Protocol Title: Pilot, single center, open, trial of rifaximin in probable Alzheimer's Disease

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Synopsis

Title:	Pilot, single center, open, trial of rifaximin in probable Alzheimer's Disease" version date Sept. 6, 2019
Study Description:	Pilot trial to examine the relationship between rifaximin therapy and change in cognition, serum neuronal markers, ammonia levels and gut microbiota in patients with mild to moderate Probable Alzheimer's Disease.
Objectives:	Primary Objective: To test if rifaximin treatment in subjects with AD is correlated with improvement in the ADAS-Cog. Secondary Objective: To test if rifaximin treatment is associated with changes in serum amyloid-beta 42, total-tau, Neurofilament light protein markers, serum pro-inflammatory markers, ammonia levels and gut microbiota in AD subjects.
Endpoints:	Primary Endpoints: Cognitive domain – Alzheimer's Disease Assessment Scale-Cognitive (ADAS-Cog)
Endpoints:	Primary Safety Endpoints will be Adverse Events Secondary Endpoints: Serum neuronal markers: Neurofilament light (NF-L) Total Tau Amyloid-beta 42 Serum pro-inflammatory markers: Interleukin (IL) 1 beta IL 2 IL 4 IL 5 IL 6 IL 8 IL 10 IL 13 Tumor necrosis factor – alpha Gut microbiota Serum Ammonia
Study Population:	Mild to moderate severity of Probable Alzheimer's Disease

Phase:	Pilot trial - 2a
Description of senior care facilities Sites/Facilities Enrolling Participants:	Single Site. Duke University Health System and affiliated
Description of Study Intervention:	Subjects will be treated with rifaximin (Xifaxan, Salix Pharmaceuticals, Bridgewater, NJ) 550 mg Orally twice a day for 12 weeks
Study Duration: analyses)	Up to 24 months (for recruitment, treatment, data
Participant Duration:	3 months treatment plus 2 month follow up

Introduction

There is an urgent need to develop novel therapies for Alzheimer's disease (AD). 10% of persons over age 65 and up to 50% over age 85 have dementia, with over 30 million people affected worldwide. The Food and Drug Administration (FDA) has approved cholinesterase inhibitors (Aricept, Exelon, Razadyne), memantine (Namenda), and a drug combining a cholinesterase inhibitor and memantine (Namzaric) to treat the cognitive symptoms of AD. These medications cannot cure AD or stop it from progressing.

There has been much speculation for decades regarding the cause of Alzheimer's Disease. Two of the many theories are the focus of our research study. The first theory posits that toxic brain concentrations of ammonia play a role in the AD pathophysiology (Seiler, 1993). There have, however, been no published trials of using ammonia lowering medications for the treatment of AD. The second and more recent theory proposes that there is a symbiotic relationship between gut microbiota and brain homeostasis and that this relationship is disrupted in patients with AD, possibly due to an imbalance in the ratio of pro-inflammatory / anti-inflammatory gut microbiota (De-Paula et al, 2018, Cattaneo et al, 2017).

Background

Rifaximin is a virtually non-absorbed antibiotic with the unique properties of lowering blood ammonia levels and altering gut flora. We hypothesize that Rifaximin will improve cognition and function in AD patients by lowering blood ammonia and / or lowering circulatory pro-inflammatory cytokines secreted by harmful gut bacteria.

We will be measuring blood ammonia levels and pro-inflammatory and anti-inflammatory compounds in the blood and analyzing gut microbiota in patients with AD before and after 3 months of Rifaximin therapy. If patients exhibit measurable improvement in cognition and function, we will analyze our data to see if their improvement correlates with a lower blood ammonia level and / or a shift towards anti-inflammatory species in the gut and a similar shift in the blood cytokine panel to favoring anti-inflammatory compounds.

Evidence supporting our hypothesis for this study is presented below.

Ammonia Neurotoxicity

Ammonia is a substrate as well as a product of 16 different enzymatic reactions in the brain. In the healthy brain, ammonia homeostasis is maintained by its combining with glutamate to form non-toxic glutamine via the glutamine synthetase pathway.
In the disease state, high brain ammonia levels adversely affect membrane potential, mitochondrial function, astrocyte morphology, energy metabolism, mRNA and protein expression, brain pH, calcium signaling and other cellular functions in the brain (Bosoi et al, 2009).

The possibility that ammonia is at least partly responsible for the pathologic changes seen in the AD brain was first proposed by Seiler in 1993, when he noted that some of the changes in the AD brain can also be seen in the brains of hyperammonemic patients (Seiler, 1993). The clinical presentation of a patient with high ammonia levels caused by congenital portal systemic shunting or an inborn error of metabolism may in fact mimic that of dementia (Miyata et al, 2009). Brain PET CT using ^{13}N ammonia showed high brain ^{13}N levels, trapped in glutamine, in the brain of patients with hepatic encephalopathy, correlating with high peripheral arterial blood ammonia levels (Keiding et al, 2005).

Ammonia as a causative factor of AD can be difficult to prove; ammonia levels cannot be quantified at autopsy because of rapid post mortem formation of ammonia. However, in-vivo studies have shown evidence of elevated ammonia levels in the AD brain. Arteriovenous sampling in early stage normoammonemic AD patients demonstrated a net release of ammonia (-25.6 micrograms / 100 g x min) from the brain compared to

net uptake of ammonia (+7.2 micrograms / 100 g x min) by the brain of young control subjects, pointing toward an endogenous source for the ammonia (Hoyer et al, 1990). This efflux of ammonia from the AD brain is most likely due to low levels of glutamine synthetase found in the AD brain (LePrince et al, 1995), causing high levels of this endogenous ammonia to build up in the extracellular fluid and diffuse across the blood brain barrier into the venous effluent. Low glutamine synthetase levels have been found to correlate with increased density of amyloid deposits in the AD brain (Le Prince et al, 1995).

The brain also receives exogenous ammonia, of which the gut is a major source. Fecal bacteria produce ammonia by fermenting protein. The aging colon contains a greater percentage of protein fermenting bacteria than is seen in the colon of younger patients (Woodmansey, 2007, Andrieux et al, 2002). The aging colon also has a longer fecal dwell time than that of younger patients, allowing higher levels of ammonia to build up before fecal evacuation (Woodmansey, 2007, Andrieux et al, 2002). Gut ammonia is absorbed into the portal venous system and detoxified in the liver to form uric acid, which is then excreted in the kidneys. The uric acid cycle of the aging liver is less efficient at detoxifying ammonia (Marchesini, 1990) allowing higher levels of ammonia to reach the systemic circulation. One study reported significantly elevated post prandial blood ammonia levels in AD patients when compared with age-matched controls (Fisman et al, 1985).

The blood brain barrier plays an important role in regulating brain ammonia metabolism (Hawkins, 2009). 20-50% of blood ammonia passes the blood brain barrier and is converted into glutamine. Glutamine and glutamate are pumped from the extracellular fluid into the endothelial cells, where glutamine is partially metabolized to ammonia and glutamate. Glutamine and ammonia then diffuse into the blood.

Breakdown of the blood brain barrier in the aging patient begins in the hippocampus, the area critical for learning and memory, and this may contribute to cognitive decline (Erdo et al, 2017). Gadolinium enhanced Brain MRI has shown that the blood brain barrier is more permeable in the aging patient when compared with younger patients (Montagne et al 2015). The blood brain barrier in the AD patient is also more permeable than that of non-cognitively-impaired age matched controls (Montagne et al, 2015). High concentrations of Gadolinium are first seen in the hippocampus, the site responsible for memory and learning, both of which are clinically impaired in AD patients.

One theory is that the blood brain barrier allows greater amounts of ammonia into the AD brain, triggering or worsening pre-existent changes.

This study aims to lower blood and brain ammonia levels in AD patients by targeting the colon. This will be accomplished by administering a virtually non-systemically absorbed antibiotic, rifaximin, which is FDA approved for hepatic encephalopathy. Rifaximin

lowers blood ammonia by altering fecal flora by blocking bacterial RNA synthesis and also by increasing small bowel glutaminase (Garcovich et al, 2012, Kang et al, 2016).

Gut Microbiota Dysbiosis

The gut harbors 95% of the total human microbiome and is made up of more than 5,000 taxa. A diverse and stable gut microbiota promotes health in humans. Gut species remain relatively constant throughout adulthood until the seventh decade, when it becomes less diverse, harboring higher numbers of Proteobacteria and lower numbers of Bifidobacteria. This change most likely accounts for chronic inflammatory disorders seen in the elderly. An imbalance in the gut bacterial species can weaken the intestinal barrier and create system wide inflammation via gut lymphoid tissue which comprises 70% - 80% of the immune system. Blood brain barrier permeability is also altered. The gut bacteria secrete pro-inflammatory compounds and neuroactive molecules that include serotonin, gamma-aminobutyric acid (GABA), catecholamines and acetylcholine which cross the blood brain barrier and cause brain inflammation and brain dysfunction. (Sochocka et al).

A growing number of pro-inflammatory compounds secreted by gut bacteria and seen in patients with AD are being discovered. These include interleukin (IL) – 6, tumor necrosis – alpha and the inflammasome complex (NLRP3). A recent study revealed higher numbers of Escherichia and Shigella in the gut of amyloid positive AD patients when compared with healthy controls and this correlated with higher levels of circulating pro-inflammatory cytokines. The AD patients also had lower numbers of gut Eubacterium rectale and this correlated with lower levels of circulating anti-inflammatory compounds than seen in controls. (Cattaneo et al). Other researchers found that a preponderance of gut Bacteroides and Blautia and reduced numbers of SMB53 and Dialister correlated with elevated levels of brain amyloid CSF biomarkers (Vogte NM et al, 2017). Interestingly, Clostridium tyrobutyricum and Bacteroides thetaiotaomicron, have been shown to actually increase the integrity of the blood brain barrier by enhancing expression of tight junction proteins and helping to maintain brain homeostasis. (Braniste et al 2014).

Recent evidence also points to a possibly more direct influence of gut bacteria on the brain. Terminal branches of the vagus nerve travel in close proximity to the intestinal barrier. Neurotransmitters and neuropeptides secreted by bacteria in the gut have been shown to activate vagal nerve ascending fibers, influencing brain function in mice. These effects ceased after vagotomy was performed. (Holzer et al 2014, Bravo et al, 2011)

Risk/Benefit Assessment

Known Risks

Risks of taking rifaximin, as listed on the package insert, are as follows.

Rifaximin is contraindicated in patients with hypersensitivity to rifaximin or rifamycin antimicrobials. Hypersensitivity reactions include exfoliative dermatitis, angioneurotic edema, and anaphylaxis. Clostridium difficile associated diarrhea is a risk whenever a patient is maintained chronically on antibiotics, with complications ranging from mild diarrhea to fatal colitis. Drug resistant bacteria can also result from long term use. There is increased systemic exposure to rifaximin in patients with severe hepatic impairment or in patients who are taking P-glycoprotein inhibitors concomitantly. Regarding use in geriatric patients, there were no reported overall differences in the safety of the drug when used in patients 65 years of age or over, when compared with younger subjects.

The most common reactions seen in patients taking rifaximin are:
Peripheral edema
Nausea
Dizziness
Fatigue
Ascites
Muscle spasms
Pruritis
Abdominal pain
Anemia
Depression
Nasopharyngitis
Arthralgia
Dyspnea
Pyrexia
Rash

Potential Benefits

Possible benefits that the subject may receive include the benefits of free medical testing and memory evaluations received at screening and during the study. Rifaximin is being used investigationally in this trial and hence it is not known if there will be any cognitive benefits. However, if there is ammonia toxicity in AD and the

drug were to be effective in lowering ammonia, then the subject may experience improvements in cognition.

Assessment of Potential Risks and Benefits

The available information suggests that the present clinical study has an acceptable risk-benefit ratio.
Regarding the risk of C. difficile Infectious Diarrhea, two large randomized Control Trials reported a 0.7% and 1.5% incidence of patients with hepatic encephalopathy taking rifaximin for 6 months. In those trials, all affected patients were at increased risk for developing C. diff because of either previous lengthy hospitalizations or long courses of antibiotics. (Bass et al, 2010, Mullen et al, 2014) suggesting the risk will be lower in healthy subjects with no such risk factors.

The safety monitoring practices employed by this protocol are adequate to protect the subjects' safety and detect expected emergent adverse effects. The approximate volume of blood (120 mL) planned for collection from each subject over the course of the entire study (not including rechecks or extra tests ordered by the PI) presents no undue risks to the subjects.

Objectives and Endpoints

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
Primary		
		ADAS-Cog is a widely used cognitive endpoint in AD clinical trials and changes on this scale are considered clinically meaningful
Secondary		
	Serum neuronal markers: Neurofilament light (NF-L)	Neurofilament light (NF-L) is a 68 kDa cytoskeletal intermediate filament

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
	Total Tau Amyloid-beta 42 Serum pro-inflammatory markers: Interleukin (IL) 1 beta IL 2 IL 4, IL 5 IL 6 IL 8 IL 10 IL 13 IL 17 Gut microbiota	protein that is expressed in neurons (Mattson et al, 2017, Rissin et al, 2010). Neurofilaments can be released in significant quantity following axonal damage or neuronal degeneration. NF-L has been shown to associate with traumatic brain injury, multiple sclerosis, frontotemporal dementia and other neurodegenerative diseases. The Simoa NF-light® assay (2) is a digital immunoassay for the quantitative determination of NF-L in serum and plasma. This ultrasensitive assay provides an analytical sensitivity of 0.6 pg/mL (10-fold greater than the 78.0 pg/mL for the standard ELISA). Plasma NFL also correlates closely with CSF concentrations. Tau is a microtubule-stabilizing protein primarily localized in central nervous system neurons, but is also expressed at low levels in astrocytes and oligodendrocytes (Dage et al, 2016). Potential movement of elevated CSF tau across the blood-brain barrier presents a possibility that measurements of tau in blood could provide a

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
		convenient peripheral window into brain/CSF status. Recent reports using digital immunoassay technology have shown elevation in peripheral tau associated with hypoxic brain injury, concussed hockey players, and repetitive minimal head injury. The Simoa™ Human Total Tau assay uses a combination of monoclonal antibodies that react with both normal and phosphorylated tau. The assay recognizes all tau isoforms. Amyloid beta 42 is a 42 amino acid proteolytic product from the amyloid precursor protein that has gained considerable attention as a biomarker correlating with cognitive disorders (Janelidze et al, 2016). Amyloid beta (Aβ) peptides (including the shorter Aβ38 and Aβ40 isoforms) are produced by many cell types in the body but the expression is particularly high in the brain. Accumulation of Aβ occurs in aging and and in the neurodegenerative process. Concentrations of Aβ42 in blood are much lower than in cerebrospinal fluid, (typically single pg/mL

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
		range), requiring very high analytical sensitivity. Serum pro-inflammatory molecules have been associated with Alzheimer's Disease pathology. (Cattaneo et al, 2017) An imbalance in the number of pro-inflammatory (e.g. Shigella, Escherichia) and anti-inflammatory (Eubacterium rectale) species in the gut may be responsible for an increase in the presence of circulating inflammatory markers (Cattaneo et al, 2017).
	Blood Ammonia	High ammonia levels in the brain may be responsible for pathological changes seen in AD.
Secondary		
	Safety and tolerability	Monitored via interviews, clinic visits, and interim phone calls. AEs and SAEs will be tabulated. Patients with AEs will be reported as per IRB and FDA guidelines.

Study Design

Overall Design

We hypothesize that treatment with rifaximin will improve cognition and will change serum neuronal markers.

This is an open label pilot study in which we will consent up to 25 subjects in order to treat 10 eligible subjects with probable mild to moderate AD. The study is single site and non-randomized. The subjects will be given rifaximin 550 mg orally twice daily for 3 months after evaluation to ensure they have no contraindications. Subjects at risk for drug interactions or tolerability issues may be started and/or maintained on a lower dose of 550 mg once a day. Individuals on concomitant drugs which are contraindicated per the package insert or in the investigators judgment will be excluded. Individuals on concomitant drugs which are not contraindicated but known to elevate blood levels SIGNIFICANTLY OR THOSE WITH MILD AEs (E.G. MILD DIARHEA) WILL receive qd dosing BASED ON CLINICIAN JUDGMENT. Consent, demographics, history, list of current medications, clinical and safety assessments, MMSE and blood tests (CBC, CMP, TSH, B-12 and Folic acid) will be performed at the Screening Visit. Medical history, physical exam, serum neuronal biomarkers and cytokines, serum ammonia level, Adverse Events and cognitive testing (ADAS-Cog-11) will be performed at the Baseline Visit. A stool sample collection kit will also be given to the subject and caregiver at the Baseline Visit, with instructions for use and how to get the sample back to the research coordinator. The stool sample will be obtained prior the subject taking the study drug. Medical history, physical exam, serum neuronal biomarkers and cytokines, serum ammonia level, Adverse Events and cognitive testing (ADAS-Cog-11) will be performed at the 3 month endpoint visit. A stool sample collection kit will also be given to the subject and caregiver at the baseline visit and 3 month Visit with instructions for use and how to get the sample back to the research coordinator. Interim safety checks will occur via phone calls one week after baseline and then every 2 weeks until the 3 month visit, which will then be followed by a 5 month phone call. Caregivers will be instructed to call the research coordinator or the PI (Dr. Suhocki after business hours or on weekends) if any change in stool is noticed at any time during the study. Unscheduled visit/labs may occur if warranted for safety based on clinical judgment. Because of a small risk of developing C. difficile up to 2 months following the last administration of rifaximin, the subjects will be followed for an additional 2 months after the 3 month treatment ends. Subjects who terminate early will receive the 3 month assessment at the time of termination.

Scientific Rationale for Study Design

This is an open label pilot study designed to provide preliminary evidence on the clinical efficacy of rifaximin in improving cognition in AD patients. We will be looking for improvement in test scores, changes in serum levels of neuronal markers and cytokines and changes in gut microbiota following treatment. We will also be collecting data

regarding the safety of long-term use of this non-absorbed antibiotic for this disease. This pilot may form the basis for a future larger randomized, double blind controlled study to further test this hypothesis. Statistical analyses will combined subjects on QD and BID for analyses as this is a pilot study and both doses may impact gut flora. In posthoc analyses we will put dosing as a covariate to examine dose effects.

Justification for Dose and Study Population

The rifaximin dosage and route of administration are the same as that used to treat hepatic encephalopathy, for which the drug and dosage are FDA approved. The dose will remain the same throughout the 3 month study of rifaximin administration unless stopped for safety reasons. The dose can be stopped or temporarily reduced for safety or tolerability reasons based on clinical judgment. The age and study sample of mild to moderate probable AD is similar to that used in AD prior trials. We will select our sample to be medically stable and free of hepatic disease and not having had recent antibiotic therapy to minimize risk for C Difficile infection.

End of Study Definition

The end of the study will occur 5 months after baseline testing and first administration of the drug. While drug administration will conclude and endpoint testing will be done at 3 months, we will continue to follow each subject for an additional 2 months, as C. difficile infectious diarrhea can occur up until 2 months after stopping an antibiotic.

Study Population

Inclusion Criteria

Subjects who meet the following criteria will be considered eligible to participate in the clinical study:

Probable Alzheimer's disease (National Institute of Neurological Disorders and Stroke (NINDS) criteria), mild to moderate severity
Ages 55-85; both genders
Mini Mental State Exam (MMSE) scores 10-22
Willing and able to comply with all scheduled clinic visits.
Stable medical health
Has a family or professional caregiver who has regular contact with subject
Ability to consent or legal guardian who can consent
Living at home or in a facility
On no AD therapies or on stable (2 months) concurrent AD therapies

Exclusion Criteria

Subjects who meet one or more of the following criteria will not be considered eligible to participate in the clinical study:

Past history of C diff infection
Individuals on concomitant drugs which are contraindicated per the package insert or in the investigators judgment will be excluded
Assessment, laboratory examination, physical examination or any other medical condition or circumstance making the volunteer unsuitable for participation in the study in the judgment of the study clinicians
Allergy to Rifaximin
Antibiotic use in the last 6 months
Hospitalization in the last 6 months
Are taking medications that interact with Rifaximin
Are taking Cyclosporine
Past or current history of bloody stools or C Diff infection
Elevated LFTs
Clinically significant abnormal hepatic or renal function
Uncorrected thyroid or B12 abnormalities
Participation in another investigational drug trial in the past 30 days
History of febrile illness within 5 days prior to the study period
Hyperammonemia caused by:
- Valproic acid
- Chemotherapy
- Lung transplant
- Bariatric surgery
- Ureterosigmoidoscopy
- Hyperalimentation
- Urinary tract infection
- Errors of metabolism
    - Urea cycle
    - Enzyme deficiencies
    - Organic acidemias
    - Fatty acid oxidation
    - Amino acid transport defects

Lifestyle Considerations

Participation in the study will not have any impact on subject lifestyle.

Screen Failures

Volunteers who are consented to participate in the clinical trial and who do not meet one or more criteria required for participation in the trial during the screening procedures will not be rescreened later.

Strategies for Recruitment and Retention

Subjects will be recruited from referrals and the community via advertisement.
The study will be posted on clinicaltrials.gov.
Subjects and caregivers will be compensated for participation in the study at $50 each per clinic visit to cover travel and time.
All tests and drug will be provided at no cost.
We expect to recruit 1 subject per month.

Study Intervention

Study Intervention Description

Rifaximin (Xifaxan, Salix Pharmaceuticals, Bridgewater, N.J.) (See Package Insert) is a drug that is approved by the FDA for use in humans for the treatment of Hepatic Encephalopathy, Traveler's Diarrhea and Irritable Bowel Syndrome. It is commercially available. It will be used in accordance with approved labeling as pertains to dosage and administration for Hepatic Encephalopathy, contraindications and warnings. However, it will be used investigationally in this trial as rifaximin is not FDA approved for the treatment of Alzheimer's Disease.

Safety, Adverse Effects reporting and Management
The AE monitoring schedule is described in the protocol. The drug will be discontinued immediately if there are emergent signs of C.Difficile (e.g. patient has watery diarrhea for longer than 24 hours, fever, abdominal pain or possible bloody stools). All AEs will be assessed by a study physician and referred for appropriate treatment. The study drug may be discontinued at any time per the study physician judgment for safety. All SAEs will be reported to the IRB within 24 hours of becoming aware of the event.

Dosing and Administration

The drug will be administered orally, under supervision of the caregiver, 550 mg twice a day, for 3 months.

Drug Acquisition and Accountability

The drug, rifaximin, will be provided by the study and acquired from the manufacturer. Compliance will be monitored at phone calls and accountability through pill counts at the endpoint study visit.

Formulation, Packaging, Appearance and Labeling
Rifaximin will be packaged in bottles of 100 tablets of 550 mg. The product will be dispensed under the supervision of the study clinician.

Product Storage and Stability

Rifaximin will be stored in the clinical study office in a double locked facility in conditions suitable for its stability.

Preparation

No special measures are required to prepare the drug for oral administration.

Measures to Minimize Bias: Randomization and Blinding

This is an open label pilot study. Objective tests and biomarkers are used to minimize bias.

Study Intervention Compliance

The subject's caregiver will be asked to keep a log of rifaximin dosage and date and time of administration. This will insure compliance and adherence to the study protocol. The compliance log and pill count will be reviewed during the endpoint visit.

Concomitant Therapy

The subject may take concomitant medications that do not interact with rifaximin. These medications will be recorded at baseline. Any new medications taken during the study period will be recorded.

Rescue Medicine. AEs will be treated as appropriate with rescue medications.

Participant Discontinuation and Participant Discontinuation / Withdrawal

Discontinuation of Study Intervention

Administration of rifaximin will be discontinued and the study will be halted if a subject develops a severe hypersensitivity to rifaximin or if a subject develops a C. difficile infectious diarrhea with complications or any other AE felt by the clinician to warrant discontinuation.

Participant Discontinuation / Withdrawal from the Study

While subjects are encouraged to complete all study evaluations, they may withdraw from the study at any time and for any reason. Every effort will be made to determine why any subject withdraws from the study prematurely. This information will be recorded. All subjects who withdraw from the study with an ongoing AE will be followed until the event is resolved or deemed stable. If a subject withdraws prematurely, all data normally collected at the 3 month Visit will be collected at the time of premature discontinuation. Subjects who had to interrupt taking the study drug for any reason may be asked to go back on drug if there are no contraindications. Subject participation may be terminated prior to completing the study for any of the following reasons:

Adverse event

Protocol violation

Loss to follow up

Subject withdrew consent at own request

Other

A Case Report Form (CRF) page will capture the date and the specific underlying reason for discontinuation of study intervention or participant discontinuation/withdrawal.

Study Assessment and Procedures

Efficacy Assessments

	V1	V2							V3	V4/End	
Procedures	Screening	Baseline	wk 1	wk 3	wk 5	wk 7	wk 9	wk 11	WK 12 3 months	WK 20 5 months	Early Term
Consent	x										
Medical History	x	x							x		x
Concomitant Medications	x	x							x		x
Vitals	x	x							x		x
VeniPuncture	x								x		x
Blood Tests	x								x		x
Stool Sample		x							x		x
Physical Exam	x								x		x
MMSE	x								x		x
ADAS-COG-11		x							x		x
Study Medication Dispensation		x									
Study Medication Accountability									x		x
AE assesment	x	x	x	x	x	x	x	x	x	x	x
Phone Assessment			x	x	x	x	x	x		x	
Subject compensation		x							x	x	x
OnCore Status update	x									x	
Phone Screening											
Bristol Stool Question		x							x		x

Unscheduled visit/labs may occur if warranted for safety based on clinical judgment.

Adverse Event Reporting

AE reporting will begin at the time of signing of the informed consent (Screening) and will continue until discharge from the study (5 months after the first administration of rifaximin).

Serious Adverse Event Reporting

The PI will notify the IRB of any SAE, whether deemed rifaximin related or not, that a subject experiences during their participation in the study within 24 hours of becoming aware of the event. Following the end of the subject's 3 month participation in the study, the PI will report SAE's spontaneously if considered at least possibly related to rifaximin. SAE reporting will follow Duke IRB requirements.

All SAE's will be followed until satisfactory resolution or until the site investigator deems the event to be chronic or the participant is stable.

Reporting Events to Participants

Subjects and their caregivers will be informed about individual or aggregate AE's, SAE's and study related results as per IRB guidelines.

Reporting of Pregnancy

NA. Subjects will not be of child bearing age.

Definition of Unanticipated Problems

Unanticipated problems in this study will be any AE's or SAE's not previously described in other investigators' research with rifaximin or not listed on the package insert for rifaximin.

Unanticipated Problem Reporting

Unanticipated problems will be reported to the IRB within 24 hours of its occurrence.

Reporting Unanticipated Problems to Participants

Unanticipated problems encountered during the study will be relayed to the subject(s) if relevant to the subject's safety as deemed appropriate by the Duke IRB.

Supporting Documentation and Operational Considerations

Regulatory, Ethical and Study Oversight Considerations

Informed Consent Process

Before each subject is enrolled in this study, written informed consent will be obtained from the subject and/or legally acceptable representative (LAR) according to local IRB requirements.

Abbreviations

AD	Alzheimer's Disease
AE	Adverse Event
FDA	Food and Drug Administration
IRB	Institutional Review Board
LAR	Legally Acceptable Representative
PI	Principle Investigator
SAE	Serious Adverse Event
US	United States

References:

1. Seiler N. Is Ammonia a pathogenetic factor in Alzheimer's Disease? Neurochemical Res. 1993;18:235-245
2. De-Paula, V et al. Relevance of gutmicrobiota in cognition, behaviour and Alzheimer's disease. Pharmacological Research 2018;136:29-34.
3. Cattaneo A, et al. Association of brain amyloidosis with pro-inflammatory gut bacterial taxa and peripheral inflammation markers in cognitively impaired elderly. Neurobiology of Aging 2017;49:60-68.
4. Braniste V et al. The gut microbiota influences blood-brain barrier permeability in mice. Sci Transl Med 2014;6:263
5. Holzer P et al. Neuropeptides and the microbiota-gut-brain axis. Adv Exp Med Biol 817:195-219.
6. Bravo JA et al. Ingestion of Lactobacillus strain regulates emotional behaviour and central GABA receptor expression in a mouse via the vagus nerve. PNAS 108:16050-16055.
7. Vogte NM et al. Gut microbiome alterations in Alzheimer's disease. Sci Rep. 2017;7:13537
8. Bosoi, CR et al. Identifying the direct effects of ammonia on the brain. Metabolic Brain Disease 2009;24:95-102.
9. Miyata K et al. Congenital portal systemic encephalopathy misdiagnosed as senile dementia. Intern Med 2009;48:321-4.

10. Keiding, S et al. Hepatic encephalopathy in cirrhosis measured by positron emission tomography. Hepatology 2006;43:42-50.
11. Hoyer, S et al. Ammonia is endogenously generated in the brain in the presence of presumed and verified dementia of the Alzheimer type. Neuroscience Letters 1990;117:358-362.
12. Le Prince, G et al. Glutamine synthetase (GS) expression is reduced in senile dementia of the Alzheimer type. Neurochemical Research 1995;7:859-862.
13. Woodmansey EJ. Intestinal bacteria and aging. Journal of Applied Microbiology 2007;102:1178-1186.
14. Andrieux C et al Metabolic characteristics of the faecal microlora in humans from three age groups. Scand J Gastroenterol 2002;37:792-8.
15. Marchesini G et al. Synthesis of urea after a protein-rich meal in normal man in relation to ageing. Ageing 1990;19:4-10.
16. Fisman M et al. Hyperammonemia in Alzheimer's Disease. Am J Psychiatry 1985;142:71-73.
17. Hawkins RA. The blood brain barrier and glutamate. Am J Clin Nutr. 2009;90:867S-74S.
18. Erdo F et al. Age-associated physiological and pathological changes at the blood-brain barrier: A review. Journal of Cerebral Blood Flow and Metabolism 2017;37:4-24.
19. Montagne, A et al. Blood-Brain Barrier Breakdown in the Aging Human Hippocampus. Neuron 2015;85:296-302.
20. Garcovich, M. Prevention and treatment of hepatic encephalopathy: Focusing on gut microbiota. World J Gastroenterol 2012;18:6693-6700.
21. Kang DJ et al. Rifaximin exerts beneficial effects independent of its ability to alter microbiota composition. Clinical and Translational Gastroenterology 2016;7:e187.
22. Sochocka M, et al. The gut microbiome alterations and inflammation-driven pathogenesis of alzheimer's disease – a critical review. Molecular Neurobiology 2018; pp 1-11, published online.Bass, N et al. Rifaximin treatment in hepatic encephalopathy. N Engl J Med 2010;362:1071-1081.
23. Mullen, K et al. Rifaximin is safe and well tolerated for long term maintenance of remission from overt encephalopathy. Clin Gastroenterol Hepatol 2014;12:1390-1397.
24. Mattson N et al. Association of plasma neurofilament light With neurodegeneration. JAMA Neurol. 2017;74(5):557-566
25. Rissin, D et al. Single-molecule EISA detects serum proteins at subfemtomolar concentrations. Nature Biotechnology 28, 595–599 (2010)

26. Dage JL et al. Levels of tau protein in plasma are associated with cognitive function in an elderly cohort. Alzheimer's & Dementia, 12, 1226-1234, 2016
27. Janelidze S et al. Plasma β-amyloid in Alzheimer's disease and vascular disease. Nature Scientific Reports, 6, 26801, 2016.
28. Package Insert, Rifaximin (Xifaxan, Salix Pharmaceuticals, Bridgewater, NJ)

https://shared.salix.com/shared/pi/xifaxan550-pi.pdf

HIGHLIGHTS OF PRESCRIBING INFORMATION
These highlights do not include all the information needed to use XIFAXAN safely and effectively. See full prescribing information for XIFAXAN.

XIFAXAN® (rifaximin) tablets, for oral use
Initial U.S. Approval: 2004

To reduce the development of drug-resistant bacteria and maintain the effectiveness of XIFAXAN and other antibacterial drugs, XIFAXAN should be used only to treat or prevent infections that are proven or strongly suspected to be caused by bacteria.

---------- INDICATIONS AND USAGE ----------

XIFAXAN is a rifamycin antibacterial indicated for:
- Treatment of travelers' diarrhea (TD) caused by noninvasive strains of *Escherichia coli* in adult and pediatric patients 12 years of age and older (1.1)
- Reduction in risk of overt hepatic encephalopathy (HE) recurrence in adults (1.2)
- Treatment of irritable bowel syndrome with diarrhea (IBS-D) in adults (1.3)

Limitations of Use
- TD: Do not use in patients with diarrhea complicated by fever or blood in the stool or diarrhea due to pathogens other than *Escherichia coli* (1.1, 5.1)

---------- DOSAGE AND ADMINISTRATION ----------

Condition	Recommended Dosage Regimen
TD (2.1)	One 200 mg tablet 3 times a day for 3 days
HE (2.2)	One 550 mg tablet 2 times a day
IBS-D (2.3)	One 550 mg tablet 3 times a day for 14 days. Patients who experience recurrence can be retreated up to two times with the same regimen.

- XIFAXAN can be taken with or without food. (2.4)

---------- DOSAGE FORMS AND STRENGTHS ----------

200 mg and 550 mg tablets (3)

---------- CONTRAINDICATIONS ----------

History of hypersensitivity to rifaximin, rifamycin antimicrobial agents, or any of the components of XIFAXAN (4)

---------- WARNINGS AND PRECAUTIONS ----------
- Travelers' Diarrhea Not Caused by *E. coli*: XIFAXAN was not effective in diarrhea complicated by fever and/or blood in the stool or diarrhea due to pathogens other than *E. coli*. If diarrhea symptoms get worse or persist for more than 24 to 48 hours, discontinue XIFAXAN and consider alternative antibiotics (5.1)
- *Clostridium difficile*-Associated Diarrhea: Evaluate if diarrhea occurs after therapy or does not improve or worsens during therapy (5.2)
- Hepatic Impairment: Use with caution in patients with severe (Child-Pugh Class C) hepatic impairment (5.4, 8.7)
- Concomitant P-glycoprotein (P-gp) inhibitors (e.g., cyclosporine): Caution should be exercised when concomitant use of XIFAXAN and a P-glycoprotein inhibitor is needed (5.5, 7.1)

---------- ADVERSE REACTIONS ----------

Most common adverse reactions:
- TD (≥2%): Headache (6.1)
- HE (≥10%): Peripheral edema, nausea, dizziness, fatigue, and ascites (6.1)
- IBS-D (≥2%): ALT increased, nausea (6.1)

To report SUSPECTED ADVERSE REACTIONS, contact Salix Pharmaceuticals, a division of Valeant Pharmaceuticals North America LLC, at 1-800-321-4576 and www.Salix.com or FDA at 1-800-FDA-1088 or www.fda.gov/medwatch.

---------- DRUG INTERACTIONS ----------

Warfarin: Monitor INR and prothrombin time; dose adjustment of warfarin may be needed to maintain target INR range. (7.2)

---------- USE IN SPECIFIC POPULATIONS ----------

Pregnancy: May cause fetal harm (8.1)

See 17 for PATIENT COUNSELING INFORMATION.

Revised: 01/2018

FULL PRESCRIBING INFORMATION: CONTENTS*

*Sections or subsections omitted from the full prescribing information are not listed

FULL PRESCRIBING INFORMATION

1 INDICATIONS AND USAGE

To reduce the development of drug-resistant bacteria and maintain the effectiveness of XIFAXAN and other antibacterial drugs, XIFAXAN when used to treat infection should be used only to treat or prevent infections that are proven or strongly suspected to be caused by susceptible bacteria. When culture and susceptibility information are available, they should be considered in selecting or modifying antibacterial therapy. In the absence of such data, local epidemiology and susceptibility patterns may contribute to the empiric selection of therapy.

1.1 Travelers' Diarrhea

XIFAXAN is indicated for the treatment of travelers' diarrhea (TD) caused by noninvasive strains of *Escherichia coli* in adults and pediatric patients 12 years of age and older.

Limitations of Use

XIFAXAN should not be used in patients with diarrhea complicated by fever or blood in the stool or diarrhea due to pathogens other than *Escherichia coli [see Warnings and Precautions (5.1), Clinical Pharmacology (12.4), Clinical Studies (14.1)]*.

1.2 Hepatic Encephalopathy

XIFAXAN is indicated for reduction in risk of overt hepatic encephalopathy (HE) recurrence in adults.

In the trials of XIFAXAN for HE, 91% of the patients were using lactulose concomitantly. Differences in the treatment effect of those patients not using lactulose concomitantly could not be assessed.

XIFAXAN has not been studied in patients with MELD (Model for End-Stage Liver Disease) scores >25, and only 8.6% of patients in the controlled trial had MELD scores over 19. There is increased systemic exposure in patients with more severe hepatic dysfunction *[see Warnings and Precautions (5.4), Use in Specific Populations (8.7), Clinical Pharmacology (12.3)]*.

1.3 Irritable Bowel Syndrome with Diarrhea

XIFAXAN is indicated for the treatment of irritable bowel syndrome with diarrhea (IBS-D) in adults.

2 DOSAGE AND ADMINISTRATION

2.1 Dosage for Travelers' Diarrhea

The recommended dose of XIFAXAN is one 200 mg tablet taken orally three times a day for 3 days.

2.2 Dosage for Hepatic Encephalopathy

The recommended dose of XIFAXAN is one 550 mg tablet taken orally two times a day.

2.3 Dosage for Irritable Bowel Syndrome with Diarrhea

The recommended dose of XIFAXAN is one 550 mg tablet taken orally three times a day for 14 days. Patients who experience a recurrence of symptoms can be retreated up to two times with the same dosage regimen.