An open-label, single-center, 6-month trial of Theracurmin for patients with Amyotrophic Lateral Sclerosis (ALS)

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1 PURPOSE/HYPOTHESES

1.1 Primary Hypothesis

The primary hypothesis is that Theracurmin treatment can decrease the rate of Revised ALS Functional Rating Scale (ALSFRS-R) progression by 50% relative to matched historical controls.

1.2 Secondary Hypotheses

Secondary hypotheses are as follows:

1. Theracurmin can increase the frequency of ALS reversals (defined by an improvement of 4 or more points in the ALSFRS-R over the course of 6 months) from 2-5% (observed spontaneously) to at least 10%.
2. Theracurmin can alter the fecal microbiome in patients with ALS.
3. The fecal microbiome of patients with ALS is significantly different from matched control subjects.
4. The fecal microbiome of patients with ALS is significantly different between subgroups with differing clinical features including sporadic vs. familial, anatomical location of symptom onset, and rate of progression as measured on the ASLFRS-R.
5. The novel features of this pilot trial will be associated with improved participant enrollment compared to prior more traditional ALS trials where enrollment is 2 participants per site per month.
6. The novel features of this pilot trial will be associated with improved participant retention compared to prior more traditional ALS trials where the dropout rate is 22% at 12 months.
7. The novel features of this pilot trial will be associated with improved participant compliance compared to prior more traditional ALS trials.

2 BACKGROUND

2.1 Amyotrophic Lateral Sclerosis (ALS) and Its Treatment

ALS is a devastating motor neuron disease that causes rapidly progressive muscle weakness, disability and premature death. In spite of a large number of attempted ALS trials, there are no significant disease-modifying therapies for this condition (1).

2.2 Failure of Previous Classically Designed ALS Trials

There are several reasons that previous ALS trials may have failed. First, the hypotheses could have been wrong. The hypotheses for many prior trials came from observations made in animal models of familial ALS (1). How well the animal models predict human ALS, the vast majority of which is not familial, has been called into question (2). Second, the dosing in previous ALS trials may not have been adequate. Indeed, most ALS trials have not even employed pharmacodynamic biomarkers to test their dosing regimen (3). In addition to these problems,

ALS trials are often challenged by slow enrollment (4) and poor retention (5). While some of the enrollment and retention issues may have to do with misconceptions on the part of enrolling clinicians and potential participants (4), patients with ALS do become more disabled over time, and study burdens, including trips to the study site, may eventually become impossible to bear.

2.3 Success of Previous Hybrid Virtual/Remote Trial

We previously conducted an open-label, single-center, hybrid-virtual 12-month trial of Lunasin for patients with ALS (6). This single-center trial featured broad inclusion criteria, historical controls, mostly virtual data collection, and real-time results. Participants measured their ALSFRS-R score, weight and perceived efficacy, and recorded these monthly on PatientsLikeMe (PLM; an online platform described section 2.7). ALSFRS-R decline was compared to historical controls. For each enrolled participant, PLM identified 3 historical controls matched according to pre-treatment ALSFRS-R progression, as in their previous Lithium study (7). We had 90% power to detect 50% slowing in decline of ALSFRS-R scores.

Although there were no significant differences in ALSFRS-R (p=0.99) between study participants and historical controls, the study's outcomes suggested that there is utility and internal validity in a virtual trial model. Enrollment rate was 9.1 participants per site per month, which is the fastest rate of any ALS trial in history. As in most ALS trials, our 50 participants were primarily white (94%) males (58%), mean age of 60, limb onset disease (82%), taking riluzole (60%). However, our participants had longer disease duration than those in most trials, and several had non-invasive ventilation, PEG and/or tracheostomy, which are traditional exclusion criteria. This trial population was therefore more representative of the population seen in ALS clinics. In spite of enrolling patients with longer and more advanced disease, the survivor retention rate was 84%, while the survivor retention rate in traditional ALS trials is 78% (5).

We also looked at the accuracy of patient recorded ALSFRS-R and weight measurements. Lin's concordance (C) showed high agreement between coordinator and participant derived ALSFRS-R and weight respectively at month 1 (C=0.9939, 0.9947) and month 12 visits (C=0.9428, 0.9905). These results suggest that participant recorded ALSFRS-R scores and weight are sufficiently accurate to be used in a clinical trial.

Compliance with study protocol (assessed by the percentage of patients who logged onto PLM every month and entered at least 2 of the 3 key outcomes) was 88% or greater for each of the first 6 months, but then began to decline, eventually reaching only 55% at 12 months. This suggests that a 6 month virtual trial design may allow for high compliance throughout the entire study duration.

2.4 Curcumin

Curcumin is a chemical compound that is one of three curcuminoids found in the culinary spice turmeric. Curcumin has been studied in a wide variety of neurologic diseases and is purported to have many bioactive properties. It is widely available as a dietary supplement in multiple formulations from many commercial companies (8). The treatment Theracurmin utilized in this study is a formulation of curcumin (see section 3.2.1).

2.5 ALS Reversals on Curcumin

ALSUntangled is a consortium of ALS clinicians and researchers that review alternative and off-label treatments on behalf of patients with ALS (9). ALSUntangled recently reported on 3 patients with a validated diagnosis of ALS, whom experienced an ALS reversal while taking a supplement regimen containing curcumin (8). Several other patients with ALS have also reported improvements on a curcumin regimens, though records were not obtainable to validate these (8).

2.6 How Curcumin Could Influence ALS

We have published a review describing multiple biological mechanisms through which curcumin could influence ALS progression (8). In this study we are going to focus Theracurmin's potential effects on the fecal microbiome.

2.6.1 Alterations of the Fecal Microbiome

As in Parkinson's disease (10), there is increasing recent evidence that abnormalities in the gut microbiome may play a role in ALS pathophysiology. In a mouse model of ALS, for example, dysbiosis and impaired intestinal tight junctions and permeability occur before symptom onset (11). Altering the mouse microbiome via germ-free conditions, broad spectrum antibiotics or introduction of specific bacteria (*Ruminococcus torques* or *Purabacteroides distasonis)* exacerbate the ALS phenotype (12). On the other hand, feeding the mice butyrate (13), a natural product of a healthy microbiome, or introduction of other specific bacteria (*Akkermansia muciniphilia*) (12) improve the ALS phenotype. Alteration of the intestinal microbiome in a worm model of ALS can also improve motor function (14). Three studies suggest that people with ALS have altered intestinal microbiomes compared to healthy controls (12, 15, 16); a fourth small study did not find such a difference (17).

The curcumin formulation used in Theracurmin has been shown to have the capability of altering the fecal microbiome in mice (18). If curcumin can lead to a more favorable fecal microbiome profile in patients with ALS, it may help to slow or reverse ALS progression.

2.7 PatientsLikeMe

PatientsLikeMe® (PLM; www.patientslikeme.com) is an online platform that has demonstrated an ability to engage patients in learning about their conditions and building a large quantitative and qualitative database about patients' experiences of their conditions. PLM is a patient network with over 300,000 members comprising over 2000 different conditions founded by a family affected by ALS, and to date has conducted over 30 published studies in ALS including studying patient use of off-label treatments. Members who join the site with ALS are asked to complete a number of data fields including demographics, disease condition history, treatments, symptoms, weight, treatment evaluations and lab measurements (e.g. weight, forced vital capacity). In addition, a unique feature of the site is the ability of patients to complete a widely used patient reported outcome, the ALS Functional Rating Scale, Revised (ALSFRS-R) which allows patients to see their disease progression in context in visual form. Data capture is facilitated by a team of PhD scientists, nurses, and pharmacists that incorporate information from various medical databases (e.g. RxNorm, ICD-10, MEDDRA). The site also provides means of social support through a message board forum, private messages, and profile commenting. Online methods of

data capture have the potential to dramatically accelerate trial recruitment, provide more convenient means of data capture for patients, and minimize trial complexity by using matched historical controls in lieu of a placebo control group. Our previous lunasin study (see section 2.3) was conducted on the PLM platform and we will continue to utilize the PLM platform for this study.

3 DESIGN & PROCEDURES:

3.1 Design Overview

This will be a 6-month, widely inclusive, largely remote/virtual, single-center, open-label pilot trial utilizing a historical control group. Following informed consent and screening, participants will take the intervention (see section 3.2.1) for 6-months. Treatment with the intervention Theracurmin HP and all study outcome measures and labs are being performed exclusively for research purposes. Collected data includes ALSFRS-R, fecal sample microbiome sampling, adverse events, and concomitant medications. Participants will be asked to register for an account on PLM with the help of the study coordinator. User IDs associated with their PLM account will be recorded by the study coordinator and shared with study staff at PLM. When registering, participants will have the opportunity to review PLM's terms and conditions (Appendix A) as well as their privacy policy (Appendix B). After the baseline video/telephone visit, participants will be asked to enter the following data: weight and height, Theracurmin treatment evaluations (Appendix C), ALSFRS-R score (Appendix D) and Thrive Questionnaires (Appendix E). Participants will be given the following study documents and materials shipped to their home:

A PLM bag (Appendix F) including:

1. Welcome letter (Appendix G)
2. Study One-Sheet (Appendix H)
3. Study drug
4. Microbiome kits
5. PatientsLikeMe gifts

3.2 Study Drug

3.2.1 Theracurmin HP

The intervention is based on the twice daily dosage of Theracurmin 90 mg capsules used in a trial of patients with mild cognitive impairment (19). This dosage is similar to that used in a trial of patients with ALS using similar bioavailable version of curcumin (20). Our 6-month intervention consists of Theracurmin HP capsules containing 90 mg curcumin each that will be taken as one capsule twice daily. Each capsule contains 300 mg Theracurmin enhanced bioavailable water-dispersible turmeric rhizome complex providing 30% curcumin (90 mg). The content of Theracurmin HP has been independently certified by NSF International under NSF/ANSI 173 (21).

3.2.2 Theracurmin HP Supply

The product Theracurmin HP (referred to as Theracurmin throughout) will be obtained from Integrative Therapeutic (22). Products will be stored in Dr. Bedlack's locked office at Duke University. At enrollment into the study, participants will be sent a 2-month supply of product. After the Week 3 video/telephone visit, participants will be sent an additional 4-month supply. Once this study ends, participants may elect to continue the Theracurmin by purchasing from third-party distributors and select health care professionals.

3.2.3 Dose Adjustments and Holidays

The investigator may temporarily reduce or stop administering the Theracurmin for adverse events. If the adverse event is mild or moderate, the dose may be reduced to 90mg once a day until the event improves. The investigator may then choose to resume the dosage of 90mg twice a day or maintain the participant at reduced dose. If a related event is serious or life threatening, the Theracurmin should be stopped and will not be re-challenged. One drug holiday will be allowed; the holiday can last no more than 7 days. All dose reductions and suspensions must be documented.

3.2.4 Compliance

The method to check compliance for study medication will be to ask the participants to record the amount of product left over at all internet PLM visits occurring after the intervention begins. No further compliance checks will be conducted.

3.2.5 Concomitant Medications

Throughout the study, investigators may prescribe any other concomitant medications or treatments deemed necessary to provide adequate supportive care, provided that they are not part of an active research study.

3.2.6 Use of Riluzole and Edaravone

The use of riluzole will be permitted during the study. Patients taking riluzole must be on a stable dose for 30 days prior to screening. The use of edaravone will also be permitted. Patients currently receiving edaravone infusions must have completed at least 2 cycles of infusions before enrolling.

3.3 Outcome Measures

3.3.1 ALSFRS-R

The ALSFRS-R will be determined at all video/telephone visits (Appendix D). 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 12 functional activities. All 12 activities are relevant in ALS. Initial validity was established in ALS patients by documenting change in ALSFRS-R scores correlated with change in strength over time, and it was closely associated with quality of life measures and predicted survival (23). 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. Recent work has shown that the measure can be conducted over the phone (24), and it can even be reliably conducted and recorded online by patients themselves (25). The ALSFRS-R will be administered to participants over video/telephone by the study coordinator at Duke on their enrollment visit. During this visit, the coordinator will also teach participants how to complete this measure themselves and to use the EM interface to record it. Calls from the coordinator will be made at weeks 1, 2 and 3 to provide additional training. Starting at week 4, participants will be asked to fill out the ALSFRS-R test on their PLM profile once every 30 days.

3.3.2 Participant Subjective Treatment Evaluation

At all PLM virtual visits, participants will be asked to evaluate the intervention. They will be asked to provide information on perceived effectiveness, side effects, adherence, burden, unexpected positive effects, and advice & tips regarding the treatment (Appendix C). They will also be asked to complete Thrive Questionnaires to assess their quality of life (Appendix E).

3.3.3 Microbiome Sequencing

The microbiome of study participants will be analyzed in saliva and stool samples at enrollment, week 4 and month 6 visits. Participants will be sent collection kits with which they will collect saliva and fecal samples and send by mail to Duke University for further analysis at each timepoint. Research personnel will deidentify the samples of any personally identifiable information (PII) and subsequently submit them to the Duke Microbiome Core (https://genome.duke.edu/cores-and-services/microbiome-shared-resource) for sample processing and preparation for biospecimens for long term archiving at the Duke University BioBank. The Duke Microbiome Core will perform DNA extraction, sequencing library preparation, and 16S rRNA sequencing on the Illumina MiSeq platform. Data analysis of the deidentified microbiome sequencing data will be conducted in collaboration with the Duke University Microbiome Center (DMC). Data will be compared within subjects with ALS to assess possible changes over the length of the study and identify microbial correlates of disease progression. Fecal microbiome data at enrollment, week 4 and month 6 will also be compared to data from healthy control participants from the same household as participants with ALS.

In addition, our collaborator Dr. Raphael Valdivia at the Duke Department of Molecular Genetics and Microbiology, will perform metagenomic analysis of deidentified selected fecal samples from patients that positively respond to Theracurmin to achieve strain level identification of microbes positively and negatively associated with improved outcomes. His group will also culture selected anaerobes (eg. *Akkermansia muciniphila*) that have been linked to ALS severity for further genetic sand phenotypic characterization.

3.3.4 Safety and Tolerability in Patients with ALS

We will record adverse and serious adverse events throughout the study. The PI will determine whether these are related to treatment. Patients will also measure and record their side effects at Month 0-6 Internet Visits on their PLM profile (See Appendix C).

3.3.5 Enrollment Rate

Enrollment rate will be calculated as the number of participants enrolled per month. A previous meta-analysis showed the mean ALS trial enrollment rate to be 2.2 participants per site per month (4). Our previous Lunasin trial had an enrollment rate of 9 participants per site per month (6).

3.3.6 Retention

Retention will be assessed by looking at the dropout rate at 6 months (the percentage of surviving enrolled patients who complete the Month 6 Visit). A previous meta-analysis of traditional clinical trials showed the mean ALS participant dropout rate to be 22% at 12 months (5). Our previous virtual trial with Lunasin had a dropout rate of 16% at 12 months (6).

3.4 Study Schedule

Following informed consent and screening, enrollment visit activities will commence and Therarcurmin treatment will be started. Treatment and all study activities will end at month 6. Participants will be required to make 4 video/telephone visits with the Duke study team (enrollment, weeks 1, 2, and 3). Participants will not be required to make any physical visits to Duke. At months 2-6, participants will make "Internet PLM visits" by measuring their own ALSFRS-R score and weight and height, and logging onto the PLM website to record it as well as perceived efficacy, perceived side effects, perceived compliance, changes in concomitant medications and Thrive questionnaires. Participants will be reminded to log into their PLM profile and record the above-mentioned data points via email and text reminders from PLM. The study coordinator will call/video participants at enrollment, weeks, 1, 2, 3 and for training and any time afterward when participants have not entered data for 2 weeks beyond expected entries. The call script for such communications can be found in Appendix I.

3.4.1 Screening/Enrollment/Baseline Video/Telephone Visits

During the enrollment visit(s) (can be one or two visits depending on patient preference and tolerance), participants will be consented. Inclusion and Exclusion Criteria will be reviewed to ensure the participant qualifies. Participants will be taught to self- administer the ALSFRS-R (23). Participants will be taught to register for an account on the website PLM and taught to enter their ALSFRS-R, perceived efficacy, compliance, adverse events and concomitant medications. The participant will complete/enter their current ALSFRS-R and weight and height in PLM during this visit. The ALSFRS-R has previously been validated for similar online use by patients (25).

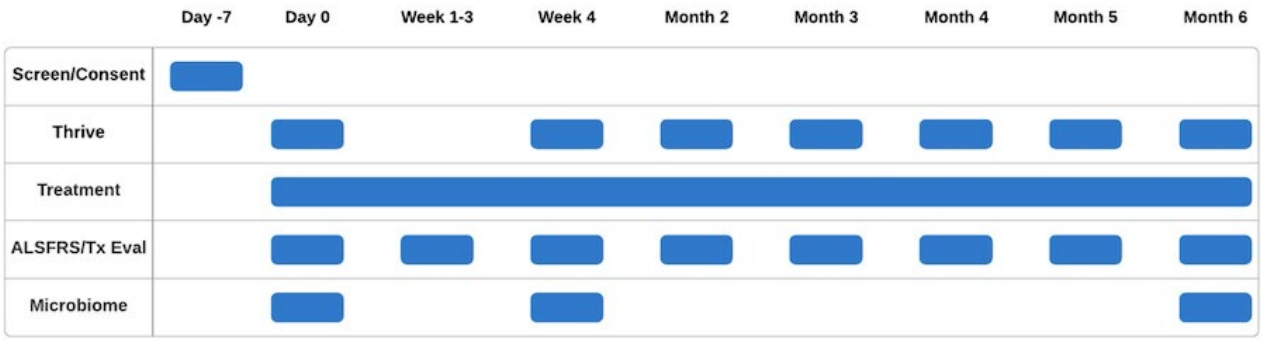
3.4.2 Week 1,2,3 Video/Telephone Visits

During the Week 1, 2, and 3 video/telephone the study coordinator will confirm that participants are logging into the PLM site and entering data without difficulty. The coordinator will measure an ALSFRS-R by phone and compare it to the participant-generated score. Differences will be reconciled as part of the teaching process.

3.4.3 Week 4 and Month 2-6 Internet PLM Visits

During the Week 4 and Month 2-6 Internet PLM Visits, participants measure their own ALSFRS-R and weight and height and will log into the PLM site to record these as well as perceived efficacy, adverse events, concomitant medications and Thrive questionnaires. If participants have not entered data within 7 days of a visit, they will receive a reminder email and text from PLM (Appendix I). If they still have not entered data after another 7 days, they will receive a reminder call from the study coordinator.

3.4.4 Study Timeline Overview Figure for Primary Participants



3.4.5 Schedule of Events Table for Primary Participants

The Table 1 shows the schedule of visits for the Primary Participants, i.e. the patients with ALS (R=research staff initiated task, P=primary participant initiated task):

Table 1: Schedule of Events for Primary Participants

	Enrollment*	Wk 1,2,3	Wk 4	Mos 2-5	Mo 6**
Inclusion and exclusion criteria review	R				
Consent	R				
Participant registers account on PLM	R				
Phone/video visits with participant to record ALSFRS-R and teach/troubleshoot as necessary	R	R			
PLM virtual visits by participant to record ALSFRS-R, anthopometrics, treatment self-evaluation, Con Meds, and AE		P	P	P	P
Long Thrive Evaluation	P		P		P
Short Thrive Evaluation		P		P	
Microbiome sample collections by participant at-home	P		P		P

*intervention begins

**intervention ends

3.4.6 Schedule of Events Table for Control Participants

The Table 2 shows the schedule of video/telephone visits for the Control Participants (R=research staff initiated task, C=control participant initiated task):

Table 2: Schedule of Events for Control Participants

	Screening/Enrollment	Wk 4 and Month 6
Consent	R	
Inclusion and Exclusion Criteria Review	R	
Microbiome sample collections by participant at-home	C	C

3.5 Participants

Primary participants will be patients with ALS (PALS) who are cared for by Dr. Bedlack, or who contact our site inquiring about this study. 50 participants will be enrolled.

3.5.1 Inclusion Criteria

Each primary participant must meet all of the following criteria at screening and baseline (unless otherwise specified) to participate in the study:

1. Male or female, aged at least 18 years.
2. Sporadic or familial ALS diagnosed as possible, laboratory-supported probable, probable, or definite as defined by revised El Escorial criteria.
3. Patient is able to understand and express informed consent (in the opinion of the site investigator).
4. Patient has access to the Internet on a desktop computer, laptop, or tablet and has a working email address.
5. Patient or caregiver is willing and able to use a computer and enter data on a secure website.
6. Patient is able to read and write English.
7. Patient is expected to survive for the duration of the trial.
8. Women must not be pregnant (will have evidence of a negative pregnancy test obtained by local physician within past 7 days or be post-menopausal)
9. Women must not be able to become pregnant (e.g., post-menopausal, surgically sterile, or using adequate birth control methods) for the duration of the study and three months after study completion. Adequate contraception includes: abstinence, hormonal contraception (oral

contraception, implanted contraception, injected contraception or other hormonal contraception, for example patch or contraceptive ring), intrauterine device (IUD) in place for ≥ 3 months, barrier method in conjunction with spermicide, or another adequate method.

3.5.2 Exclusion Criteria

Primary Participants will be excluded for any of the following:

1. Patient is taking other experimental treatments for ALS (those that are part of an active research study).
2. Prior side effects from curcumin or turmeric containing products
3. Patient has a medical or psychiatric illness that could in the investigator's opinion interfere with the patient's ability to participate in this study.
4. Pregnant women or women currently breastfeeding.
5. Life expectancy shorter than the duration of the trial.
6. Taking an antiplatelet agent or anticoagulant (due to the theoretically increased risk of bleeding from curcumin products)

3.5.3 Control Subjects

In addition to the 50 primary participants described above, we will seek to enroll 50 healthy control participants. We will attempt to enroll one control subject from each enrolled primary participant's home, preferably a spouse or partner of similar age if possible. We plan to use this data to compare the control participants to the ALS participants at baseline, week 4 and month 6. We will not conduct further follow-up or collect additional samples with the control subjects. There are no specific inclusion or exclusion criteria for control subjects except living in the same household and free of any neurodegenerative disease. This is an observational only study for the control subjects that constitutes minimal risk for the control subjects.

3.6 Recruitment & Compensation

Patients will be mainly recruited from Dr. Bedlack's Duke ALS Clinic or will have called our center to inquire about the study. Since Dr. Bedlack's clinic and our phone lines are open to all relevant demographic groups, all groups will have access to this study. In addition we will post the following on the Duke ALS Clinic website and Twitter feed: "Duke ALS Curcumin Study is now open. Call name at telephone # for details." No compensation will be provided.

3.7 Consent Process

Consent will be obtained by Dr. Bedlack, or by his study coordinator over video/telephone services. Potential participants will be given all the time they need to review the written consent and ask questions about it. No study procedures will occur prior to the consent form being signed.

3.8 Capacity to Give Consent

Only potential participants with appropriate capacity to provide informed consent will be offered a consent form.

3.9 Risk Assessment

Curcumin appears reasonably safe. Large meta-analyses indicate that participants have not been recorded to have had a major adverse event. Side effects are infrequent (<10%) mostly gastrointestinal such as nausea, constipation, diarrhea, indigestion, and abdominal pain. Some participants also experienced hot flashes (26-28). This side effect profile is fairly similar to that experienced by a small American pilot trial utilizing Theracurmin in patients with mild cognitive impairment (19).

Risk associated with participation in the Duke or PLM database include loss of confidentiality. Risks of logging into Internet for any website including PLM include inadvertently downloading malicious software such as viruses that could put personal information or computer integrity at risk. These risks are minimized by the properties of the PLM website (see below).

Participants will be provided with contact numbers for the research staff. All sites will have an appropriate staff member available 24 hours a day, 7 days a week in the event of an emergency. Participation in this research study is purely for research purposes, and is not intended to provide any direct benefit to the volunteers other than altruism (knowledge that they are helping investigators develop better treatments, and potentially helping other patients down the road). No at risk populations such as minors, prisoners, pregnant women, or cognitively impaired adults will be included in this study. Patients who elect not to participate will have standard of care options for their ALS including participation in Dr. Bedlack's multi-disciplinary ALS clinic and the drug riluzole.

3.10 Costs to Subjects

There is no cost to subjects for participating in this study.

4 DATA ANALYSIS & STATISTICAL CONSIDERATIONS

4.1.1 Historical Controls

For each enrolled participant, three matched historical controls will be identified from the PatientsLikeMe database. Participants will be matched according to their ALSFRS-R progression rate before they start on the Theracurmin (estimated by assuming their score was normal at 48 on the date of symptom onset). Full details of the matching process are described in a previous paper (7).

4.1.2 Primary Hypothesis and Sample Size

For the primary hypothesis, we will run a t-test comparing the ALSFRS-R progression rate in our participants to a group of matched historical controls identified from the PatientsLikeMe community. This type of analysis has previously been used to look for treatment effects in

patients with ALS taking lithium (7), dexpramipexole and various forms of sodium chlorite (29) and lunasin (6). We made the following assumptions to calculate a sample size requirement for this analysis:

1. The mean ALS-FRS progression in 12 months is 11 points (rate of ~0.9 points/month) and the standard deviation at 12 months is ~8 ALS-FRS points (estimates based on unpublished data from PatientsLikeMe.)
2. The effect size we are looking for is to halve the mean progression rate
3. The treatment is not having a harmful effect (allows us to use a one-tailed comparison)
4. There is a 5% chance of seeing an effect this big by chance alone
5. We desire a 90% chance of finding an effect this big, assuming it is real
6. There will be a dropout rate of 15% over 6 months. We had a 16% survivor drop-out rate over 12 months in our prior Lunasin trial (6).

Based on these assumptions we will again need to enroll 50 participants (and compare them to 50 matched historical controls).

4.1.3 ALS Reversals

To look for an increase in the frequency of ALS reversals, we will count the number of participants who have an ALSFRS-R score that improves by 4 points or more over 6 months. The observed frequency of spontaneous ALS reversals defined in this way is 2-5% (30). We will look for an increase in this frequency to at least 10% (1 in 10).

4.1.4 Enrollment

To determine whether the participant enrollment rate in this study is greater than in studies with more traditional designs, we will compare our enrollment rate (participants enrolled per month) to the mean ALS trial enrollment rate of 2 (4) using a chi-squared test.

4.1.5 Retention

To determine whether participant retention in this study is greater than in studies with more traditional designs we will compare our dropout rate (percentage of living enrolled participants that complete the Month 6 Visit) to the mean ALS participant dropout rate of 22% at 12 months (5) using a chi-squared test.

4.1.6 Microbiome Analyses

We will compare saliva and stool microbiome samples between patients with ALS and healthy controls at every sampling point. We will compare the changes in the saliva and stool microbiome over time in patients with ALS on Theracurmin to the changes observed in untreated healthy controls. We will compare the enrollment saliva and stool microbiome across patients with different ALS origins (sporadic versus familial), onset regions (limb versus bulbar) and ALSFRS-R progression rates.

Raw, 16S microbiome sequencing data will undergo processing & strict quality control using the Qiime2 pipeline (31). Dada2 (32) will be used to infer and quantify amplicon sequence variants (ASVs). Taxonomy information will be assigned to the ASVs using the q2-feature-classifier (33) against the SILVA 132 database (34) Initial analyses of the data will focus on associations between the diversity of the microflora environment to ALS patients relative to the control samples as well as between the different forms of ALS. We will employ four different metrics for calculating within-sample diversity (alpha diversities: Faith's phylogenetic diversity, Shannon index, Pielou's evenness, and the total number of ASVs) and four different metrics for calculating between-sample diversities (beta diversities: Bray-Curtis dissimilarity, Jaccard dissimilarity, UniFrac, and weighted UniFrac). To identify associations between alpha diversity metrics and ALS, we will employ linear mixed-effects models with household as a random effect. Beta diversity indices will also be evaluated using mixed-effect modeling using the *vegan* package from the R statistical programming environment. The *PhyloSeq* (35) package will be employed to test for differential abundance of individual taxa between ALS patients and the control samples. In brief, raw ASV counts will be agglomerated in Family, Genus, and Species level data before being normalized by the 'poscounts' function from DESeq2 (36). Negative binomial linear models will then be used to identify differential abundance of microflora at the varied taxonomic ranks. Performing all analyses in a linear model framework will allow us to control for potential cofactors such as patient antibiotics usage, age, and gender. The false discovery rate will be used to control for multiple hypothesis testing.

5 SAFETY AND ADVERSE EVENTS

5.1 Adverse Events Monitoring

All adverse events (AEs), whether observed by the Investigator, elicited from the participant or volunteered by the participant, and whether ascribed to the drug or not, will be recorded. This will include the following: a brief description of the event, the date of onset, the date of resolution, the duration and type of the event, the severity, contributing factors and any action taken with respect to the study drug. This recording will commence with the institution of protocol-specific procedures (including any pretreatment procedures) and continue at each study visit or telephone contact until 4 weeks following the last study related visit.

For each adverse event, the relationship to the study drug will be recorded as one of the choices on the following scale:

> **DEFINITE** Causal relationship is certain (i.e., the temporal relationship between drug exposure and the adverse event onset/course is reasonable, there is a clinically compatible response to de-challenge, other causes have been eliminated and the event must be definitive pharmacologically or phenomenologically using a satisfactory re-challenge procedure if necessary).
>
> **PROBABLE** High degree of certainty for causal relationship (i.e., the temporal relationship between drug exposure and the adverse event onset/course is reasonable, there is a clinically compatible response to de-challenge [re-challenge is not required] and other causes have been eliminated or are unlikely).

POSSIBLE Causal relationship is uncertain (i.e., the temporal relationship between drug exposure and the adverse event onset/course is reasonable or unknown, de-challenge/re-challenge information is either unknown or equivocal and while other potential causes may or may not exist, a causal relationship to the study drug does not appear probable).

UNLIKELY Not reasonably related, although a causal relationship cannot be ruled out (i.e., while the temporal relationship between drug exposure and the adverse event onset/course does not preclude causality, there is a clear alternate cause that is more likely to have caused the adverse event than the study drug).

NOT RELATED No possible relationship (i.e., the temporal relationship between drug exposure and the adverse event onset/course is unreasonable or incompatible, or a causal relationship to study drug is implausible).

The severity of each adverse event must be recorded as one of the choices on the following scale:

MILD No limitation of usual activities

MODERATE Some limitation of usual activities

SEVERE Inability to carry out usual activities

The expectedness of an AE must be indicated when reporting adverse events. An unexpected adverse event is any adverse experience for which the specificity or severity of the event is not consistent with the known side effects of Theracurmin (or similar curcumin products), as described in section 3.9 above.

5.1.1 Reporting of Serious Adverse Events

A serious adverse drug event (SAE) is defined as any adverse event that occurs during the study that results in any of the following outcomes: death, a life- threatening adverse event (i.e., the participant was at immediate risk of death from the event as it occurred; does not include an event, that had it occurred in a more severe form, might have caused death), inpatient hospitalization or prolongation of existing hospitalization (hospitalizations scheduled before enrollment for an elective procedure or treatment of a pre-existing condition which has not worsened during participation in the study will not be considered a serious adverse event), a persistent or significant disability/incapacity (substantial disruption of one's ability to conduct normal life functions), a congenital anomaly/birth defect, a medically important event or required medical intervention to avoid one of the above outcomes. In addition to the above procedures for AEs, all SAEs will be reported to the IRB within 24 hours of recording. All serious adverse event information will be followed until resolution or an appropriate end point is reached. This may involve contacting other clinicians responsible for the participant’s care to obtain information on diagnoses, investigations performed and treatment given

Fatal or life-threatening, unexpected adverse events will be reported to the FDA by telephone, facsimile, or in writing as soon as possible, but no later than 7 calendar days after first knowledge by the Sponsor-investigator. Serious, unexpected adverse events that are not fatal or

life-threatening will be reported to the FDA by telephone, facsimile, or in writing as soon as possible, but no later than 15 calendar days after first knowledge by the sponsor-investigator.

5.2 Data & Safety monitoring

Adverse events will be tracked throughout the study as described above and below. There is no formal safety monitoring plan or DSMB, nor are there any formal stopping rules. PI will review and sign off on all adverse events and promptly report these to the IRB.

5.2.1 Data Handling and Record Keeping

The Site Investigator (SI) is responsible to ensure the accuracy, completeness, legibility, and timeliness of the data reported. Data reported in the eCRF derived from source documents should be consistent with the source documents and discrepancies should be explained.

5.2.2 Global Unique Identifier (GUID)

A patient Global Unique Identifier (GUID) will be used as an identifier. The GUID is an 11-character string that is generated using encryption technology and algorithms licensed by the NCRI from the National Institutes of Health (NIH).

The GUID is generated on a secure website that utilizes 128-bit Secure Socket Layer (SSL). The GUID is generated using an irreversible encryption algorithm – it accepts twelve identifying data elements, (e.g. last name at birth, first name at birth, gender at birth, day, month and year of birth, city and country of birth, etc.), and produces a unique random-generated character string, or GUID. No identifying information is stored in the system; it is simply used to generate the GUID. If the same information is entered again, the same GUID will be returned.

Participants will be given the option to opt out of sharing their de-identified information across studies.

5.3 PatientsLikeMe

5.3.1 General Technical Information on the PatientsLikeMe Website

The PatientsLikeMe website is one of the oldest and most complex (in terms of lines of code) Ruby on Rails commercial website in the world. PLM manages over 700,000 users, 2,400 conditions, and over 20 million medical datapoints of information. PLM's published uptime target is 99.97% uptime; they have beaten this benchmark for the past three years.

For 2014, the uptime number was 99.998%. PLM counts unplanned downtime against the uptime number, but actual planned downtime is very small. They push out releases almost on a daily basis, only a handful of which require any downtime at all, and the average downtime is under 2 minutes.

5.3.2 Security Information

We require that patient data be stored on physical servers with clearly documented physical and network security protocols. We minimize accounts and access to servers with patient data on them. All individual machines that handle patient or industry partner data are encrypted and subject to audit according to our SOP's. In addition, all PatientsLikeMe employees undergo background checks as a condition of employment. On the topic of physical security, our servers are hosted by RackSpace, a recognized leader in data center security. Physical access to the servers is tightly controlled and monitored. At PatientsLikeMe's offices, all means of entry to the building are locked and controlled. Team members must use a key, key card, or input a door code to enter the building. To date, we are unaware of any unauthorized access to our building and have not experienced any issues with break-in, theft, and so forth. Our building is monitored by security personnel 24 hours a day, 7 days a week.

On the topic of network security, we maintain strict firewall rules. Virtual private network access is required to access any part of our server infrastructure. We maintain separate virtual networks for production, testing, and client access environments to further enhance our network security.

Please refer to the whitepaper covering Security and Privacy Policies included as Appendix J within this document.

5.4 Good Clinical Practices and Human Subjects Protection Training

The investigator and coordinator involved with the conduct of this study will be certified in Good Clinical Practices (GCP) and Human Subjects Protection training. Human Subjects Protection training certification will be obtained by completing approved training, such as the online computer based training offered by the NIH Office of Human Subjects Research (http://ohsr.od.nih.gov).

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