability of large-caliber motor neurons. This finding also hints at the therapeutic potential of modulating neurofilament content and/or organization to slow the disease. The sensitivity of motor neurons to altered axonal dynamics is underscored by mutations in kinesins [47], peripherin [48], and dynactin [49] that cause motor neuron degeneration. Although these findings are suggestive, no clear mechanism of injury or therapeutic approaches have emerged from preclinical studies yet.

2.5. Rationale for protein mishandling hypothesis

Protein aggregates form in several neurodegenerative diseases but there is debate over whether they are causative, harmless epiphenomena, or potentially beneficial sinks to sequester toxic abnormal proteins in disease states. Ubiquinated protein aggregates are found in upper and lower motor neurons in sporadic as well as familial forms of ALS [50,51]. In transgenic models, SOD1-immunoreactive inclusions accumulate in motor neurons and astrocytes before the onset of motor dysfunction [52]. These aggregates also include neurofilaments, glial fibrillary acidic protein, two neuronal glutamate transporters, bcl-2, and proteins involved in chaperone and proteosome functions [53,54], potentially linking these aggregates to a number of pathogenetic mechanisms including axonal dysregulation, glutamate mishandling, apoptosis, and impaired folding of functional proteins essential to motor neurons and/or clearance of potentially dysfunctional ubiquitin-tagged proteins. In addition, aggregations within mitochondria may contribute to impaired cellular energetics and potentially set off disease-initiating damage within motor neurons [55,56]. The toxicity of aggregates is suggested by the finding that over-expression of heat-shock proteins, molecular chaperones, can reduce mutant SOD1 aggregation and enhance the survival of cultured motor neurons [57–59]. Induction of heat-shock proteins by arimoclomol increased the life span of SOD1G93A mice by 22% [60], prompting the current clinical Phase II trial in humans (www.clinicaltrial.gov). Up-regulating the expression of individual or combinations of heat-shock proteins and their co-factors may be a valid neuroprotective strategy for motor neuron disease, especially in light of the characteristically high threshold motor neurons have for mounting a heat shock response to stress [61].

2.6. Rationale for mitochondrial dysfunction hypothesis

Evidence that mitochondria may participate in ALS comes chiefly from model systems. Neuropathologic studies of motor neurons in SOD1 transgenic mice reveal mitochondrial vacuolation [9,62] prior to cell loss [63]. SOD1 accumulates in vacuolated mitochondria [64]. Mitochondrial proteins and lipids display oxidative damage and respiratory chain complexes show reduced activity and ATP synthesis [65]. Apoptotic regulation within motor neurons may be disrupted due to release of mitochondrial cytochrome C into the cytosol [1,66], or trapping bcl-2 within mitochondrial SOD1 aggregates [53]. Expression of mutant SOD1 in the NSC34 motor neuronal cell line results in swollen mitochondria, impaired activity of respiratory chain complexes II and IV, diminished ATP production, and protein alterations [67,68], strengthening the impression that respiratory defects could be cell-autonomous in vivo. Observations in sporadic ALS support a role for mitochondrial dysfunction, including altered mitochondrial morphology in liver [69,70] and muscle [71]; a high frequency of mitochondrial DNA mutations in cortical motor neurons [72]; multiple mutations and decreased mitochondrial DNA in muscle and spinal cord [73], and reduced complex IV activity in spinal motor neurons [74]. Mitochondrial DNA mutations, accumulated over time in affected tissues, have been postulated to play in role in the pathogenesis of ALS, however, evidence diverges over whether these mutations in fact impair mitochondrial respiration [75,76]. Taken together, the evidence implicating mitochondrial dysfunction in ALS is indirect. It is important to stress, however, that mitochondria sit at the nexus of several cell processes that may be relevant to motor neuron disease, including cytochrome C-mediated apoptosis, impaired cell bioenergetics, and the production of reactive oxygen species. Thus, compounds with mitochondrial effects have the potential to be rewarding targets for therapeutic development.

2.7. Rationale for apoptotic hypothesis

An increasing body of evidence suggests that the final cell death pathway for motor neurons in ALS is apoptotic. Studies of human post-mortem tissue demonstrate TUNEL-positive degenerating motor neurons [77,78]; increased levels of apoptosis related molecules [79]; significantly increased levels of caspase 1 and 3 activity in spinal cord [77], and altered balance between pro- and anti-apoptotic regulatory proteins favoring apoptosis [80]. Cellular disease models suggest that motor neurons expressing mutant SOD1 are more likely to undergo apoptosis under conditions of oxidative stress [81]. In the mutant SOD1 mouse model, caspase 1 and caspase 3 expression is increased in the spinal cord of symptomatic mice, and the balance of bcl2 protein family members shifts in a direction favoring apoptosis [82,83]. The therapeutic potential of apoptotic inhibitors is underscored by neuroprotective effects of genetic manipulations in SOD1 transgenic mice to over-express anti-apoptotic molecules [84], exposure to caspase inhibitors in cellular models [85], and intraventricular administration of a broad spectrum caspase inhibitor to mutant SOD1 mice which demonstrated that chronic caspase inhibition can significantly prolong survival [82]. Pure caspase inhibitors have not been clinically assayed. Compounds that attenuate cell death through other means potentially include minocycline, which has been show to inhibit release of mitochondrial...
cytochrome C and inhibit cell death in vitro and in vivo [66], and ritonavir, which abolishes apoptotic response to oxidative stress in neurons [86]. Minocycline and ritonavir are currently being evaluated in clinical studies with ALS patients.

2.8. Rationale for inflammatory hypothesis

The presence of activated microglia is a histological feature of ALS and SOD1-mediated murine disease [87,88]. In mice, microglial activation precedes clinical weakness and motor neuron loss [89], and chronic stimulation of innate CNS immunity exacerbates motor neuron disease in mice [90]. Activated microglia elaborate several potentially neurotoxic molecules, including reactive oxygen and nitrogen species, proteases, and pro-inflammatory cytokines that amplify inflammation. The profile of inflammatory cytokines and enzymes up-regulated in the spinal cord or CSF of ALS patients (IL-6, IL-1β, cyclo-oxygenase 2 (COX2), and prostaglandin E2 (PGE2)) [91–93] and in the spinal cord of mutant SOD1 mice (IL-1β, TNFa, COX2, PGE2) [94] tends to implicate microglial involvement. Microglia conditioned by CSF from ALS patients act to increase the vulnerability of cultured neurons to glutamate toxicity, an effect blocked by minocycline [95]. Further, minocycline, an inhibitor of microglial activation, slows disease progression in mutant SOD1 mice [96–99]. Non-neuronal cells, including microglia, may be especially relevant to SOD1-mediated disease. Restricted neuronal expression of mutant SOD1 does not produce motor neuron degeneration in animals [100,101]. In chimeric mice, motor neurons expressing mutant SOD1 are unaffected if surrounded by normal non-neuronal cells, whereas normal motor neurons surrounded by non-neuronal cells expressing mutant protein developed ubiquitinated intraneuronal deposits, a feature typical of diseased neurons [102]. Preclinical studies of inflammatory mediators in motor neuron degeneration have furnished a number of cellular and molecular targets. Current clinical initiatives to address inflammatory aspects of ALS include the glutamic acid derivative thalidomide, which reduces the synthesis of TNF-α and minocycline. Whether immunomodulation has a beneficial effect on human disease remains to be seen.


In vitro systems have refined our insight into disease mechanisms and provided useful platforms to identify potential therapeutic approaches. These include purified primary cultures of motor neurons from embryonic rat [105], co-culture systems of motor neurons from embryonic mouse plated on a glial feeder layer of cortical astrocytes [106], and organotypic spinal cord slices from post-natal rats [31,107]. Additionally, cell lines expressing mutant SOD1 have been developed with motor-neuron like cells (NSC34) [108] and HeLa cells [109]. Prior to 1995, some 50 drugs and trophic factors were evaluated in culture systems [110], furnishing preclinical data for a number of investigational new drug trials. Currently, an expanding range of receptor and signaling targets together with the development of combinatorial chemistry, compound libraries, and high-throughput screening techniques stand to increase the number of potential drugs exponentially-far beyond the range of what can actually be tested. Thus libraries to be tested in high-throughput screening assays will have to be rationally designed in order to yield practical results. An initial cost-efficient approach is to screen compounds that are already FDA-approved for other indications. The Neurodegeneration Drug Screening Consortium, for example, tested 1040 FDA-approved drugs in cell-based models of neurodegeneration [111], an effort which established groundwork for the pending clinical trial of certriaxone in ALS. Other kinds of compound libraries can be constructed, based on any number of desired properties-CNS-penetration, for example. Multiple-well microtiter formats for automated high throughput screens can be designed to detect changes in target protein levels by chemiluminescent reporters, like luciferase. Lower throughput manual assays may permit greater complexity of experimental design, including more sensitive quantitation of cell death. Potential limitations of in vitro models may include biochemical or structural differences between adult and embryonic cell types, for example if disease-relevant proteins or their post-translational modification are developmentally regulated. Additionally, all culture systems with the possible exception of spinal cord explants do not replicate the metabolic interactions likely to be operating in vivo. This is especially relevant to cell death since it is increasingly apparent that microglia, motor neurons, astrocytes, and endothelial cells likely form a metabolic unit that contributes to the demise of motor neurons in disease states [112]. Overall, the power of in vitro models to investigate disease mechanisms and search for promising therapies far outweighs potential limitations.

4. Strengths and weaknesses of animal model for drug discovery

Several experimentally induced mutations – G93A, G37R and G85R – have been developed in transgenic mouse models, however, the SOD1G93A mutant is primarily used for therapeutic research. The SOD1G93A mutant generally recapitulates human disease, including cardinal findings of motor neuron disease. The major weakness of animal models for ALS thus far has been the de facto failure of these models to predict response in humans. Riluzole, first demonstrated to be effective in the SOD1G93A mouse model. As experience as shown, the converse does not always hold and a number of compounds found to be effective in this model failed in humans, including vitamin E [113], gabapentin [114], topiramate [115], celecoxib [116], and creatine [117]. The reasons for discordant results between mouse and human trials are not fully understood but may relate to inherent differences between the mouse and human disease. For example, the vacuolar degeneration seen in SOD1 models is not a feature of human disease. Unlike ALS patients, transgenic mice over-express mutant protein 5- to 15-fold, and life span
can vary with level of protein expression [118]. Additionally, species-specific differences may be important, such as differences in microglial activation. Comparability of pharmacokinetics, routes of delivery, timing of treatment, and relevance of the familial disease model to sporadic disease are all issues of potential importance for interpreting and applying data from mouse studies to humans. Despite its track record of poor predictive ability, the transgenic mouse model remains a critically important tool both to unravel the complex stages of motor neuron disease, and to pursue new therapeutic approaches.

5. Translation to humans

5.1. Preclinical toxicology

The FDA currently requires toxicology studies in animals for any investigational new drug application to support the safety of a new compound for use in humans (see Content and Format of Investigational New Drug Applications (INDs) for Phase I Studies of Drugs Including Well Characterized, Therapeutic, Biotechnology-Derived Products). Information about toxicities and toxicokinetics in animals guides subsequent trial design, for example by determining criteria to exclude patients who would be at high risk for developing anticipated toxicity and incorporating appropriate monitoring. The cost of acquiring these data can mount to $1,000,000 or more, making these studies prohibitive for any but the most promising compounds. Thus, the number of compounds that can ever be brought to this stage of development is limited by the availability of resources.

5.2. Phase I

Phase I trials introduce a new investigation compound into humans. Typically these are small trials (less than 30 subjects), designed to assess safety, dose-ranges, and pharmacokinetics. Starting dose selection is a critical issue in translating preclinical findings into human trials. Factors that may affect optimal dose determination relate to a compound’s pharmacokinetics and bioavailability, which may vary between species or with route of administration (e.g., oral versus intraperitoneal). Interactions with other commonly encountered medications in humans may also affect metabolism and thus dose selection.

Animal data tend to be extrapolated to estimate a starting dose with some minimal activity that also has minimal risk of side effects. Human Equivalent Dose (HED) calculations, an algorithm to determine the maximum recommended starting dose, are normally based on body surface area conversions mg/m². Other parameters for drug extrapolation are just as appropriate and, in specific instances where there are similar mg/kg doses across species, the HED rules stipulate that body weight may be used. The calculated dose can change dramatically depending on which parameter is used. For example, the most effective neuroprotective dose of creatine in the SOD1G93A mouse studies was 2% of the diet [119], corresponding to 30–35 g/d in ALS patients weighing 70 kg. Subsequent negative trials of creatine in humans [117,120], however, started at doses of 5 and 10 gm/kg based on body surface area conversions, legitimately raising the question of whether these studies may have been under dosed.

Ideally, several different doses would be selected for evaluation in sequential dose-escalation cohorts with careful safety monitoring during inter-cohort intervals, or alternately by randomization of subjects by dose. Investigating a single dose runs the risk of missing adverse events that could surface in later trials, as well as the more remote risk of missing large effects. Small phase I dose-ranging trials in “typical” ALS patient cohorts (heterogeneous, on riluzole, etc.) may provide the most useful set of data for planning subsequent clinical trials, however, dose-ranging trials are often performed in healthy controls rather than in the target study population to assess subacute toxicity and pharmacokinetic parameters.

5.3. Phase II trial design

Phase II studies are designed to assess long term safety and refine pharmacokinetic data, determine optimal dose, and gather preliminary data on efficacy. Designing good phase II studies in ALS is challenging due to inherent variability between subjects that can obscure small treatment effects, the relative insensitivity of current outcome measures, and the fact that the length of treatment needed to observe even preliminary efficacy is not known in advance. Traditional phase II trials typically require 100–150 subjects, usually randomized and placebo-controlled, studied over 9–12 months. Because phase II trials are quite resource intensive, several innovations meant to increase the efficiency of these trials are being actively explored by a number of research communities, including proof-of-concept testing, lead-in periods, futility designs and statistical modifications that attempt to minimize the chances of missing an effective therapy.

By virtue of its lethality and rapid progression, ALS is appropriate for proof-of-concept testing of potential therapeutics, where a drug is administered for a short period and effects on measurable markers of disease are sought. What constitutes a reasonable length of treatment, though, can be difficult to decide in advance. Additionally, the inherent variability between subjects may obscure a smaller response to disease-modifying therapy especially in the short run, so that these trials still require large numbers of subjects and a control group. Finally, identifying and validating markers of neurodegeneration that vary with disease progression and disease-modifying treatment are necessary first steps to instituting proof-of-concept trials. While no validated surrogate markers exist for ALS, promising work in MR-spectroscopy, neurophysiologic data, and proteomics and metabolomics suggest that appropriate markers may be on the horizon (see below).

Lacking valid surrogate markers, certain functional measures are employed as study endpoints and have proven to be quite useful for ALS clinical trials. Newer trial designs that incorporate a lead-in period off-drug may improve the accuracy of functional measures. Lead-in design is well-suited to outcome measures that have linear characteristics and correlate with disease outcome, such as the ALS Functional Rating Scale (ALSFRS) or forced vital capacity (FVC) [121,122]. Observing
the behavior of a given measure during a lead-in period can allow slope estimation for individual subjects, thus facilitating detection of a treatment response. Because lead-in studies are more efficient at detecting treatment effects, smaller samples sizes can be used.

In cancer drug development, open-label, single-arm phase II trials are used to assess safety and determine whether an agent shows sufficient efficacy to warrant a full phase III evaluation. An assessment of no effect, or no “worthwhile” effect, of a given compound essentially demonstrates the futility of proceeding with further clinical testing. In futility analyses it is important to minimize Type I errors, i.e., false negatives, in order to minimize the risk of missing a potentially effective therapy. Multi-arm futility designs evaluating several different therapies may speed up the process of identifying “minimally worthwhile” compounds for further study, thus potentially compressing the research and patient time taken up by compounds that do not work.

Another way to minimize the risk of missing a potentially good drug is to modify the P value conventionally used for statistical significance [123]. Setting a standard two-sided P value at 0.05 effectively minimizes the risk of taking a truly ineffective drug forward to about a 1 in 40 chance. Alternatively, setting a one-sided p at 0.10 minimizes the risk of abandoning a good drug in phase II testing. A one-sided alternative hypothesis assumes that there is a directional difference in outcome (drug A improves outcome more than drug B), whereas two-sided does not specify the direction (treatment could be better or worse with respect to primary outcome). Thus, for ALS clinical trials, it may be reasonable to statistically structure phase II trials with one-sided P values of 0.10 to avoid the worst-case scenario of missing a good drug, understanding that this confers a 10% chance of proceeding to phase III with an ineffective drug. It is of course disappointing and resource-intensive when initial efficacy findings from phase II are not confirmed at later stage evaluation, as was the case for gabapentin [114] and BDNF [124]. The severity and fatality of ALS, however, may warrant reconsideration of statistical biases that increase the likelihood of missing the next effective therapy to treat this disease.

Data from well-designed early phase trials is critical planning later-stage efficacy trials. Conventional phase II studies provide data on drug activity, magnitude of effect size, optimal dose, and higher resolution toxicity information. Concerns about the efficiency of phase II studies have prompted re-evaluation of optimal design strategies which await use and validation by the ALS community.

5.4. Phase III trial design

Phase III trials are large trials intended to determine whether a treatment is effective and to establish safety data. Although relevant to other phases as well, the important variables monitored in phase III trials are outcome measures, safety, and prognostic factors. Outcome, or efficacy, variables define the end point of the study. Prolonging life is obviously a desirable therapeutic goal, but using survival as an endpoint can be problematic. To demonstrate a meaningful effect on mortality, trials must enroll large numbers of ALS patients (250 or more subjects per arm) and follow them over long periods (12 to 18 months), thus limiting the number of compounds that can be tested. And while survival seems unequivocal as an endpoint, it may be affected by the use of non-invasive ventilatory techniques, nutritional support via gastrostomy tube, or care within a multi-disciplinary ALS clinic. Constrained resources, limited numbers of eligible patients, and variations in survival due to ancillary support have motivated researchers to define meaningful surrogate outcome measures that are objective, reproducible, and valid across trials of varying duration [122]. At present, no validated surrogate measure directly correlates to pathophysiologic changes nor is it known whether any given surrogate marker is sensitive to disease-modifying therapy [125]. Several clinical markers are used, including various muscle tests, pulmonary testing (FVC), and a standardized functional rating scale (ALSFRS). An analysis of subjects enrolled in a clinical trial of topiramate suggested that declines in FVC and ALSFRS were linear and independent predictors of survival [122]. ALSFRS was also found to correlate with survival in a study of ALS clinic patients [126]. A recent negative trial of pentoxifylline in patients with ALS, however, found a significant discrepancy between survival and changes in functional measures including ALSFRS, casting some doubt on the adequacy of these measures as surrogate endpoints in phase III trials [127]. Potential pathophysiologic markers include estimation of motor unit number (MUNE), transcranial magnetic stimulation (TMS), and magnetic resonance spectroscopy (MRS) of motor cortex. Experience with MUNE suggests that it can show a higher percentage change than clinical measures and can decrease earlier than clinical measures like grip strength, thus making it a potentially excellent surrogate endpoint for efficacy trials [128,129]. TMS and variations in MR spectra, while promising, are not yet appropriate to use for trial endpoints. Finally, proteomics and metabolomics, comprehensive inventories of proteins and small molecules present in cells and tissue, will likely identify biomarkers in many disease states. Proteomic studies in CSF [130] and metabolomic studies in plasma [131] from subjects with ALS suggest that characteristic profiles may exist for the disease, however, whether significant change occurs with disease progression is not yet known.

Baseline data includes information to determine whether the subject is representative of the study population and meets inclusion/exclusion criteria for the study. These data usually include variables that may correlate with overall disease progression and serve as prognostic factors of disease outcome. In ALS, variables like gender, age at onset, duration of disease, bulbar features, for example, may be of prognostic value. Randomization typically ensures that each group of subjects is on average alike, to minimize the possibility that an observed treatment effect is due to inherent differences between groups. Stratifying patients with similar influencing variables allows for subgroup analyses to determine the relationship between prognostic variables and outcomes, and permits a balancing
out of the potential impact of prognostic variables on perceived study outcomes. Failing to take account of prognostic variables may result in perceived effects unrelated to treatment. For instance, enrolling a disproportionate number of elderly subjects or subjects with significant bulbar dysfunction can result in poorer trial outcomes that do not truly reflect on the efficacy of the trial drug. Furthermore, drop-out rates can affect the balancing effect of randomization, thus it is important for study monitoring to periodically review baseline variables in treatment and control arms to identify potential inequities in prognostic variables between the groups that could affect the interpretation of test results.

5.5. Effect size

The difference in a primary outcome between placebo and drug is considered the effect size. With limited resources, one tends to look for larger effect sizes to reduce the number of patients needed to reach significance and to eliminate drugs with no significant impact on disease. The number of failed trials in ALS perhaps prompts reconsideration of this bias. Smaller effect sizes may be a more realistic goal for efficacy trials in ALS. For example, a 15–20% reduction in the rate of decline of FVC may be a meaningful treatment effect because it correlates to a measurable increase in survival.

5.6. Patient selection

Recruiting adequate numbers of the right patients is critical for clinical study at all phases. Underpowered early studies provide insufficient data about toxicity, and negative results in underpowered early phase studies can divert research interest from potentially promising compounds. The incidence and prevalence of ALS can impact recruitment. Additionally, regional variations in time-to-diagnosis, referral practices, and survival rates can affect the composition of the study groups. For example, delays in diagnosis in some countries may bias patient samples towards those with more advanced disease, making determination of therapeutic potential more difficult. Such regional differences can also pose challenges to conducting international collaborative trials. Variations in the use of nutritional support, non-invasive ventilatory techniques, and riluzole between national or international centers can affect patient survival and therefore study outcomes, suggesting that enrollment between centers must be balanced. Narrow inclusion and exclusion criteria generally capture a relatively homogeneous population. While stratified or very homogenous groups have smaller variability, and thereby the advantage of requiring fewer patients, a disadvantage of narrowly defining inclusion/exclusion criteria is that it may limit the generalizability of study results across the entire disease population.

5.7. Placebo

Placebo-controlled studies have been considered an absolute requirement for determination of real effects in clinical trials. Uncontrolled trials may make determination of both drug benefits and side effects difficult, and undermine the value of early clinical findings. For example, a pronounced positive effect found during early studies of thyroid-releasing hormone was disconfirmed in subsequent controlled testing [132]. The chance of being randomized into a placebo arm, however, is emotionally difficult for this patient population given the utter lack of effective treatment. The use of historical controls from past trials has been limited by the amount and quality of data available on these subjects, and worries that the “natural history” of the disease may have changed due to changes in supportive measures like PEG, NIPPV, and multidisciplinary care. The Northeast ALS consortium has initiated a program to store all placebo data from ALS trials in a common NINDS data bank. Thus, going forward the ALS community has the opportunity to develop, validate, and eventually utilize a “virtual” placebo group in future ALS trials.

6. Conclusion

ALS remains a severe, life-threatening disease for which there is no treatment to significantly slow deterioration or repair clinical function. Preclinical research suggests that several levels of cellular dysfunction contribute to selective motor neuron death, whether in tandem or locked-step sequence is not known. Thus, targeting major and minor pathways identified by experimental injury remains the basis of drug selection for human trials. Drug assessment in humans is a lengthy and resource-intensive process. In the setting of constrained resources, candidate compounds can be eliminated in early phase studies not only on the basis of toxicity data, but also potentially on the basis of efficacy with appropriately modified designs. Emerging surrogate markers of disease progression have the potential to further shorten efficacy assessments if any marker turns out to be sensitive to disease-modifying therapy. Until that point, large, well-designed, multi-center efficacy trials remain the standard approach to reducing trial length and pursuing new drugs that may have a clinically significant impact on ALS.

References


