The Effects of Organic Triphala and VSL#3 Probiotic Supplementation on Stool Microbiome Profiles and Inflammation in Healthy Elderly Subjects

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UCSD Human Research Protections Program
New Biomedical Application
RESEARCH PLAN

Instructions for completing the Research Plan are available on the HRPP website.
The headings on this set of instructions correspond to the headings of the Research Plan.
General Instructions: Enter a response for all topic headings.
Enter "Not Applicable" rather than leaving an item blank if the item does not apply to this project.

Version date: 7/10/2020

1. PROJECT TITLE

The Effects of Organic Triphala and VSL#3 Probiotic Supplementation on Stool Microbiome Profiles and Inflammation in Healthy Elderly Subjects ("TRIPH2017")

2. PRINCIPAL INVESTIGATOR

Paul J. Mills, PhD

3. FACILITIES

UC San Diego Core Laboratory of Dr. Paul Mills (MTF-418)
UC San Diego Alzheimer's Disease Research Center (ADRC)
UC San Diego School of Medicine Building 4 Clinical Rooms

4. ESTIMATED DURATION OF THE STUDY

2 years

5. LAY LANGUAGE SUMMARY OR SYNOPSIS (no more than one paragraph)

Normal aging can lead to loss of gut microbial biodiversity which is linked to inflammaging and immunosenescence or the loss of immunocompetence. Probiotics, such as VSL#3®, and certain herbal supplements such as *Triphala* are associated with restoration of gut community architecture, increased gut barrier function and decreased inflammation. The present project will examine the potential benefits of a synbiotic (which denotes a prebiotic plus probiotic, and in this study, is an herbal prebiotic plus probiotic) intervention (8 weeks of supplementation) on gut microbiome profiles assessed via stool, inflammatory blood markers, and questionnaires about gastrointestinal health and mood. In this exploratory study, we will examine psychological and physical functioning at baseline and after 8 weeks of supplementation with synbiotic, *Triphala* alone, or placebo.

6. SPECIFIC AIMS

Primary Aim: In healthy, cognitively intact elderly subjects, determine the effects of an 8-week synbiotic supplement intervention on the stool microbiome (pre- and post-intervention) as compared to *Triphala* alone and placebo.

Secondary Aim: In healthy elderly subjects, examine the effects of an 8-week synbiotic supplementation intervention on inflammatory blood markers compared to herb alone or placebo.

Exploratory Aim 1: In healthy elderly subjects, examine the effects of an 8-week synbiotic intervention on gastrointestinal and psychological health via questionnaires compared to herb alone or placebo.

7. BACKGROUND AND SIGNIFICANCE

The elderly (persons aged 65 years or older) represent the fastest growing segment of the human population (15% of total US population). Among health care costs for older Americans, 95% are related to chronic diseases. The average senior spends about 3 times more in personal health care compared to working age adults [1]. The elderly are susceptible to inflammaging, or chronic inflammation resulting from a dysregulated balance between pro- and anti-inflammatory processes, as well as immunosenescence (reduced immune responsiveness) that leads to increased susceptibility to infection. The elderly are also known to be susceptible to damaging effects of free-radicals that may be produced in greater quantities in the aged due to chronic inflammatory processes.

Herbal remedies represent some of the most ancient medicines and are considered powerful means of maintaining human health and homeostasis. *Triphala* is a well-recognized Ayurvedic polyherbal medicine containing three beneficial plant species with known benefits that include the reduction of inflammation through

powerful antioxidant properties and immunomodulatory effects. The increased popularity of herbal remedies such as *Triphala* has led to dramatic improvements in the processing of crude plant materials that serve to maximize the absorption of otherwise poorly absorbed plant components. Despite these improvements, these preparations still display pronounced variability in efficacy which is likely related to the natural variation in composition of gut microbiota species that catalyze the biotransformation of herbal components. This response variability is not unique to herbs and in fact may be the case for virtually all health-promoting compounds ingested by humans (e.g. polyphenolic compounds derived from plants). For example, it is known that phytochemicals in *Triphala* such as quercetin and gallic acid promote the growth of Bifidobacteria and Lactobacillus while inhibiting the growth of undesirable gut residents such as *E. coli*. In addition, lactic acid bacteria possess enzymatic activity (e.g. tannase) to degrade plant tannins such as gallic acid contained in *Triphala*. There is extensive research on *Triphala*, which has demonstrated both safety and efficacy [2].

Our administered probiotic formulation co-administered with *Triphala* (the synbiotic) will contain species from 2 bacterial taxa that are generally lost in the elderly gut microbiota in genera Bifidobacteria and Lactobacillus. Both species have been approved for use by FDA (GRAS or generally regarded as safe) and have general association with human health; the particular probiotic formulation to be administered in this project is the widely clinically studied VSL#3® probiotic capsule, which contains both Lactobacillus and Bifidobacteria [3-5]. To our knowledge, no synbiotic (combination therapy of prebiotic and probiotic) developments targeting the elderly population have been described. The benefits of these taxa for the elderly are numerous. The widespread capacity of the Bifidobacteria to metabolize polyphenolic compounds (e.g. esters, glycosides or polymers) into bioactive metabolites with strong antioxidant activities and their impact on human health make them an attractive target. Both Lactobacillus and Bifidobacteria have been shown to increase lifespan in animal models as well as reduce inflammation in both humans and animal models [6-13]. Among many health benefits, Lactobacillus prevents oxidative DNA damage in human-derived colon cells and produces metabolites that are important mediators in the prevention of colorectal cancer [14-15]. Changes of the gut microbiota in elderly appear to involve a reduction in numbers of Lactobacilli and Bifidobacteria and an increase in numbers of potentially pathogenic species that lead to gastrointestinal disorders, inflammation, and infection [16]. Elevated Bifidobacteria levels are beneficial at all ages, including boosting the declining immune systems of the elderly [17]. Indeed, the level of Bifidobacteria in the gut is anti-correlated with serum levels of inflammatory TNFα and IL-6. Probiotics containing Bifidobacteria have been shown to positively modulate the microbiota of the elderly. Similarly, prebiotic treatments have led to similar effects [18-20]. For example, galacto-oligosaccharide prebiotic treatment of elderly subjects resulted in an increase in Bifidobacteria levels in the gut [21]. Inulin type fructans (i.e. prebiotics) are naturally occurring polysaccharides that are indigestible by humans. Inulins and similar fructans in *Triphala* induce a bloom of Bifidobacteria and Lactobacillus in the gut and have therefore been the subject of intensive study. Similarly, oligofructose prebiotic treatment of human subjects decreased the colonization by *Clostridium difficile* and was accompanied by reductions of relapse and hospital stay duration [22]. Thus, opportunities to improve human health and immunity through the modulation of the gut microbial communities by dietary supplement intervention remain highly promising for anti-aging and reducing morbidity and mortality associated with infection in the aged.

Our overarching research goal is to help reduce early morbidity from aging and mortality associated with infection in the aged by developing probiotics with the potential to enhance the health benefits of *Triphala* to reverse chronic inflammation and stimulate immune robustness. Our project is based on the hypothesis as well as *in vitro* data from our laboratory which demonstrate that the bioactivity of *Triphala* is elicited by the gut microbiome to generate a widened spectrum and abundance of anti-inflammatory compounds. We reason that the *Triphala*-induced benefits to both the elderly as well as persons of all ages can be enhanced by co-administration of specific probiotic species. *Thus, we hypothesize that probiotic formulations consisting of bacterial species capable of mediating the increased digestion, bioabsorption and bioactivity of Triphala will increase and make more uniform the response and impact of Triphala treatment on human populations. We hypothesize that the potent anti-inflammatory Triphala when co-administered with VSL#3 will further reduce inflammation and promote a healthier gut community architecture in the elderly.*

8. PROGRESS REPORT

N/A

9. RESEARCH DESIGN AND METHODS

Recruitment. A total of 75 participants will be recruited from UCSD ADRC. We aim to recruit approximately 20 eligible individuals per group (1. synbiotic, 2. herb alone, and 3. placebo control) from UCSD ADRC.

Screening. After written consent, patients will undergo a medical history intake to determine eligibility. Along with study participants' referring providers, ADRC will provide oversight in determining whether potential subjects are eligible according to the following criteria:

Inclusion criteria:
1) Cognitively intact;
2) Able to give informed consent in English;
3) Medical clearance by their doctor;
4) > 60 years of age.

Exclusion criteria:
1) Alzheimer's Disease or other neurodegenerative disease such as Parkinson's Disease;
2) Current use or use in the past 3 months of antimicrobial or steroidal drugs;
3) Medical conditions affecting immune status (e.g., heart failure, inflammatory bowel disease, hepatitis C, HIV);
4) International travel in the past 3 months.
5) Previous known side effect or negative reaction to VSL#3 or *Triphala.*

Note that heart failure, inflammatory bowel disease, hepatitis C, and HIV patients are excluded due to these diseases featuring variable and significantly altered immune status and gut microbiome, which are the main outcome measures of the study. This pilot study seeks to identify relatively healthy elderly subjects to assess these markers in the context of dietary supplementation.

Assessment visits. Assessment personnel will be naive to the participant group assignment. Testing will take place at SOM Building 4. At T1 (baseline): participants will 1) review their study responsibilities with the opportunity to reconsider participation 2) provide a stool and small blood sample for biomarker assessment; 3) complete psychosocial measurements via questionnaires; 4) receive group assignment instructions at the end of the visit. Measures will be repeated at T2 (post-intervention period).

Randomization. A computer-generated randomization algorithm will determine group assignment to ensure that participant demographics are evenly distributed across study conditions.

Measures of gut microbiome and treatments received.
Treatments: Two grams of organic *Triphala* powder (Banyan Botanicals, Inc.) alone or with 1 capsule VSL#3® (VSL Pharmaceuticals, Inc.) probiotic taken with a few ounces of room temperature water in the morning and at bedtime, or placebo capsule taken morning and evening. Participants will be provided their synbiotic or placebo capsules during the T1 session. VSL#3® is a clinically validated probiotic that is commonly prescribed by UCSD GI physicians to help restore gut microflora.
Gut microbiome assessment: Participants will be mailed a stool collection kit prior to T1 and asked to bring their morning stool sample during the T1 and T2 assessment sessions.
Blood marker assessment: Participants will donate 1 vial of blood during the T1 and T2 assessments with the licensed phlebotomist.

Experimental Conditions:
Interested patients will provide informed consent and then be randomized to participate in the synbiotic, *Triphala* alone, or placebo alone group. Subjects will receive stool kits and a set of questionnaires to fill out at T1. Subjects will bring their morning stool sample and fill out the questionnaires again at T2.

Synbiotic Group. Subjects will be provided both written and verbal instructions and given a kit containing encapsulated Organic *Triphala* powder (Banyan Botanicals, Inc.) and VSL#3® (VSL Pharmaceuticals, Inc.) capsules. Subjects will take 2 grams of encapsulated organic *Triphala* powder and 1 capsule of VSL#3 with room temperature water in the morning and at bedtime.
Herb Only Group. Subjects will be provided both written and verbal instructions and given a kit containing encapsulated Organic *Triphala* powder (Banyan Botanicals, Inc.). Subjects will take 2 grams of organic *Triphala* powder with a few ounces of room temperature water in the morning and at bedtime.
Placebo Group. Subjects will be provided both written and verbal instructions and given a kit containing placebo capsules. Subjects will be instructed to take 2 (inert) capsules with room temperature water in the morning and at bedtime.

Assessment Outcomes
Outcomes Hypothesis: Feasibility, treatment expectancy/satisfaction, retention, and adherence.
Recruitment rates will be recorded and new recruitment strategies will be formulated to overcome barriers presented by decliners for a future study. Treatment expectancy and satisfaction - 6 item credibility/ expectancy questionnaire (CEQ) will be utilized. Retention (% of drop-outs) will be recorded, and exit interviews will be administered to determine reasons for drop-out. Adherence to intervention will be derived from T1 and T2 presence.
Outcomes Hypothesis: Objective measures of gut microbiome health.
16S rDNA Sequencing: Stool samples will be analyzed to identify alterations in the gut microbiota community structure by 16S rDNA sequencing. This experimental design will allow us to assess the efficacy of *Triphala* and probiotic species to positively modulate inflammaging and immunosenescence. We will also assess a panel of 30 cytokine/chemokines/lymphokines for this purpose. Finally, our study design permits us to evaluate potential synergistic interactions between the probiotic species and *Triphala*.
Outcomes Hypothesis: Objective measures of inflammatory status.
Blood pressure (BP): 3 resting automated BPs will be taken (Dinamap 1846SX) and averaged.
Biomarkers: Blood samples will be obtained by a licensed phlebotomist from a mobile phlebotomy service, Veni-Express in SOM Building 4's Clinical Rooms. Serum heparin or EDTA-preserved plasma will be stored at -80^{o} C until assay in Dr. Mills' laboratory space MTF-418. Inflammatory biomarkers will be assessed by ELISA (MSD, Minneapolis, MN).

For those participants who specifically consent to tissue banking in the ICF, their samples will remain in storage in Dr. Mills's laboratory space MTF-418 after the end of this study. These blood and stool samples will remain under the sole control of the PI and may be investigated later for other inflammation-related biomarkers and gut microbes.

Exploratory Outcomes Hypothesis: Health-related behaviors.
Pittsburgh Sleep Quality Index (PSQI): A self-report of sleep quality and disturbances over a 1-month period. Nineteen items generate seven "component" scores. The sum of scores yields one global score;
Fruit/Vegetable/Fiber Screen and Fat Screen: Rapid screeners with high correlations for total fat, saturated fat, cholesterol, fruit/vegetable intake.
Leisure Time Exercise Questionnaire (LTEQ): By Godin and Shephard, a valid and reliable self-administered questionnaire designed to measure an individual's regular physical activity level.
Alcohol Use Disorders Identification Test (AUDIT): A simple screening method to assess alcohol use.
Exploratory Outcomes Hypothesis: Psychological health.
Beck Depression Scale (BDI)-II: A 21-item self-report scale used to measure depression symptom changes.
Perceived Stress Scale (PSS): Assesses unpredictability, uncontrollability, and overwork/stress.
Perceived Benefits Scale (PBS): Yields subscales of enhanced self-efficacy, increased community closeness, increased compassion, increased faith, lifestyle changes, enhanced family closeness, and material gain.
Positive and Negative Affect Schedule (PANAS): Two 10 item scales of positive and negative emotionality.
Satisfaction with Life Scale (SWLS): Assesses satisfaction with life as a whole and has high internal consistency and temporal reliability.
*The Mindful Eating Questionnaire (MEQ): M*easures five constructs related to mindfulness of eating behavior:

disinhibition (inability to stop eating when full); external cues (eating in response to environmental cues); emotional eating (eating in response to emotional state); awareness (being aware and appreciative of effects of food on senses) and distraction (focus on other activities while eating) with 28 items scored on a 4 point scale - “Never/Rarely,” “Sometimes,” “Often,” and “Usually/Always.” This instrument has a good internal consistency reliability (Cronbach’s alpha = 0.64), and construct validity.[132]

Ayurvedic Health Forms (AHF): The Chopra Center’s 102-item Mind/Body Quiz is used to assess body and mind constitution and state of imbalance in Clinical Ayurveda on a 3-point scale – “Never”, “Occasionally”, “Very often.” The *Ojas/Ama* Quiz is a 20-item assessment of Yes/No questions to determine level of *Ojas* and *Ama*, which are Ayurvedic Medical classifications of immune strength and toxic burden.

Additional Measures

Age, gender, socio-economic status, ethnic group, relationship status, education, body-mass Index and waist-hip circumference will be recorded. If for medical reasons subjects alter medications, it will be recorded via a monthly tracking form that the subject can submit.

Statistical Issues

Analytic Approach. Summary statistics (means, SDs, ranges, frequencies) will be computed for all variables of interest and compared between the treatment arms at baseline using 2-sample tests to test if randomization achieved balance in key covariates across groups. In the event of pre-existing variable group differences associated with dependent or relevant clinical variables, the nuisance variables will be statistically controlled. Outcome measures (e.g. health-related behaviors, psychological health, and objective measures of microbiome health and inflammation) will be transformed as needed to better approximate Gaussian distributions. The proposed study period is 2 months; hence we anticipate little loss-to-follow-up. However, subjects may drop out for a variety of reasons. It is important to take missing data into account when testing hypotheses of interest. Mixed-models can be applied even if some observations are missing as long as the data are “missing at random”. Missing data (e.g., drop-outs) will be tested to determine if it is informative, and the methods to test for completely random dropouts will be applied. If needed, pattern-mixture models will be developed which take "missingness" into account when estimating parameters of interest using the methods of Park & Lee.

Analysis Plan. Two sample *t*-tests will be used to compare change scores (post-treatment minus pretreatment) in each outcome. Given the multiple outcomes, we will also use O'Brien's test which can be used to compare multiple endpoints across groups; we will apply O’Brien’s test to the multiple outcomes for each hypothesis (e.g., psychological health via positive and negative affect, depressive symptoms; health behaviors via diet, physical activity, medication adherence, and alcohol usage). The O'Brien test statistic is a weighted linear combination of the t-statistics for each outcome. Finally, we will fit mixed effects models, with a subject-specific intercept included to allow for individual variability in outcome. Main covariate effects will be treatment group, time (pre-treatment and immediately post-treatment), and the group X time interaction. Likelihood ratio tests will be used to test the significance of these covariates. A significant group X time interaction will indicate that outcome trajectories differ across treatment groups.

Sample Size and Power. We hypothesized a Cohen’s d effect-size (standardized mean difference between groups) of 0.75 based on previous studies. With at least 60 subjects completing 2 visits, we will have 80% power (2-sided alpha=0.05) to detect a 0.75 effect-size (Cohen’s d). To be conservative, we aim to recruit 75 subjects to this study, allowing for a 20% drop-out/loss-to-follow-up rate and a total of 60 subjects completing the 2 visits. Note that our calculations did not control the Type I error for the multiple endpoints. Our objective in this small pilot study is to obtain early evidence of the putative efficacy of this novel intervention on a multitude of outcomes, psychosocial and biological, in the elderly. We expect our findings to inform the design of a larger future clinical trial in elderly subjects.

10. HUMAN SUBJECTS

This project will recruit 75 and test a total of 60 healthy elderly subjects from UCSD ADRC. We aim to test 60 participants (20 per group, 3 groups) from UCSD.

Patients who are elderly and cognitively intact will be identified, screened, and recruited from UCSD ADRC following the procedures outlined in Section 11 below.

Study testing visits will take place at SOM Building 4.

Along with the referring physician, Dr. Guerry Peavy will oversee determination of whether potential subjects fit eligibility criteria according to the criteria outlined below.

Inclusion criteria:
1) Cognitively intact;
2) Able to give informed consent in English;
3) Medical clearance by their doctor;
4) > 60 years of age.

Exclusion criteria:
1) Alzheimer's Disease or other neurodegenerative disease such as Parkinson's Disease;
2) Current use or use in the past 3 months of antimicrobial or steroidal drugs;
3) Medical conditions affecting immune status (e.g., rheumatoid arthritis, heart failure, hepatitis C, HIV);
4) Individuals diagnosed with Diabetes Mellitus
5) International travel in past 3 months.
6) Previous known side effect or negative reaction to VSL#3 or *Triphala*

Obtained research material will include vital signs, peripheral blood and plasma (processed and stored in Core Laboratory until assay), stool, and psychological and health behavior questionnaires.

All data will be obtained from recruited subjects for research purposes only. Data will be collected specifically for the proposed research project.

Inclusion of Women and Minorities
In the general population, inflammaging and immunosenescence is not gender-specific and we will recruit both men and women for this project.

Race/Ethnicity will be studied in a relative proportion to the ethnic composition of the San Diego area. San Diego has a diverse ethnic population. We will study English-speaking Hispanic and Latino as well as non-Hispanic and non-Latino participants.

11. RECRUITMENT AND PROCEDURES PREPARATORY TO RESEARCH

Overview. A total of 75 participants who are generally healthy and otherwise meet our study criteria will be recruited and randomly assigned to either an 8-week synbiotic group, herb alone, or placebo control with a goal to obtain 60 patients who complete the study (20 in each group) after an estimated 20% attrition. Placebo control subjects will be given the option of having the 8-weeks of herbal synbiotic for directed personal use upon conclusion of the study. Dr. Guerry Peavy has volunteered to help identify individuals who meet criteria for this study. Additional staff members responsible for assisting with recruitment are listed elsewhere in this application.

With this application, we are requesting a Waiver of Documented Consent to conduct the telephone screens. Asking interested individuals whether they meet the basic study criteria over the telephone (age, immune disorder diagnosis, medication use) presents no more than minimal risk of harm and involves no procedures for which written consent is normally required outside of the research context. Written consent will be obtained at the initial, in-person visit before any study questionnaires are administered or research procedures are performed.

With this application, we are also requesting a partial waiver of HIPAA authorization so that our study staff may help clinical staff identify potentially eligible participants using EPIC. When a member of our research team identifies a patient who may be eligible, he or she will then send a secure EPIC email asking the provider if they agree that a given patient is a good candidate for the study and asking the provider to speak with his or her patient about our study. The provider will provide a brief overview of the study during the patient's clinical

visit. The provider will additionally ask the patient if they would like a follow-up phone call from study staff to learn more details about the research study and see if they qualify (i.e., Telephone Screen). If the patient verbally assents, the provider will reply to the study staff via a secure EPIC email indicating that the patient is willing to be called about the study and study staff will then call the patient—only with their verbal permission. If the patient is not willing to be contacted, the provider will also indicate that via secure EPIC email, so that uninterested patients are not contacted by study staff. Using this secure communication method, there is minimal risk that any PHI could be lost or disclosed outside the designated research staff and University. All individuals with access to the PHI have been trained in privacy procedures. The PHI will remain at UCSD, and all the University and Privacy and Data Security rules will be followed. Participation in research is entirely voluntary. Interested volunteers may refuse to participate in the phone screen or opt out at any time without penalty or loss of benefits to which they are entitled at UCSD. The project could not practicably be conducted without a waiver because without a partial waiver of consent we would fail to confirm the most basic eligibility criteria (age, English-speaking, not taking antimicrobial or steroidal medication, etc.). It would not be feasible to obtain full consent and authorization from each patient in order to determine the much smaller group of patients that would ultimately meet study inclusion/exclusion criteria. Once we have identified via phone screen this smaller group who are likely eligible, it will be the patients interested in our research who will be consented for the study.

We hereby, then, request with this application a HIPAA waiver to prescreen using eMR records and a waiver of documented consent for phone screening. The research presents no more than minimal risk of harm to subjects and involves no procedures for which written consent is normally required outside of the research context.

a. This prescreening involves minimal risk because we will utilize the eMR records to identify potential subjects. EPIC will be viewed only by authorized personnel who have completed all necessary privacy training courses. PHI will not be shared or disclosed for any other purposes;

b. The waiver or alteration will not adversely affect the rights and welfare of the subjects because potential research subjects who are informed about the study by authorized study staff have no obligation to participate, or even be further screened for the study;

c. The research could not practicably be carried out without the waiver because there are hundreds of new and potentially eligible patients seen annually at UCSD and it would not be feasible to obtain full consent and HIPAA authorization from each patient in order to determine the much smaller group of patients that would be eligible for the study. Once we have identified this smaller group, it will be the patients interested in our research who will be consented for the study; and

d. The subjects will be provided with additional pertinent information after being identified as potentially eligible such as (a) the opportunity to read the ICF prior to deciding whether or not to participate (b) the opportunity to speak with authorized study staff about what participation would entail, including the study's risks and benefits, and (c) an opportunity to read and ask questions about the Research Subject's Bill of Rights.

All identifiers will be protected in accordance with University policies. These include:
1. The particular SI that will be used in this study will be clinical information in the patient's medical record file, including demographic information, laboratory tests, echocardiogram results, medication lists, problem lists, clinic notes, and other medical records.
2. All SI described will be used according to the study protocol, i.e. to determine the clinical characteristics of patients and clinical outcomes over time.
3. Only the PIs of the study, designated and approved study personnel as indicated in the protocol will have access to the SI. No person outside of the authorized study personnel will have access to the SI.
4. In the event of a real or suspected breach of security, University officials will be notified as soon as possible.

The project could not practicably be conducted without a waiver because without a partial waiver of HIPAA and consent, potentially eligible patients could not be efficiently identified due to the fact that there are hundreds of potentially eligible patients seen annually. It would not be feasible to obtain full consent and authorization from each patient in order to determine the much smaller group of patients that would ultimately meet study inclusion/exclusion criteria. Once we have identified via prescreening this smaller group who are potentially eligible, it will be the patients interested in our research who will be consented for the study. As an example, because we are looking for individuals who are over the age of 60, who have not been diagnosed with a disorder affecting their immune system, and who are not taking antimicrobial or steroidal medication, it would be extremely inefficient to approach and consent individuals who do not meet at least these basic inclusion criterion.

The project could not practicably be conducted without use of PHI because EPIC uses MRNs, name and birth date to identify patients. Without having access to this PHI, we cannot review medical records. PHI to be used for prescreening would be: name, MRN, demographic data, medical record data, including clinical data related to medical conditions, medication list, and problem list.

In addition to recruiting new study patients from the ADRC, we will also conduct a retrospective review of our previous research participants who have indicated on ICFs for other studies that they are willing to be contacted for future research studies. For these participants, we are also requesting a partial HIPAA and consent waiver for prescreening purposes only to review the eMR to ensure they appear to meet our basic inclusion/exclusion criteria before contacting the patient, in an effort not to squander the study staff or patient's resources, namely time. When contacting individuals who participated in our previous studies and agreed to be contacted, we will follow the submitted telephone script.

12. INFORMED CONSENT

Informed consent will be obtained by the PI or one of his trained staff-members prior to participant enrollment or any study procedures. The consent procedures will involve a thorough explanation of the study and associated procedures, as well as a review of the ICF on a point-by-point basis in a private office at UCSD. All consent is voluntary; records are kept confidential and coded by identifying participant numbers in the computer database, which requires authorized study staff to use their Active Directory login to access the password protected files. Participants will receive a copy of the ICF that they have signed and originals will be maintained by the study coordinator in a locked file cabinet.

We are requesting a Waiver of Documented consent for conducting telephone screens with potentially eligible candidates who have discussed the study with their clinical provider. If their clinical provider ascertains that the patient would like to learn more about the study and the patient verbally assents to receiving a phone call from study staff, the provider will send a secure EPIC reply to the research staff indicating such, and study staff will contact the patient by phone to explain the study and conduct the telephone screen. We are requesting that research staff be permitted to verify basic inclusion and exclusion criteria prior to the laboratory testing visit, such as inquiring whether the potential participant is over the age of 60 and does not have (a) Alzheimer's Disease or other neurodegenerative disease such as Parkinson's Disease; (b) Current use or use in the past 3 months of antimicrobial or steroidal drugs; (c) Medical conditions affecting immune status (e.g., rheumatoid arthritis, hepatitis C, HIV); (d) International travel in past 3 months; (e) Previous known side effect or negative reaction to VSL#3 or *Triphala*. At the end of the call, the research assistant will schedule the interested and preliminarily eligible individual for initial study visit. This phone screening presents no more than minimal risk of harm to subjects and involves no procedures for which written consent is normally required outside of the research context. The written informed consent process will occur at the beginning of the initial in-person session and thus, the research assistant will obtain a written informed consent prior to any further data collection.

If an individual does not pass the screening, he or she will be thanked for their interest. If they do pass the screening, they will be scheduled for their initial study visit. At this in-person visit, the patient will review the ICF with the research staff and provide written informed consent to participate in the study. Once the potential

participant has been informed s/he is eligible there will be discussion about the research, risks/benefits, and alternatives to the study. If the participant wishes to delay the treatment initiation for 1 week after consent to consider alternatives, that will be permitted. The option of a week delay should remove undue influence..

13. ALTERNATIVES TO STUDY PARTICIPATION

The alternative to participation is not to participate in this study. Participation is entirely optional and has no bearing on other rights to which a patient is entitled at UCSD or UCSD ADRC.

14. POTENTIAL RISKS

Participation in this study may involve some added risks or discomforts. These include:

a. Pain, risk of fainting, lightheadedness and dizziness associated with the blood draw.
c. Mild soreness and/or a bruise at the needle site.
d. A small risk also exists that the vein may form a clot at the needle stick, which will gradually clear over one to two weeks.
e. Risk of local infection at blood draw site.
f. There is risk of loss of confidentiality. This will be minimized by using an identifier number rather than patient's name on all of the information gathered for the study.
g. There may be mild psychological discomfort answering personal questions.
h. There may be mild psychological discomfort providing a stool sample.
i. There is an additional risk of bleeding for people taking blood thinning medications. Though considered to be a low risk, participants may want to discuss this with their physician prior to enrolling in the study.

Since this is an experimental treatment, there may be some unknown risks that are currently unforeseeable.

There are no known drug interactions or medical risks known with taking *Triphala;* a side effect of *Triphala* includes potential slightly looser bowel movement that is transient or resolves upon reduction of dose or cessation of *Triphala*. VSL#3 may cause a side effect of possible mild abdominal bloating in the first few days of consumption, which is a transitory phenomenon due to changing intestinal microflora that is self-resolving or can be reduced by decreasing the daily probiotic dose.

15. RISK MANAGEMENT PROCEDURES AND ADEQUACY OF RESOURCES

Healthy elderly subjects will be recruited at UCSD ADRC and be under the care of their physician.
The protocol will be approved by the IRB. All of the investigators and study staff have completed the federal human subjects certification.

Written consent will be obtained from each subject to review available medical records. We will make every effort to minimize the potential risks from the blood or stool sampling and the behavioral and psychosocial testing.

Subjects will be assigned an identification number, which will be used for data analysis purposes. The PI and Project Manager will have the link between the subject number and the subject's name. This information will be locked in a filing cabinet in the Project Manager's office.

Questionnaires will be labeled with subject numbers and locked in a filing cabinet in the Project Manager's office. Research records will be maintained in confidence and released only upon written consent of the subject, or as required by law.

All data analyses will be performed on aggregate data without identifiers for individual subjects. All study data will be kept confidential.

Each eligible subject's physician will be mailed a letter informing them of their patient's interest in participation in the study. Once enrolled into the study, all patients will continue to receive their usual health care from their doctors.

Data Safety Monitoring Plan

This is a low risk protocol. At the request of the IRB, the study investigators have appointed Mike Ziegler, Professor of Medicine, as the Data Safety Monitoring Officer (DSMO) who will help ensure adequate progress, patient's safety and confidentiality of personal information. Dr. Ziegler is not otherwise connected with the proposed project, however his research interests are similar to those included in the present study and he has been involved in similar types of studies. Dr. Ziegler will be in charge of evaluating and monitoring aspects of the study including participants' physical/medical safety, side effects and adverse events. Dr. Ziegler is familiar with the herbs, study operations, and data collection procedures found in the present study. The DSMO will monitor participants per the following:

1. Review and assessment of the performance of the study operations.
2. Examination of interim results of the study for evidence of efficacy or adverse effects.
3. Possible termination of the study because of early attainment of study objectives.
4. Identifying safety concerns or inadequate performance by staff.
5. Ensuring that any paper records, which reflect electronic data or relationships of those data, are kept in locked cabinets. Each PC workstation's operating system has a password protection scheme that allows startup of the system only by those with knowledge of the designated password.
6. The PIs will be charged with the reporting of all deaths and serious adverse events to both the DSMO and the IRB. This notification is submitted in writing to the IRB within 24 hours. Other adverse events will be reported within 10 working days.
7. Each participant's health status will be monitored and reported by the Project Coordinator to the Safety Monitoring Board on a monthly basis. Formal Safety Monitoring Board reports will be submitted to the IRB with each continuing review application during the active phase of the project.
8. Review any adverse events if any occur. PI will ask the Project Coordinator to break the code and have the statistician in our study determine if adverse events occur significantly more frequently in any one condition.

16. PRIVACY AND CONFIDENTIALITY CONSIDERATIONS INCLUDING DATA ACCESS AND MANAGEMENT

Participation in this study may involve a loss of privacy, but information will be handled as confidentially as possible

In case of an adverse event (such as injuries or chest pain) the referring physician will be notified. Further, this information may be shared with the IRB, according to the institutions reporting requirements.

All research records will be labeled with a subject number. The code key will be kept in a locked file in the research team's office. Any research records that personally identify participants will be kept only as paper records in a secure UCSD location, or as files behind the secure UCSD computer firewall. The study database is password protected and available only to trained study staff.

Blood and stool samples collected as part of the study will not be labeled with any information that could personally identify subjects. Blood and stool samples will be stored in locked freezers until analysis by trained research staff. For subjects who consented to tissue banking, their unused blood and stool samples will remain in storage in MTF-418 under the PI's control after the end of this study.

Any presentations or publications from this information will report data in aggregate and not identify individual participants.

We will keep confidential all research and medical records that identify subjects to the extent allowed by law. However, there are some circumstances in which we may have to show personal information to other people. For example, the IRB may look at or copy portions of records that identify study participants.

17. POTENTIAL BENEFITS

The potential benefits of the research to the subjects themselves may be minimal. There are no known long-term benefits to the subject. The theoretical benefits may be scientific in gaining greater understanding of

reversing the increased inflammation and reduced immune robustness observed in the elderly. The low potential risks to subjects are reasonable in related to the anticipated potential anti-inflammatory benefits to subjects and in relation to the importance of the knowledge that may be reasonably expected as a result of the study.

18. RISK/BENEFIT RATIO

Both the risks and the benefits to individual participants are relatively low. There are no known drug interactions or medical risks known with taking *Triphala*; a side effect of *Triphala* includes potential slightly looser bowel movement that is transient or resolves upon reduction of dose or cessation of *Triphala*. VSL#3 can cause a side effect of possible mild abdominal bloating in the first few days of consumption, which is a transitory phenomenon due to changing intestinal microflora that is self-resolving or can be reduced by decreasing the daily probiotic dose.

19. EXPENSE TO PARTICIPANT

There will be no charges for study procedures, however subjects may incur expenses due to lost work or childcare as part of their participation for which they will be responsible.

20. COMPENSATION FOR PARTICIPATION

Participants will receive $25 after the first and second laboratory testing sessions (T1 & T2) for a possible total of $50 for completing the 2 testing sessions.

UCSD will provide necessary medical treatment should a subject be injured by participation in this research.

21. PRIVILEGES/CERTIFICATIONS/LICENSES AND RESEARCH TEAM RESPONSIBILITIES

Paul Mills, PhD: Principle Investigator. Dr. Mills is a Professor of Family Medicine at UCSD and Co-Director of Translational Research Technologies laboratories program at the UCSD Clinical Translational Research Institute. Dr. Mills will provide direct support to Dr. Peterson for general oversight of the project as well as overseeing the assays conducted in his UCSD Core Laboratory, and assist with study implementation, data management, statistical analyses and manuscript preparation.

Christine Tara Peterson, PhD: Co-Investigator. Dr. Peterson is a Research Fellow in Dr. Mills' lab that will oversee the project as well as perform the microbiome assays and analysis. She will be managing the study design, data, and manuscript preparation.

Guerry Peavy, PhD: Project Scientist. Dr. Peavy is a licensed neuropsychologist and faculty member of the UCSD Department of Neurosciences. She has worked at the ADRC since 1990 and focused on cognitive functioning in patients with Alzheimer's disease. She will be assisting with recruitment of healthy subjects from ADRC and assisting with study implementation.

Chris Pruitt, BS: Research Associate. Chris Pruitt is an employee in Dr. Mills' UCSD Core Laboratory and will be responsible for running the inflammatory marker assays on de-identified samples for this project.

Meredith A. Pung, PhD: Research Associate. Dr. Pung will serve as the project's lead Study Coordinator, coordinating with the recruiters and clinical staff, following up with patients identified as eligible, obtain informed consent, complete laboratory testing sessions, and administer the depression and other behavioral and psychosocial instruments. She will be responsible for IRB application and amendments, R&D paperwork, and data safety updates. Dr. Pung will also be responsible for initial data entry, data cleaning and analyses.

Kathleen Wilson, MS: Research Associate. Ms. Wilson will obtain informed consent, complete laboratory testing sessions, and administer the depression and other behavioral and psychosocial instruments. She will also assist with initial data entry, data cleaning and analyses.

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23. FUNDING SUPPORT FOR THIS STUDY

A Private Foundation, Samuel Lawrence Foundation, has provided funding in the amount of $100,000 to Dr. Peterson for the total costs for this project. The funds are being administered by UC San Diego.

24. BIOLOGICAL MATERIALS TRANSFER AGREEMENT

N/A

25. INVESTIGATIONAL DRUG FACT SHEET AND IND/IDE HOLDER

N/A

26. IMPACT ON STAFF

No UCSD clinical nursing staff will be involved with this project.

27. CONFLICT OF INTEREST

The UCSD COI Office has reviewed this project and has determined that no conflict exists for this project. Please see uploaded letter from the COI for more information.

28. SUPPLEMENTAL INSTRUCTIONS FOR CANCER-RELATED STUDIES

N/A

29. OTHER APPROVALS/REGULATED MATERIALS

N/A

30. PROCEDURES FOR SURROGATE CONSENT AND/OR DECISIONAL CAPACITY ASSESSMENT

N/A