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Study Title: Safety and Therapeutic Potential of the FDA-approved Drug Metformin for C9orf72 ALS/FTD

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FULL STUDY TITLE

A Single-Center, Open Label Study to Assess the Safety and Tolerability of Metformin in Subjects with C9orf72 Amyotrophic Lateral Sclerosis over 24 Weeks of Treatment

SHORT STUDY TITLE

Safety and therapeutic potential of the FDA-approved drug Metformin for C9orf72 ALS

Protocol number: UF2019-001

Protocol Version: 0.9
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Sponsor:
University of Florida

Phase 2

IND Number: IND144443

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1. PROTOCOL APPROVAL SIGNATORY PAGE

Study Title: A Single-Center, Open Label Study to Assess the Safety and Tolerability of Metformin in Subjects with C9orf72 Amyotrophic Lateral Sclerosis over 24 Weeks of Treatment

SAFETY APPROVAL

I have read this protocol and agree that the design of the protocol adequately protects the safety of the patients.

_____ _____

Date:

2. INVESTIGATORS AND FACILITIES

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University of Florida

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Clinical Laboratory:	University of Florida
Pharmacokinetic/Statistical Facility:	University of Florida
CRO Safety (Pharmacovigilance) Reporting:	University of Florida

3. SYNOPSIS

Title	A Single-Center, Open Label Study to Assess the Safety and Tolerability of Metformin in Subjects with C9orf72 Amyotrophic Lateral Sclerosis over 24 Weeks of Treatment.
Objectives	The primary objective is to assess the safety and tolerability of Metformin in subjects with C9orf72 amyotrophic lateral sclerosis administered for 24 weeks. Preclinical studies conducted in the Ranum lab show that Metformin improves molecular, behavioral and neuropathological phenotypes in a mouse model of C9orf72 ALS. These effects are thought to occur because Metformin prevents the activation of the protein kinase R (PKR) pathway and reduces the production of mutant proteins made from the C9orf72 expansion mutation. The overall objective is to determine if Metformin is safe in C9orf72 ALS patients and is a potentially viable therapeutic treatment for C9-ALS that reduces repeat-associated non-ATG (RAN) proteins that are produced by the C9orf72 repeat expansion mutation.

Experimental Design	This is a single-center, open label safety and tolerability trial. Approximately sixteen (16) individuals with C9orf72 amyotrophic lateral sclerosis (ALS) will be enrolled. Subjects will be screened to determine eligibility according to the specified inclusion/exclusion criteria. Following informed consent, screening, and enrollment, during study visit 1, subjects will undergo clinical examinations and questionnaires including the validated amyotrophic lateral sclerosis functional rating scale-revised (ALSFRS-R). Subjects will have blood work performed to monitor clinical safety, RAN proteins and biomarker analyses. Lumbar punctures will be performed to allow measurements of RAN protein levels in the cerebrospinal fluid (CSF) and for molecular and biomarker analyses. MRI/DTI imaging will be obtained at the beginning of the study and at the end of the drug treatment phase. Subjects will return on Day 1 for swallowing and respiratory evaluation. The subjects will then be given a first dose of Metformin (with escalating dose of the drug. The initial dose during week one will begin at 500mg/day, in the following weeks this will increase to 1000 mg/day (week two), 1500 mg/day (week three) and 2000mg per day (weeks 4 to 24). The subjects will begin by taking the first dose in the morning during week one and then morning and evening doses in weeks 2-24 with an interim assessment at days 42, 84 and final evaluation at 168 days (+/- ~7) to assess the safety and tolerability of Metformin.
Interventions	Metformin is a widely used, well-tolerated drug that has been used for decades as a first-line defense for treating type 2 diabetes. Its safety in the general population has been well established. Subjects will begin treatment with Metformin at a dosage of 500 mg with an escalation of dosage by 500 mg every week to a maximal dosage of 2000 mg. Dosing will be once daily during week one and twice daily from weeks 2-24. Due to COVID-19 the Metformin dosing may be altered with an additional dispensing to allow for continuation of the drug to week 36 (and discontinued at Week 36). This will allow for additional time to coordinate lumbar puncture at a remote location and the collection of CSF and blood sample while the participant is still taking Metformin. For participants continuing on study drug from Week 24-36, safety labs will be collected at an alternative site located near the participants home area.

Study Duration	The anticipated time from study enrollment until completion of data collection and analyses is approximately 10-18 months.
Participant Duration:	Total study participant time is expected to be approximately 26 weeks from time of screening to completion of study trial phase and then an additional 26 weeks of follow-up phase (total 52 weeks).
Study Population and Sample Size	Approximately sixteen (16) subjects with *C9orf72* ALS will be enrolled. The inclusion criteria are intended to provide a cohort of subjects with ALS who are appropriate candidates for treatment with Metformin. Subjects must be suitable candidates in the investigator's judgment and fulfill Inclusion/Exclusion criteria. Inclusion will require a diagnosis of probable, or definite ALS in accordance with the Revisited El-Escorial Criteria (Cedarbaum, 1999), positive testing for the C9orf72 expansion mutation, no allergies or contraindications to barium, Metformin, or inactive ingredients* in the study medication, and no contraindications to study procedures. Female subjects must not be pregnant or breast-feeding.
Phase	2

Inclusion Criteria	1. Male or female subjects between 18-80 years of age, inclusive. 2. Subjects have a diagnosis of probable or definite ALS in accordance with the Revisited El-Escorial Criteria (Cedarbaum, 1999). 3. Subjects have a diagnosis of C9orf72 positive ALS. 4. Subjects must be currently on an oral diet and able to take foods, pills and liquids by mouth equivalent to a score of 4 or above on the Functional Oral Intake Scale (Crary, 2005). 5. Subjects must have no known allergy to Metformin. 6. Subjects or subject's legally authorized representative must be willing and able to complete informed consent/assent and HIPAA authorization. 7. Ability of the study subject or representative to comprehend and be informed of the nature of the study, as assessed by the PI or Co-Investigators. 8. Availability to participate for the entire study duration. 9. Female subjects of childbearing potential must have a negative urine pregnancy test prior to a videofluoroscopic swallowing study (VFSS) exam during Visit 1, 3, and 4.

Exclusion Criteria:

1. Subjects who score 3 or below on the Functional Oral Intake Scale (FOIS) (Crary, 2005).
2. Subjects who do not carry the C9orf72 hexanucleotide repeat expansion as determined by previous laboratory analyses.
3. Allergy to barium sulfate or Metformin
4. Subjects with a history of clinically significant liver disease, renal disease, or any other medical condition judged to be exclusionary by the investigator.
5. Subjects who are unwilling to sign informed consent or subjects who for any other reason in the judgment of investigator are unable to complete the study.
6. Female subjects who have a positive urine pregnancy test (βhCG) at screening or visit 1, are trying to become pregnant or are breastfeeding.
7. Subjects with active cancer within the previous 2 years, except treated basal cell carcinoma of the skin.
8. Subjects who have taken any experimental drug within 30 days prior to enrollment or within 5 half-lives of the investigational drug –whichever is the longer period.
9. Subjects with known history or presence of moderate or severe renal impairment as defined by an eGFR value below 30 mL/min/1.73 m2.
10. Subjects with hepatic impairment as defined by baseline elevations of serum aminotransferases greater than 5 times upper limit of normal or evidence of liver dysfunction (e.g., elevated bilirubin).
11. Use of potentially hepatotoxic drugs: (e.g., allopurinol, methyldopa, sulfasalazine).
12. Subjects with clinically significant abnormal laboratory values in the judgment of the investigator.
13. Subject with implanted electrical device (i.e. cardiac pacemaker or a neurostimulator), metal or metallic clip(s) in their body (i.e. an aneurysm clip in the brain) that will be damaged by participation in the MRI portion of the study.

14. Anything else that, in the opinion of the investigator, would place the subject at increased risk or preclude the subject's full compliance with or completion of the study.

Assessments	Dr. James Wymer: • Disease Documentation • Comprehensive/brief physical exam • Comprehensive/brief neurologic exam • ALS Functional Rating Scale – Revised (ALSFRS-R) • BDI-Fast Screen • Eating Assessment Tool-10 (EAT-10) • Functional Oral Intake Scale (FOIS) • Forced Vital Capacity (FVC) • Peak Cough Flow • Body Mass Index • Adverse events • Clinical laboratory assessment, blood and urine • Lumbar puncture for CSF collection (20 ml volume on each study visit) • Vital signs/height and weight • Medical history • Concomitant medication • Inclusion/exclusion criteria Dr. David Vaillancourt: • MRI and DTI imaging of corticolumbar and corticobulbar tracts Nicole Herndon, M.S., CCC-SLP, BCS-S: • Videofluoroscopic Swallow Study (VFSS)

Endpoints	The primary endpoints of this study are: • To evaluate the tolerability and safety of Metformin administered over 24 weeks in subjects with ALS • To test the hypothesis that metformin can reduce RAN protein levels in patients with C9orf72 ALS
Statistical Analysis	The following safety and tolerability information will be included as summary statistics: • Type, incidence, and severity of adverse events • Physical and neurological examinations • Vital signs (heart rate, blood pressure, respiration rate, temperature), Height/Weight • Clinical laboratory tests (hematology, serum chemistry and urinalysis) • ALS Functional Rating Scale – Revised (ALSFRS-R) • Eating Assessment Tool -10 • Functional Oral Intake Scale (FOIS) • BDI-Fast Screen Depending on the study enrollment rate, the Sponsor may elect to perform an interim analysis using available data from the subset of subjects who have completed the study at that time. The results of this interim analysis will be used only for internal planning and possibly for sharing with regulatory authorities. The results of the interim analysis will have absolutely no influence on the subsequent conduct of the study.

4. LIST OF ABBREVIATIONS

Abbreviation	Definition
AE	adverse event
ALS	amyotrophic lateral sclerosis
ALSFRS-R	ALS Functional Rating Scale-Revised
ALT	Alanine Aminotransferase Test
AST	Aspartate Aminotransferase Test
BDI-FS	BDI-Fast Screen
BUN	Blood Urea Nitrogen
CBC	Complete Blood Count
CDE	Common Data Elements
CFR	Code of Federal Regulations
CMSU	Clinical Materials Services Unit
CRF	case report form
CS	clinically significant
DM	data management
DIGEST	Dynamic Imaging Grade of Swallowing Toxicity Scoring System
EAT-10	Eating Assessment Tool -10
eCRF	electronic case report form
EDC	electronic data capture
FOIS	Functional Oral Intake Scale
FDA	Food and Drug Administration
FVC	Forced Vital Capacity
GCP	Good Clinical Practice
HIPAA	Health Insurance Portability and Accountability Act
ICF	Informed Consent Form
ICH	International Council on Harmonization
MedDRA	Medical Dictionary for Regulatory Activities
NINDS	National Institute of Neurological Disorders and Stroke

RAN proteins	Repeat Associated Non-ATG (RAN) proteins
REDCap	Research Electronic Data Capture
SAE	Serious Adverse Event
SIT	Speech Intelligibility Test
SOA	Schedule of Activities
VFSS	Videofluoroscopic Swallow Study

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6. BACKGROUND AND PHARMACOKINETICS

Metformin is an inexpensive, well tolerated, orally active biguanide that is known to cross the blood brain barrier. Dr. Ranum's laboratory has discovered that Metformin can reduce RAN protein levels in cell culture and a *C9orf72* BAC transgenic mouse model, and improved disease phenotypes and motor neuron survival in the C9orf72 ALS mice. This study is designed to evaluate the safety and tolerability of Metformin in the treatment of C9ORF72-expansion positive patients with amyotrophic lateral sclerosis. The study will also test the hypothesis that metformin can reduce RAN protein levels in patients with C9orf7 ALS.

6.1. Rationale

The discovery that a G_4C_2 repeat expansion in the C9orf72 gene is the most common cause of both familial and sporadic amyotrophic lateral sclerosis (C9-ALS) presents an opportunity to better understand the molecular mechanisms of ALS and more than 40 similar microsatellite expansion diseases. In addition to ALS, these repeat expansion diseases also include Huntington's disease, myotonic dystrophy types 1 and 2, fragile X syndrome and more than ten different spinocerebellar ataxias. Currently there are no effective treatment strategies for ALS or any of these disorders. Research in the Ranum laboratory identified two fundamental mechanisms in spinocerebellar ataxia type 8 (SCA8) that are now thought to play major roles in C9-ALS and other microsatellite expansion diseases. These mechanisms include: 1) bidirectional expression of the expanded repeat mutation resulting in the expression of sense and antisense expansion transcripts; and 2) that expansion mutations express toxic proteins in all three reading frames without the canonical AUG start codon through a novel process called repeat associated non-AUG (RAN) translation. The accumulation of RAN proteins in C9-ALS and a growing number of diseases highlights the need to develop drugs that effectively block their production.

More recently, the Ranum lab has generated unpublished preclinical data (Zu et al., 2019, manuscript in revision, APPENDIX H) showing RAN translation can be regulated both in vitro and in vivo through the protein kinase R (PKR) pathway. In cells, steady state levels of several types of RAN proteins are increased by PKR activation and decreased by PKR inhibition. Additionally, the Ranum lab previously generated a *C9orf72* BAC (C9-BAC) transgenic mouse model that mimics the neuromuscular, neurological and molecular features of both C9-ALS. Using these C9-BAC mice, they show that inhibiting PKR by AAV expression of a dominant-negative form of the protein, PKR-K296R decreases RAN protein pathology in vivo and improves behavioral phenotypes. Additionally, the Ranum group has data showing Metformin, a widely used FDA-approved diabetes drug, also decreases PKR activation. Treatment of the C9orf72 BAC transgenic ALS mice with Metformin also decreased RAN protein levels, reduced neuroinflammation and improved behavioral and increased motor neuron survival in their C9-BAC mice. Based on these promising preclinical data, we now propose a study to test the safety and potential efficacy of Metformin in the treatment of C9-ALS patients.

Metformin is a widely used, well-tolerated drug that has been used for decades as a first-line defense for treating type 2 diabetes. Recently, it has also been shown to have beneficial effects on aging and neurodegenerative disorders. In Fragile X syndrome (FXS), Metformin normalizes protein translation

by inhibiting the MAPK/Erk pathway indicating a connection with protein translation. Metformin also improved survival and behavioral phenotypes in Huntington's disease mice, a microsatellite expansion disorder that also involves RAN protein accumulation.

6.2. Mechanism of Action

While there are multiple ongoing therapeutic strategies aimed at targeting individual C9-RAN proteins, there are no effective strategies targeting the production of these proteins. Structured RNAs are a common theme in RAN translation as multiple expanded repeat motifs, including the C9 G_4C_2 and G_2C_4 motifs, are known to express RAN proteins and form hairpin or G-quadruplex structures. The increased propensity of longer repeat tracts to adopt RNA structures may explain why longer repeat tracts are typically associated with higher levels of RAN protein accumulation and expression of RAN proteins in multiple reading frames. In investigating the effects of cellular stress pathways on RAN translation, we have discovered that hairpin-forming repeat expansions induce the phosphorylation of PKR which leads to an upregulation of RAN translation. Additionally, by studying compounds that can alter RAN translation, we have discovered that Metformin decreases RAN translation by inhibiting levels of phosphor-PKR in cell culture models. Additionally, we have evidence that Metformin reduces RAN translation and ameliorates phenotypes in our C9-ALS mouse model. Taken together these data suggest that Metformin may be effective in the treatment of C9-ALS.

6.3. Pharmacokinetics

We will be using Metformin 500 mg ER from Amneal Pharmaceutical or an equivalent supplier.

Absorption
The absolute bioavailability of a metformin hydrochloride 500 mg tablet given under fasting conditions is approximately 50% to 60%. Studies using single oral doses of metformin hydrochloride 500 mg to 1,500 mg and 850 mg to 2,550 mg, indicate that there is a lack of dose proportionality with increasing doses, which is due to decreased absorption rather than an alteration in elimination. At usual clinical doses and dosing schedules of metformin hydrochloride, steady state plasma concentrations of metformin are reached within 24 to 48 hours and are generally <1 mcg/mL. Following a single oral dose of metformin hydrochloride extended-release tablets, C is achieved with a median value of 7 hours and a range of 4 to 8 hours. Peak plasma levels are approximately 20% lower compared to the same dose of metformin hydrochloride tablets, however, the extent of absorption (as measured by AUC) is comparable to metformin hydrochloride tablets.

At steady-state, the AUC and C are less than dose proportional for metformin hydrochloride extended-release tablets within the range of 500 mg to 2,000 mg administered once daily. Peak plasma levels are approximately 0.6, 1.1, 1.4 and 1.8 mcg/mL for 500, 1,000, 1,500, and 2,000 mg once-daily doses, respectively. The extent of metformin absorption (as measured by AUC) from metformin hydrochloride extended-release tablets at a 2,000 mg once-daily dose is similar to the same total daily dose administered as metformin hydrochloride tablets 1,000 mg twice daily. After repeated administration of metformin hydrochloride extended-release tablets, metformin did not accumulate in plasma. Effect of food: Food decreases the extent of absorption and slightly delays the absorption of metformin, as shown by approximately a 40% lower mean peak plasma concentration (C), a 25%

lower area under the plasma concentration versus time curve (AUC), and a 35-minute prolongation of time to peak plasma concentration (T) following administration of a single 850 mg tablet of metformin hydrochloride with food, compared to the same tablet strength administered fasting. Although the extent of metformin absorption (as measured by AUC) from the metformin hydrochloride extended-release tablets increased by approximately 50% when given with food, there was no effect of food on C and T of metformin. Both high and low fat meals had the same effect on the pharmacokinetics of metformin hydrochloride extended-release tablets.

Distribution
The apparent volume of distribution (V/F) of metformin following single oral doses of metformin hydrochloride 850 mg averaged 654 ± 358 L. Metformin is negligibly bound to plasma proteins. Metformin partitions into erythrocytes, most likely as a function of time.

Metabolism
Intravenous single-dose studies in normal subjects demonstrate that metformin is excreted unchanged in the urine and does not undergo hepatic metabolism (no metabolites have been identified in humans) nor biliary excretion.

Elimination
Renal clearance is approximately 3.5 times greater than creatinine clearance, which indicates that tubular secretion is the major route of metformin elimination. Following oral administration, approximately 90% of the absorbed drug is eliminated via the renal route within the first 24 hours, with a plasma elimination half-life of approximately 6.2 hours. In blood, the elimination halflife is approximately 17.6 hours, suggesting that the erythrocyte mass may be a compartment of distribution.

6.4. Safety

Metformin is contraindicated in patients with severe renal impairment (eGFR below 30 mL/min/1.73 m2), hypersensitivity to Metformin, and acute or chronic metabolic acidosis, including diabetic ketoacidosis, with or without coma.

See attached product information sheet (Appendix H) regarding the warnings and precautions (lactic acidosis, vitamin B12 deficiency, and hypoglycemia.

The most commonly observed AEs associated with the use of Metformin are listed in table 1 of the product information sheet and include diarrhea, nausea/vomiting, flatulence, asthenia, indigestion, abdominal discomfort, and headache. Diarrhea lead to discontinuation in 6% of patients. Postmarketing experience has identified cholestatic, hepatocellular, and mixed hepatocellular liver injury (see package insert attached).

7. STUDY OBJECTIVES

7.1. Primary Objectives

Metformin is an inexpensive, well-tolerated, orally active biguanide that is known to cross the blood brain barrier. Preclinical data generated in the Ranum lab show that Metformin can reduce RAN protein levels in cell culture and in a C9orf72 BAC transgenic mouse model. Additionally, they show that Metformin treatment improves behavioral phenotypes including DigiGait performance, decreases neuroinflammation and increases motor neuron survival in these mice. These data provide the rationale for testing the therapeutic potential of Metformin in C9orf72 ALS patients. Our primary objective is to evaluate the safety and tolerability of Metformin in the treatment of C9orf72-expansion positive patients with amyotrophic lateral sclerosis. An additional primary outcome measure will be to test if metformin reduces RAN protein levels which are produced from the C9orf72 expansion mutation in blood and CSF from C9orf72 ALS patients being treated with metformin.

8. STUDY DESIGN

Approximately 16 subjects with C9orf72 positive ALS will be enrolled. The subjects will be instructed in the use of Metformin and receive the first dose of Metformin under supervision of Dr. Wymer during Visit 1.

Subjects will then continue on Metformin per the dosing schedule for 24 weeks; with interim safety assessments at days 42, 84, 126 and 168 (+/- ~7 days).

Subjects will be screened to determine eligibility according to the specified inclusion/exclusion criteria.

9. SELECTION AND ENROLLMENT OF SUBJECTS

9.1. Inclusion Criteria and Exclusion Criteria

9.1.1. Inclusion Criteria:

Subjects meeting all the following criteria may be included in the study:

1. Male or female subjects between 18-80 years of age, inclusive.
2. Subjects have a diagnosis of possible, probable or definite ALS in accordance with the Revisited El-Escorial Criteria
3. Subjects must be currently on an oral diet and able to take foods and liquids by mouth equivalent to a score of 4 or above on the Functional Oral Intake Scale (Crary, 2005).
4. Subjects must have no known allergy to barium sulfate or Metformin.

5. Subjects or subject's legally authorized representative must be willing and able to complete informed consent/assent and HIPAA authorization.
6. Ability to comprehend and be informed of the nature of the study, as assessed by the PI or Sub-Investigator.
7. Availability to participate for the entire study duration.
8. Female subjects of childbearing potential must have a negative urine pregnancy test at Screening and Visit 1, 3, and 4.

9.1.2. Exclusion Criteria:

Potential subjects meeting any of the following criteria will be excluded:

1. Subjects who score 3 or below on the Functional Oral Intake Scale (Crary, 2005).
2. Subjects who do not carry the C9orf72 hexanucleotide repeat expansion as determined by previous laboratory analysis.
3. Subjects with known allergy to barium sulfate or Metformin.
4. Subjects with a history of clinically significant liver disease, renal disease, or any other medical condition judged to be exclusionary by the investigator.
5. Subjects who are unwilling to sign informed consent or subjects who for any other reason in the judgment of investigator are unable to complete the study.
6. Female subjects who have a positive urine pregnancy test (βhCG) at screening or visit 1, 2, 3, and 4, or are trying to become pregnant or are breastfeeding.
7. Subjects with active cancer within the previous 2 years, except treated basal cell carcinoma of the skin.
8. Subjects who have taken any experimental drug within 30 days prior to enrollment or within 5 half-lives of the investigational drug – whichever is the longer period.
9. Subjects with known history or presence of moderate or severe renal impairment as defined by an eGFR value below 30 mL/min/1.73 m2.
10. Subjects with hepatic impairment as defined by baseline elevations of serum aminotransferases greater than 5 times upper limit of normal or evidence of liver dysfunction (e.g., elevated bilirubin).
11. Use of potentially hepatotoxic drugs: (e.g., allopurinol, methyldopa, sulfasalazine).
12. Subjects with clinically significant abnormal laboratory values in the judgment of the investigator.

13. Subject with implanted electrical device (i.e. cardiac pacemaker or a neurostimulator), metal or metallic clip(s) in their body (i.e., an aneurysm clip in the brain) that will be damaged by participation in the MRI portion of the study.
14. Anything else that, in the opinion of the investigator, would place the subject at increased risk or preclude the subject's full compliance with or completion of the study.

Inclusion in the study is entirely voluntary and subjects may withdraw at any time for any reason. Possible reasons for withdrawal could include serious adverse events, adverse events, and subject's choice. Subjects wishing to stop medication will immediately discontinue medication. Subjects who elect to discontinue medication will be asked to comply with end of study assessments (visit 4 on Table 3 - Schedule of Activities table) at a follow-up visit in an outpatient setting (to be determined by Investigator or Staff) at 14 +/- days after the last treatment Visit. Subjects who withdraw due to an adverse event will be followed whenever possible until resolution of the adverse event. It may be appropriate for the subject to return to the site 2 weeks after the subject is off study to evaluate for resolution of any adverse events and to receive further information about options for future clinical care. The Investigator will decide the course of action.

9.2. Study Enrollment Procedures

The following screening procedures will be conducted on each potential subject.

- Obtain informed consent as evidenced by potential subject or subject's legally authorized representative signing an informed consent. Informed consent will be signed prior to any study-related procedures and prior to screening per protocol. Informed consent will be obtained by one of the investigators and/or his or her designee.
- Record medical history
- Documentation of disease and C9orf72 mutation
- Obtain vital signs (temperature, respiratory rate (RR), heart rate (HR), blood pressure (BP)) and weight/height.
- Collect blood and urine for clinical laboratory evaluation (hematology, chemistry, liver function tests, urinalysis) and for additional molecular studies including RAN protein assessment, and urine for pregnancy test (females of childbearing potential only). For a complete list of all tests to be performed (Section 12.3.7, *Laboratory Evaluations*.)

- COVID nasal collection and testing will be completed to determine if the participant is COVD-19 negative. The results from the testing will be provided to the participant the next day when results have been placed in EPIC.
- Complete physical and neurological examinations
- ALS Functional Rating Scale – Revised (ALSFRS-R)
- Eating Assessment Tool – 10 (EAT-10)
- Functional Oral Intake Scale (FOIS)
- BDI-Fast Screen
- Collection of pretreatment AEs, if the subject is receiving Metformin pre-study
- Principal Investigator's/Sub-Investigator's review of Inclusion/Exclusion criteria and all screening results/data to assess eligibility of each potential subject
- Record/Review concomitant medications

9.2.1. Subject Recruitment and Retention

Details for recruitment procedures are provided in the protection of human subjects section. Briefly, ALS patients will be primarily identified and recruited from the University of Florida-Gainesville. Due to the rarity of the C9orf72 gene mutation, if insufficient patients are recruited, physicians at the University of Florida we are in frequent contact with physicians at other ALS centers in Florida and other parts of the US (Mayo-JAX, UF Jacksonville, Baptist Health, USF, and Miami, Johns Hopkins and Washington University) or other institutions and organizations. Generally, patients with the C9orf72 gene are well-known to their attending neurologists. The PI, co-investigators, and study coordinator will be able to work with treating neurologists at these sites to identify potential participants. C9 ALS subjects currently taking Metformin or previously prescribed Metformin may be considered. If a patient known to have the C9orf72 mutation is interested, contact information will be offered to the patient so that they can communicate with local study staff at will. We have a target recruitment schedule of only 16 patients and it is expected that there will be minimal need to reach out to other sites. All other sites, if referring patients, will ONLY BE USED FOR RECRUITMENT purposes. All recruited patients will be seen at UF Gainesville. The study will be advertised through clinicaltrials.gov and IRB approved UF listings.

9.2.2. Screening Logs

Screening logs to document reasons for ineligibility and reasons for nonparticipation of eligible subjects will be maintained to document number of subjects referred, referral source,

number screened, number failed due to ineligibility or nonparticipation of eligible subjects, and reasons for ineligibility or nonparticipation of eligible subjects.

9.2.3. Randomization/Treatment Assignment

Randomization is not applicable. This is an open label safety and tolerability trial.

10. DRUG PRODUCT

10.1. Drug Information

Table 2 Drug Information

Treatment	
Drug Name	Metformin SR
Strength	500mg
Dosage Form	Oral tablet
Manufacturer	
Dose	1 x 500 mg/day during week 1 2 x 500 mg/day during week 2 3 x 500 mg/day during week 3 4 x 500 mg/day during weeks 4-24 4 x 500 mg/day during Weeks 24-Week 36 (Optional dosing)

11. INVESTIGATIONAL PRODUCT

11.1. Handling of Study Drug, Packaging and Labeling

The Investigative team will obtain a sufficient quantity of the study drug from the UF Pharmacy to allow completion of this study including 10% extra for replacements if pills are lost or misplaced by study subjects.

The study drug will be sent to the Investigator. Each individual bottle with drug will be labeled in English containing at minimum: Protocol Number, Drug Name, Strength, Does and Route of Administration, and cautionary statements required by the FDA about the use of this FDA approved drug to test the safety and tolerability of the drug in C9orf72 ALS patients, "Keep Out of Reach of Children", storage conditions and batch/lot number.

The site pharmacy will keep a log of study drug kits received and dispensed identified by subject ID, subject initials, and date dispensed. Study drug will be stored at room temperature as directed by institutional SOP. The site pharmacist or coordinator as indicated in the delegation log will dispense study drug at the study visit #1, visit #2, visit 3 and visit 4. Subjects will begin treatment with Metformin at a dosage of 500 mg / day with an escalation of dosage by 500 mg every week to a maximal dosage of 2000 mg. Dosing will be twice daily from week 2 to week 24. In accordance with local regulatory requirements, the investigator, designated site staff, or head of the medical institution (where applicable) must document the amount of investigational product dispensed to study subjects, the amount received, and the amount destroyed upon completion of the study. The investigator is responsible for ensuring product accountability records are maintained throughout the course of the study. The inventory record will include details of Metformin study drug received and dispensed to subjects, batch, and ID numbers will be maintained by the Investigator.

At the completion of the study, all unused study drug, including spares, will be retained by the study site. An accounting must be made of any drug deliberately or accidentally destroyed.

11.2. Concomitant Interventions

All concomitant medications received by a subject during participation in the study will be recorded on the appropriate source documents.

11.2.1. Required Concomitant Medications/Interventions

No concomitant medications or interventions are required per protocol.

11.2.2. Prohibited Medications/Interventions

Concomitant medications and interventions will be reviewed at screening. The investigator may choose to exclude a subject from participation if a concomitant medication or intervention is judged likely to interfere with the study objectives or to affect the subject's safety.

The following drugs are known to interact with Metformin tablets (US Package Insert; revision 05/2018) and should not be administered with Metformin:

- Agents that may increase Metformin blood concentration;
- Agents that may decrease Metformin plasma concentrations;
- Agents that may increase the risk of lactic acidosis; Carbonic anhydrase inhibitors may increase risk of lactic acidosis.
- Agents that may impact glucose control and cause hyper or hypo glycemia; Taking Metformin with other drugs that lower blood sugar can raise your risk of hypoglycemia (low blood sugar).
- Potentially hepatotoxic drugs: (e.g., allopurinol, methyldopa, sulfasalazine)
- Drugs that reduce metformin clearance (such as ranolazine, vandetanib, dolutegravir, and cimetidine) may increase the accumulation of metformin.
- Medications that interact with Metformin include digoxin, cimetidine, furosemide, nifedipine, amiloride, ranitidine, triamterene, morphine, quinidine, vancomycin, trimethoprim and procainamide.
- Alcohol can potentiate the effect of metformin on lactate metabolism and should not be used in excess while taking Metformin.

11.3. Subject Compliance

Subject compliance will be assessed at all visits with direct and open questioning about compliance and adverse events. All returned medication will be counted. Greater than 10% noncompliance will prompt re-education of subject by the investigator on the importance of medication compliance.

12. STUDY PROCEDURES

12.1. Schedule of Activities

The schedule of study activities is presented in Table 3.

Timeline:

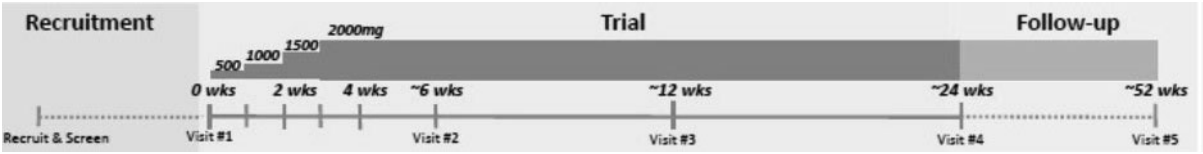

Table 3. Schedule of Activities:

Evaluation	Recruit & Screen	Visit 1	Visit 1	Visit 2	Visit 3	Phone Call	Visit 4	Visit 4	Phone Call	Phone Call
		Day 1	Day 2	6 weeks	12 weeks	18 Weeks	Day 1 24 weeks	Day 2 24 weeks	36 Weeks	52 weeks
		PM	AM	(42 days)	(84 days)	(126 days)	(168 days)	(168 days)	(252 days)	(365 days)
Informed Consent	X									
Inclusion/Exclusion Review	X									
Documentation of Disease	X									
Record/Review Concomitant Medications	X		X	X	X	X	X		X	
Medical History/Demographics	X		X			X			X	
Vital Signs/Height and Weight	X		X	X	X		X			
Comprehensive Physical and Neurological Exams	X				X		X			
Brief Physical and Neurological Exam			X	X						
C9+ and RAN protein blood draw	X		X	X	X		X			
Clinical Labs for safety (hematology, chemistry, liver function and urinalysis)	X		X	X	X		X			
Urine Pregnancy Test (females of childbearing potential)	X		X	X	X		X			
Lumbar Puncture (for RAN proteins and biomarkers)			X	X	X		X			
MRI/DTI imaging			X					X		
VFSS Penetration-Aspiration Scale		X			X			X		
Functional Oral Intake Scale (FOIS)		X			X	X		X	X	X
ALS Functional Rating Scale – Revised (ALSFRS-R)	X		X	X	X	X	X		X	X
Eating Assessment Tool-10 (EAT-10)		X			X	X		X	X	X
Forced Vital Capacity (FVC)		X			X			X		
Peak Cough Flow		X			X			X		
Body Mass Index		X			X			X		
BDI-FastScreen	X			X	X		X			
Adverse Event			X	X	X	X	X		X	X
Dispense Study Drug			X	X	X					

Drug Accountability/Compliance				X	X		X			

Outcome Measures:

Primary: 1. ALSFRS-R score

Secondary: 2. Soluble GP in blood; 3. Swallowing physiology (PAS, DIGEST); 4. PFT's (FVC, PCF); 5. MRI/DTI outcomes.

12.2. Timing of Study Activities

No study procedures will be performed prior to the signing of the Informed Consent Form (ICF). All subjects will sign an ICF prior to undergoing any study tests or procedures.

Visit windows are consecutive calendar days and the target visit dates are calculated from the Baseline Visit, which should occur within 14 to 28 days of the Screening Visit. All clinic visits and calls after the Baseline visit will have a +/- 7 work day window.

Inclusion in the study is entirely voluntary and subjects may withdraw at any time for any reason. Possible reasons for withdrawal could include serious adverse events, adverse events, and subject's choice. Subjects wishing to stop medication will immediately discontinue test article. Subjects who elect to discontinue medication will be asked to comply with end of study assessments (visit 4 on Table 3 Schedule of Activities) at a follow up visit in an outpatient setting (to be determined by Investigator or Staff) at 14 +/- days after the last treatment dose. Subjects who withdraw due to an adverse event will be followed whenever possible until resolution of the adverse event. It may be appropriate for the subject to return to the site 2 weeks after the subject is off study to evaluate for resolution of any adverse events and to receive further information about options for future clinical care. The Investigator will decide the course of action.

12.2.1. Screening procedures

At the screening visit, potential subjects will be informed about study procedures and will then sign an informed consent form. Inclusion/exclusion criteria will be reviewed and a medical history and comprehensive physical and neurological examination will be completed. Vital signs, height, weight, and concomitant medications will be recorded. Safety laboratory tests will be performed including complete blood count (CBC) with differential, electrolytes, BUN, creatinine, ALT, AST, total bilirubin, albumin, and a urine pregnancy test for women of childbearing potential. and COVID-19 nasal collection and testing.

The following procedures will be performed during the screening visit:

- Informed consent
- Inclusion/exclusion assessment

- Documentation of Disease
- Medical history/Demographics
- Record concomitant medications
- Clinical labs for safety (hematology, chemistry, liver functions, and urinalysis)
- C9+ and RAN protein blood draw
- Urine Pregnancy Test (females of childbearing potential)
- COVID-19 nasal collection and testing - Due to COVID-19 Pandemic nasal collection and testing of the participants has been implemented to determine if the participant is COVID negative. The collection is done at UF Springhill collection site and testing is completed at Rocky Point Labs overnight with results placed in EPIC. The results of the testing will be provided to the participant once results are posted in EPIC. This will collection and testing will continue throughout the participants participation in the study at this visit.
- Vital signs
- Height and weight
- Comprehensive physical and neurological examinations
- ALS Functional Rating Scale – Revised (ALSFRS-R)
- BDI-Fast Screen

As part of the screening visit, the investigator will determine the competence of caregiver to administer the study drug at home. Teaching will begin on the importance of compliance and proper handling of study drug. All inclusion and exclusion criteria and safety laboratory tests will be reviewed by the investigator prior to scheduling the baseline visit (visit 1 on schedule of activities table). A paper log will be kept at the site to record all subjects screened for entry into the study. This information will also be captured electronically in the electronic data capture system (EDC) using REDCap. REDCap (Research Electronic Data Capture) is a secure, Web-based application designed to support traditional case report form data capture. Demographic characteristics of all subjects who are screened will be recorded whether or not they qualify for entry into the study. The reason for non-qualification will be recorded for all subjects who are not eligible. The reason for nonparticipation will also be recorded for subjects who are eligible but choose not to participate in the trial. If a subject fails screening for the study, the subject can be re-screened if the investigator determines it is appropriate to do so. At any time during the study, repeat laboratory tests can be obtained if the investigator or central laboratory believes that a laboratory test result is in error. Additional laboratory tests may also be obtained at any time during the study, at the discretion of the investigator.

ALS Functional Rating Scale – Revised (ALSFRS-R) Questionnaire

The ALSFRS-R is a quickly administered (5 minute) ordinal rating scale (ratings 0-4) used to determine subjects' assessment of their capability and independence in 12 functional activities/questions (Cedarbaum, 1999). All 12 activities are relevant in ALS. Initial validity was established by documenting that in subjects with ALS, change in ALSFRS-R scores correlated with change in strength over time, was closely associated with quality of life measures, and predicted survival (**Appendix A**). The test-retest reliability is greater than 0.88 for all test items. The advantages of the ALSFRS-R are that the categories are relevant to ALS; it is a sensitive and reliable tool for assessing activities of daily living function in subjects with ALS; and it is quickly administered. In a recent trial employing the ALSFRS as a secondary outcome measure, placebo-treated patients showed a decline of 0.92 units per month, with a standard error of 0.08. With appropriate training the ALSFRS-R can be administered with high inter-rater reliability and test retest reliability.

Beck Depression Inventory-Fast Screen (BDI-FS) (Appendix D) is a brief self-report inventory designed to evaluate depression in patients with medical illness. It has been found to be an efficient and effective tool for depression screening in the ALS population. The symptoms and prognosis of ALS have an immense impact on a patient's life as well as their family and caregivers. Depression, when present, can significantly affect the life of patients and is more commonly seen in situations involving the threat of loss. BDI-FS is a short 7 question self-report that can assist the investigator and staff in evaluating if patients are dealing with depression. The BDI-FS is evaluated at the time of the subject's study visit by study staff. Each answer is scored on a scale of 0-3. Measures of 4-6 correspond to mild depression, 7-9 indicate moderate depression, and 10-21 indicates moderate-severe depression. Any measurement indicating depression, or any verbal suggestion of suicidality, will be reported to the investigator immediately. The investigator will address the results with the subject prior to their leaving the facility. Any interventions deemed necessary will be addressed by a qualified physician or investigator at that time.

12.2.2. On-Study/On-Intervention Evaluations/Procedures

12.2.2.1. Baseline, Visit 1, Day 1 pm and Visit 1, Day 2 am:

The baseline visit (Visit 1, Day 1 pm and Visit 1, Day 2 am), will cover 2 days and include the first dose of study drug, and study drug dispensing. It will be scheduled within 14 to 28 days after the screening visit. At this visit inclusion/exclusion criteria and concomitant medications will be reviewed. Vital signs, brief physical and neurologic examination, as well as study questionnaires and testing will be performed as noted in the schedule of activities. To lessen travel for participants during the COVID-19 pandemic, and after the pandemic, the Visit 1, Day 1 and Visit 1, Day 2 may take place in the same week as the Screening visit. If these visits are scheduled to take place in the same week, the *clinical labs for safety and *blood draw for C9+ and RAN proteins, and *urine pregnancy test, will not be repeated.

The following documentation and procedures will be performed at Baseline (Visit 1, Day 1):

- FOIS
- BDI-Fast Screen (BDI-FS)
- VFSS – Penetration Aspiration Scale
- EAT – 10
- Forced Vital capacity (FVC)
- Peak Cough Flow
- Body Mass Index

The following documentation and procedures will be performed at Baseline (Visit 1, Day 2):

- Adverse event review
- Inclusion/exclusion assessment
- Record/review Concomitant medications
- Medical history
- Vital signs; weight and height
- Brief physical exam
- Brief neurologic exam
- Clinical labs for safety (hematology, chemistry, liver functions, and urinalysis)
- Blood draw for C9+ and RAN proteins
- Lumbar puncture for RAN proteins and biomarkers
- Urine Pregnancy Test (females of childbearing potential)
- MRI/DTI imaging
- ALSFRS-R
- Dispense study drug

Eating Assessment Tool -10 (EAT-10)
EAT-10: The Eating Assessment Tool-10, is a validated 10-item patient report scale of perceived swallowing impairment (Belafsky et al. 2008). The EAT-10 uses a 5-point ordinal rating scale where a patient rates their perceived degree of impairment for each item from 0 (no impairment) to 4 (severe impairment) for a total range of scores between 0 (no perceived swallowing impairment) to 40 (severe impairment) (**Appendix B**). One of the former PIs of this study (Dr. Plowman) recently determined that the EAT-10 was sensitive to identify ALS patients with severe dysphagia including aspiration. A Receiver Operating Curve analysis revealed that a cut point of 8 on the EAT-10 yielded a sensitivity of 86%, specificity of 72%,

likelihood ratio of 3.1, and negative predictive value of 95.5% for detecting aspiration in ALS (Plowman, et al., 2016).

Functional Oral Intake Scale (FOIS)
The FOIS is a simple 7-point scale (Crary, 2005 – see **Appendix C**) that describes the subject's functional status with respect to oral intake, ranging from 1 (No oral intake) to 7 (total oral intake with no restrictions).

12.2.2.2. Study Visit 2, Week 6:

All subjects will return at week 6 for a brief evaluation. The visit window is to be scheduled at 6 weeks (42 days) plus or minus 3 days. Participants will be seen for assessment of any adverse events, and concomitant medications. Study drug accountability and compliance will be assessed. Vital signs, brief physical and neurologic examination, as well as study questionnaires and testing will be performed as noted in the schedule of activities.

The following procedures will be performed at Visit 2, Week 6:

- Adverse events review
- Record/review Concomitant medications
- Vital signs, weight and height
- ALSFRS-R
- Brief physical exam
- Brief neurologic exam
- Clinical labs for safety (hematology, chemistry, liver functions, and urinalysis)
- Blood draw for C9+ and RAN proteins
- Lumbar puncture for RAN proteins and biomarkers
- Dispense study medication
- Drug Accountability/Compliance
- BDI-Fast Screen (BDI-FS)
- Urine Pregnancy Test (females of childbearing potential)

12.2.2.3. Study Visit 3, Week 12:

All subjects will return at week 12 for a mid-study evaluation. The visit window is to be scheduled at 12 weeks (84 days) plus or minus 3 days. Participants will be seen for assessment of any adverse events, and concomitant medications. Study drug accountability and compliance will be assessed. Vital signs, physical and neurologic examination, as well as study questionnaires and testing will be performed as noted in the schedule of activities.

The following procedures will be performed at Visit 3, Week 12:

- Adverse events review
- Record/review concomitant medications
- Vital signs, weight and height
- ALSFRS-R
- Comprehensive physical exam
- Comprehensive neurologic exam
- Clinical labs for safety (hematology, chemistry, liver functions, and urinalysis)
- Blood draw for C9+ and RAN proteins
- Urine Pregnancy Test (females of childbearing potential)
- COVID-19 nasal collection and testing may take place at this visit if travel time allows for the participant - Due to COVID-19 Pandemic, nasal collection and testing of the participants has been implemented to determine if the participant is COVID negative. The collection is done at UF Springhill collection site and testing is completed at Rocky Point Labs overnight with results placed in EPIC. The results of the testing will be provided to the participant once results are posted in EPIC. This collection and testing may continue throughout participation in the study at this visit.
- Lumbar puncture for RAN proteins and biomarkers
- FOIS
- VFSS – Penetration Aspiration Scale
- EAT – 10
- Forced Vital capacity (FVC)
- Peak Cough Flow
- Body Mass Index
- BDI-Fast Screen (BDI-FS)
- Dispense study medication
- Drug Accountability/Compliance

12.2.2.4. Phone Call, Week 18:

Subjects will be contacted by phone at Week 18 and the following study related evaluations will be completed:

- ALSFRS-R
- EAT-10
- FOIS

- Adverse event review
- Record/review concomitant medications

12.2.2.5. Study Visit 4, Week 24 Day 1 or Day 2:

All subjects will return at week 24 for a two-day end of study evaluation. The visit window is to be scheduled at 24 weeks (168 days) plus or minus 3 days. Participants will be seen for assessment of any adverse events, and concomitant medications. Study drug accountability and compliance will be assessed. Vital signs, physical and neurologic examination, as well as study questionnaires and testing will be performed as noted in the schedule of activities.

The following documentation and procedures will be performed at Visit 4, Week 24, on Day 1 or Day 2:

- Adverse events
- Record/review concomitant medications
- Vital signs, weight and height
- ALSFRS-R
- Comprehensive physical exam
- Comprehensive neurologic exam
- Clinical labs for safety (hematology, chemistry, liver functions, and urinalysis) – Due to the COVID-19 Pandemic, and only for the participants currently enrolled (3) in this study, the clinical labs for safety will be drawn at a local lab or physician's office and will be shipped as needed. If there is any charge for the draw the participant may either pay the invoice and be reimbursed or have the lab contact the study team in the ICF to pay the invoice directly. This modification to the protocol is to prevent potential exposure to COVID-19 by minimizing participant travel to our site.
- Blood draw for C9+ and RAN proteins – Due to the COVID-19 Pandemic, and only for the participants currently enrolled (3) in this study, additional blood samples will be collected and will be shipped to the Ranum Lab for analysis. The kits, drawing instructions, and shipping documents will be sent to the physician or the participant to complete this procedure. If there is a charge for the blood draw the participant may either pay the invoice and be reimbursed or have the lab contact the study team in the ICF to pay the invoice directly. This modification to the protocol is to prevent potential exposure to COVID-19 by minimizing participant travel to our site.
- Urine Pregnancy Test (females of childbearing potential)
- COVID-19 nasal collection and testing may take place at this visit if travel time allows for the participant - Due to COVID-19 Pandemic, nasal collection and testing of the participants has been implemented to determine if the participant is COVID

negative. The collection is done at UF Springhill collection site and testing is completed at Rocky Point Labs overnight with results placed in EPIC. The results of the testing will be provided to the participant once results are posted in EPIC. This collection and testing may continue throughout participation in the study at this visit.

- Lumbar puncture for RAN proteins and biomarkers – Due to the COVID-19 Pandemic, and only for the participants currently enrolled (3) in this study, this procedure may be completed in the participant's home area and the samples shipped to the Ranum Lab for analysis. The procedure will be completed by a licensed physician who is qualified in the proper procedures and has agreed to complete the lumbar puncture per the protocol. The lumbar puncture kit and needle will be shipped to the physician for this procedure. This modification to the protocol is to prevent potential exposure to COVID-19 by minimizing participant travel to our site.
- Drug Accountability/Compliance
- FOIS
- VFSS – Penetration Aspiration Scale
- EAT – 10
- Forced Vital capacity (FVC)
- Peak Cough Flow
- Body Mass Index
- BDI-Fast Screen (BDI-FS)
- MRI/DTI imaging

12.2.2.6. Phone call, Week 36:

Subjects will be contacted by phone at Week 36 and the following study related evaluations will be completed:

- Adverse events review
- Record/review concomitant medications
- ALSFRS-R
- EAT – 10
- FOIS

12.2.2.7. Phone call, Week 52 (Follow-up):

Subjects will be contacted by phone at week 52 for an end of study evaluation. The phone call window is to be scheduled at 52 weeks plus or minus 3 days.

- Adverse events review

- Record/review concomitant medications
- ALSFRS-R
- EAT – 10
- FOIS

Missed Visits: If a subject fails to appear for a scheduled visit, the site will contact the subject. The site will stress the importance of the evaluation and reschedule within a window of +/- 3 days if possible.

12.2.2.8. Study Medication/Intervention Discontinuation Evaluations/Procedures

The investigator must document within 24 hours any subject permanently discontinuing study drug, documenting the reasons for discontinuation in the CRF and on the appropriate source documents. Subjects will be asked to comply with end of study assessments (visit 4 on schedule of activities table) at a follow-up visit in an outpatient setting (to be determined by Investigator or Staff) at 14 +/- days after the last treatment dose. The outcome measures will be documented at the study visit(s) following discontinuation of study drug. Subjects who have discontinued study drug will be removed from ongoing participation in the study.

Inclusion in the study is entirely voluntary and subjects may withdraw at any time for any reason. Possible reason for withdrawal could include serious adverse events, adverse events, and subject's choice. Subjects wishing to stop medication will immediately discontinue test drug. Subjects who elect to discontinue medication will be asked to comply with end of study assessments (visit 4 on schedule of activities table) at a follow up visit in an outpatient setting (to be determined by Investigator or Staff) at 14 +/- days after the last treatment dose. Subjects who withdraw due to an adverse event will be followed whenever possible until resolution of the adverse event. It may be appropriate for the subject to return to the site 2 weeks after the subject is off study to evaluate for resolution of any adverse events and to receive further information about options for future clinical care. The Investigator will decide the course of action.

All serious adverse events (SAEs), whether or not the event is deemed drug-related, will be reported on the SAE Report Form.

12.2.2.9. Final On-Study Evaluations

Subjects who elect to discontinue medication will be asked to comply with end of study assessments (see visit 4 on schedule of activities table) at a follow-up visit in an outpatient setting (to be determined by Investigator or Staff) at 7 +/- days after the last treatment visit. Subjects who withdraw due to an adverse event will be followed whenever possible until resolution of the adverse event. It may be appropriate for the subject to return to the site 2 weeks after the subject is off study to evaluate for resolution of any adverse events and to

receive further information about options for future clinical care. The Investigator will decide the course of action.

12.2.2.10. Off-Study Requirements

Study medication will be distributed at Week 24 (due to COVID-19 pandemic and travel difficulties). Drug will be discontinued and collected at Week 36. Study drug will be ordered by the Co-Investigator, dispensed by Investigational Drug Service and a study team member will ship it to the participants (3). There are no additional risks of extending the medication by 12 weeks. The subject will be contacted by phone at weeks 18 and 36 to evaluate for resolution of any adverse events and to receive further information about options for future clinical care. In addition to this contact there will be another phone contact at weeks 36 and 52 to monitor long term health and follow-up on clinical outcome. This phone call will be at the discretion of the Investigator. Subjects may be contacted for follow-up safety evaluations, including possible blood draw for further study.

12.2.2.11. Pregnancy

Women who are pregnant will not be enrolled in the study. Metformin has not been well studied in pregnancy and the Metformin US Package Insert notes that based on animal studies Metformin was not teratogenic. A recent review of RCT data reveal no evidence of an increase in congenital malformations or miscarriages when Metformin is started before pregnancy and continued to term (Hyer et al 2018).

Following administration of study drug, any known cases of pregnancy in female subjects or female partners of male subjects, will be reported until 30 days after the subject completes or withdraws from the study. (Section 13.3; *Procedures for Reporting Adverse Events*)

12.3. SPECIAL INSTRUCTIONS AND DEFINITIONS OF EVALUATIONS

12.3.1. Protocol Deviations

Missed visits and any procedures not performed (not attempted) for any reason will be reported as protocol deviations. Details regarding any deviations will be documented.

12.3.2. Documentation of Amyotrophic Lateral Sclerosis

To be eligible for participation in this trial, participants must have ALS defined as probable or definite ALS in accordance with the Revisited El-Escorial Criteria (Cedarbaum,1999). These criteria will be clearly designated on source documentation and in the electronic case report forms (eCRFs).

12.3.3. Medical History

A comprehensive medical history will be obtained at the screening visit and reviewed for any exclusionary criteria.

12.3.4. Treatment History

Medications will be reviewed for concomitant therapies that would be exclusionary.

12.3.5. Concomitant Medications/Treatments

Current medications will be reviewed for contraindicated therapies.

12.3.6. Clinical Assessments

Patient Reported Outcomes: The Eating Assessment Tool 10[91] (**Appendix B**) and the ALSFRS-R (Appendix A) will be administered at each time point to track self-reported bulbar impairment profiles. To facilitate efficiency, questionnaires will be sent to patient's homes one-week prior to appointments for completion.

12.3.7. Laboratory Evaluations

Blood specimens will be collected by qualified study center personnel at Screening, Visit 1, 2, 3, and Visit 4 as described under study procedures and sent to Shands for analysis for the following safety laboratory evaluations: hematology (complete blood count with differential), chemistry (electrolytes, BUN, creatinine, ALT, AST, total bilirubin, albumin). Urine will be collected for urinalysis.

In females, of childbearing potential, urine will be collected for pregnancy test during recruitment/screening, visit 1, visit 3 and visit 4. Prior to each VFSS, women of childbearing potential will be asked to perform the urine pregnancy test in a private bathroom located near the exam room.

C9orf72 testing will be performed using the repeat-primed PCR assay and other molecular assays as needed in the Ranum lab.

RAN protein levels: Isolation of peripheral blood lymphocytes (PBLs) for protein and other molecular studies will be performed in the Ranum and the Garrett laboratories. C9 RAN proteins present in the blood will be analyzed using highly sensitive MesoScale Discovery (MSD) electrochemiluminesence assays using custom antibodies generated and characterized by the Dr. Ranum's group. The MSD assays has been optimized for the detection of C9 RAN proteins. The remainder of the samples will be stored in the Center for NeuroGenetics bank for additional molecular or biochemical studies for an indefinite period of time.

Abnormal laboratory test results and positive pregnancy test results will be flagged by the laboratory. All clinically important abnormal laboratory tests occurring during the study will be followed until they resolve (return to normal or baseline values) or stabilize, or until they are considered by the Investigator to be no longer clinically significant.

COVID-19 nasal collection and testing may take place at this visit if travel time allows for the participant - Due to COVID-19 Pandemic, nasal collection and testing of the participants has been implemented to determine if the participant is COVID negative. The collection is done at UF Springhill collection site and testing is completed at Rocky Point Labs overnight with results placed in EPIC. The results of the testing will be provided to the participant once results are posted in EPIC. This collection and testing will continue throughout participation in the study.

12.3.8. Lumbar Puncture Analysis:

CSF fluid will be drawn from the patient for the following disease evaluations:

Inflammation and RAN protein levels: Neuroinflammation markers and/or C9 RAN proteins present in CSF will be analyzed using a highly sensitive MesoScale Discovery (MSD) electrochemiluminesence assays and other assays. For some of these studies custom antibodies generated and characterized by the PI will be used. The RAN MSD assays have been optimized for the detection of the C9 GP RAN proteins. These and other molecular studies will be done in the Ranum. Any remaining CSF will be stored indefinitely in the Center for NeuroGenetics bank for future studies.

Due to the COVID-19 Pandemic, and only for the participants currently enrolled (3) in this study, this procedure may be completed in the participant's home area and the samples shipped to the Ranum Lab for analysis. The lumbar puncture will be completed by a licensed physician who is qualified to perform this procedure and agrees to complete the procedure per the protocol.

Metabolomics: The level of Metformin in the CSF and levels of various metabolomics components will be analyzed by traditional liquid chromatography tandem mass spectrometry of (UPLC/MS/MS) in the Garrett lab.

12.3.9. Speech and Swallowing Function:

The following will be performed at the Fixel Institute for Neurological Diseases, in room 1010.6 as directed by Nicole Herndon, a Certified Speech Language Pathologist. The following procedures and outcomes will be performed and derived:

- ***Videofluoroscopic Swallow Study (VFSS):*** VFSS will be performed using a Phillips BV Endura fluoroscopic C-arm unit (GE OEC 8800 Digital Mobile C-Arm system type 718074) that is housed at Fixel and used for clinical and research purposes. Fluoroscopic data will be captured in high resolution at 30 frames per second and in the lateral field of view using a TIMS Dicom (Version 3.2, TIMS Medical, TM, Chelmsford, MA). Patients will be seated and will complete a standardized bolus protocol consisting of: 1) three 5ml thin liquid barium; 2) a regular sip of thin liquid taken from a cup containing 90cc of thin liquid; 3) consecutive cup sip of remaining barium; 4) three teaspoons of thin honey consistency barium; 5) two teaspoons of pudding-thick barium; 6) graham cracker with pudding-thick barium; and a 7) 13mm barium tablet (EZ-Disk). To ensure patient safety, a bail out criterion will be utilized and the VFSS will cease on the third episode of frank aspiration or residue greater than 75% of either vallecular or pyriform housing that is unable to be cleared with strategies. Once the study is completed, recordings will be saved and digitally backed up for subsequent analyses.
- ***VFSS Outcomes:*** Two independent, blinded and trained raters will perform analyses on the data for every bolus using validated procedures. Discrepancies between raters will be flagged and resolved at a consensus meeting and three swallows from each evaluation will be repeat rated at random for evaluation of intra-rater reliability. VFSS measures are summarized below and include indices of Global Swallowing Function (Appendix E) and Swallowing Safety (Appendix F).
- ***Global Swallowing Function*:** The validated Dynamic Imaging Grade of Swallowing Toxicity (DIGEST) will be performed on all collected videofluoroscopic swallowing studies to assess global swallowing function (Hutcheson and Fuller, 2015). The DIGEST total score is determined using the composite of individual airway safety and bolus efficiency subscores (range: 0-4). The DIGEST total is rated on a 5-point ordinal score ranging from 0 (no dysphagia) to 4 (life-threatening dysphagia). For statistical analysis, DIGEST total scores will be compared within individuals between the Visit 1 (baseline), Visit 3 (12 weeks) and Visit 4 (24 weeks). DIGEST total scores of <2 indicate functional swallowing and scores ≥ 2 indicate dysphagia. The DIGEST scoring schema is provided in Appendix E.
- ***Swallowing Safety:*** The Penetration-Aspiration scale [(PAS), (Rosenbek et al., 1996)] is a validated eight-point ordinal scale indexing the degree of airway invasion during swallowing, the participant's response, and whether the invasive material is successfully ejected from the airway. PAS scores of 1 and 2 are considered safe, while scores between 3 and 5 indicate penetration of material in the upper airway at or above the level of the true vocal folds.

PAS scores greater than 6 indicate aspiration of material below the level of the true vocal folds into the airway. For statistical comparisons across airway safety status levels the convention is as follow: safe swallowing = PAS $\leq$2 and unsafe swallowing = PAS$\geq$3.

12.3.9.1 Pulmonary Function

The following will be performed by trained clinical research coordinators at the Fixel Institute for Neurological Diseases:

- ***Voluntary Peak Cough Flow Testing:*** Participants will be seated with a nose clip in place and instructed to take a deep breath in and "cough hard like you have something stuck in your throat". To reduce the potential for labial air leakage due to reduced orofacial muscle tone, a rubber flanged mouthpiece (MTH6400), will be attached to the end of a Mini-Wright CE0120 analogue peak flow meter device. The patient will perform three trials inter-spaced with a one-minute rest break. Outcomes will include peak expiratory flow (L/min) (PEF). The highest obtained values will be used.
- ***Pulmonary Function Testing****:* Forced vital capacity (% predicted) will be assessed using a hand-held digital spirometer (MD Spiro Micro 1 Spirometer).

12.3.10. Imaging

The following will be performed on the Siemens or Phillips 3T system at the University of Florida under the direction of Dr. Vaillancourt:

- ***Measurement of brain structure and brain function*****:** This experiment will require the participant to lie down on a platform that will move into an enclosed magnetic resonance imaging system for both functional magnetic resonance imaging (fMRI) and structural imaging (diffusion, T1 and T2 weighted). fMRI is a technique used to non-invasively measure changes in brain activity in relation to performance of a specific task. T1, T2, and diffusion imaging examine the macrostructure and microstructure of the brain tissue non-invasively. In brain structure measures, the participant will lie still in the MRI without performing a task. In brain function measures, the participant will perform a motor task. The participant will be asked to practice the experimental tasks described below until they feel comfortable performing them. During this task the participant will use a small pinch grip device that they will hold between the thumb and index finger. Once inside the scanner, the participant will be asked to lie still while the study staff perform a scan to provide an anatomical picture of the brain. The participant will be asked to

complete a series of tasks during which study staff will obtain pictures of the functional changes in brain activity. During this task the participant will move their hand under different conditions. In one condition the participant will be asked to perform the task with no sensory information about the task. Study staff will refer to this task as a "self-initiated" task. No stimulus will be presented in this condition. In another condition the participant will be asked to respond to a stimulus, which will give them information regarding the timing of their movement or the extent to which they should contract their muscles. Study staff will refer to this task as an "auditory cued task" or a "visually cued task". The participants' goal during all movement conditions will be to move as quickly as possible.

12.3.11. BDI-Fast Screen

The BDI-Fast Screen, which assesses depression (**Appendix D**), will be administered at Visit 2, Visit 3, Phone call at 18 weeks, Visit 4, and Phone call at week 36. Subject indicating depression will be counseled by a physician prior to leaving the clinic if they are at UF and may be referred to a mental health professional.

12.3.12. Subject Adherence Assessments

Adherence to study requirements and compliance with medication will be assessed at the study visit through direct questioning by staff personnel.

13. SAFETY AND ADVERSE EVENTS

The AE definitions and reporting procedures provided in this protocol comply with all applicable International Conference on Harmonization (ICH) guidelines. Study staff will carefully monitor each subject throughout the study for possible AEs. All AEs will be documented on CRFs designed specifically for this purpose. It is also important to report all AEs, especially those that result in permanent discontinuation of the investigational product being studied, whether serious or non-serious.

Safety Review Plan and Monitoring- Oversight of participant safety includes review of adverse events as well as study progress, data integrity and study outcomes. The study will engage a physician safety monitor to discuss issues regarding side effects and adverse events. The safety monitor is external to the clinical study and is free of any conflicts of interest to maintain independence and objectivity.

Safety and study progress reviews

1. Adverse events will be reviewed by the Principal Investigator, the Co-Principal Investigator(s) and the Safety Officer

2. Study progress will be reviewed by the study investigators on a quarterly basis (e.g. Recruitment, retention, protocol adherence).

3. For each Continuing Review period, a report will include

a. list and summary of adverse events
b. whether adverse events rates are consistent with pre-study assumptions
c. Summary of recruitment and retention and reason for withdrawals
d. whether the study is on track to be completed and accomplish the stated aims.

13.1. Adverse Event Reporting and Follow Up

Subjects will be instructed to inform clinic personnel of any untoward medical symptoms and/or events that may arise during the course of the study. If an adverse event is experienced by a subject, then the subject will be questioned concerning symptoms that may have occurred after the administration of the study drug. The incidence, severity and duration of all AEs will be recorded according to the following scale:

Mild	Adverse event resulting in discomfort, but not sufficient to cause interference in normal daily activities
Moderate	Adverse event resulting in discomfort that is sufficient to cause interference in daily activities.
Severe	Adverse event resulting in discomfort causing an inability to carry out normal daily activities

Subjects who withdraw due to an adverse event will be followed whenever possible until resolution of the adverse event. It may be appropriate for the subject to return to the site 2 weeks after the subject is off study to evaluate for resolution of any adverse events and to receive further information about options for future clinical care. The Investigator will decide the course of action.

13.2. Assessing Relationship to Study Drug

The Principal Investigator/Sub Investigators will assess the relationship of all adverse reactions to the drug, using the following scale:

Probable	A clinical event, including laboratory test abnormality, with a reasonable time sequence to drug administration, unlikely to be attributed to concurrent disease or other drugs or chemicals, and which follows a clinically reasonable response on withdrawal.
Possible	A clinical event, including laboratory test abnormality, with a reasonable time sequence to drug administration, but which could also be explained by concurrent disease or other drugs or chemicals. Information on drug withdrawal may be lacking or unclear.
Unlikely	A clinical event, including laboratory test abnormality, with a temporal relationship to drug administration which makes a causal relationship improbable, and which other drugs, chemicals or underlying disease provide plausible explanation.
Unrelated	This category is applicable to AEs which are judged to be clearly and incontrovertibly due to extraneous causes (diseases, environment, etc.) and do not meet the criteria for drug relationship listed for the above-mentioned conditions.

Subjects who withdraw due to an adverse event will be followed whenever possible until resolution of the adverse event. It may be appropriate for the subject to return to the site 2 weeks after the subject is off study to evaluate for resolution of any adverse events and to receive further information about options for future clinical care. The Investigator will decide the course of action.

13.3. Procedures for Reporting Adverse Events

Subjects will be instructed to inform clinic personnel of the AEs that may arise during the course of the study. Treatment of any AEs will be administered under the direction of the Investigator.

All symptoms will be recorded by clinic staff and will be reviewed by the Investigator or Sub-Investigators prior to any subsequent dosing.

When appropriate, medical tests and examinations will be performed to document resolution of the event(s).

Adverse events will be coded into the Preferred Term (PT), classified according to the current version of Medical Dictionary for Regulatory Activities (MedDRA) with System Organ Classification (SOC) and reported with severity, duration, onset time and relationship to study drug and action taken.

Female subjects of child-bearing potential must have a negative pregnancy test at all visits where VFSS will be performed. Following administration of study drug, any known cases of pregnancy in female subjects will be reported until 30 days after the subject completes or withdraws from the study. The pregnancy will be reported immediately by phone and by faxing/emailing a completed Pregnancy Report to the Principal Investigator or study team within 24 hours of knowledge of the event. The pregnancy will not be processed as a serious adverse event (SAE); however the Principal Investigator will follow the subject until completion of the pregnancy and must assess the outcome in the shortest possible time but not more than 30 days after completion of pregnancy. If the outcome of the pregnancy meets the criteria for immediate classification of an SAE (e.g., spontaneous or therapeutic abortion [any congenital anomaly detected in an aborted fetus is to be documented] stillbirth, neonatal death, or congenital anomaly), the Principal Investigator will report the event by phone and by faxing a completed SAE form to the IRB and the FDA within 24 hours of knowledge of the event.

All serious adverse events (SAEs), whether or not the event is deemed drug-related, will be reported on the SAE Report Form

The Investigator will be responsible for notifying the IRB and regulatory agencies.

If a subject experiences a non-serious Adverse Event:

All clinical adverse events are recorded in the CRFs designed specifically for this purpose. It is important to report all AEs, especially those that result in permanent discontinuation of the investigational product being studied, whether serious or non-serious.

The site should fill out the CRF and enter the non-serious AE information into the online Adverse Event Reporting System within 5 working days/7 calendar days of the site learning of a new AE or receiving an update on an existing AE.

Expected adverse events for Metformin are provided in Section 6.4; *Safety.*

14. CRITERIA FOR INTERVENTION DISCONTINUATION

Subjects will be discontinued from the study under the following circumstances:

- If the subject is hemodynamically unstable
- If discontinuation suspension is recommended by treating physician
- At the subject's request

15. STATISTICAL CONSIDERATIONS

15.1. General Design Issues

This open label study has been designed to assess the safety and tolerability of Metformin in individuals with C9orf72 positive ALS. The study will be performed at the University of Florida. The anticipated time from study enrollment until completion of data analyses is approximately 10-18 months. The inclusion criteria are intended to provide a cohort of subjects with ALS who are appropriate candidates for treatment with Metformin. Exclusion criteria prohibit enrollment of subjects with certain medical conditions, allergies to study drug or females that are pregnant or breastfeeding.

15.2. Outcomes

15.3. Primary and Secondary Outcomes:

The primary objective of this study is to assess the safety and tolerability of Metformin administered twice daily.

Additional outcomes:

- Type, incidence, and severity of adverse events
- Physical and neurological examinations
- Vital signs (heart rate, blood pressure, respiration rate, temperature), Height/Weight
- Clinical laboratory tests (hematology, serum chemistry and urinalysis)
- BDI-Fast Screen
- Impact on swallowing
- Impact on respiratory testing
- Impact on RAN protein levels
- Impact on neuroimaging

15.4. Sample Size and Accrual

Using a conservative value, screening 50 individuals affected by ALS should yield the 16 study subjects for this protocol. Participants will be recruited from UF and multiple clinical sites in the state of Florida and throughout the US. Interested participants will be contacted by the investigators or their staff and invited to participate. These recruitment strategies will include a mechanism by which the subjects can provide their contact information.

15.5. Data Monitoring

Data Monitoring will be conducted according to Good Clinical Practice and applicable government regulations. The investigator agrees to allow monitors access to the clinical supplies, dispensing and storage areas, and the clinical files of the study subjects and, if requested, agrees to assist the monitors.

Safety monitoring will include careful assessment and appropriate reporting of adverse events. Medical monitoring will include contemporaneous assessment of serious adverse events.

15.6. Data Analyses

15.7. Data analyses will be performed by the investigative team including individuals working in the Ranum laboratory with the exception that Dr. Ranum will not perform statistical analyses. General data analysis including impact on swallowing and respiration, and manuscript writing will also be performed by Co-Investigator Emily K. Plowman at Ohio State University. Statistical Analysis

Statistical analysis will be performed by investigators in the laboratories of Drs. Wymer, Ranum, Vaillancourt or Garrett. Because Dr. Ranum has a potential conflict of interest as an inventor of a pending patent and as a founder of RanTran that may license this technology from UF in the future, she will not perform any of the specific statistical tests of the data generated during the course of this study. Summary statistics will be reported for safety and tolerability endpoints as listed above (Section 15.2.1) as well as subject characteristics (demographics, ALS Functional Rating Scale – Revised (ALSFRS-R), RAN protein levels, respiratory, and bulbar dysfunction).

Depending on the study enrollment rate, the PI may elect to perform an interim analysis using available data from the subset of subjects who have completed the study at that time. The results of this interim analysis will be used only for internal planning and possibly for sharing with regulatory authorities. The results of the interim analysis will have absolutely no influence on the subsequent conduct of the study.

The Suicidal Ideation Score is a numerical score derived from the categories 1-7 on BDI-FastScreen (BDI-FS) present at the assessment. It will be reported as a numerical score and will be provided in the listing as well. If no ideation; then score is zero.

No formal statistical tests will be performed to compare Screening vs. Visit 1 vs Visit 2 vs Visit 3 on tolerability and safety endpoints.

Summary statistics will also include concomitant medications. No efficacy analysis will be performed.

Additional statistical and alternate tests may be performed, if necessary.

16. ETHICAL CONSIDERATIONS

16.1. Basic Principles

This research will be carried out in accordance with Good Clinical Practice (GCP) as set out by the International Council for Harmonization (ICH), the basic principles defined in the U.S. Code of Federal Regulations (21 CFR Part 312), the Belmont Report, Directive 2001/20/EC (Europe), The Tri-Council Policy Statement and the principles enunciated in the most recent version of the World Medical Association Declaration of Helsinki.

16.2. Institutional Review Board Review and Informed Consent

The protocol and the informed consent document and any subsequent modifications will be reviewed and approved by the IRB responsible for oversight of the study. A signed consent form, approved by the IRB, will be obtained from the subject by Study Investigators and/or their IRB-approved designee.

For subjects who cannot provide consent for themselves, such as those below the legal age, a parent, legal guardian, or person with power of attorney, must sign the consent form; additionally, the subject's assent must also be obtained if he or she is able to understand the nature, significance, and risks associated with the study.

Written informed consent will be obtained from each participant before any study-specific procedures or assessments are performed and after the aims, methods, anticipated benefits and potential hazards are explained. The Investigator will keep the original consent forms and copies will be given to the participants. It will also be explained to the participants that they are free to refuse entry into the study and free to withdraw from the study at any time without prejudice to future treatment. Written and/or oral information about the study in a language understandable to the participant will be given to all participants. HIPAA guidelines for confidentiality and the principles of medical ethics will be adhered to during the study.

16.2(a) Informed consent procedure:

The investigator will inquire if the patient is interested in hearing about the study. If the patient wants to hear information, the staff member will introduce himself/herself to the patient and/or legally authorized representative, if applicable. The informed consent session will take place in a private setting. The consent form may be read aloud to the potential subject(s) or the potential subject(s) may be asked to read the consent in the presence of the staff member. Adequate time must be given to the potential subject or legal representative to read the consent form and to ask any questions.

All questions must be answered regarding the research before the document is signed. The person obtaining consent will acknowledge that the potential subject/legal representative verbalizes understanding of the research and the research related procedures.

A copy of the fully signed consent form will be given to the potential subject/legal representative, the original signed consent form will be filed in the potential subject's research file, and the consent form will be scanned into the patient's electronic medical record.

16.3. Revisions and/or Amendments to the Protocol

All revisions and/or amendments to this protocol must be documented and approved in writing by the Investigator. If the revision/amendment will affect subject safety and/or study design, then the amendment will be re-submitted to the IRB for approval. Administrative changes (i.e., typographical errors, discrepancies, clarifications) will also be submitted to the IRB, but may not require approval. A copy of the IRB's approval documents will be included in the final report.

For revisions or amendments to the protocol that substantially alter the study design after initiation of the study, the Investigator will decide whether a revised and approved ICF will be needed for continued participation.

16.3(a) Signing of revised informed consent forms

If a consent form is revised while patients are enrolled in the study, and the IRB states that patients need to be re-consented, all study subjects currently enrolled and participating in the study must be advised of the changes, have their questions answered, and sign the revised version of the consent form if they wish to continue their participation in the study. The staff member who advised the subject of the changes and re-consented the subject must also sign the consent form.

A copy of the signed revised consent form will be given to the subject/legal

representative, the original revised consent form will be filed in the subject's binder, and the revised consent form will be scanned into the subjects electronic medical record unless the study is of a sensitive nature and the IRB has approved not scanning the consent into the subject's medical record.

16.4. Study Termination

The Investigators reserve the right to discontinue the study at the clinical study site for safety or administrative reasons at any time. Should the study be terminated and/or the clinical study site closed for any reason, all documentation and study medication pertaining to the study must be retained at the site.

16.5. Delegation of Investigator Tasks

The Investigator may delegate tasks as appropriate to individuals who are qualified by education, training and experience (and state licensure where relevant) to perform the delegated task, as described in the FDA Guidance for Industry on Investigator Responsibilities – Protecting the Rights, Safety, and Welfare of Study Subjects, October 2009.

16.6. Subject Confidentiality

All laboratory specimens, evaluation forms, reports, video recordings, and other records that leave the clinical study site will be identified only by the study specific Subject Identification Number (SID) to maintain subject confidentiality. All records will be kept in a locked file cabinet. All computer entry and networking programs will be done using study specific SIDs only. Clinical information will not be released without written permission of the subject, except as necessary for monitoring by IRB and the FDA.

17. REFERENCES

Cedarbaum JM, Stambler N, Malta E, Fuller C, Hilt D, Thurmond B, et al. The ALSFRS-R: a revised ALS functional rating scale that incorporates assessments of respiratory function. BDNF ALS Study Group (Phase III). J Neurol Sci 1999;169(1-2):13-21.

Crary MA, Carnaby-Mann GD, Groher ME. Initial psychometric assessment of a functional oral intake scale for dysphagia in stroke patients. *Arch Phys Med Rehabil* 2005;86:1516-1520.

Rosenbek JC, Robbins JA, Roecker EB, Coyle JL, Wood JL. A penetration-aspiration scale. Dysphagia 1996;11(2):93-8.

Watanabe H, Atsuta N, Nakamura R, Hirakawa A, Watanabe H, Ito M, Senda J, Katsuno M, Izumi Y, Morita M and others. Factors affecting longitudinal functional decline and survival

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APPENDIX A

ALS Functional Rating Scale – Revised

ALSFRS-R

(ALS Functional Rating Scale)

** Please circle the number of your answer

Patient Name_____ **MRN#**_____ **Date**_____

1. Speech 4 normal speech processes 3 detectable speech disturbance 2 intelligible with repeating 1 speech combined with non-vocal communication 0 loss of useful speech	**7. Turning in bed and adjusting bed clothes** 4 normal 3 somewhat slow or clumsy, needs no help 2 can turn alone or adjust sheets with great difficulty 1 can initiate, cannot turn or adjust sheets 0 helpless
2. Salivation 4 normal 3 slight but definite excess of saliva in mouth, may have nighttime drooling 2 moderately excessive saliva, may have minimal drooling 1 marked excess of saliva with some drooling 0 marked drooling, requires constant tissue	**8. Walking** 4 normal 3 early ambulation difficulties 2 walks with assistance 1 non-ambulatory functional movement only 0 no purposeful leg movement
3. Swallowing 4 normal eating habits 3 early eating problems, occasional choking 2 dietary consistency changes 1 needs supplemental tube feedings 0 NPO (exclusively parenteral or enteral feedings.	**9. Climbing Stairs** 4 normal 3 slow 2 mild unsteadiness or fatigue 1 needs assistance 0 cannot do
4. Handwriting 4 normal 3 slow or sloppy, all words legible 2 not all words legible 1 able to grip pen, unable to write 0 unable to grip pen	**R-1. Dyspnea (difficult or labored breathing; breathlessness or shortness of breath)** 4 none 3 occurs when walking 2 occurs with one or more: eating, bathing, dressing 1 occurs at rest, either sitting or lying 0 significant difficulty, considering mechanical support
5a. Cutting food and handling utensils (patients <u>without</u> gastrostomy) 4 normal 3 somewhat slow and clumsy, needs no help 2 can cut most foods, slow or clumsy, some help needed 1 foods cut by someone else, can still feed slowly 0 needs to be fed	**R-2. Orthopnea (difficult or labored breathing that occurs when laying flat and is relieved by elevating the head and chest with two pillows)** 4 normal 3 some difficulty sleeping, d/t shortness of breath, does not routinely use more than two pillows 2 needs extra pillows to sleep (>2) 1 can only sleep sitting up 0 unable to sleep
5b. Cutting food and handling utensils (patients <u>with</u> gastrostomy) 4 normal 3 clumsy, able to perform manipulations 2 some help needed with closures and fasteners 1 provides minimal assistance to caregiver 0 unable to perform any aspect of task	**R-3. Respiratory Insufficiency** 4 none 3 intermittent use of BiPAP 2 continuous use of BiPAP at night 1 continuous use of BiPAP day and night 0 invasive mechanical ventilation by intubation/trach
6. Dressing and hygiene 4 normal 3 independent self care with effort or decreased efficiency 2 intermittent assistance or substitute methods 1 needs attendant for self care 0 total dependence	**Total Score _____/ 48**

APPENDIX B

Eating Assessment Tool (EAT-10)

Please answer each of the ten questions listed below by **circling the appropriate number** that you feel best describes how you feel, with: 0= no problem at all; 4= severe problems.

Circle the appropriate response:

To what extent are the following scenarios problematic for you?	0= No Problem 4=Severe Problem				
1. My swallowing problem has caused me to lose weight.	0	1	2	3	4
2. My swallowing problem interferes with my ability to go out for meals.	0	1	2	3	4
3. Swallowing liquid takes extra effort.	0	1	2	3	4
4. Swallowing solids takes extra effort.	0	1	2	3	4
5. Swallowing pills takes extra effort.	0	1	2	3	4
6. Swallowing in painful.	0	1	2	3	4
7. The pleasure of eating is affected by my swallowing.	0	1	2	3	4
8. When I swallow food sticks in my throat.	0	1	2	3	4
9. I cough when I eat.	0	1	2	3	4
10. Swallowing is stressful.	0	1	2	3	4
				Total EAT-10	

APPENDIX C

FUNCTIONAL ORAL INTAKE SCALE (FOIS)

Functional Oral Intake Scale[1]

TUBE DEPENDENT (levels 1-3)

1 No oral intake
2 Tube dependent with minimal/inconsistent oral intake
3 Tube supplements with consistent oral intake

TOTAL ORAL INTAKE (levels 4-7)

4 Total oral intake of a single consistency
5 Total oral intake of multiple consistencies requiring special preparation
6 Total oral intake with no special preparation, but must avoid specific foods or liquid items
7 Total oral intake with no restrictions

[1] Crary MA, Carnaby-Mann GD, Groher ME. Initial psychometric assessment of a functional oral intake scale for dysphagia in stroke patients. *Arch Phys Med Rehabil* 2005;86:1516-1520.

APPENDIX D

BDI®-FastScreen for medical patients

Today's Date:

Name: _____ Marital Status: _____ Age: _____ Sex: _____

Occupation: _____ Education: _____

BDI–FastScreen

This questionnaire consists of groups of statements. Please read each group of statements carefully, then pick out the one statement in each group which best describes the way you have been feeling during the past 2 weeks, including today! Circle the number beside the statement you picked. If several statements in the group seem to apply equally well, circle the statement which has the largest number.

1.
- **0** I do not feel sad.
- **1** I feel sad much of the time.
- **2** I am sad all the time.
- **3** I am so sad or unhappy that I can't stand it.

2.
- **0** I am not discouraged about my future.
- **1** I feel more discouraged about my future than I used to be.
- **2** I do not expect things to work out for me.
- **3** I feel my future is hopeless and will only get worse.

3.
- **0** I do not feel like a failure.
- **1** I have failed more than I should have.
- **2** As I look back, I see a lot of failures.
- **3** I feel I am a total failure as a person.

4.
- **0** I get as much pleasure as I ever did from the things I enjoy.
- **1** I don't enjoy things as much as I used to.
- **2** I get very little pleasure from the things I used to enjoy.
- **3** I can't get any pleasure from the things I used to enjoy.

5.
- **0** I feel the same about myself as ever.
- **1** I have lost confidence in myself.
- **2** I am disappointed in myself.
- **3** I dislike myself.

6.
- **0** I don't criticize or blame myself more than usual.
- **1** I am more critical of myself than I used to be.
- **2** I criticize myself for all of my faults.
- **3** I blame myself for everything bad that happens.

7.
- **0** I don't have any thoughts of killing myself.
- **1** I have thoughts of killing myself, but I would not carry them out.
- **2** I would like to kill myself.
- **3** I would kill myself if I had the chance.

NOTICE: This form is printed with both green and black ink. If your copy does not appear this way, it has been photocopied in violation of copyright laws.

_____ Total

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PEARSON

22 23 24 A B C D E

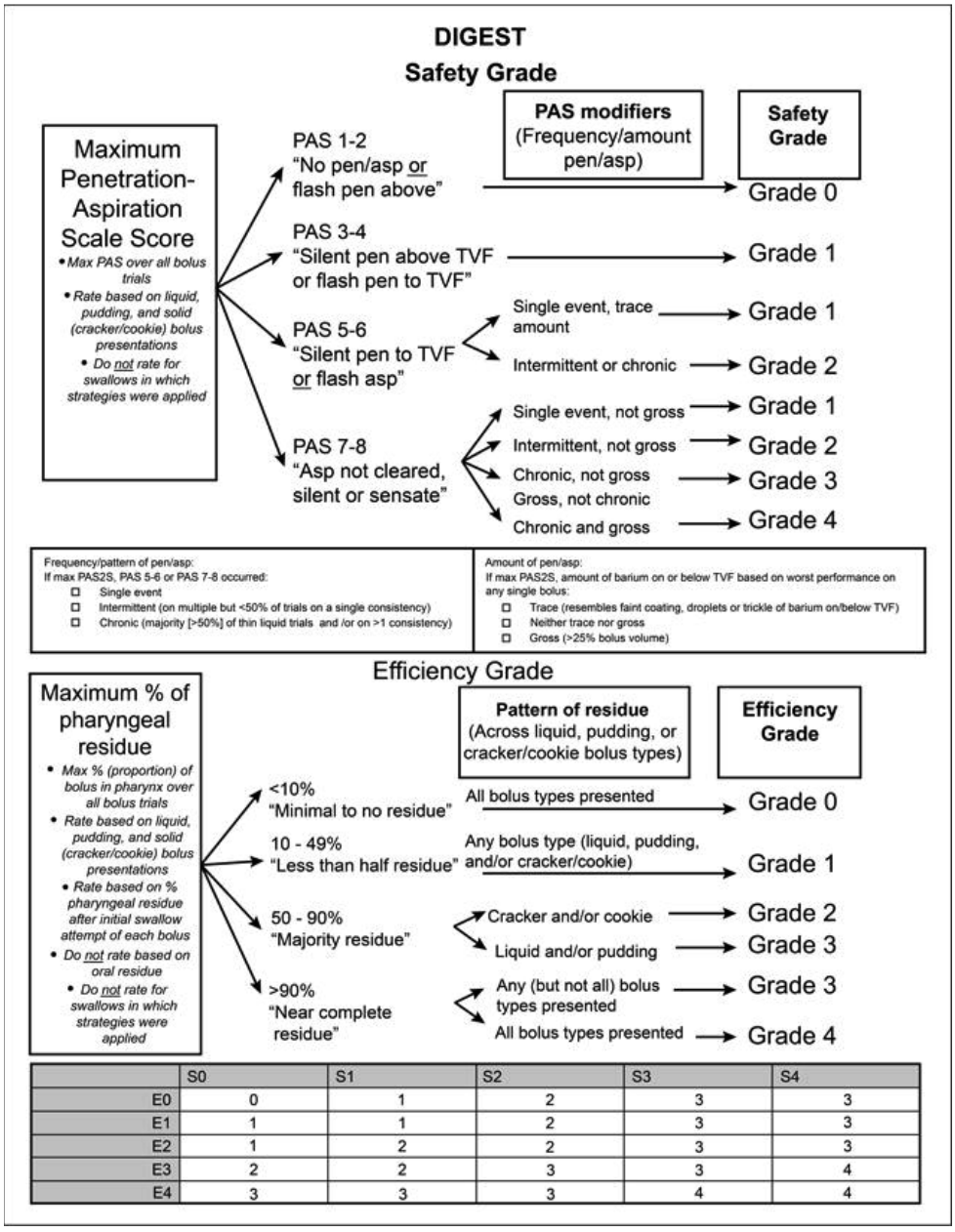
Pearson Executive Office 5601 Green Valley Drive Bloomington, MN 55437
800.627.7271 www.PsychCorp.com

PsychCorp

Product Number 0158019431

APPENDIX E

DIGEST Scoring System



	S0	S1	S2	S3	S4
E0	0	1	2	3	3
E1	1	1	2	3	3
E2	1	2	2	3	3
E3	2	2	3	3	4
E4	3	3	3	4	4

APPENDIX F

Penetration-Aspiration Scale (PAS)

Score	Description of Events
1	Material does not enter airway.
2	Material enters the airway, remains above the vocal folds.
3	Material enters the airway, remains above the vocal folds, and is not ejected from the airway.
4	Material enters the airway, contacts the vocal folds, and is ejected from the airway.
5	Material enters the airway, contacts the vocal folds, and is not ejected from the airway.
6	Material enters the airway, passes below the vocal folds, and is ejected into the larynx or out of the airway.
7	Material enters the airway, passes below the vocal folds, and is not ejected from the trachea despite effort.
8	Material enters the airway, passes below the vocal folds, and no effort is made to eject.

APPENDIX G

Metformin inhibits RAN translation through PKR pathway and corrects ALS/FTD phenotypes in C9orf72 mouse model

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MANUSCRIPT ATTACHED

APPENDIX H

Drug Pamphlet for Metformin 500 mg ER from Asend Laboratories, LLC and Amneal Pharmaceuticals, LLC attached