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Review

β_2-Adrenoceptor agonists as novel, safe and potentially effective therapies for Amyotrophic lateral sclerosis (ALS)

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ABSTRACT

Amyotrophic lateral sclerosis (ALS) is a chronic and progressive neuromuscular disease for which no cure exists and better treatment options are desperately needed. We hypothesize that currently approved β_2-adrenoceptor agonists may effectively treat the symptoms and possibly slow the progression of ALS. Although β_2-agonists are primarily used to treat asthma, pharmacologic data from animal models of neuromuscular diseases suggest that these agents may have pharmacologic effects of benefit in treating ALS. These include inhibiting protein degradation, stimulating protein synthesis, inducing neurotrophic factor synthesis and release, positively modulating microglial and systemic immune function, maintaining the structural and functional integrity of motor endplates, and improving energy metabolism. Moreover, stimulation of β_2-adrenoceptors can activate a range of downstream signaling events in many different cell types that could account for the diverse array of effects of these agents. The evidence supporting the possible therapeutic benefits of β_2-agonists is briefly reviewed, followed by a more detailed review of clinical trials testing the efficacy of β-agonists in a variety of human neuromuscular maladies. The weight of evidence of the potential benefits from treating these diseases supports the hypothesis that β_2-agonists may be efficacious in ALS. Finally, ways to monitor and manage the side effects that may arise with chronic administration of β2-agonists are evaluated. In sum, effective, safe and orally-active β_2-agonists may provide a novel and convenient means to reduce the symptoms of ALS and possibly delay disease progression, affording a unique opportunity to repurpose these approved drugs for treating ALS, and rapidly transforming the management of this serious, unmet medical need.

Contents

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1. Introduction

Amyotrophic lateral sclerosis (ALS; also known as Lou Gehrig's disease) is a chronic, progressive neuromuscular disease that primarily involves a gradual loss of function and eventual death of motor neurons in the spinal cord and brain, as well as significant atrophy of the muscles they innervate. Clinically, ALS is characterized by stiff and/or twitching muscles and significant muscle weakness due to gradual muscle wasting, resulting in difficulty ambulating, speaking, swallowing, and eventually breathing. The average survival from diagnosis to death is three to four years, though about 10% of ALS patients may survive 10 years or more. There is no cure, with few treatment options currently available. Riluzole reportedly can extend life expectancy about two to three months and non-invasive ventilation may temporarily improve quality and length of life. For all these reasons, efforts continue to try to identify and develop more effective treatment options.

β-Adrenergic agonists share structural and pharmacological similarities with epinephrine. While these agents have been developed and approved primarily for the treatment of bronchial ailments such as asthma and COPD (chronic obstructive pulmonary disease), accumulating evidence demonstrates that their effects extend beyond smooth muscle relaxation and bronchodilation. Among the varied pharmacologic effects of β-agonists, they may enhance skeletal muscle structure and function, significantly increasing muscle repair, bulk and strength by stimulating protein synthesis and inhibiting protein degradation while significantly reducing body fat via the well-established 'repartitioning effect' (Lynch and Ryall, 2008). While these effects were initially exploited by the livestock industry, this use quickly expanded to include human body builders and strength-training athletes. In more recent years, experimental interest has further expanded to include clinical tests of β-agonists in the treatment of a wide range of muscle-wasting and neuromuscular diseases. These novel pharmacological effects, if induced safely and reliably in humans, might significantly improve the status of ALS patients by reducing the magnitude of symptoms and possibly slowing disease progression.

This paper reviews data collected from various animal models of muscle and neural degeneration that supports the use of β-agonists in treating these pathologies, and examines the signaling pathways induced by β_2-agonists that may be responsible for these effects. However, the major focus is a detailed review of nearly three dozen clinical publications reporting on the efficacy of β-agonists in a number of human neuromuscular disorders, including ALS. Collectively, these papers offer intriguing evidence supporting the hypothesis that β_2-agonists may be therapeutically valuable for treating ALS, as each of the diseases share one or more important pathologies with ALS. The review concludes with suggestions for monitoring and mitigating the potential side effects that may occur following chronic β_2-agonist administration, to help assure that the hypothetical risks might be acceptable relative to the anticipated benefits.

2. β_2-Adrenoceptors: multiple signaling pathways produce diverse pharmacologic benefits

There are two groups of adrenergic receptors, α and β, each composed of several subtypes ($\alpha_{1A,B,D}$, $\alpha_{2A,B,C}$; β_1, β_2 and β_3). Within skeletal muscles, α_1-adrenoceptors are primarily located on the vascular smooth muscle of arterioles, where they control blood flow through the skeletal muscle (Lynch and Ryall, 2008), but otherwise do not affect skeletal muscle function. In rodent skeletal muscles, β_2 adrenoceptors are the dominant subtype, accounting for 80–90% of the total adrenoceptor content. The other isoform found in this tissue is the β_1-subtype, accounting for the remaining 10–20% of total adrenoceptor content (Jensen et al., 1995; Kim et al., 1991). The β adrenoceptor population in human skeletal muscles is almost exclusively comprised of the β_2-subtype (Liggett et al., 1988), although the presence of low densities of β_3-adrenoceptors has been suggested (Chamberlain et al., 1999).

β_2-Adrenoceptors are widely expressed on neurons throughout the human central nervous system, particularly in the hippocampus, and the prefrontal, parietal, temporal and motor cortices (Joyce et al.,

1992; Russo-Neustadt and Cotman, 1997). In contrast, only low densities of β_2-adrenoceptors are found in the dorsal and ventral horns of rat spinal cord (Atlas and Melamed, 1978; Mizukami, 2004; Patterson and Hanley, 1987), with β-adrenoceptors homogeneously distributed throughout the gray matter of the human spinal cord (Manaker et al., 1988). While there have been few published studies characterizing β-adrenoceptors on spinal cord motor neurons in the ventral horn (a region critically degraded in ALS) β_2-adrenoceptors do exist in many brain regions whose degeneration has been shown to be important to ALS (e.g., motor neocortex, frontal cortex, temporal cortex, hippocampus, and cerebellum, among others) (Tsermentseli et al., 2012). This might be especially important in view of ALS links to frontal-temporal dementia. The extensive distribution of β_2 adrenoceptors throughout the CNS is consistent with their being ideal pharmacological targets for treating the upper motor and cognitive manifestations of ALS. In addition to their location on skeletal muscles, which suggests a role in maintaining peripheral pathways essential for proper motor function, the presence of β_2-adrenoceptors on astrocytes, microglia and immunocytes in ventral horn argues for a possible inter-cellular explanation for evidence that β_2-agonists can protect spinal cord neurons and restore/preserve their function.

Stimulation of β_2-adrenoceptors activates many signaling pathways linked to a variety of changes in different tissues and cell types (Fig. 1, Table 1). This section provides an overview of the major β_2-signaling pathways that may be relevant to the treatment of ALS. More detailed information may be found in other recent reviews (Joassard et al., 2013; Lynch and Ryall, 2008).

2.1. β_2-Adrenoceptor activation of the cAMP/PKA/CREB pathway

β_2-Adrenoceptors belong to the G protein-coupled receptor family and activate the canonical cAMP-dependent pathway. Following β_2-adrenoceptor stimulation, the receptor couples to the Gαs subunit and activates adenylate cyclase, generating cAMP. This is a master signaling pathway, with elevated cAMP levels driving multiple parallel signaling pathways that play critical roles in regulating skeletal muscle, CNS and immunocyte morphology and function (Fig. 1). A major effect of cAMP is the activation of cAMP-dependent protein kinase A (PKA), which then enters the cell nucleus and phosphorylates the ubiquitous transcription factor CREB. CREB increases the expression of cAMP-inducible genes containing the cAMP response element (CRE) sequence in mitochondria, myocytes, neurons, astrocytes, microglia and immunocytes.

2.1.1. Enhancing protein metabolism

The hypertrophic effects of β_2-agonists on skeletal muscle appear to be mediated by activation of the cAMP/PKA/CREB pathway (Joassard et al., 2013). Multiple β_2-adrenoceptor induced signaling pathways stimulate protein synthesis within cells, including CREB which regulates the expression of numerous genes involved in skeletal myocyte differentiation, the enhancement of protein synthesis and inhibition of protein degradation (Joassard et al., 2013; Lynch and Ryall, 2008); see Fig. 1. Moreover, neuronal β_2-adrenoceptor activation of CREB has been shown to promote the synthesis of proteins mandatory for neuronal homeostasis (Gelinas and Nguyen, 2005; Zhou et al., 2013), and while not yet empirically confirmed in ALS, the presence of β_2-adrenoceptors on neurons throughout several brain regions affected by ALS raises the possibility of neuroprotection and functional neurorestoration, as well.

2.1.2. Inhibiting proteolysis induced by the glutamate-calcium pathogenic cascade

Glutamate toxicity is commonly recognized as an important pathogenic event in ALS, resulting in aberrantly high, toxic levels of intracellular calcium. Calpains are a family of unique, non-lysosomal, Ca^{2+}-dependent proteases optimally active under neutral pH conditions. In response to normal, regulated Ca^{2+}-fluxes, calpains initiate the controlled degradation of contractile proteins in skeletal muscle, and are implicated in neuronal synapse formation, maintenance and remodeling. However, under conditions of calcium dysregulation, uncontrolled calpain-mediated proteolysis has been implicated as an important pathogenic player in the glutamate-calcium cascade in a number of diseases, including ALS. Unregulated calpain activation has been shown to cause significant damage in many cell types, including muscle and neurons due to uncontrolled proteolysis (Bartus, 1997). Consistent with this idea, elevated calpain activity is observed in the brain and spinal cord of ALS patients (Yamashita et al., 2012). β_2-Agonists have been shown to inhibit abnormal Ca^{2+}-dependent calpain proteolysis in muscle (Koopman et al., 2010; Lynch and Ryall, 2008), by increasing the transcription of calpastatin, the endogenous inhibitor of calpain activity, via CREB (Goncalves et al., 2012; Sensky et al., 2006), and/or by the direct activation of constitutive calpastatin by PKA (Bartus, 1997; Lynch and Ryall, 2008; Navegantes et al., 2001). While these effects of β-agonists have not yet been studied in neurons, the same pathways exist in many CNS neurons expressing β_2 receptors, and may therefore produce the same benefits.

2.1.3. Improving mitochondrial function

PGC-1α is a master regulator of mitochondrial biogenesis and metabolism, and is enriched in skeletal muscle (Ruas et al., 2012). β-Adrenergic signaling may increase PGC-1α activity directly by stimulation of PGC-1α (Chinsomboon et al., 2009; Peterson et al., 2013) or indirectly through the activation of SIRT1 by PKA (Fig. 1) (Gerhart-Hines et al., 2011). Regardless of the mechanism, acute stimulation of β_2-adrenoceptors rapidly increases PGC-1α levels in rodent skeletal muscle (Koopman et al., 2010; Pearen et al., 2009). Moreover, β_2-agonists increase expression of mitochondrial genes targeted by PGC-1α and induce mitochondrial biogenesis (Koopman et al., 2010). Recently, alternative splice variants of PGC-1α have been identified (Ruas et al., 2012), with the PGC-1α1 isoform associated with the promotion of mitochondrial biogenesis, while PGC-1α4 induces muscle hypertrophy independent of mitochondrial biogenesis (Ruas et al., 2012). Hence, β_2-agonists may activate PGC-1α1 driven mitochondrial biogenesis while promoting the synthesis of other proteins by activating PGC-1α4.

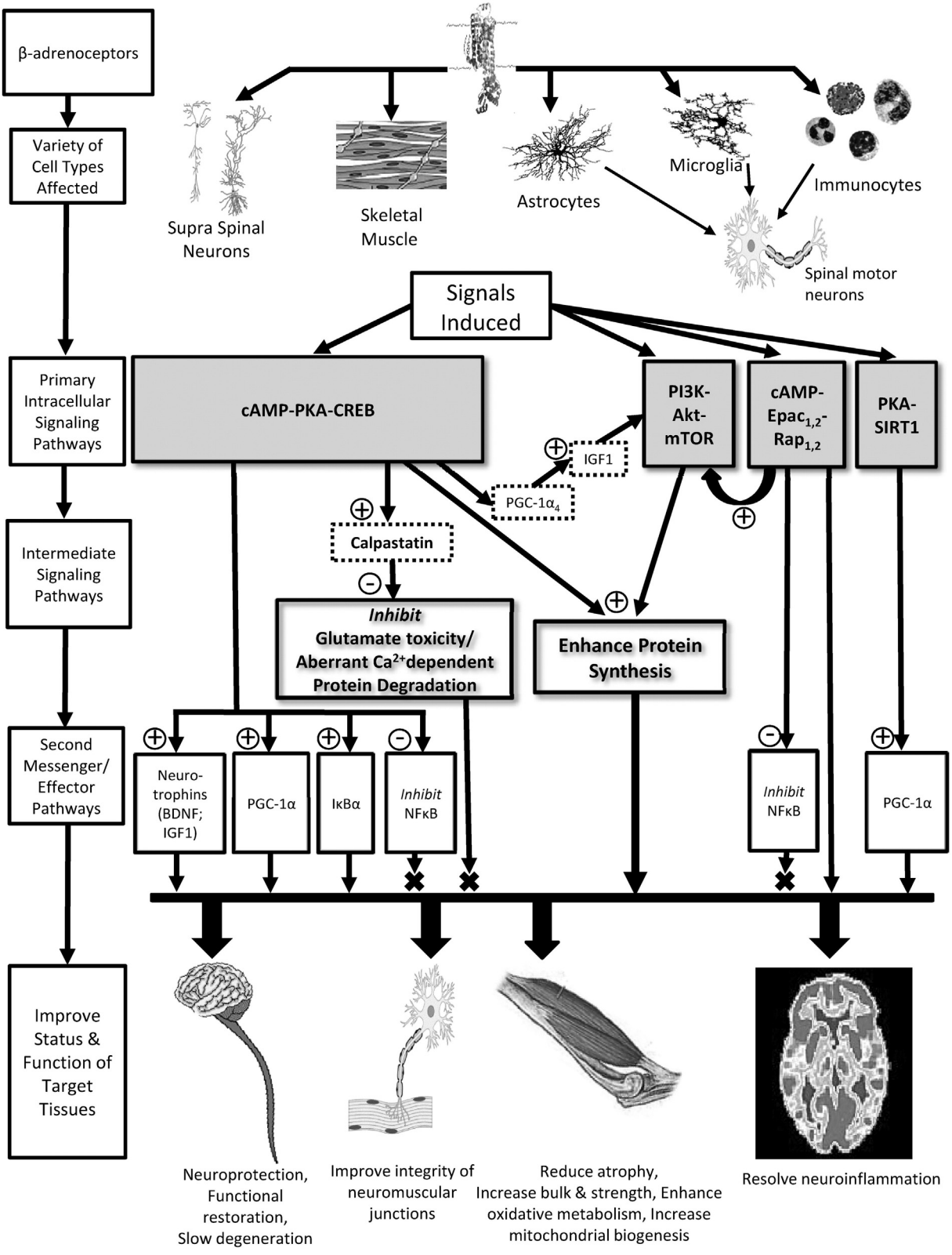
2.1.4. Effects on neurotrophic factors in neurons

Stimulation of β_2-adrenoceptors in neurons directly promotes the synthesis and release of neurotrophic factors via the cAMP/PKA/CREB pathway (Fig. 1). For example, β_2-adrenergic stimulation activates NGF and BDNF pro-survival pathways in rat neurons (Counts and Mufson, 2010). IGF-1 is a neurotrophic factor that prevents decrements in motor function and motor neuron loss while increasing survival in the G93A-SOD1 mouse model of ALS (Kaspar et al., 2003). In muscles, IGF-1 is induced by CREB induced elevations of a PGC-1α isoform (Fig. 1). IGF-1 then promotes protein synthesis via autocrine activation of the IGF-1 receptor and downstream PI3K-Akt signaling (Jesinkey et al., 2014b; Rommel et al., 2001; Ruas et al., 2012). Hence, activation of neuronal β_2-adrenoceptors may provide neuroprotective benefits to ALS patients by increasing neurotrophic factors.

2.1.5. Effects on inflammation and trophic factor signaling in astrocytes

Astrocytes possess both β_1- and β_2-adrenoceptors (De Keyser et al., 1999; Mantyh et al., 1995; Sutin and Shao, 1992), with the latter stimulating the synthesis and release of neurotrophic and anti-inflammatory factors in the CNS. The transcription factor NFκB is a major mediator of astrocyte-mediated neuroinflammatory responses (Fig. 1) (Gavrilyuk et al., 2002). Activation of the cAMP/PKA/CREB pathway decreases the transcriptional activity of NFκB (Gerlo et al., 2011) and mediates the clenbuterol induced suppression of NFκB inflammatory responses in rat brain (Ryan et al., 2013). Astrocytic β_2-receptor driven elevations of cAMP also promote the synthesis and release of the trophic factors BDNF, NGF, TGF-β1 and bFGF, producing anti-apoptotic and neuroprotective actions in rodent models of cerebral ischemia and excitotoxicity (Culmsee et al., 2007; Culmsee et al., 1999; Day et al., 2014; Gleeson et al., 2010; Riva et al., 1996).

Astrocyte involvement is a common pathologic feature in ALS, with disease severity often correlating with the degree of astroglial activation. There remains disagreement in the ALS field as to whether activation of astrocytes is a driving pathogenic event, or merely a response to ongoing tissue destruction caused by unrelated factors. There is also data suggesting that astrocytes derived from ALS patients are toxic to motor neurons, and other evidence that a reduction in astrocyte-derived transporter molecules (EAAT) in ALS contributes to glutamate-mediated toxicity. A number of studies using stem-cell derived astrocytic implants have been conducted in non-clinical models of ALS and spinal cord injury (Nicaise et al., 2015). The results of these studies are inconsistent, offering at best temporary improvements in motor function. This may reflect the complex nature of the astrocytic transplants and the early stages of development of this therapeutic modality. Programs



investigating human embryonic stem cell derived astrocytes for ultimate use in Phase I/II studies have recently been funded, and may yield valuable results in the future. Finally the so-called "Q trial", administering glia-derived precursor cells that theoretically are differentiated in vivo to astrocytes is ongoing in an effort to protect motor neurons (https://clinicaltrials.gov/ct2/show/NCT02478450?term=Q-therapeutics&rank=1). Additionally, it is known that β_2-adrenoceptor density is highly elevated in reactive astrocytes derived from the spinal cords of ALS patients (De Keyser et al., 1999), and that stimulation of β_2-adrenoceptors confers selective neurotrophic and neuroprotective properties to astrocytes. Thus, agonist treatment of β_2-adrenoceptors on endogenous astrocytes may be effective in promoting motor neuron survival and nerve regrowth in ALS patients.

2.1.6. Modulating microglia and immunocytes

Noradrenaline influences many immune cell functions, including cytokine production, cell proliferation and antibody secretion (Elenkov et al., 2000; Kin and Sanders, 2006). Most immunocytes express β_2-adrenoceptors, the activation of which triggers potent anti-inflammatory and immunosuppressive effects (Carnevale et al., 2007). Accordingly, β_2-agonists are potent inhibitors of pro-inflammatory cytokine release from most immune cells (Theron et al., 2013), primarily by the cAMP/PKA/CREB pathway, but also by an alternate cAMP-dependent pathway (cAMP-Epac1/2-Rap1/2, Fig. 1). In particular, β_2-activation of the cAMP pathway inhibits proinflammatory cytokine release by T-helper 1 (Th1) cells, while promoting the synthesis of anti-inflammatory cytokines, favoring a protective Th2 response (Carnevale et al., 2007). This effect may be of particular relevance in ALS, where early, beneficial immune responses shift to deleterious Th1-driven immune responses with disease progression (Zhao et al., 2013).

Microglia are another immune cell type activated in ALS disease and implicated as an important pathogenic event in motor neuron degeneration (Turner et al., 2004; Zhao et al., 2013). Microglia express high levels of β_2-adrenoceptors and cAMP elevation following β_2-stimulation suppresses microglial proliferation and hypertrophy attendant to inflammatory processes (Fujita et al., 1998; Tanaka et al., 2002). β_2-activation increases IκBα levels and downstream cAMP signaling blocks microglial release of inflammatory cytokines and free radicals (Colton and Chernyshev, 1996; Dello Russo et al., 2004; Madrigal et al., 2005; Mori et al., 2002; Prinz et al., 2001). Consequently, β_2-supression of microglial activation reduces microglia-induced neuronal cell death (Madrigal et al., 2006).

2.2. Non-cAMP/PKA/CREB pathways induced by β2-adrenoceptor stimulation

Direct and indirect activation of the cAMP/PKA/CREB pathway by β_2-agonists results in an array of cellular responses. However, other pathways directly activated by β_2-agonists have potential in treating ALS and deserve mention. β_2-Adrenoceptors can switch coupling to initiate alternate signaling events involving the PI3K–Akt–mTOR pathway (Fig. 1) (Joassard et al., 2013). The ability of clenbuterol to attenuate loss of muscle mass in rat models of muscular atrophy has been linked to activation of protein synthesis pathways involving mTOR (Kline et al., 2007). Other alternate signaling pathways are illustrated in Fig. 1.

2.3. Summary: β_2-adrenoceptor modulated signaling pathways

It is difficult to determine the relevance that an intracellular event may have to a disease state outside the well-controlled conditions of in vitro or ex vivo testing. The role of β_2-adrenoceptor stimulation in ALS is no exception. However, in a variety of cell types impacted by ALS, β_2-adrenoceptor activation modulates a number of intracellular signaling pathways that maintain or enhance the viability of cells under conditions of physical stress. The consistency of these effects suggest that pharmacologically inducing these pathways using β_2-agonists could protect against the pathologic changes associated with ALS and other neurodegenerative syndromes in intact animal models. The following section reviews the literature supporting this hypothesis.

3. Evidence for the neuroprotective effects of β-agonists in animal models

Over 25 years of animal research has established that β-agonists positively affect muscle function in vivo. β-agonists consistently increase muscle strength and mass, reverse muscle wasting, and delay the onset of motor deficits in a wide range of animal models of muscle degeneration (Joassard et al., 2013; Lynch and Ryall, 2008; Ryall and Lynch, 2008). Similarly, the diverse signaling pathways induced by β-agonists can protect neurons and promote neural repair following an array of insults. While animal models absolutely predictive of therapeutic efficacy in ALS remain to be established (Rothstein, 2003), the pathways induced by β-agonists might yield biologically-relevant improvements in whole animals manifesting pathologies in common with ALS.

While a number of studies provide evidence of the neuroprotective effects of β_2-agonists, only two have been tested in putative models of ALS. In the G93A-SOD1 mouse model of ALS (Teng et al., 2006), a single dose level of the β-agonist clenbuterol produced a robust delay in the onset of hind limb weakness as measured by performance on a rotarod test. A modest, but statistically significant slowing of disease progression as measured by Kaplan-Meier survival curves was also observed, albeit solely in the female cohort, with a non-significant trend toward increased motor neuron survival. It remains uncertain whether more robust responses may have been achieved if more than the single dose level had been tested. A more recent report (Paik et al., 2015) tested the non-selective β-agonist terbutaline sulfate in zebrafish overexpressing the mutant TDO-43 (Q331K), which develops motor axon degeneration and defective neuromuscular junctions (NMJ). Treatment with terbutaline at 9h post-fertilization, before the onset of axonal outgrowth, prevented the development of defects in axons and the NMJ. When administered later post-fertilization, after motor neuron defects were apparent, terbutaline effected a significant rescue of motor axons and NMJs. The authors predicted that terbutaline was a promising candidate for ALS based on their retrospective analysis of historic patient records and drug–drug interactions using a mathematical model. These studies support the concept that the signaling pathways induced by β-agonists may produce biological changes that benefit the whole animal, and may be of therapeutic relevance to ALS.

Data from other animal models of neurodegeneration provide greater support for a role for β_2-agonists protecting or repairing degenerating motor neurons in ALS. Clenbuterol treatment delayed the development

Fig. 1. Summary of signaling pathways of particular relevance to ALS that are modulated by activation of β_2-adrenoceptors. β_2-Adrenoceptors are ubiquitously located on many of the cell types involved in ALS, including skeletal muscle, glia, immunocytes and many neurons, with the possible exception of spinal cord motor neurons (very few published results could be found). Nonetheless, as depicted in the figure, spinal cord motor neurons are directly impacted by glia and immunocytes expressing β_2 receptors. Agonist activation of β_2-adrenoceptors stimulates a cascade of intracellular signaling pathways in these tissues, perhaps most prolific being the cyclic adenosine monophosphate (cAMP)/protein kinase A (PKA)/cAMP-response element binding protein (CREB) pathway. As depicted in the figure, a large and diverse array of pathways are activated, in turn inducing an even greater variety of second messenger/effector pathways. In addition to enhancing calpastatin, which inhibits aberrant calcium-dependent/calpain-mediated proteolysis (a key pathogenic event in glutamate toxicity) and enhancing protein synthesis, these pathways can inhibit nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB), or activate effectors including neurotrophins such as brain derived neurotrophic factor (BDNF) and nerve growth factor (NGF), peroxisome proliferator-activated receptor gamma co-activator (PGC-1α), and inhibitor of NFκB alpha (IκBα). All of these effectors can broadly enhance and/or protect the metabolic and functional performance of skeletal muscle and neurons, as well as exert anti-inflammatory effects on immunocytes, and anti-inflammatory and potential neurotrophic effects through glia.

of motor deficits in the chronic motor neuron degeneration (mnd) mouse model, while reducing the proportion of motor neurons with eccentric nuclei (a marker of synaptic dysfunction) (Zeman et al., 2004). In addition, 6 weeks of clenbuterol significantly reduced spinal cord damage in a dose-related fashion following contusion injury in rats (Zeman et al., 1999), while dose-dependent protection of the spinal cord from ischemia and reperfusion injury was observed following clenbuterol administration to rabbits (Chen et al., 2013). Other studies show that β_2-agonists protect neurons in vivo and in vitro by inhibiting microglial activation (Qian et al., 2011), while suppressing the chronic-relapsing pathology in the rodent EAE (experimental autoimmune encephalomyelitis) model of multiple sclerosis (MS) by modulating autoimmune pathologies (Wiegmann et al., 1995). Specifically, terbutaline reduced both the onset and recurrence of experimental MS by inhibiting monocyte production, which plays a key role in pathogenesis of MS. Terbutaline also suppressed experimental allergic neuritis in Lewis rats by reducing demyelination and Wallerian degeneration, while improving electrophysiological and functional motor endpoints (Kim et al., 1994).

Further evidence of the neuroprotective properties of β_2-agonists, possibly mediated by the release of neurotrophic factors, was observed using transgenic models of monogenetic neurodegenerative diseases. The β_2-agonist formoterol significantly improved the synaptic density and complexity of newly formed dentate granule neurons in the hippocampus, with significant improvements in cognitive function in the Ts65Dn mouse model of Down's syndrome (Dang et al., 2014). In the Mecp2 null mouse model of Rett syndrome, characterized by neuromuscular dysfunction and degeneration, clenbuterol treatment improved behavioral function and restored a microRNA-mediated pathway downstream of BDNF, which affects normal IGF-1 expression (Mellios et al., 2014). Several investigations have demonstrated that IGF-1 protects degenerating spinal cord motor neurons in the SOD1-over expressing model of ALS (Franz et al., 2009; Kaspar et al., 2003; Lepore et al., 2007). Using β_2-agonists to increase IGF-1 levels (Fig. 1) may have similar effects. Finally, β_2-agonists such as formoterol may induce mitochondrial biogenesis, restore the expression and function of mitochondrial proteins and increase the copy number of multiple genes involved in the mitochondrial electron transport chain, presumably through activation of down-stream transcription pathways (Jesinkey et al., 2014a; Wills et al., 2012). Given the importance of mitochondria in neural function and repair, and the role of mitochondrial dysfunction in the pathogenesis of ALS (Cozzolino and Carri, 2012), this attribute could offer yet another important pathway by which β_2-agonists improve the status of ALS.

3.1. Summary of the in vivo effects of β-agonists in animal models

β_2-Adrenoceptor stimulation induces a range of positive effects in animal models of neuromuscular degeneration. While none of these models are predictive of therapeutic efficacy in human diseases let alone ALS, they indicate that the signaling pathways induced by β_2-agonists improve function and reduce or protect against tissue damage in a number of organ systems. These changes could act synergistically to suppress pathogenic elements of ALS, providing further evidence that β_2-agonists may reduce symptom severity and slow disease progression in ALS.

4. Clinical experience: tests of β-agonists in human neuromuscular disorders

The clinical efficacy of β_2-agonists has been tested in a variety of neuromuscular diseases. Currently, only a single, open label trial has tested β_2-agonists in ALS. Nonetheless, these nearly three dozen clinical studies testing the therapeutic efficacy of β-agonists in treating different neuromuscular maladies may offer insights into their potential for treating ALS. Despite their clear differences relative to ALS in etiology, pathogenesis, pathology and certain symptoms, their commonalities with ALS can be more important than their differences when defining opportunities for correcting ALS pathologies. For example, some of these neuromuscular diseases have a primary neurologic pathology (often including degeneration of motor neurons) that impacts the musculature innervated by the degenerating neurons, as in ALS (Table 1). Other diseases are characterized by degeneration and dysfunction of the NMJ (Table 2), negatively impacting both the host muscle and the innervating motor neuron. Finally, the remaining disorders have a primary myopathology that also impacts the NMJ and indirectly, the innervating neuron (Table 3). By comparing and contrasting the ability of β_2-agonists to overcome these varying pathologies, one can gain insight into the likelihood that similar disturbances in ALS might be similarly improved.

4.1. Neuromuscular disorders with a primary neuropathology

The progressive neurodegeneration of upper and lower motor neurons is generally recognized as the hallmark of ALS, with weakened, atrophied muscles considered a secondary effect of axonal denervation. Though the study and use β-agonists as anabolic agents for muscle has a history longer and richer (Ryall and Lynch, 2008) than their possible benefit against neurodegeneration, a reasonable number of clinical tests have nonetheless also been conducted in human diseases with a primary neuronal genesis. These offer compelling, albeit preliminary support for the hypothesis that β_2-agonists may protect and/or restore function and morphology of degenerating motor neurons in ALS (Tables 1 and 2).

4.1.1. Amyotrophic lateral sclerosis

In the only study conducted testing a β_2-agonist in ALS, clenbuterol was administered at a single dose level to 16 subjects diagnosed with progressive ALS (functional rating score ranging from 4–40) (Soraru et al., 2006). In the 14 subjects completing this pilot study, the mean composite myometer score for the upper limbs significantly improved from baseline by 20% at 3 months and 23% at 6 months ($p = 0.01$), and for the lower limbs by 22% at 3 months and 27% at 6 months ($p = 0.0002$). The mean composite MRC (Medical Research Center) score of upper and lower limb strength trended toward improvement, but was not statistically significant. The mean forced vital capacity (FVC) score trended toward improvement at 3 months (5%; $p = 0.28$) and was significant at 6 months (10%; $p = 0.03$). Despite these effects, no change was observed in the ALS Functional Rating scale at either assessment time point. Side effects were mild and always occurred within the first few days of starting dosing, quickly resolving without altering dose. The most common complaints were hand tremors (2 patients), cramps, fasciculations (3 patients), and nervousness (3 patients). In no patient was heart rate increased or palpitations observed. It should be noted that the mean dose of ~86 μg/kg/day is well below the efficacious doses of clenbuterol in preclinical models (Ryall and Lynch, 2008). Therefore, while interpreting the results of this pilot study is difficult, it nonetheless supports the idea that further effort to test higher doses in ALS is warranted, possibly titrating doses to limit the development of side effects, which based on other evidence, may produce more robust and consistent effects than reported here.

4.1.2. Spinal bulbar muscular atrophy (SBMA)

SBMA is a debilitating neurodegenerative disorder of motor neurons in the brain stem and spinal cord. It therefore shares with ALS an important pathogenic neuronal deficit as well as a clinical phenotype of muscle cramps and progressive weakness.

A study in 20 SBMA patients (Querin et al., 2013) employed clenbuterol to induce both Akt and IGF-1, based on prior research in motor neurons transduced to mimic SBMA showing that Akt and IGF-1 co-activation increased neuron survival (Palazzolo et al., 2007). In the 16 patients completing the study, a significant and sustained increase

Table 1
Clinical trials in neuromuscular disorders with primary neuropathology.

DsX	Paper	Num.	Drug	Dose/day	Length	Design	Efficacy endpoints	Notes
ALS	Soraru et al. (2006)	16 (14)	Clen	60 μg	6 mo	Uncontrl	Composite myometer improved; MRC not improved	
SBMA	Querin et al. (2013)	20 (16)	Clen	20 μg for 2 d; then 40 μg/day	12 mo	Uncontrl	6 min walk; fVC	CK increase related both to clen. & SBMA
SMA	Kinali et al. (2002)	13	Albut	3 mg–8 mg	6 mo	Uncontrl	Myometry, fVC, MRC, lean body mass	Dose was age-dependent
SMA	Pane et al. (2008)	23	Albut	3 mg 1 wk., then 6 mg	12 mo	Uncontrl	Hammersmith scale improved at 6 & 12 mo	
SMA	Tan (2011))	2	Albut	6 mg	6 mo	Uncontrl, case rep	Neck & lung strength fVC	
Mus atrophy/SC injur	Signorile et al. (1995)	10	Meta-pro	80 mg	1 mo	Blinded/ controlled	Muscle size & strength	
Mus Atrophy/SC Injur	Murphy et al. (1999))	3	Albut	4 mg	2 wks/group	Blinded/ X-over	Muscle mass muscle work output	
Denervated mus atrophy	Jiang et al. (2011)	71	Clen	120 μg	3 mo	Blinded/ controlled	Muscle atrophy improvement	
MS	Makhlouf et al. (2001)	16	Albut	4 mg: 1st wk.; then 12 mg: 2nd wk	1 wk	Uncontrl	Reduced IL-12 producing monocytes	
MS	Khoury et al. (2010)	44	Albut	4 mg	1 & 2 yrs	Blinded/ controlled	Increased time to relapse; improved MS functional comp. at 6 mo & 1 yr. (primary endpoint: 2 yrs)	Both active and placebo received glatiramer

Definitions—ALS: amyotrophic lateral sclerosis, Alb: albuterol, blinded: double blind treatment, CK: creatine kinase, Clen: clenbuterol, controlled: randomized & placebo-controlled, denervated musc atrophy: muscle atrophy after denervation, FVC: forced vital capacity, HMF Hammersmith motor functional scale, Meta: metaproterenol, MRC: manual muscle testing with the Medical Research Council scale, MS: multiple sclerosis, musc atrophy/spinal cord inj: muscle atrophy after spinal cord injury, N: total subjects enrolled, (number of subjects completing all protocol assessments); SBMA: spinal bulbar muscular atrophy; SMA: spinal muscular atrophy, uncontrl: uncontrolled; no placebo group; X-over: cross-over design (can be blinded or not), but all patients alternatively receive both treatment and placebo. Note: for "Num" (i.e., number of subjects), the larger number refers to total subjects enrolled; number in parenthesis refers to number of subjects that completed all protocol assessments (i.e., excludes subjects who withdrew from the protocol for any reason).

in distance walked in 6 min was seen, as well as improved forced vital capacity ($p < 0.001$). Side effects (primarily tremor and cramps) were mild, transient and typically occurred early in the dosing schedule. In two subjects the tremors caused early withdrawal. This open label study, showing statistically significant improvement of neuromuscular performance in a serious motor neuron disease offers circumstantial evidence supporting the hypothesis that oral β2-agonists may be effective in ALS.

4.1.3. Spinal muscular atrophy (SMA)

SMA is an autosomal recessive disease caused by a mutation in the SMN1 gene, which encodes SMN, a protein necessary for motor neuron survival. Four forms of SMA (types I-IV), distinguished by disease severity, are commonly recognized. All share some semblance to the degeneration and death of motor neurons characteristic of ALS.

Three small, open label clinical studies in patients with type II SMA have been reported, all suggesting therapeutic benefit. The first treated 13 children with SMA II and III with albuterol (also called salbutamol, especially in Europe) (Kinali et al., 2002). A significant increase in myometry scores, forced vital capacity and lean body mass was observed ($p < 0.05$). Another open label trial testing albuterol in 23 young patients with SMA type II (Pane et al., 2008) observed a significant improvement in the Hammersmith motor functional scale at both 6 months and 1 year of treatment ($p = 0.006$). This was followed by another open label study in two wheel-chair bound children with SMA type II (Tan, 2011). Albuterol significantly increased forced lung capacity, and improved muscle strength so that they could, for the first time, perform several important daily activity tasks. Side effects were not an issue in any of these studies. A potential mechanism for albuterol's efficacy in SMA patients was identified (Tiziano et al., 2010). Albuterol was orally administered to 12 SMA type II-III subjects and SMN2 full-length transcript levels were elevated in direct proportion to the patients' SMN2 gene copy number. These data suggest that albuterol (and likely other β-agonists), may act by elevating SMN2 protein levels by a downstream signaling pathway stimulating SMN2 gene transcription or splicing. While SMN2 has not been implicated as a pathogenic variable for ALS, it remains to be seen whether this mechanism of action may nonetheless benefit ALS patients. That is, it might be interesting to test whether elevating SMN2 to *supraphysiological levels* could help improve the survival of degenerating motor neurons, either in SOD1-over expressing mice or ALS patients, much as many past investigators have argued for neurotrophic factors.

4.1.4. Muscle atrophy following spinal cord injury

Two double-blind, placebo controlled studies have administered β-agonists to patients following serious spinal cord injuries. The first investigation was a small, randomized crossover design testing metaproterenol versus placebo in paralyzed patients for four weeks (Signorile et al., 1995). Objective measures of muscle strength and size (expressed as cross-sectional area) were increased ($p < 0.001$). In the second study, three patients with spinal cord injuries were given either albuterol or placebo for two weeks while engaged in a functional electrical stimulation (FES) cycling program (Murphy et al., 1999). Increases in mass of several muscles were seen, though contractile function of the quadriceps muscle was not improved. Total work output significantly increased 64% with albuterol treatment compared to 27% with training alone. Side effects were mild and transient. It is not clear how much this improvement was due to muscle-only effects, versus a β_2-agonist recovery of spinal cord neuron function (as seen in certain animal studies).

4.1.5. Muscle atrophy following peripheral denervation

A randomized, double-blind, placebo-controlled study administering either clenbuterol or placebo was performed on 71 patients suffering from brachial plexus injuries (Jiang et al., 2011). As observed in animal studies employing the same injury, clenbuterol ameliorated denervated muscle atrophy in these patients. Since loss of muscle tone, bulk and function occurs in ALS, due in part to denervation caused by the

axonopathy of ALS, these double-blind, controlled data provide further support for a possible benefit of β_2-agonists in ALS.

4.1.6. Multiple sclerosis (MS)

Multiple Sclerosis (MS) is the most common autoimmune disorder causing chronic neurological disease. The autoimmune response damages oligodendrocytes, causing axonal demyelination and functional disability. Microscopically, the disease is characterized by perivascular infiltrates of mononuclear cells. Some produce IL-12, which stimulates T-cell proliferation as an important step in the pathogenesis of MS.

β_2-Agonists are immunomodulators (Theron et al., 2013) and decrease IL-12 expression by monocytes through the cAMP signaling pathway (Panina-Bordignon et al., 1997). In one of two studies testing β_2-agonists in ALS, 16 progressive patients (aged 18–60 years) were given daily albuterol (Makhlouf et al., 2001), which significantly reduced levels of IL-12-producing monocytes. Later, a double-blind clinical trial tested whether albuterol improved clinical symptoms in relapsing MS patients (Khoury et al., 2010). Forty-four subjects were randomized to receive either albuterol-plus-glatiramer acetate (an immunomodulator or 'immune-decoy' approved for MS) or placebo-plus-glatiramer acetate. A delay to first relapse ($p = 0.03$), as well as an improvement in the Multiple Sclerosis Functional Composite (MSFC) test was seen at 6 months ($p = 0.005$) and 12 months ($p = 0.04$) in the albuterol group. However, this improvement was lost by 24 months. Adverse effects were generally mild and dropout rates between groups were equivalent. The authors concluded that albuterol combined with glatiramer acetate improved MS over glatiramer alone. While they did not comment on the loss of efficacy by 24 months, it seems plausible that it may have been due to β_2-adrenoceptor desensitization, which could have been prevented with either drug holidays or increases in dose (see Section β-adrenoceptor desensitization). No effort was made to monitor or control for this possibility.

4.2. Neuromuscular disorders characterized by the pathology of the neuromuscular junction (NMJ)

Evidence strongly suggests that ALS progresses as an axonopathy, wherein neurons degenerate in retrograde fashion from the NMJ toward the cell body in the spinal cord. Therefore, protecting the interface of the motor neuron terminals and muscle fibers seems prudent. Moreover, because the pathogenesis of ALS may begin with the NMJ (Dupuis and Loeffler, 2009), restoring and protecting NMJ integrity and function may be necessary, although not sufficient, for treating ALS. The following section reviews a decade-long effort testing the effects of β_2-agonists in various myasthenic diseases, all characterized by defects in the NMJ (Table 3). The benefits reported offer further support for the hypothesis that β_2-agonists might provide a novel therapy for ALS.

Diseases characterized by the loss of transmission from motor neurons to muscle and defects to the NMJ are grouped as myasthenia, or myasthenia gravis (MG) for more severe forms. MG may be caused by a congenital mutation or an acquired autoimmune component. β-agonists have been tested in MG patients with mutations to DOK-7 (a muscle-specific protein essential for NMJ formation) and MuSK (muscle specific kinase, responsible for signaling the motor neuron terminal to dock with the motor endplate), as well as mutations or deficiencies in acetylcholinesterase (AchE) or the acetylcholine receptor in the endplate. Because the clinical designs of these studies vary markedly, so does the strength of the data generated. Therefore, the results are grouped on the following basis: (1) small case reports, (2) open-label retrospective observations, (3) open-label prospective trials, and (4) double-blind, controlled trials.

4.2.1. Small case studies

The first publication testing a β-agonist in MG was a case involving two severely impaired children (age 10 and 11) with a mutation-mediated AChE deficiency who were given the non-specific α/β-agonist, ephedrine in (Bestue-Cardiel et al., 2005). One subject was able to climb a few steps but could only walk 200 m without rest; the second could not lift her arms to a horizontal position, stand on one foot or walk normally. Both patients responded "dramatically" to ephedrine, with results persisting beyond 24 months. No serious adverse effects were reported. Another case study (Sadeh et al., 2011) involved two congenital myasthenic syndrome (CMS) patients with a mutation in the acetylcholine receptor who had a limited response to conventional therapies (pyridostigmine; 3,4 diaminopyridine). Albuterol produced "a dramatic

Table 2
Clinical trials in neuromuscular disorders characterized by a pathology of the NMJ.

DX	Publication	# subj	Drug	Total dose/day	Length	Design	Efficacy	Other notes
MG	Liewluck et al. (2011)	3 + 15	Albut	Adults: 4–12 mg child: lower doses	1–25 mo	Uncontrl	Walking distance & number of steps climbed	Subjects had either DOK7 mutations or AChE deficiency
MG	Burke et al. (2013)	9	Albut	3 m to 8 mg	1.5+ yrs	Uncontrl	Functional ability (walk. dis. & reduced fatigue)	Escalating dosing schedule; DOK7 mutations
MG	Gallenmuller et al. (2014)	2	Albut	8–12 mg	NA	Uncontrl, Case Rep	Walking and stair-climbing	MUSK mutation
MG	Lorenzoni et al. (2013)	5	Albut	6 mg	12 mo	Uncontrl	QMG scores	DOK7 mutations
MG	Finlayson et al. (2013)	1	Albut	6 mg	12 mo	Uncontrl, case rep	QMG score	Slow channel myasth syndrome
MG	Soliven et al. (2009)	8	Terb	7.5 mg	2 wks each leg	Blinded; X-over	QMG score (1° endpoint) & neuromus trans. positive	Patients clinically Dx'd: MG no change in FVC or grip strength
MG	Mihaylova et al. (2008)	5	Ephe	2.2 to 3 mg/kg/day	NA	Case rep	Improved motor symptoms	Patients all had COLQ mutations
MG	Bestue-Cardiel et al. (2005)	2	Ephe	150–200 mg (doses fractionated)	24 mo	Uncontrl, case rep	Mobility improved	AChE deficiency
MG	Sadeh et al. (2011)	2	Albut	6 mg	6 mo and 1 yr	Uncontrl, case rep	Improved strength and in activities-of-daily-living	Ach receptor mutation
MG	Lashley et al. (2010)	12 (10)	Ephe	15 to 90 mg/day	8 mo	Uncontrl	QMG, strength and mobility scores improved	DOK7 mutation
MG	Schara et al. (2009)	8	Ephe	25 mg then up to 75–100 mg	12 to 24 mo	Uncontrl	Muscle strength and exercise tolerance; MRC scale	Dose escalation: start at 25; gradually increase to 75 to 100 mg; DOK7 mutation

Definitions — All subjects have major pathologies of the neuromuscular junction. ACh: acetylcholine, AChE: acetylcholinesterase; Alb: albuterol, blinded: double blind treatment, Eph: ephedrine, FVC: forced vital capacity, MG: myasthenia gravis, including congenital myasthenic syndromes, MRC: manual muscle testing using Medical Research Council scale, N: total subjects enrolled, (number of subjects completing all protocol assessments); QMG: Quantitative myasthenia gravis score, Terb: terbuterol, uncontrl: uncontrolled; no placebo group, X-over: cross-over design (can be blinded or not), but all patients alternatively receive both treatment and placebo Note: for "Num" (i.e., number of subjects), the larger number refers to total subjects enrolled; number in parenthesis refers to number of subjects that completed all protocol assessments (i.e., excludes subjects who withdrew from the protocol for any reason).

improvement" in strength and in activities-of-daily-living with no adverse effects. In another single-patient case report (Finlayson et al., 2013), a 62 year old CMS patient suffering motor impairments due to acetylcholine receptor dysfunction was, following a second respiratory arrest, given albuterol (along with the patient's normal fluoxetine which was regarded beforehand as only marginally effective, alone). A year later, the Quantitative Myasthenia Gravis (QMG) score showed "a striking reduction" (nearly 40%) in severity of symptoms. A final case report studied two brothers who had inherited a defect in MuSK (Gallenmuller et al., 2014). At ages 13 and 16, both manifested serious motor impairments. While esterase inhibitors were ineffective, within a week of albuterol treatment a wheelchair bound patient was able to climb stairs with a bannister while the other patient no longer required any aids to walk, with further improvement occurring as the dose was raised.

4.2.2. Open-label retrospective observations

In one of three retrospective trials, ephedrine was given to five patients with mutations in the acetylcholinesterase (AChE) gene (Mihaylova et al., 2008), and found to reduce motor deficits more effectively than AChE inhibitors and 3, 4-diaminopyridine. A second, retrospective study (Liewluck et al., 2011) evaluated 15 patients with DOK-7 mutations and 3 patients with endplate AChE deficiency. Albuterol produced significant improvements in a 9-point activities-of-daily-life questionnaire in both diagnostic groups ($p < 0.001$). One patient experienced atrial flutter at 2 weeks, causing treatment to be stopped. All other side effects, including muscle cramps, jitteriness, tremor, insomnia and hypertension, were reported to be mild, tolerable, transient, and expected. A final retrospective study (Burke et al., 2013) reported that albuterol produced progressive improvement in all nine children (6 to 15 years old) treated within one month of dosing; the 3 non-ambulatory patients all resumed walking with assistance. Improvements were seen in a number of timed tests as well as a functional test-of-daily-living, with a plateau in improvement reached within one to 1.5 years. No major side effects were reported.

4.2.3. Open-label prospective trials

Four prospective clinical trials were conducted testing β-agonists against myasthenia, one of which was controlled. The first was an open-label, dose-escalation paradigm testing ephedrine in 8 patients with DOK mutations (Schara et al., 2009). Dose-escalation continued until either improvement or side effects were observed. Within weeks, clinical assessments and reports from patients and families established improved muscle strength and exercise tolerance in all patients, particularly in their proximal muscles. No improvement was seen in FVC or neurophysiological tests involving repetitive stimulation. The drug was well tolerated. Another prospective study in 12 patients with a DOK-7 mutation showed progressive improvement in the QMG score ($p = -0.009$) and mobility scores ($p = 0.0006$) over the 6 to 8 month assessment period (Lashley et al., 2010). Proximal muscles were most robustly improved, as were activities of daily living. Ten of the 12 patients tolerated ephedrine (15 to 90 mg/day); 1 had intolerable insomnia and the other elevated blood pressure, but apparently no effort was made to temporarily lower the dose until tolerance to these side effects might occur (see section on controlling side effects). The third, open-label prospective study tested albuterol in 5 patients with a DOK-7 mutation (Lorenzoni et al., 2013). QMG scores at 3, 6, 9 and 12 months showed a "clear beneficial response". Albuterol was generally well tolerated, though some mild side effects (e.g., tremor, occasional palpitations and headache) were initially seen but soon dissipated.

Finally, a most-noteworthy, randomized, double-blind, placebo-controlled cross-over study in 8 subjects with generalized MG tested the selective β-agonist terbutaline (Soliven et al., 2009). Despite the small sample size (and lack of statistical power), the QMG score (the predesignated primary endpoint) showed a significant improvement over placebo ($p = 0.03$), as did neuromuscular transmission, based on repetitive nerve stimulation ($p < 0.05$). On the other hand, the functional disability scale, grip measurements and FVC did not show statistically significant improvement. Terbutaline was well tolerated in all study subjects.

4.2.4. Summary of β-agonist trials in myasthenia gravis

Eleven separate clinical studies have been published by eleven independent groups of investigators administering β-agonists to patients with MG of varying etiologies and pathologies (Table 2). Despite these differences, their single common quality is that stimulating β2-adrenoceptors may provide important symptomatic relief. While only a few of the studies offer conclusive or convincing evidence of efficacy (particularly the uncontrolled trials), collectively they provide intriguing evidence that β-agonists may have some efficacy in treating MG. Though a clear mechanism of action has yet to be elucidated, the fact that such varied etiologies of MG show evidence of possible clinical benefit offers support for the hypothesis that β-agonists might well benefit other diseases where degeneration and dysfunction of the NMJ is an important component of their pathogenesis, such as ALS. The ability of β-agonists to provide apparent benefit to MG, despite the pathogenic diversity of NMJ degeneration provides support for the hypothesis that they may also provide benefit for whatever pathogenic variable(s) are most responsible for the NMJ degeneration in ALS.

4.3. Neuromuscular disorders with a primary myopathology

These disorders differ fundamentally from ALS in that their major pathologies are driven by muscle abnormalities. Nonetheless, evidence that β-agonists may improve their symptoms suggests a possible benefit for ALS, given that gradual loss of muscle strength and function is an important part of the ALS phenotype. Therefore, the efficacy of β-agonists in treating diseases that primarily involve degeneration of the musculature suggests utility in treating ALS. However, because it does not directly address the main deficiencies of ALS, a review of the individual studies will be more cursory, complemented by individual listings in Table 3.

4.3.1. Muscular dystrophy (MD)

Muscular dystrophy refers to a group of muscle diseases that share the important clinical phenotype of a weak and progressively deteriorating musculoskeletal system, caused by defects in muscle proteins and degeneration and death of muscle cells, clinically accompanied by significant impairments in locomotion. Duchenne is the most common childhood form of muscular dystrophy and is caused by mutations or deletions in the DMD-gene, which expresses dystrophin (a cytoplasmic protein essential for the integrity of the muscle cell).

Three small, open label trials were conducted with albuterol. In two of the three (Fowler et al., 2004; Skura et al., 2008), a significant increase in lean body mass and reduced fat mass was achieved, though no differences were found in isometric knee, manual muscle tests, or time to run/walk 30 ft. Importantly, in another small study of four different types of muscular dystrophy, clenbuterol was beneficial to muscles whose mass was more preserved, but failed to improve the most atrophic muscles. This suggests that β-agonists may be most effective in the early stages of muscular dystrophies and atrophies.

4.3.2. Facioscapulohumeral dystrophy (FSHD)

FSHD is an autosomal dominant disease caused by a dual genetic error involving a 'toxic gain of function' of the DUX4 gene (a homeodomain transcription factor), along with deletion of D4Z4 repeats. It usually affects the skeletal muscles of the face, scapula and upper arms.

Four clinical trials tested albuterol in FSHD, three of which were randomized, double-blind controlled designs (Kissel et al., 2001; Payan et al., 2009; van der Kooi et al., 2004). Collectively, the results of these tests of albuterol in FSHD are mixed, in that 3 of the 4 studies

Table 3
Clinical trials in neuromuscular disorders with a primary myopathology.

DX	Publication	# subj	Drug	Total dose/day	Length	Design	Efficacy	Other notes
DMD	Fowler et al. (2004)	9	Albut	4 mg for 4 d then 8 mg	12 wks	Blinded; X-over	Isometric knee extensor and MMT improved	
DMD	Skura et al. (2008)	14	Albut	12 Mg: Sustained release	3 mo each arm	Blinded; X-over	Time to walk 30 m and lean body mass improved	Isometric knee and MMT showed no effect
MD mixed	Oya et al. (2001)	4	Clen	30 and 40 μg	6–18 mo	Uncontrl	Muscles mass increased	Benefits only early in disease
FSHD	Kissel et al. (1998)	15	Albut	8 mg: 1 wk. then 16 mg	3 mo	Uncontrl	Lean body mass increased and MVICT improved	
FSHD	Kissel et al. (2001)	90 (84)	Albut	8 mg to 32 mg (dose escalation/wk: 8 mg; 16 mg; 16 mg–32 mg)	13 mo	Blinded/ controlled	Muscle mass and strength improved	MVICT and MMT showed no effect
FSHD	van der Kooi et al. (2004)	65 (61)	Albut	8 mg for 2 wks then 16 mg	6.5 mo	Blinded/ controlled	Muscle volume increased and MVICT improved	
FSHD	Payan et al. (2009)	56	Albut	16 mg: Sustained release	6 mo	Blinded/ controlled	No sig. Benefit on QMT, MMT or timed motor tasks	Dosing schedule: 3 wks on; 1 wk. off
Post-surgery	Maltin et al. (1993)	20	Clen	40 μg	1 mo	Blinded/ Controlled	Leg strength improved	
Cachx	Greig et al. (2014)	13 (7)	Form	80 μg	2 mo	Uncontrl	Muscle size/function increased; quad &grip strength improved	Also given appetite stimulant (megestrol)
AMD	Angelini et al. (2004)	5	Albut	6 to 12 mg/day IV for 10 days; then monthly	6 mo	Uncontrl	Improved muscle mass and global functional tests	Long-term follow up for over 3 years
CCD	Schreuder et al. (2010)	1	Albut	8 mg	1 yr	Uncontrl, case rep	Improved FEV; increased strength	Central core disease, plus mitochondrial dysfunction
CCD	Messina et al. (2004)	13 (10)	Albut	8 mg	6 mo	Uncontrl	Improved myometry; MRC scores, FVC	

Definitions — Alb: albuterol, AMD: acid maltase deficiency, CCD: central core disease, Clen: clenbuterol, controlled: randomized & placebo-controlled, DMD: Duchenne's and Becker's muscular dystrophy, FSHD: facioscapulohumeral dystrophy, Form: formoterol; MD: muscular dystrophy, FEV: forced expiratory capacity, FVC: forced vital capacity, MMT: manual muscle test, MRC: Medical Research Council, MVICT: maximum voluntary isometric contraction, N: total subjects enrolled (number of subjects completing all protocol assessments); post-surgery: post surgical muscle recovery, QMT: quantitative muscle testing, uncont: uncontrolled; no placebo group, X-over: cross-over design (can be blinded or not), but all patients alternatively receive both treatment and placebo.

produced some evidence for enhanced strength, while one of three randomized, double-blind, placebo-controlled trials was able to achieve statistical significance on its a priori-defined, primary outcome measure (van der Kooi et al., 2004). These results are therefore intriguing, but must be considered inconclusive. Uncertainty remains as to whether more robust and consistent effects might be achieved with more potent, long-lasting and selective β_2-agonists (e.g., clenbuterol, formoterol), as well as the use of a dosing schedule designed to minimize transient side effects and β_2-adrenoceptor desensitization.

4.3.3. Acid maltase deficiency (AMD)

Acid maltase deficiency (also called Pompe disease and Glycogen Storage Disease type II) is a lysosomal storage disease resulting from a genetic defect that causes a lack of acid maltase, which normally breaks down glycogen. This causes muscular wasting, accompanied by a slowly progressing weakness. Like ALS, respiratory complications are the main cause of death.

An open-label, prospective study (Angelini et al., 2004) tested intravenous albuterol in 5 adult AMD patients. A late-stage elderly patient who had been diagnosed 40 years earlier required an overnight respirator and had the worst functional score. He could not tolerate i.v. albuterol and was not included in the analysis. The other four subjects showed varying improvements in the functional scale score and the MRC Score (Medical Research Council) composite.

4.3.4. Central core disease (CCD) and multi-mini core disease

Central core disease is an autosomal dominant congenital myopathy disease caused by mutations in the gene that expresses the ryanodine receptor 1 (essential for skeletal muscle function), while multi-minicore disease shares somewhat similar clinical and pathologic features. Both are manifested by a loss in muscle tone and weakness, which, while typically not progressive, is nonetheless persistent.

In a case report, a 9-year old boy diagnosed with central core disease plus mitochrondrial dysfunction (Schreuder et al., 2010) was given albuterol for a year. Significant progress was seen in activities of daily living (e.g. self-care, sitting up, raising arms above shoulders, independent feeding, improved speech and writing), compared to years prior to albuterol treatment. Improved FEV and increased muscle strength were also noted.

An open-label study of 18 patients with central core and multi-minicore diseases tested albuterol (Messina et al., 2004). Two patients with central core disease withdrew after 4 months because they did not note any improvement, while one with minicore disease withdrew due to increased tremors and palpitations. The remaining 10 subjects completed the 6 months study without any significant adverse effects, showing significant increases in myometry, MRC scores and FVC.

4.3.5. Post-surgical muscle recovery

Twenty otherwise healthy patients requiring medial meniscectomy were enrolled into a placebo-controlled, double-blind trial, given either clenbuterol (20 μg b.i.d.) or placebo for 4 weeks, followed by a two week wash out period (Maltin et al., 1993). Clenbuterol produced a more rapid recovery of strength in knee extensor muscles in the operated leg, and in the non-operated leg strength was increased above baseline ($p = 0.01$). The authors concluded that clenbuterol "has therapeutic potential in the treatment of muscle-wasting conditions".

4.3.6. Cachectic patients with advanced malignancy

Cachexia (or wasting syndrome) involves a significant loss of weight, muscle atrophy, fatigue, weakness and loss of appetite that often accompanies late-stage malignancies, as well as AIDS and several other diseases. Thirteen patients with advanced malignancy and involuntary weight loss were treated with formoterol. Six patients withdrew before 8 weeks due to the frail, comorbid condition of the patient population (according to the authors). One withdrew due to supraventricular tachycardia. Other formoterol side effects were mild to moderate, transient, and tolerable, involving tremor and peripheral edema. Of the 7 patients who remained in the study for 8 weeks, 7/8

(86%) achieved what was described as a major response for muscle size and/or function, including an increase in quadriceps volume ($p = 0.004$) and a trend toward increased quadriceps and grip strength.

5. Summary of pharmacological experience with β_2-agonists in neuromuscular degeneration

Though relatively little attention has been given to a possible therapeutic role of β_2-agonists for ALS, the cumulative weight of evidence suggests that this class of pharmaceuticals may benefit a wide range of neural and/or muscular pathologies characteristic of several other human neuromuscular diseases. Importantly, each of these diseases shares important pathologies with ALS. The broad signaling pathways and pharmacologic responses induced by β_2-agonists suggest that the pathologies of ALS might similarly benefit, despite clear mechanistic differences in the pathogenesis. The strongest evidence argues that appropriate dosing of selective β_2-agonists should enhance muscle bulk and muscle strength in ALS patients. While this may have indirect benefits on degenerating motor nerve terminals (by promoting a healthier terminal target), there is clear evidence that β_2-agonists may directly protect and restore motor neurons and NMJ in animal models and human diseases sharing some of the same degenerative changes as ALS. Finally, β_2-agonists suppress pathogenic inflammation and induce lipolysis, resulting in greater glucose energy metabolism while enhancing mitochondrial biogenesis and function. Any one of these pharmacological effects might be expected to improve the status of ALS patients, while several of them working synergistically might constitute a transformative therapy for ALS.

The availability of relatively long-lasting, orally active, safe and selective β_2-agonists, such as clenbuterol and formoterol, makes further tests and possible treatment for ALS convenient, practical and compelling.

6. Monitoring and managing potential side effects and toxicity.

The majority of the side effects of β_2-agonist therapy were mild, transient and consistent with known adrenergic pharmacology (below). Unfortunately, the majority of human trials have used non-specific β-agonists with a greater spectrum of adverse effects, although more current animal studies have observed greater efficacy and fewer side effects using more selective β_2-agonists. Additionally, few clinical studies incorporated a dose titration regimen, which may have allowed tolerance to β_2-adrenoceptor stimulation to develop. This may have significantly limited the number and intensity of side effects, as demonstrated in recent animal studies. Notwithstanding these limitations in dosing schedules, most clinical studies reported side effects as either non-existent or mild, transient, and tolerable.

6.1. Minor, common sympathetic-mediated side effects

The most common side effects reported involve sympathetic-like responses, including: muscle tremors, fasciculations and cramps, tachycardia, palpitations, increased blood pressure, insomnia, excitability, irritability, nervousness, facial flushing, nausea, headache and sweating. When these occur, they typically appear quickly, peak within the first 3 to 4 days of β_2-agonist use and are transient, often dissipating with continued drug exposure (i.e., 'tolerate out') within days of dosing. Importantly, they are generally dose-related and quickly reversible when doses are lowered. Except for rare exceptions, these events are described as mild and tolerable. However, it is important to note between-subject variability exists. While the majority of studies report no, or mild, transient, and tolerable adverse events, patients withdrew from the protocol on rare occasions because of tremors and palpitations. Therefore, careful monitoring of individual patients for unusual sensitivity seems prudent.

Because the side effects are clearly dose-related, if the patient cannot tolerate a side effect or wait for the development of tolerance, temporarily lowering the dose should lessen or eliminate the side effect. One practical means of reducing the probability of an adverse event is to employ a dosing protocol starting with relatively low doses and then increasing the dose over the course of several days to a week or more. Careful monitoring by a physician familiar with each patient may be important.

In the event that these side effects persist at doses believed essential for neuromuscular benefit, then concomitant medication can be used to manage each symptom. For example, NSAIDs or acetaminophen can be used to treat headaches; muscle cramps can often be avoided by staying well-hydrated or by consuming bananas and oranges. For more serious cramps, prescription potassium medication with superior bioavailability is available as a safe and effective option. Over-the-counter taurine supplementation (at 3–5 g per day) reportedly helps to prevent or minimize cramping. If insomnia persists, over-the-counter sleep aids can be tried, and if necessary, a prescription sedative might be helpful as a temporary solution. Similarly, prescribed medications, accompanied by careful patient monitoring can be considered for treating nervousness, irritability and nausea.

6.2. Potential cardiovascular toxicity

Some rat studies report cardiac hypertrophy following very high, chronic doses of β_2-agonists (Lynch and Ryall, 2008); (Ryall and Lynch, 2008). Importantly, the cardiac hypertrophy was transient, in that a 4-week washout period was sufficient to return the heart back to baseline sizes. There is also evidence that cardiac hypertrophy can be minimized or prevented by dose titration and/or employing a drug holiday (Lynch et al., 1999). By testing a wide range of doses in rats, the anabolic effects of formoterol on skeletal muscle were found to occur at far lower doses than those required to induce cardiac hypertrophy (Ryall et al., 2006), presumably because of that drug's greater selectivity for β_2-adrenoceptors, which comprise about 90% of the β-adrenoceptors in rat skeletal muscle (Jensen et al., 1995). For treating ALS with selective β_2-agonists this advantage is even greater, because the population of β-adrenoceptors in human skeletal muscle is nearly exclusively the β_2 subtype (Elfellah et al., 1989). Moreover, while the human heart contains some β_2 adrenoceptors (20–40%), the majority are β_1 receptors, which should not be targeted by formoterol (Brodde et al., 2001). Most importantly, no hint of cardiac hypertrophy was seen in any of the clinical studies reported, to date.

Tachycardia is one of the first indications that β_2-agonists are having a biological effect, and may be used as a surrogate marker for dose adjustment and the avoidance of cardiotoxicity. One blinded, crossover, placebo-controlled study tested a wide dose range of oral formoterol (40 μg to 320 μg) and albuterol (2 to 18 mg) (Lofdahl and Svedmyr, 1989). Heart rate, diastolic blood pressure and skeletal muscle tremor showed no significant changes at lower doses of either drug; the two drugs showed parallel dose–response effects on all measures with increasing doses. For the purposes of this discussion, the greatest shortcoming of the data is that only an acute, single dose was tested, with observations lasting only 8-h. Therefore, the well-established tolerance to side effects that often occurs after 3 to 4 days of β_2-agonist dosing had no chance to occur. No significant increases in heart rate were reported with oral formoterol at or below 180 μg per day for a week, with only a modest, statistically significant increase occurring at 320 μg per day (Lee et al., 2013). Overall, nearly three dozen published human trials have tested β2-agonists against a wide variety of neuromuscular maladies with no problematic cardiovascular effects noted, except for single patients in each of two studies who displayed a ventricular tachycardia and an atrial flutter, respectively (Greig et al., 2014; Liewluck et al., 2011).

In conclusion, while the apparent cardiovascular risks of chronic, high-dose systemic β_2-agonists are often mentioned in the literature, the current experience with these compounds in a variety of human neuromuscular disorders suggests a much lower liability. However, a single case report of an otherwise healthy 17-year-old body builder in Eastern Europe may be worth noting as an exception to that point.

The patient self-administered clenbuterol (20 mg b.i.d. on a rotating schedule of two days on/two days off), claimed he took no other illicit drugs or steroids, and suffered a nonfatal coronary infarction (Kierzkowska et al., 2005). The authors of the case report acknowledged they had no means of confirming the patient's reported drug use. More importantly, the clenbuterol dose was approximately 500-times higher than that administered in the therapeutic clinical studies. Therefore, it seems possible that more serious cardiovascular effects may occur with extremely high doses of β-agonists, consistent with the appearance of cardiac hypertrophy in high dose animal studies. Nonetheless, the doses recommended for neuromuscular therapy are far below the cardiotoxic doses. Moreover, possible cardiovascular toxicity may be avoided by monitoring heart function to assure that tachycardia or hypertension does not persist. If noted, a judgment weighing the possible risks and dose de-escalation might be employed, keeping in mind that relatively mild and transient changes in these cardiovascular parameters often occur at the onset of dosing or soon after dose escalation. If these events are relatively mild, time might be allowed for tolerance to develop, under the close supervision of a physician.

6.3. β-Adrenoceptor desensitization

Continuous activation of β-adrenoceptors results in the gradual attenuation of the biological response (i.e., tachyphylaxis), a process precipitated in large part by receptor desensitization. Following β-adrenoceptor phosphorylation, the receptor is internalized for dephosphorylation and subsequent recycling to the membrane surface, or for degradation. In the case of β_2-adrenoceptor down-regulation, a number of possible complications may arise, ranging from the loss or reduction of β-adrenoceptor mediated effects impairing sympathetic function, to overcompensation of other systems in response to decreased sympathetic tone. Anecdotally, body builders have routinely implemented 'drug holidays' to avoid a 'wearing off' of the anabolic effects of β-agonists. A recent study in rats administered clenbuterol (Sirvent et al., 2014) observed increased muscle fatigue over time (an observation occasionally reported by others with continuous, chronic use), impaired intracellular Ca^{2+} signaling and increased calcium-dependent calpain proteolysis. The authors concluded that chronic clenbuterol treatment might impair calcium homeostasis that could contribute to remodeling and functional impairment of fast twitch skeletal muscle fibers. These observations argue for the inclusion of 'drug holidays' into chronic dosing schedules and the importance of carefully monitoring patients for changes in status to differentiate diminished pharmacological response due to receptor desensitization versus possible disease progression accompanied by loss of function.

Another potential concern is that once the β-adrenoceptor system has achieved a highly desensitized state, secondary problems such as a rapid 'rebound response' could occur following abrupt discontinuation of β-agonist administration. Addressing this issue requires careful selection of the dose and dosing schedule, along with regular and thorough patient monitoring. This poses the question, 'What dosing schedule might be applied to mitigate problems associated with β_2-adrenoceptor desensitization?' It is generally acknowledged that minimizing receptor desensitization requires a dosing schedule that alternates periods of drug administration with 'drug holidays'. Because the optimal dosing schedule will vary depending on the specific β-agonist, its dose level and the individual, whatever dosing regimen is selected must be complemented with careful observation of the patient and possible modifications to dose in an attempt to assure that desensitization has not occurred. If desensitization is strongly suspected, changes to the dosing regimen should be considered, ranging from increasing the dose (assuming intolerable side effects are not induced) to inserting an extra one-to-two week drug holiday, after which dosing is resumed while careful monitoring by a physician continues.

6.4. Drug interactions

In addition to the possible side effects resulting from β_2-adrenoceptor stimulation, there exists the possibility of unwanted side effects arising from interactions with other drugs taken by ALS patients. While few studies in the literature warn of serious drug–drug interactions with β-agonists, it is always wise for the prescribing physician to monitor and manage possible interactions. It has been noted that β-agonists may potentiate the effects of theophylline and anticholinergics, while an increased risk of arrhythmias may occur with concomitant administration of β-agonists and MAO-inhibitors or tricyclic antidepressants. Caution and careful, regular monitoring should be employed in these cases, as well as with those patients with underlying cardiopathologies and/or the use of β-agonists or β-antagonists for other medical reasons. Risks of complications from possible drug interactions need to be weighed against the intended benefits of treating a progressive, life-threatening disease for which there currently is no adequate therapy.

7. Discussion and conclusions

This review explores the hypothesis that stimulation of β_2-adrenoceptors may constitute a novel, safe and effective therapy for ALS. Research in animal models has established that β-agonists can provide a wide variety of beneficial pharmacological effects, mediated via the plethora of signaling pathways and transcription factors that alter the structure and function of skeletal muscle, neurons, glia and immunocytes in ways that could improve the status of ALS patients. Moreover, over 30 clinical studies, several of which were double-blind and placebo-controlled, tested β-agonists in different neuromuscular diseases that share one or more pathologies with ALS. While the ALS community has so far largely ignored β-agonists as potential therapies, the 'weight of evidence' from studies involving other neuromuscular disorders provides intriguing, albeit preliminary evidence that β_2-agonists may correct many of the serious neural and muscular deficiencies also seen in ALS. The possibly of synergistic interactions between the multiple pathways induced by β_2-agonists provides even more compelling support for the hypothesis.

The fact that several long-lasting, orally active and selective β_2-agonists have been approved and used safely in humans for decades provides a convenient, practical and compelling means for more direct and definitive testing of this hypothesis. Positive results obtained in ALS patients tested in appropriately controlled and sufficiently powered clinical trials could quickly lead to 'repositioning' or 'repurposing' oral β_2-agonists for treatment of ALS. This circumstance conceivably creates an unprecedented opportunity for rapidly transforming the treatment of this serious, unmet medical need.

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