A Clinical Investigation of the Safety and Efficacy of Clenbuterol on Motor Function in Individuals with Amyotrophic Lateral Sclerosis

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4 List of Abbreviations

Adverse Event (AE)
ALS Functional Rating Scale (ALSFRS-R)
Alanine Aminotransferase (ALT)
Amyotrophic Lateral Sclerosis (ALS)
Aspartate Aminotransferase (AST)
Creatine Kinase (CK)
Enzyme Replacement Therapy (ERT)
Forced Vital Capacity (FVC)
Institutional Review Board (IRB)
Late-Onset Pompe Disease (LOPD)
Left Ventricular Assist Devices (LVAD)
Left Ventricular (LV)
Maximum Voluntary Ventilation (MVV)
Microgram (mcg)
Not Applicable (NA)

5 Study Summary

Title	A Clinical Investigation of the Safety and Efficacy of Clenbuterol on Motor Function in Individuals with Amyotrophic Lateral Sclerosis
Short Title	Clenbuterol in ALS
Protocol Number	NA
Phase	Phase ½
Methodology	Single arm
Study Duration	24 weeks
Study Center(s)	Single-center
Objectives	The goals are to determine safety and efficacy with regard to motor function of oral clenbuterol in subjects with ALS
Number of Subjects	25
Diagnosis and Main Inclusion Criteria	1. Diagnosis of ALS according to the El Escorial criteria 2. Age: 18+ years at enrollment.
Study Product, Dose, Route, Regimen	Clenbuterol (Spiropent): 40 to 80 mcgs BID per oral (up to 160 mcg per day maximum)
Duration of administration	24 weeks
Reference therapy	Standard of care at baseline
Statistical Methodology	The critical test of treatment efficacy will be the comparison of the ALSFRS-R slope during treatment to the estimated pre-treatment slope.

6 Introduction

This document is a protocol for a human research study. This study is to be conducted according to US and international standards of Good Clinical Practice (FDA Title 21 part 312 and International Conference on Harmonization guidelines), applicable government regulations and Institutional research policies and procedures.

6.1 Background

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease that lacks effective treatment, proceeding to respiratory failure and premature death for affected individuals. The neuropathy of ALS involves both upper motor neurons (UMN) and lower motor neurons (LMN) with critical secondary muscle involvement (KINSLEY AND SIDDIQUE 1993). UMN signs include hyperreflexia, Babinski sign, increased muscle tone, and weakness. LMN signs include weakness, muscle wasting, hyporeflexia, muscle cramps, and fasciculations. Affected individuals typically present with either asymmetric weakness of the extremities (stumbling or poor handgrip), termed "limb onset" or bulbar findings (dysarthria, dysphagia). Limb onset is more frequent than bulbar onset. Once it begins, the weakness progresses, ultimately leading to total disability and dependence and shortened survival. The mean age of onset is 56 years in individuals with no known family history and 46 years in individuals with more than one affected family member (familial ALS or FALS). Average disease duration is about three years, but it can vary significantly. Death usually results from compromise of the respiratory muscles. Multiple confirmed genetic and suspected environmental causes have been described (KINSLEY AND SIDDIQUE 1993). Current ALS treatments have little effect on disease progression and are mainly directed at optimizing quality of life (Oskarsson and Staff 2018).

The lack of effective therapy to reverse weakness or arrest ALS disease progression represents a critical unmet medical need. Clenbuterol's ability to promote muscle hypertrophy and improve muscle function make it an attractive option for filling this need. Clenbuterol has been shown to stimulate muscle hypertrophy through inducing expression of insulin-like growth factor (Igf) -1 and Igf-2 and their receptors (MATSUMOTO *et al.* 2006).

Clenbuterol is a selective β2 agonist used for the treatment of asthma in Europe with a well-established record of safety. In asthmatic patients with exercise induced bronchospasm a dose of 20 mcg of clenbuterol given intravenously over 3-4 minutes resulted in negligible cardiac effects (DEL BONO *et al.* 1979). Subjects with asthma given 80 mcg of clenbuterol had a mild increase in heart rate, but no arrhythmia or significant electrocardiographic changes, tremor and headache has also been noted in asthma patients as well (WHITSETT *et al.* 1981). Multiple other studies in asthmatics and patients with chronic airway obstruction, found none or very mild AE (PASOTTI *et al.* 1979; TSCHAN *et al.* 1979; DI GIOACCHINO *et al.* 1987). These and other studies reassure us regarding the potential use of clenbuterol in ALS (BIRKS *et al.* 2006; KAMALAKKANNAN *et al.* 2008).

Clenbuterol has been well tolerated and had beneficial muscle effects in a Phase I/II clinical trial (160 mcg/day) that enrolled patients with late-onset Pompe disease who were previously treated with ERT (KOEBERL *et al.* 2018). Similar dosages have increased muscle strength or increased muscle mass in other studies. Patients with chronic heart failure developed increased lean muscle

mass after taking clenbuterol at 80 mcg/day for 12 weeks (KAMALAKKANNAN *et al.* 2008). A small group of patients with Duchenne muscular dystrophy developed increased power and volume of well-preserved muscle following 18 months of taking clenbuterol, 30-40 mcg/day (OYA *et al.* 2001). Adverse events (AEs) associated with clenbuterol may include tremor, muscle cramps, nervousness, palpitations, tachycardia, and headache (Table 1), and are typically well-tolerated. These studies demonstrated the benefits of clenbuterol's effects on muscle, and its safety and tolerability in a variety of conditions other than ALS.

A previous small open-label pilot trial of clenbuterol in patients with ALS showed very promising results (SORARU *et al.* 2006). Sixteen patients with ALS were started on 60 mcg/day, and fourteen completed 6 months of treatment. Significant improvements in limb muscle strength and forced vital capacity (FVC) occurred in these patients, and a functional measure called the ALS functional rating scale score was stable over the course of the trial. Improvements and stability on these recognized ALS outcome measures are not typical of the natural history of this disease. Again, clenbuterol was found to be safe and well-tolerated (**Table 1**). The dramatically positive results of this pilot trial need to be replicated.

Table 1: Long-Term Clinical Studies with Clenbuterol

Study	**Study Design**	**Number of Subjects**	**Dosage and Regimen**	**Adverse Events**
(DI GIOACCHINO *et al.* 1987)	Open label study of clenbuterol in exercise induced asthma	14 patients	20 mcg bid for 60 days	Small increase in heart rate
(ISHIKO *et al.* 2000)	Randomized study of clenbuterol and physiological therapy (PT) in stress incontinence	61 patients assigned to three groups A-clenbuterol only B- PT only C - both	20 mcg bid for 12 weeks in 41 patients (group A and C)	1) Two patients with headache 2) One patient with mild hepatopathy 3) One patient with rash 4) One patient with numbness
(SORARU *et al.* 2006)	6 month open label dosing in patients with **ALS** already on riluzole	16 patients	20 mcg TID for 6 months	Tremor, nervousness, cramps, fasciculations (details not provided)
(KAMALAKKANNAN *et al.* 2008)	Single site, randomized, double blind placebo controlled in subjects with class 2 or 3 congestive heart failure	19 subjects -- 10 placebo 9 clenbuterol	20 mcg BID, increased to 40 mcg BID after 7 days – 12 weeks total	1) Two clenbuterol subjects withdrew, one with asymptomatic ventricular tachycardia, and one with muscle cramps without elevated CK 2) Six clenbuterol and two placebo subjects with muscle cramps 3) CK elevated in 5

				subjects on clenbuterol, and in 4 on placebo 4) Tremors in 5 subjects on clenbuterol and two on placebo
(KOEBERL *et al.* 2018)	52 week double-blind, placebo controlled, prospective study in LOPD patients stably treated with ERT for >1 year	13 randomized, 11 completed	40 mcg for 1 week, 40 mcg BID for 5 weeks, 80 mcg AM + 40 mcg PM for 1 week, 80 mcg BID for 40 weeks	4 subjects with tremor 4 subjects with insomnia 4 subjects with muscle spasms

6.2 Investigational Agent

ACTIONS AND MODE OR MECHANISMS OF ACTIONS
Clenbuterol is a broncholytic agent which acts through selective stimulation of the β_2-receptors. It differs from other β_2-specific sympathomimetics by its low dose, long biological half-life and rapid and complete absorption by oral administration. It has no significant effects on β_1-cardiac receptors.

PHARMACOLOGY
Pharmacokinetics: Invasion half-life of clenbuterol is 1 hour, and the distribution corresponds to an open two compartment model with a side-compartment. Elimination from plasma takes place in 2 phases, the half-life of the a-phase being 1 hour and that of β-phase 34 hours. Elimination is primarily renal (87% after 168 hours) 43% of clenbuterol is recovered unchanged in the urine. Dose intervals of 12 hours suffice to maintain a plasma level. Five metabolites have been found in humans.

INDICATIONS
Prophylaxis and therapy of bronchospasm in bronchial asthma, acute and chronic bronchitis associated with emphysema.

CONTRAINDICATIONS
Thyrotoxicosis, idiopathic hypertrophic subvalvular aortic stenosis, tachycardia, tachyarrhythmia. Known sensitivity to sympathomimetic amines.

SIDE EFFECTS/ADVERSE REACTIONS
Side effects rarely occur at the recommended doses. Some patients could have, at the beginning of the treatment, tremor, nervousness, extrasystoles, tachycardia, palpitations, vertigo, anxiety, headache and nausea. In these cases the dose should be reduced.

PRECAUTIONS/WARNINGS

Warnings: Although animal studies have shown no teratogenic effect, clenbuterol should not be given during the first three months of pregnancy.

Precautions: Care must be exercised when additional sympathomimetic agents or IMAO drugs are administered. Clenbuterol should be used with caution in patients with hypertension, coronary heart disease, hyperthyroidism and diabetes. Caution should be used when treating patients with recent cardiac infarction.

6.3 Preclinical Data

Teng and colleagues investigated the effect of clenbuterol upon longevity in a transgenic murine model of familial ALS (TENG et al. 2006). Clenbuterol treated mice demonstrated improved neuromuscular function through better Rotarod performance, slower disease progression, and reduced loss of lumbar motor neurons and body weight. These benefits are attributable to clenbuterol's induction of muscle Igf -1 expression and the associated muscle hypertrophy (MATSUMOTO et al. 2006), because the expression of Igf-1 with gene therapy in muscle has also improved muscle function and survival in mice with ALS (KASPAR et al. 2003). These data suggest that clenbuterol will have beneficial effects both through muscle hypertrophy and neuroprotection in ALS. The main symptoms of ALS stem from muscle weakness, and respiratory muscle weakness is the main cause of mortality.

6.4 Dose Rationale and Risk/Benefits

The selected dose (80 mcg BID) is based upon the experience with the long-term administration of clenbuterol, specifically the beneficial muscle effects in a Phase I/II clinical trial that enrolled patients with late-onset Pompe disease who were previously treated with ERT (KOEBERL *et al.* 2018).

The potential benefits outweigh anticipated risks for daily clenbuterol administration in patients with ALS. Clenbuterol treatment has been associated with specific AEs that may include tremor, muscle cramps, nervousness, and headache (Table 1), as observed in our Phase I/II study in patients with Pompe disease (KOEBERL *et al.* 2018). Cardiovascular adverse effects of clenbuterol may include palpitations associated with an increase in heart rate. However use of clenbuterol in conservative doses (up to 720 mcg/day) is well tolerated. In a pilot study of patients with congestive heart failure (CHF), clenbuterol was given at 40 mcg twice daily for 12 weeks in 19 patients (n=10 clenbuterol, n=9 placebo) with chronic heart failure in addition to their standard CHF therapy. *The drug was well tolerated and there were no serious AEs in that study*. Only two patients required discontinuation of study drug (one due to asymptomatic slow ventricular tachycardia seen on a 24-hour electrocardiogram and the other due to muscle cramps). Muscle cramps occurred in 6 clenbuterol subjects; only one subject required dose reduction. CK was elevated in 6 clenbuterol subjects (range of peak CK was 300-664 mg/dl, normal range 51-294 mg/dl) and mild tremors were reported in five subjects. No subjects required dose reduction due to elevation of CK or tremors in that study.(KAMALAKKANNAN *et al.* 2008) In our study in Pompe disease two subjects required dose reduction to 120 mcg from 160 mcg twice daily, due to minor AEs that resolved subsequently (KOEBERL *et al.* 2018).

In a pilot study of patients with congestive heart failure (CHF), clenbuterol was given at 40 mcg BID per oral for 12 weeks in 19 patients (n=10 clenbuterol, n=9 placebo) with chronic heart failure in addition to their standard CHF therapy. The drug was well tolerated and there were no serious adverse effects in any of the study subjects. Only two patients required discontinuation of study drug (one due to asymptomatic slow ventricular tachycardia seen on a 24- hour electrocardiogram and the other due to muscle cramps). Muscle cramps occurred in 6 clenbuterol subjects; only one subject required dose reduction. CK was elevated in 6 clenbuterol subjects (range of peak CK300-664 mg/dl, normal range 51-294 mg/dl) and mild tremors were reported in five subjects. No subjects required dose reduction due to elevation of CK or tremors (KAMALAKKANNAN *et al.* 2008).

Other studies generated data about the use of clenbuterol in patients with cardiac disease (Table 2). Clenbuterol in high doses (up to 2100 mcg/day) has been administered to 19 patients with end-stage HF supported with an LVAD, without significant arrhythmia or AE (YACOUB 2001; BIRKS *et al.* 2006). A study of clenbuterol in seven patients with implanted left ventricular assist devices (LVAD), a maximal dose of 720 mcg per day was given over 3 months. All seven patients were treated with and continued a maximum tolerated dose of metoprolol prior to initiation of clenbuterol. This dose of clenbuterol was well tolerated in all subjects. Serum creatine kinase (CK) was elevated in four subjects on clenbuterol, peak range (314-1497mg/dl). Mild tremors were seen in three subjects; mild muscle cramps were reported in four. No dose reduction was required for AE and there were no arrhythmic events (GEORGE *et al.* 2006). In another study of 15 patients with LVAD in heart failure were treated with clenbuterol. The first stage of treatment was four drugs, lisinopril, carvedilol, spironolactone, and losartan. The second phase of treatment was initiated when carvedilol was replaced with bisoprolol, and clenbuterol was started at a dose of 40 mcg three times daily and titrated to a maximal dose of 700 mcg TID (2100 mcg/day). Mild tremor was seen in all patients, muscle cramps in 3, but without elevated CK, and 1 patient had diaphoresis (BIRKS *et al.* 2006). A prospective study in 2011 looked again at this same pharmacological therapy of 20 patients with continuous flow LVAD with heart failure again showing that clenbuterol was well tolerated, with 4 patients developing muscle cramps and 2 developing mild tremor (BIRKS *et al.* 2011).

Clenbuterol has also been shown to be well tolerated when used to treat urinary incontinence in Germany and Japan (Table 2). In a controlled double blind study of 39 patients with motor urge incontinence, 20 were treated with 10 mcg clenbuterol three times a day and the other 19 were treated with flavoxate hydrochloride for 6 weeks. Adverse events consisted of 4 patients with trembling fingers and tachycardia, 3 patients with nervousness and 1 patient stopped due to a drug interaction with an alpha stimulating, antihypertensive drug, clonidine which caused dizziness (GRUNEBERGER 1984). In a comparison study of three groups of women all with stress incontinence, Group A got clenbuterol only, Group B got physiological therapy and Group C got both. Clenbuterol was given at a dose of 20 mcg twice daily for 12 weeks. Adverse events consisted of 2 patients with headache, 1 patient with mild hepatopathy, 1 patient with rash and 1 patient with numbness (ISHIKO *et al.* 2000).

Table 2: Other Clinical Studies Using Clenbuterol

Heart Failure Subjects (Reference)	**Study Design**	**Number of Subjects**	**Dosage and Regimen**	**AEs**
(BIRKS *et al.* 2006)	All received LV assist device and were in severe heart failure, primary pharm regiment and the secondary regiment of clenbuterol	15 subjects	40 mcg BID, titrated up to 40 mcg TID then titrated up to 700 mcg TID	Mild tremor in all 4 with muscle cramps without elevated CK Diaphoresis in 1
(GEORGE *et al.* 2006)	All with LV assist devices with heart failure	7 subjects	Started at 40 mcg TID titrated up to 720 mcg a day over 6 weeks, 12 weeks of total treatment	4 with elevated CPK, all had increases in CPK 3 with tremors 4 with muscle cramps
(BIRKS *et al.* 2011)	All received LV assist device and were in severe heart failure, primary pharm regiment and the secondary regiment of clenbuterol	20 subjects	16 of the 20 made it to stage 2 to receive clenbuterol started at 40 mcg BID and increased to 700 mcg TID	4 with muscle cramps 2 with mild tremors 2 with ventricular tachycardia that was not clenbuterol
Asthma/ Chronic Airway obstruction Subjects (Reference)	**Study Design**	**Number of Subjects**	**Dosage and Regimen**	**AEs**
(WHITSETT *et al.* 1981)	Single oral doses of clenbuterol compared to placebo, randomized double blind crossover in subjects with reversible obstructive airway disease	13 subjects, 10 finished study	Multiple single oral doses in 40, 60 and 80 mcg	Tremor and headache

(TSCHAN *et al.* 1979)	Dose response study of clenbuterol in subjects with obstructive lung disease	12 subjects	6,12, 24 and 48 mcg given qd for 4 days	No side effects noted
(PASOTTI *et al.* 1979)	Double blind comparison of clenbuterol and salbutamol in subjects with asthma	30 subjects	15 clenbuterol 30 mcg BID for 3days and then 20 mcg BID	No side effects noted
(DI GIOACCHINO *et al.* 1987)	Open label study of clenbuterol in exercise induced asthma	14 subjects	20 mcg BID for 60 days	Small increase in heart rate
(BRUSASCO *et al.* 1980)	Single blind cross over study comparing clenbuterol and terbutaline in subjects with chronic airway obstruction	16 subjects	20 to 30 mcg TID for 2 weeks	5 with tremors
(DEL BONO *et al.* 1979)	Single blind placebo controlled cross over study comparing IV clenbuterol and IV terbutaline in asthmatic subjects	9 subjects	20 mcg daily by IV for 4 days	No adverse reactions were observed with clenbuterol

Incontinence Subjects (Reference)	Study Design	Number of Subjects	Dosage and Regimen	AEs
(GRUNEBERGER 1984)	Controlled double blind study of Flavoxate vs Clenbuterol in motor urge incontinence	39 subjects total 20 subjects in Clenbuterol arm and 19 in Flavoxate arm	20 subjects treated for 6 weeks with 10 mcg clenbuterol three times a day	4 with trembling fingers and tachycardia 3 with nervousness 1 subject stopped due to a drug interaction with an alpha stimulating, antihypertensive drug, clonidine that caused dizziness
(ISHIKO *et al.* 2000)	Randomized study of clenbuterol and physiological therapy (PT) in stress incontinence	61 subjects assigned to three groups A-clenbuterol only B- PT only C - both	20 mcg BID for 12 weeks in 41 subjects (group A and C)	2 with headache 1 with mild hepatopathy 1 with rash 1 with numbness

7 Study Objectives

Primary Objective

To assess the safety and tolerability of clenbuterol in subjects with ALS

Secondary Objective

To assess the efficacy of clenbuterol with regard to motor function in subjects with ALS

8 Study Design

8.1 General Design

This is a 24 week Phase I/II study of clenbuterol in patients with ALS (Table 3, Section 11). All subjects will be evaluated at Week 0 to establish a baseline and return to be evaluated for safety and efficacy during the Week 4, 12 and 24 visits. The drug will be initiated at the Week 0 visit in a staged manner (first once daily and later BID), and the dose will be increased at the Week 6 visit in a similarly staged manner to minimize AEs and related attrition. All subjects will return for a final visit after a total of 24 weeks in the study.

The initial dose of clenbuterol will be 40 mcg per oral each morning for one week, followed by 40 mcg BID for the next 5 weeks until the week 6 visit. If the 40 mcg BID per oral is well tolerated, the dose will be increased to 80 mcg each morning/40 mcg each evening for one week, followed by 80 mcg BID for the next 5 weeks until the Week 12 visit. If 80 mcg BID is tolerated at Week 12, the subject will continue on that dose until Week 24. The subject will have several phone visits to evaluate safety, tolerability and ALSFRS-R score. These will occur at Week 1, 6, 16, and 20.

The efficacy of clenbuterol administration will be evaluated with the revised ALS Functional Rating Scale (ALSFRS-R) and other secondary endpoints.

8.2 Primary Study Endpoints

The primary endpoint is safety of clenbuterol at 80 mcg BID. Adverse and serious AEs will be systematically gathered as described in section 8 below.

8.3 Secondary Study Endpoints

Secondary endpoints will include the ALSFRS-R, forced vital capacity (FVC), sniff nasal inspiratory, isometric muscle strength, and sub-maximum handgrip fatigue as described (SHEFNER *et al.* 2016).

9 Subject Selection and Withdrawal

9.1 Inclusion Criteria

1. Diagnosis of possible or more definite ALS according to the El Escorial criteria
2. FVC >50% of predicted for age, height and gender. If FVC testing is restricted due to COVID-19, then a most recent FVC within the past 6 months of 80% or greater will be allowed.
3. At least four of 12 ALSFRS-R questions scored as 2 or 3 at screening.

4. Diminished but measurable grip strength (1) in at least one hand (females:10–50 pounds; males, 10–70 pounds).
5. Taking riluzole at a stable dose or not taking riluzole at screening.
6. On Radicava at a stable dose for at least 30d or not taking this
7. Life expectancy at least 6 months
8. Able to swallow tablets without crushing.
9. Age: 18+ years at enrollment.
10. Subjects are capable of giving written consent.
11. If sexually active, must agree to use contraceptive or abstinence for duration of treatment
12. Females of child bearing age must have negative pregnancy test at screening

9.2 Exclusion Criteria

1. Concurrent illness or laboratory abnormalities that could confound the measurement of ALS progression or interfere with the ability to complete the study.
2. Taking any investigationa1 study drug within 30 days of screening or five half-lives of the prior agent.
3. No previous exposure to clenbuterol.
4. Pregnancy
5. Clinically relevant EKG abnormality (arrhythmia, cardiomyopathy)
6. Tachycardia (resting heart rate greater than 100 beats per minute)
7. History of seizure disorder
8. Hyperthyroidism
9. Pheochromocytoma
10. Pregnancy
11. Have any other co-morbid conditions that in the opinion of the study investigator, places the participant at increased risk of complications, interferes with study participation or compliance, or confounds study objectives
12. History of hypersensitivity to β2-agonist drugs such as albuterol, levalbuterol (Xopenex), bitolterol (Tornalate), pirbuterol (Maxair), terbutaline, salmeterol (Serevent).
13. The use of the following concomitant meds is prohibited during the study:
    i. diuretics (furosemide, Lasix)
    ii. digoxin (digitalis, Lanoxin);
    iii. β-blockers such as atenolol (Tenormin), metoprolol (Lopressor), and propranolol (Inderal);
    iv. tricyclic antidepressants such as amitriptyline (Elavil, Etrafon), doxepin (Sinequan), imipramine (Janimine, Tofranil), and nortriptyline (Pamelor);
    v. MAO inhibitors such as isocarboxazid (Marplan), phenelzine (Nardil), rasagiline (Azilect), selegiline (Eldepryl, Emsam), or tranylcypromine (Parnate); or
    vi. other bronchodilators such as albuterol (Ventolin), levalbuterol (Xopenex), bitolterol (Tornalate), pirbuterol (Maxair), terbutaline (Brethine, Bricanyl), salmeterol (Serevent), isoetherine (Bronkometer), metaproterenol (Alupent, Metaprel), or isoproterenol (Isuprel Mistometer).

9.3 Subject Recruitment and Screening

Patients from Dr. Bedlack's clinic will be approached by study staff to determine their interest in participating in this clinical study. The consent form will be reviewed at the subject's routine clinic visit. Following consent, research study appointments will be made. No compensation is being provided for participation in this study.

9.4 Early Withdrawal of Subjects

When and How to Withdraw Subjects
If the subject decides not to participate or to withdraw from the study, this action will not involve any penalty or loss of benefits to which the subject is entitled, and will not affect the subject's access to health care at Duke. *If the subject* decides to withdraw, the subject will be asked to contact the investigator to notify him. In addition, the subject will be asked to return all unused study drug to the investigator or his staff. The subject will be told about any new information that may affect his or her health, welfare, or willingness to stay in this study.

The investigator may decide to take any subject off this study if his or her condition gets worse, if he or she has serious side effects, or if the investigator determines that it is no longer in the subject's best interest to continue. If the study were halted by the sponsor or a regulatory agent, the subjects will be notified and other options will be discussed.

Data Collection and Follow-up for Withdrawn Subjects
Subjects who withdraw from the study prior to completion of activities will be called in order to determine subject's overall health status and ascertain any adverse effects, and will be asked to return to a follow-up visit for evaluation including medical history, vital signs, and physical examination.

10 Study Drug

10.1 Description

The active study drug will be 20 mcg Spiropent tablets.

10.2 Treatment Regimen

Dosage will initially 40 mcg daily for one week, 40 mcg BID per oral daily for the next 5 weeks. If the 40 mcg BID per oral is well tolerated, the dose will be increased to 80 mcg each morning/40 mcg each evening for one week, followed by 80 mcg BID per oral for the remainder of the study.

10.3 Method for Assigning Subjects to Treatment Groups

All subjects will be assigned to the study drug.

10.4 Preparation and Administration of Study Drug

The drug will be stored and dispensed from the Investigational Drug Service at Duke University Health Systems (beth.mclendon@duke.edu).

10.5 Subject Compliance Monitoring

Compliance (pill counts) will be measured at the Week 4, Week 12, and Week 24 visits. The subject will have phone visits during Week 1, 7, 12, 16 and 20, and compliance will be discussed then. Subjects who miss >6 doses of the study drug, will be considered non-compliant and withdrawn from the study (Table 3).

10.6 Prior and Concomitant Therapy

The use of the following concomitant meds is prohibited during the study:

- digoxin (digitalis, Lanoxin);
- diuretics (furosemide, Lasix);
- β-blockers such as atenolol (Tenormin), metoprolol (Lopressor), and propranolol (Inderal);
- tricyclic antidepressants such as amitriptyline (Elavil, Etrafon), doxepin (Sinequan), imipramine (Janimine, Tofranil), and nortriptyline (Pamelor);
- MAO inhibitors such as isocarboxazid (Marplan), phenelzine (Nardil), rasagiline (Azilect), selegiline (Eldepryl, Emsam), or tranylcypromine (Parnate); or
- other bronchodilators such as albuterol (Ventolin), levalbuterol (Xopenex), bitolterol (Tornalate), pirbuterol (Maxair), terbutaline (Brethine, Bricanyl), salmeterol (Serevent), isoetherine (Bronkometer), metaproterenol (Alupent, Metaprel), or isoproterenol (Isuprel Mistometer).

10.7 Packaging

The Investigational Drug Pharmacy at Duke will package drug for this study.

10.8 Blinding of Study Drug

Not applicable.

10.9 Receiving, Storage, Dispensing and Return

10.9.1 Receipt of Drug Supplies

Spiropent (PZN#01980325) will be purchased from Boehringer Ingelheim.

Upon receipt of the study treatment supplies, an inventory must be performed and a drug receipt log filled out and signed by the person accepting the shipment. It is important that the designated study staff counts and verifies that the shipment contains all the items noted in the shipment inventory. Any damaged or unusable study drug in a given shipment (active drug) will be documented in the study files. The investigator must notify study sponsor of any damaged or unusable study treatments that were supplied to the investigator's site.

10.9.2 Storage

Storage will be at ambient temperature in the Duke Hospital Pharmacy.

10.9.3 Dispensing of Study Drug

The Investigational Drug Service will dispense study drug (clenbuterol 20 mcg) to the study team at the Screening/Baseline/Week 0 visit. Subsequently, additional supply of study drug will be shipped before Week 18 to allow completion of the study. The Investigational Drug Service will maintain records of drug dispensing.

10.9.4 Return or Destruction of Study Drug

Unused drug will be destroyed by the Investigational Drug Service at the end of the study.

At the completion of the study, there will be a final reconciliation of drug shipped, drug consumed, and drug remaining. This reconciliation will be logged on the drug reconciliation form, signed and dated. Any discrepancies noted will be investigated, resolved, and documented prior to return or destruction of unused study drug. Drug destroyed on site will be documented in the study files.

11 Study Procedures

1. Research Activities: Medical History: Reviewed prior to study drug initiation.
2. Vital Signs: Completed at Screening/Baseline, Week 4, Week 12, and Week 24.
3. Physical Examination: Performed at Screening/Baseline, Week 4, Week 12, and Week 24.
4. AE Review: Completed at Week 4, Week 12, Week 24, and telephone visits.
5. Blood Samples: Blood samples will be obtained at Screening/Baseline, Week 4, Week 12, and Week 24. for the following analyses:
    - Safety Labs: Hematology and Chemistry (5 ml) to consist of:
        a. Complete Blood Count (CBC)
        b. Chemistry panel (including electrolytes, BUN, creatinine, glucose)
        c. Liver panel (including albumin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and total bilirubin)
        d. Creatine kinase (CK)

6. Electrocardiogram: Performed at Screening/Baseline, Week 4, Week 12, and Week 24.
7. Forced Vital Capacity (FVC) Testing: Performed at Screening/Baseline, Week 4, Week 12, and Week 24
8. ALSFRS-R: Performed at Screening/Baseline, Week 4, Week 12, Week 16, Week 20 and Week 24.
9. Muscle strength testing: Myometer testing (SORARU *et al.* 2006) and isometric muscle strength, and sub-maximum handgrip fatigue (SHEFNER *et al.* 2016), performed at Screening/Baseline, Week 4, Week 12, and Week 24.
10. Telephone visits: Performed during Week 1, 6, 16 and 20.
11. Serum pregnancy testing at Screening/Baseline. Urine pregnancy testing at Weeks 4, 12 and 24.
12. Thyroid function (T4, TSH) at Screening/Baseline.

Table 3: Testing Schedule

	Screening/ Baseline (Week 0)	Phone Visit (Week 1)	Study Visit (Week 4)	Phone Visit (Week 6)	Study Visit (Week 12)	Phone Visit (Week 16)	Phone Visit (Week 20)	Final Visit (Week 24)
Medical History	X							
Vital Signs	X		X		X			X
Physical Exam	X		X		X			X
Obtain Consent	X							
Study Drug Dispensed	X		X		X			
AE Review		X	X	X	X	X	X	X
Blood Sample	X				X			X
Safety testing	X		X		X			X
Thyroid function testing	X							
Pregnancy test								
Blood	X							
Urine			X		X			X
EKG	X		X		X			X
Pulmonary function testing	X		X		X			X
ALSFRS-R	X		X		X	X	X	X
Muscle strength testing	X		X		X			X

12 Statistical Plan

12.1 Sample Size Determination

Due to the number of patients with ALS who are followed in Dr. Bedlack's clinic, we estimate that 25 subjects can be enrolled and complete the study within 1 year.

Power Analysis

The ALSFRS-R is a rating utilized for monitoring the progression of disability in patients with ALS. The decline of the rating is generally linear in time and current research focuses on reducing the slope of ALSFRS-R with respect to time through novel treatments. Alfredo E. Farjat, Ph.D. performed a simulation study to assess the effectiveness for detecting specific changes in the slope of ALSFRS-R with respect to time (Table 4). The goal was to estimate the power for future clinical trials.

Table 4: Power Percentage and Standard Deviation Assuming 25 Patients

Number of observations (per patient)	Slope percentage change (compared to pre-trial rate of decline)				
	16%	**17%**	**18%**	**19%**	**20%**
4	35.4 (1.5)	43.6 (1.6)	50.1 (1.6)	54.0 (1.6)	57.7 (1.6)
5	70.2 (1.4)	74.7 (1.4)	81.3 (1.2)	83.4 (1.2)	88.5 (1.0)
6	89.8 (1.0)	95.0 (0.7)	95.9 (0.6)	97.3 (0.5)	98.6 (0.4)
7	98.8 (0.3)	99.1 (0.3)	99.6 (0.2)	98.8 (0.1)	>99.9 (<0.1)

The results indicate that a 20% change in the slope, compared to pre-trial rate of decline, can be detected with power >99.9 (<0.1) with 7 observations (six months trial - baseline observation plus one observation monthly).

12.2 Statistical Methods

Descriptive statistics (N, mean, median, standard deviation, and range) will be provided as appropriate. Continuous data will be summarized by using means, medians, standard deviations and ranges for each group and visit. Categorical data, including AE analysis, will be summarized by using frequencies and distributions. Secondary endpoints will include the ALSFRS-R, FVC, isometric muscle strength, and sub-maximum handgrip fatigue as described (SHEFNER *et al.* 2016) and myometer testing as described (SORARU *et al.* 2006).

The critical test for efficacy will be comparison of the slope of the ALSFRS-R during treatment compared to pre-treatment. Pre-treatment slope for each participant will be estimated as follows: (48-enrollment ALSFRS-R)/months since symptom onset. ALSFRS-R is a quickly administered (five minute) ordinal rating scale (ratings 0-4) used to determine patients' assessments of their capability and independence in 13 functional activities. All 12 activities are relevant in ALS. Initial validity was established in ALS patients by documenting that change in ALSFRS-R scores correlated with change in strength over time, was closely associated with quality of life measures, and predicted survival. The test-retest reliability is greater than 0.88 for all 13 items. The ALSFRS-R declines linearly with time over a wide range during the course of ALS. The minimum clinically significant change in this scale is said to be 20%. The measure can be reliably conducted over the phone. A secondary measure of efficacy will be the comparison of the change in lower limb total myometry observed between Baseline and Week 24. A statistically significant treatment effect will be determined by a two-tailed, paired t-test, with a critical p value $< .05$. Tertiary analyses will include a mixed design (between and within) ANOVA of the lower limb total myometry across visits as well as a comparison of the slope of percent predicted FVC during treatment versus pre-treatment . Pre-treatment slope for each participant will be estimated as follows: (100%-enrollment percent predicted FVC)/months since symptom onset. Multiple exploratory comparisons will be made to identify potential length of treatment effects.

12.3 Subject Population(s) for Analysis

All subjects who complete the study will be included in the analysis, providing non-compliance is not documented.

13 Safety and Adverse Events

13.1 Definitions

13.1.1 Adverse Event

An ***adverse event*** (AE) is any symptom, sign, illness or experience that develops or worsens in severity during the course of the study. Intercurrent illnesses or injuries should be regarded as AEs. Abnormal results of diagnostic procedures are considered to be AEs if the abnormality:

- results in study withdrawal
- is associated with a serious AE
- is associated with clinical signs or symptoms
- leads to additional treatment or to further diagnostic tests
- is considered by the investigator to be of clinical significance

13.1.2 Serious Adverse Event

Adverse events are classified as serious or non-serious. A ***serious adverse event*** is any AE that is:

- fatal
- life-threatening
- requires or prolongs hospital stay
- results in persistent or significant disability or incapacity
- a congenital anomaly or birth defect
- an important medical event

Important medical events are those that may not be immediately life threatening, but are clearly of major clinical significance. They may jeopardize the subject, and may require intervention to prevent one of the other serious outcomes noted above. For example, drug overdose or abuse, a seizure that did not result in in-patient hospitalization, or intensive treatment of bronchospasm in an emergency department would typically be considered serious.

All AEs that do not meet any of the criteria for serious should be regarded as ***non-serious adverse events***.

13.1.3 Adverse Event Reporting Period

The study period during which AEs must be reported is normally defined as the period from the initiation of any study procedures to the end of the study treatment follow-up. For this study, the study treatment follow-up is defined as 30 days following the last administration of study treatment.

13.1.4 Preexisting Condition

A preexisting condition is one that is present at the start of the study. A preexisting condition should be recorded as an AE if the frequency, intensity, or the character of the condition worsens during the study period.

13.1.5 General Physical Examination Findings

At screening, any clinically significant abnormality should be recorded as a preexisting condition. At the end of the study, any new clinically significant findings/abnormalities that meet the definition of an AE must also be recorded and documented as an AE.

13.1.6 Post-study Adverse Event

All unresolved AEs should be followed by the investigator until the events are resolved, the subject is lost to follow-up, or the AE is otherwise explained. At the last scheduled visit, the investigator should instruct each subject to report any subsequent event(s) that the subject, or the subject's personal physician, believes might reasonably be related to participation in this study. The investigator should notify the study sponsor of any death or AE occurring at any time after a subject has discontinued or terminated study participation that may reasonably be related to this study. The sponsor should also be notified if the investigator should become aware of the development of cancer or of a congenital anomaly in a subsequently conceived offspring of a subject that has participated in this study.

13.1.7 Abnormal Laboratory Values

A clinical laboratory abnormality should be documented as an AE if <u>any one of the following</u> conditions is met:

- The laboratory abnormality is not otherwise refuted by a repeat test to confirm the abnormality
- The abnormality suggests a disease and/or organ toxicity
- The abnormality is of a degree that requires active management; e.g. change of dose, discontinuation of the drug, more frequent follow-up assessments, further diagnostic investigation

13.1.8 Hospitalization, Prolonged Hospitalization or Surgery

Any AE that results in hospitalization or prolonged hospitalization should be documented and reported as a SAE unless specifically instructed otherwise in this protocol. Any condition responsible for surgery should be documented as an AE if the condition meets the criteria for an AE.

Neither the condition, hospitalization, prolonged hospitalization, nor surgery are reported as an AE in the following circumstances:

- Hospitalization or prolonged hospitalization for diagnostic or elective surgical procedures for a preexisting condition. Surgery should ***not*** be reported as an outcome of an AE if the purpose of the surgery was elective or diagnostic and the outcome was uneventful.
- Hospitalization or prolonged hospitalization required to allow efficacy measurement for the study.
- Hospitalization or prolonged hospitalization for therapy of the target disease of the study, unless it is a worsening or increase in frequency of hospital admissions as judged by the clinical investigator.

13.2 Recording of Adverse Events

At each contact with the subject, the investigator must seek information on AEs by specific questioning and, as appropriate, by examination. Information on all AEs should be recorded immediately in the source document, and also in the appropriate AE module of the case report form (CRF). All clearly related signs, symptoms, and abnormal diagnostic procedures results should recorded in the source document, though should be grouped under one diagnosis.

All AEs occurring during the study period must be recorded. The clinical course of each event should be followed until resolution, stabilization, or until it has been determined that the study treatment or participation is not the cause. SAEs that are still ongoing at the end of the study period must be followed up to determine the final outcome. Any SAE that occurs after the study period and is considered to be possibly related to the study treatment or study participation should be recorded and reported immediately.

13.3 Reporting of Serious Adverse Events

13.3.1 IRB Notification by Investigator

Reports of all SAEs (including follow-up information) must be submitted to the IRB within 10 working days. Copies of each report and documentation of IRB notification and receipt will be kept in the Clinical Investigator's binder.

13.3.2 FDA Notification by Sponsor

The IND sponsor shall notify the FDA by telephone or by facsimile transmission of any serious, unexpected and study related experience associated with the use of the drug as soon as possible but no later than 15 calendar days from the sponsor's original receipt of the information. In addition, any fatal or life-threatening experience associated with the use of the drug shall be reported as soon as possible but no later than 7 calendar days from the sponsor's original receipt of the information.

If a previous AE that was not initially deemed reportable is later found to fit the criteria for reporting, the IND sponsor will submit the AE in a written report to the FDA as soon as possible, but no later than 15 calendar days from the time the determination is made.

13.4 Unblinding Procedures

Not applicable.

13.5 Definitions

13.5.1 Adverse Event

An **adverse event** (AE) is any symptom, sign, illness or experience that develops or worsens in severity during the course of the study. Intercurrent illnesses or injuries should be regarded as AEs. Abnormal results of diagnostic procedures are considered to be AEs if the abnormality:

- results in study withdrawal
- is associated with an SAE
- is associated with clinical signs or symptoms
- leads to additional treatment or to further diagnostic tests
- is considered by the investigator to be of clinical significance

13.5.2 Serious Adverse Event

Adverse events are classified as serious or non-serious. A **serious adverse event** is any AE that is:

- fatal
- life-threatening
- requires or prolongs hospital stay
- results in persistent or significant disability or incapacity
- a congenital anomaly or birth defect
- an important medical event

Important medical events are those that may not be immediately life threatening, but are clearly of major clinical significance. They may jeopardize the subject, and may require intervention to prevent one of the other serious outcomes noted above. For example, drug overdose or abuse, a seizure that did not result in

in-patient hospitalization or intensive treatment of bronchospasm in an emergency department would typically be considered serious.

All AEs that do not meet any of the criteria for serious should be regarded as **non-serious adverse events**.

13.6 Medical Monitoring

13.6.1 Internal Data and Safety Monitoring Committee

The study will be monitored throughout the trial by a Data and Safety Monitoring Committee (DSMB) consisting of a 4 Duke University School of Medicine faculty members who are unaffiliated with the study. The DSMB will convene during the study at approximately 6 month intervals to review summaries of accrual and enrollment information, results for EKGs and PFTs, and safety (AEs, withdrawal from study). The DSMB will provide summaries to the PI's. Safety will be monitored on an ongoing basis as descriptions of SAEs will be communicated to the DSMB Chair within 24 hours of their occurrence.

14 Data Handling and Record Keeping

14.1 Confidentiality

Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). The subject will not be identified by name, social security number, address, telephone number, or any other direct personal identifier in study records disclosed outside of Duke University Health System (DUHS). Study records and samples disclosed outside of Duke will be identified only with the subject's initials, date of birth and unique code number. The key to the research code will be kept in a locked file in Dr. Koeberl's research office. Test results will be recorded in the subject's password-protected research record (located on the Division of Medical Genetics' division website, maintained by Duke OIT) and medical record.

14.2 Source Documents

Source data is all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents Examples of these original documents, and data records include: hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files, and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical trial.

14.3 Case Report Forms

The study case report form (CRF) is the primary data collection instrument for the study. All data requested on the CRF must be recorded. All missing data must be explained. If a space on the CRF is left blank because the procedure was not done or the question was not asked, write "N/D". If the item is not applicable to the individual case, write "N/A". All entries should be printed legibly in black ink. If any entry error has been made, to correct such an error, draw a single straight line through the incorrect entry and enter the correct data above it. All such changes must be initialed and dated. DO NOT ERASE OR WHITE OUT ERRORS. For clarification of illegible or uncertain entries, print the clarification above the item, then initial and date it.

14.4 Records Retention

The research record will be retained for 6 years following the completion of the study. Any research information in the subject's medical record will be kept indefinitely.

15 Auditing, and Inspecting

15.1 Auditing and Inspecting

The investigator will permit study-related monitoring, audits, and inspections by the Ethics Committee (EC) or Institutional Review Board (IRB), the sponsor, government regulatory bodies, and University compliance and quality assurance groups of all study related documents (e.g. source documents, regulatory documents, data collection instruments, study data etc.). The investigator will ensure the capability for inspections of applicable study-related facilities (e.g. pharmacy, diagnostic laboratory, etc.).

Participation as an investigator in this study implies acceptance of potential inspection by government regulatory authorities and applicable University compliance and quality assurance offices.

16 Ethical Considerations

This study is to be conducted according to US and international standards of Good Clinical Practice (FDA Title 21 part 312 and International Conference on Harmonization guidelines), applicable government regulations and Institutional research policies and procedures.

This protocol and any amendments will be submitted to a properly constituted independent EC/IRB, in agreement with local legal prescriptions, for formal approval of the study conduct. Any protocol amendments will also be submitted to the FDA as per 21 CFR Part 312.30 (in addition to the IRB).

All subjects for this study will be provided a consent form describing this study and providing sufficient information for subjects to make an informed decision about their participation in this study. This consent form will be submitted with the protocol for review and approval by the EC/IRB for the study. The formal consent of a subject, using the EC/IRB-approved consent form, must be obtained before that subject is submitted to any study procedure. This consent form must be signed by the subject or legally acceptable surrogate, and the investigator-designated research professional obtaining the consent.

17 Study Finances

17.1 Funding Source

Departmental (Research Fund of Dr. Richard Bedlack)

17.2 Conflict of Interest

Any investigator who has a conflict of interest with this study (patent ownership, royalties, or financial gain greater than the minimum allowable by their institution, etc.) must have the conflict reviewed by a properly constituted Conflict of Interest Committee with a Committee-sanctioned conflict management plan that has been reviewed and approved by the IND sponsor (Dwight Koeberl, MD PhD) prior to participation in this study.

18 Publication Plan

Neither the complete nor any part of the results of the study carried out under this protocol, nor any of the information provided by the sponsor for the purposes of performing the study, will be published or passed on to any third party without the consent of the study sponsor. Any investigator involved with this study is obligated to provide the sponsor with complete test results and all data derived from the study.

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