U.S. Study to Protect Brain Health through Lifestyle Intervention to Reduce Risk: Microbiome Ancillary Study

Short Study Name:

POINTER-Microbiome

Protocol Version 1.0
29 Oct 2020

In Memoriam: Martha Clare Morris, ScD

POINTER-Microbiome Principal Investigator

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U.S. POINTER Parent Trial Coordinating Center Principal Investigators

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ABBREVIATIONS

PROTOCOL SYNOPSIS

ABBREVIATIONS

ACOCC	**ADMINISTRATIVE AND CLINICAL OPERATIONS COORDINATING CENTER**
AD	**ALZHEIMER'S DISEASE**
ADMC	**ALZHEIMER'S DISEASE METABOLICS CONSORTIUM**
AE	**ADVERSE EVENT**
AMP-AD	**ACCELERATED MEDICINE PARTNERSHIP FOR ALZHEIMER'S DISEASE**
BVA	**BASELINE VISIT A**
BVB	**BASELINE VISIT B**
CC	**COORDINATING CENTER**
CFR	**CODE OF FEDERAL REGULATIONS**
CRP	**C-REACTIVE PROTEIN**
DSMB	**DATA SAFETY AND MONITORING BOARD**
FINGER	**FINNISH GERIATRIC INTERVENTION STUDY TO PREVENT COGNITIVE IMPAIRMENT AND DISABILITY**
GCP	**GOOD CLINICAL PRACTICE**
HDL	**HIGH DENSITY LIPOPROTEIN**
HIPAA	**HEALTH INSURANCE PORTABILITY AND ACCOUNTABILITY ACT**
ICH	**INTERNATIONAL CONFERENCE ON HARMONIZATION**
IRB	**INSTITUTUIONAL REVIEW BOARD**
MIND	**MEDITERRANEAN-DASH INTERVENTION FOR NEUROGENERATIVE DELAY**
NCRAD	**NATIONAL CENTRALIZED REPOSITORY FOR ALZHEIMER'S DISEASE AND RELATED DEMENTIAS**
OHRP	**OFFICE OF HUMAN RESEARCH PROTECTIONS**
PHI	**PROTECTED HEALTH INFORMATION**
PI	**PRINCIPAL INVESTIGATOR**
PID	**PARTICIPANT IDENTIFICATION**
SAE	**SERIOUS ADVERSE EVENT**

SG	**SELF-GUIDED**
STR	**STRUCTURED**

PROTOCOL SYNOPSIS

STUDY TITLE	U.S. Study to Protect Brain Health Through Lifestyle Intervention to Reduce Risk of Alzheimer's Disease Microbiome Ancillary Study
STUDY NAME	U.S. POINTER Microbiome Ancillary Study
ABBREVIATED STUDY NAME	POINTER-Microbiome
PRINCIPAL INVESTIGATORS	POINTER-Microbiome PI: Ali Keshavarzian U.S. POINTER Coordinating Center PIs: Laura Baker, Mark Espeland
STUDY SPONSOR	National Institute on Aging (NIA)
STUDY DESIGN	Ancillary study to the U.S. POINTER lifestyle intervention trial that adds in-home collection of fecal samples and plasma donation from the POINTER biospecimen repository for approximately 1000 parent trial participants at Baseline and Month 24.
DURATION OF STUDY PARTICIPATION	At least 24 months (duration of the parent trial)
SUMMARY OF KEY ASSESSMENTS	• Fecal sample collection (at home) for analysis of gut microbiota and AD metabolic signatures • Plasma donation (from parent trial biospecimen repository) for analysis of AD metabolic signatures
INCLUSION/ EXCLUSION CRITERIA	All participants must be enrolled in the parent trial; there are no additional inclusion/exclusion criteria
PRIMARY OUTCOME MEASURES	• Alzheimer metabolic signatures in blood including (but not limited to) phospho- and sphingolipids, amino acids, secondary bile acids and acycarnitines (targeted and untargeted metabolomics) • Gut microbiome composition (shotgun metagenomic analysis)
SECONDARY OUTCOME MEASURES	Molecular signatures that may predict cognitive trajectory and other biological responses (e.g., brain imaging outcomes) to the lifestyle intervention

1 INTRODUCTION

Epidemiological studies report that dietary interventions predict cognitive trajectory including the development of Alzheimer's disease (AD) in older adults. The U.S. POINTER Microbiome Ancillary Study, referred to as POINTER-Microbiome, is supported by the NIA U19 Alzheimer Gut Microbiome Project (PI: Rima Kaddurah-Daouk, Duke University). The ancillary study will test the effects of the POINTER lifestyle intervention, particularly the diet component, on gut microbiome composition/function and blood and fecal metabolomes as they relate to cognitive trajectory, brain imaging structure/function and other relevant trial outcomes. POINTER-Microbiome will also investigate the mechanism(s) and interrelations between molecular signatures that relate to these effects. It has been well-establshed that diet impacts the gut microbiota community structure and function, which in turn can have a wide variety of biological effects on metabolism and the immune system. Recent studies suggest that gut microbiota may also affect brain function. The Alzheimer's Disease Metabolomics Consortium (ADMC) and the Accelerated Medicine Partnership for Alzheimer's Disease (AMP-AD) have identified metabolic signatures that correlate with markers of AD and AD progression. POINTER-Microbiome will expand on the work of the ADMC and AMP-AD by examining the effect of the POINTER lifestyle intervention on the gut microbiome and its metabolic signatures to understand how these effects relate to cognitive decline and AD risk reduction. This ancillary study is expected to enhance our understanding of AD pathogenesis and potential treatments by emphasizing the pivotal role of diet on the microbiota-brain axis.

1.1 U.S. POINTER Parent Trial Summary

U.S. POINTER was launched to test the generalizability of the Finnish Geriatric Intervention Study to Prevent Cognitive Impairment and Disability (FINGER) findings in a geographically and racially diverse cohort of Americans. U.S. POINTER uses a similar multimodal intervention approach that has also proven successful for cardiovascular disease and diabetes prevention. The parent study will investigate whether random assignment to a Self-Guided (SG) versus a Structured (STR) lifestyle intervention influences cognitive trajectory over 2 years in 2000 older adults (age 60-79 yrs). POINTER participants are cognitively normal but *at-risk* for cognitive decline and dementia due to factors such as sedentary lifestyle, poor diet, suboptimum cardiovascular health, and family history of significant memory impairment. Participants assigned to the SG arm attend facilitated group meetings 2-3 times per year for education and support, and complete blood testing every 6 months and an annual physical exam. Participants assigned to the STR arm complete a more intensive lifestyle intervention that includes structured physical and cognitive exercise programs, a modified Mediterranean diet, and frequent physical exams, blood testing, and health coaching for cardiometabolic risk management. Intervention effects on 2-year trajectories of a global cognitive composite score and other outcomes are assessed at 6-month intervals.

1.2 U.S. POINTER Microbiome Ancillary Study Summary

POINTER-Microbiome will enroll approximately 1000 of the 2000 parent trial participants at five clinical sites. The microbiome ancillary study adds in-home fecal sample collection at Baseline and Month 24 of the parent trial, and donation of stored Baseline and Month 24 plasma samples (from the parent trial biospecimen repository) for ancillary study enrollees. Samples will be profiled using metagenomics and metabolomics technologies to identify the effects of the MIND Diet and other lifestyle intervention domains on the gut microbiome and metabolome, and relate these outcomes to pre-defined AD metabolic signatures, brain structure and function (in partnership with the POINTER Brain Imaging Ancillary Study), and cognition (assessed in the parent trial).

1.3 Aims

Aim 1: Test the hypothsis that predefined blood metabolic signatures of AD characterized by metabolites including (but not limited to) phospho- and sphingolipids, amino acids, secondary bile acids and acycarnitines are modified by the POINTER lifestyle intervention.

Aim 2: Relate pre- and post-intervention changes in the gut microbiome to metabolomics data, to brain structure and function (including cognition), and to AD blood biomarkers.

Aim 3: Define molecular signatures that correlate with or predict cognitive outcomes associated with the lifestyle intervention.

Aim 4: Using system analyses techniques, characterize lifestyle-induced changes in the brain-gut microbiome network that mediate protection of cognitive function in at-risk older adults.

2 BACKGROUND AND SIGNIFICANCE

2.1 Rationale for Studying the Influence of Controlled Diets on Gut Microbiome, Metabolome and Cognitive Function

Consumption of plant based diets (high in fiber) have been widely demonstrated to be beneficial for health and this effect may be mediated (at least in part) through changes in the gut microbiota. It is well-established that diet impacts the gut microbiota and their metabolites, referred to as the gut microbiome. The gut microbiome is a community of bacteria, archaea, viruses and eukaryotic microbes including 40 trillion microorganisms with more than 3 million genes and a 1000 species of bacteria alone. It plays a vital role in human physiology, including nutrition, metabolism, hormone production, inflammation, neural function, and the development of disease. Recent studies suggest microbiota-derived metabolites may also affect brain function and behavior through their effects on intestinal barrier function and signaling via neural, immune and hormonal gut-brain communication channels.[1-3]

There is no “ideal” microbiome but the Western lifestyle (sedentary lifestyle, consumption of diets high in fat/sugar and low in fiber) is commonly characterized by decreased microbial diversity, increased abundance of pro-inflammatory bacteria and pathobionts, and decreased abundance of putative benefical bacteria.[4,5] In contrast, consumption of diets rich in varied plants are associated with a benefical microbiota profile characterized by increased microbial diversity, low abundance of pro-inflammatory bacteria, and high abundance of benefial commensal bacteria.[6] The gut microbiota is robustly influenced by diet including diversity and composition of bacteria in the adult gut, especially over months to years that are relevant for beneficial effects of lifestyle interventions.[7] However, the mechanisms by which diet-microbiome interactions can cause, prevent, or even reverse disease have yet to be identified. The MIND diet, a component of the POINTER lifestyle intervention, was developed to incorporate green leafy vegetables, berries and nutrients, and reduce consumption of certain food products (e.g., saturated fats, simple sugar) to produce a gut bacterial profile that is anti-inflammatory and anti-atherogenic, with lower CRP and triglycerides and higher HDL.[8] In prospective studies, the MIND diet is associated with protection of brain health in the aging brain.[9,10]

The U.S. POINTER Microbiome Ancillary Study will build on work of the ADMC and the AMP-AD by examining lifestyle intervention effects on the gut microbiome and on cognitive health and protection against the development of AD in older at-risk adults.

2.2 Rationale for Collection of Fecal Samples

POINTER-Microbiome aims to understand the role of the gut microbiome in AD pathogenesis. Samples collected as part of the project will be analyzed using metagenomic and metabolomic techniques, in an attempt to define the bidirectional biochemical communication between the brain and the gut, and to improve the understanding of neurodegenerative disease in the context of external influences including diet and other lifestyle exposures, and the microbiome.

3 POTENTIAL RISKS AND BENEFITS

3.1 Potential Benefits

There continues to be mounting evidence from epidemiological studies that cognitive decline and risk of developing AD may be decreased by ingesting a diet comprised plant-based foods such as the MIND diet. The beneficial effects could involve the mutation to the compostion and function of the gut microbiome and the downstream impact on brain function. This mechanism underscores the need to identify the effects of dietary interventions on gut microbiome composition and function, blood and fecal metabolomes and cognitive decline that could have important consequences for the prevention of AD and other related disorders.

Participants will be compensated $50 per fecal sample collected, for a total of $100 (Baseline, Month 24).

3.2 Potential Risks and Protection against Risks

3.2.1 Potential Risks

The sampling technique for fecal specimen collection has been used for decades with minimal side effects.

3.2.2 Protection against Risks

NIH guidelines will be followed to protect participant confidentiality. The data will be collected for research purposes only, and will be managed by the parent trial Data Coordinating Center (DCC), the National Centralized Repository for Alzheimer's Disease and Related Dementias (NCRAD), and Duke University. Safety monitoring will be provided by the U.S. POINTER Coordinating Center Safety Monitor and Committee, and the U.S. POINTER Data Safety and Monitoring Board (DSMB).

4 STATISTICAL ANALYSES

Data collected in POINTER-Microbiome will be pooled with two other diet intervention studies that are included as part of the larger supported project (Project 2 of U19 Alzheimer Gut Microbiome Project, PI: Rima Kaddurah-Daouk). Data analyses will be conducted by the U19 investigators and shared with U.S. POINTER. The statistical analytic plan will be approved by the parent trial biostatistical team prior to data analysis.

5 STUDY POPULATION

POINTER-Microbiome will enroll approximately 1000 parent trial participants.

5.1 Inclusion and Exclusion Criteria

Parent trial inclusion and exclusion criteria apply.

5.2 Recruitment and Screening

U.S. POINTER participants will be approached to assess their interest in POINTER-Microbiome participation during the scheduling phone call for their first baseline visit (BVA). Interested participants will be provided with an informational brochure to review. Participants with continued interest will be consented at the second parent trial baseline visit (BVB).

5.3 Inclusion of Women and Minorities

No candidate will be excluded for reasons of sex, race, or ethnic group. Consistent with enrollment goals for the parent study, POINTER-Microbiome will target enrollment of 50% women, and a minimum of 23%

from racial/ethnic minority groups, which reflects the demographics of the U.S. population as reported by the Census Bureau in 2016.

6 POINTER-MICROBIOME ASSESSMENTS

6.1 Fecal Sample Collection

Fecal samples will be collected from study participants at two different time points using non-invasive procedures once after receipt of the collection kit at the Baseline visit and once after receipt of the collection kit at the Month 24 visit. Kits are designed by the American Gut Project and include: (1) a 30mL collection tube containing 15mL of 95% ethanol, (2) a disposable spatula (double-ended, with Spoon & Scoop) and (3) a stool collection paper. Kit boxes are designed to comply with IATA and DOT regulations. Samples will be sent directly to NCRAD by the participant using shipping materials and pre-paid mailing labels provided by study staff.

Following consent, participants will receive training and printed instructions regarding fecal sample collection and will be provided with a collection kit to take home, which will include shipping materials.

6.2 Stored Plasma Donation from Parent Trial Biospecimen Repository

Frozen plasma from the U.S. POINTER biospecimen repository will be sent to NCRAD for analysis for each enrolled POINTER-Microbiome participant. No additional blood will be collected in this ancillary study.

6.3 Specimen Collection, Shipment, Receipt and Processing

The U19 has developed customized at-home fecal collection kits for the project and will leverage the exceptional resources of NCRAD to aggregate, homogenize, aliquot and distribute samples. Kits are provided to microbiome ancillary study participants at the Baseline and Month 24 clinic visits. After collecting the sample at home, participants will mail the sample directly to NCRAD for processing and storage until analysis. Stored frozen plasma for ancillary study participants will be sent from the POINTER Biospecimen Repository to NCRAD.

NCRAD utilizes 2D data matrix scannable barcode labels which can be stored electronically in OnCore, a secure, web-based enterprise research management system hosted by Indiana University, ensuring that every specimen aliquot is uniquely identified and tracked at all times. Labels include specimen type, a unique specimen number, barcode, and study name. All specimens are checked in upon receipt; the barcodes on the tubes are verified to match the barcode in OnCore, and any non-conformance issues are noted in OnCore. Storage location and sample quality measures are stored and tracked.

7 TEMPORARY DISCONTINUATION

Temporary discontinuation of study assessments is treated differently than study withdrawal (see **Section 8**). In the event of temporary study discontinuation due to circumstances such as travel or unavailability, a study coordinator will work with the participant to schedule the fecal sample collection in a timely manner. Temporary discontinuation of intervention activities and withdrawal from POINTER-Microbiome activities are independent. Specifically, fecal sample collection may continue in the event of temporary intervention discontinuation. Decisions regarding continuation and discontinuation in the parent and ancillary studies are made on a case-by-case basis by the U.S. POINTER Coordinating Center, in collaboration with the ancillary study and Site PIs. If the participant discontinues the POINTER intervention but agrees to continue with parent trial assessments, the participant will be permitted to remain in POINTER-Microbiome.

Reason(s) for temporary discontinuation in POINTER-Microbiome will be captured in the parent trial web-based data entry system.

8 STUDY WITHDRAWAL

Participants may withdraw their consent to participate in POINTER-Microbiome at any time. Withdrawals will be tracked in the parent trial data management system. If a POINTER-Microbiome participant withdraws consent for the parent trial, this participant will also be withdrawn from the microbiome ancillary study. Participants who withdraw from the parent trial but agree to an early termination outcomes assessment visit will also be offered an early termination microbiome assessment.

Reasons(s) for study withdrawal will be coded as follows:

- Participant is unwilling or unable to participate
- U.S. POINTER is terminated
- Lost to follow-up: participant could not be recalled to the clinic for follow-up assessments
- Death

9 PERSONNEL REQUIREMENTS

9.1 Coordinating Center Personnel

- POINTER-Microbiome Coordinating Center Project Manager: The Coordinating Center (CC) Project Manager is responsible for ensuring that the microbiome assessments are carried out at each site as per protocol, for management of accounting, subcontracts and supply acquisitions, and for oversight and tracking of IRB-related activities.The CC Project Manager will also oversee POINTER-Microbiome data monitoring at clinical sites and will provide support as needed to POINTER-Microbiome Site Study Coordinators.

9.2 Site Personnel

- Parent Trial Site Principal Investigator: The parent trial Site PI is responsible for overseeing all POINTER-Microbiome ancillary activities at the site. The Site PI will work with the POINTER-Microbiome Site Study Coordinator to ensure that fecal collection is carried out as per protocol.
- Parent Trial Site Lead Project Manager: The Site Lead Project Manager oversees all day-to-day participant activities for the parent trial and ancillary studies. The Site Lead Project Manager will supervise the POINTER-Microbiome Site Study Coordinator to ensure that ancillary study activities are appropriately integrated within parent trial activities.
- POINTER-Microbiome Site Study Coordinator: The Study Coordinator is supervised by the parent trial Lead Project Manager and the Site PI. The Study Coordinator is responsible for carrying out day-to-day POINTER-Microbiome study activities including consenting, scheduling, participant training and communication, participant tracking, fecal sample collection, and kit management. The Study Coordinator serves as the main liaison between the site and the U.S. POINTER Coordinating Center.

10 POINTER-MICROBIOME INTEGRATION WITH THE PARENT TRIAL

10.1 Study Organization

Ancillary study leadership include the ancillary study PI (Ali Keshavarzian, MD of Rush University), PIs of the parent trial Coordinating Center (Laura Baker, PhD; Mark Espeland, PhD), and the parent trial Executive and Steering Committees. Leadership is also provided by the PI of the larger U19 Alzhiemer Gut Microbiome Project (Rima Kaddurah-Daouk, PhD of Duke University).

10.1.1 POINTER-Microbiome Integration with Parent Trial Leadership

Ancillary study investigators are members of the parent trial Steering Committee that meets monthly to discuss leadership issues related to trial conduct. The Steering Committee includes several Core Workgroups that focus on specific aspects of trial conduct (e.g., recruitment, clinical operations), and POINTER-Microbiome investigators are members on one or more of these Cores. All major scientific decisions for the parent trial and its ancillary studies will be determined by majority vote of the Steering Committee.

10.1.2 POINTER-Microbiome Integration with Parent Trial Coordinating Center

The CC assumes the primary responsibility for ensuring proper regulatory oversight, setting up and managing subcontracts with sites, overseeing implementation of all study procedures including intervention, outcomes assessments, training and certification of staff, and data monitoring, tracking and analysis. A POINTER-Microbiome CC Project Manager will work closely with other CC staff to ensure seamless integration of POINTER-Microbiome procedures with those of the parent trial at the clinical sites. The CC Project Manager will also serve as the liaison with clinical sites to resolve issues as they arise and to oversee timely communication with participants when questions arise regarding fecal sample collection.

10.2 Schedule of Fecal Sample Collections

Fecal samples will be collected for microbiome assessment following the Baseline and Month 24 clinic visits.

10.3 Masking to Intervention Assignment

Participants enrolled in POINTER-Microbiome, regardless of intervention group assignment, will provide fecal samples and blood samples (from the respository) for assessment.

10.4 Study Coordination

POINTER-Microbiome will be coordinated by the U.S. POINTER Coordinating Center to facilitate efficient use of study resources, data security and data sharing.

10.5 U.S. POINTER Coordinating Center

The parent trial CC is comprised of two branches, the Administrative and Clinical Operations Coordinating Center (ACOCC) and the Data Coordinating Center (DCC).

10.5.1 Administrative and Clinical Operations Coordinating Center

The ACOCC will oversee operations related to participant-facing POINTER-Microbiome activities. Examples of relevant operations are provided below.

1. Finances, including development and management of agreements with sites as well as tracking invoicing
2. Regulatory, including development of IRB applications and tracking of approvals
3. Training and certification of Site Study Coordinators to properly carry out the protocol
4. Safety monitoring, including reporting of protocol deviations and adverse events to the IRB
5. Site auditing

10.5.2 Data Coordinating Center

The DCC will carry out data-related operations for POINTER-Microbiome as outlined below.

1. Forms development for data collection and tracking

2. Tracking of microbiome ancillary study blood aliquots, fecal sample assessment kits, shipped kits and completed assessments
3. Monitoring site performance (e.g., recruitment, microbiome assessment completion rates)
4. Generate reports as requested for the parent trial Steering Committee and DSMB, and for the NIA
5. Develop a web-based data system for data capture, secure storage, reporting and disaster recovery
6. Ensuring confidentiality of participants' Protected Health Information throughout the lifespan of data collection, storage and sharing
7. Coordinate sharing of data with other parent trial ancillary studies for operational, safety, and scientific purposes, and with national databases for future scientific investigation

10.6 POINTER-Microbiome Clinical Sites

Personnel at each site that will assist with POINTER-Microbiome protocol implementation include parent trial personnel (Site PI, Lead Project Manager) and the POINTER-Microbiome Study Coordinator. Site responsibilities to support POINTER-Microbiome are listed below.

1. Submit IRB materials as necessary for local IRB site review before submitting to the U.S. POINTER central IRB
2. Recruit and confirm eligibility of participants
3. Obtain informed consent from interested parent trial participants
4. Review fecal sample collection procedures with participants
5. Provide collection kits and shipping materials to participants
6. Contact participants to review collection procedures and shipping instructions
7. Receive and store fecal sample collection kits
8. Enter tracking information into the POINTER central database
9. Resolve database queries as needed
10. Invoice and follow-up to ensure site and participant payments are received
11. Ensure the safety of participants

11 SAFETY MANAGEMENT

11.1 Potential Risks

POINTER-Microbiome poses minimal risk.

11.2 Adverse Events and Serious Adverse Events: Definitions and Reporting

POINTER-Microbiome adverse events (AE) are collected as part of the parent study and are defined as clinically relevant unfavorable or unintended health events that occur during the intervention delivery, intervention-related activities, or outcomes assessments at the clinic. Expected risks described in the consent form will be recorded and reported to the DSMB but not logged as AEs. All AEs will be classified using MedDRA coding.

Serious Adverse Events (SAEs) are defined per the Code of Federal Regulation Title 21 Part 312 (http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/cfrsearch.cfm?fr=312.32) and include: death, a life-threatening adverse experience, inpatient hospitalization or prolongation of existing hospitalization, or persistent or significant disability/incapacity. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered as SAEs if they have the potential to jeopardize participant safety or to require medical or surgical intervention to prevent a more serious outcome listed above.

Adverse events and serious adverse events will be recorded on the electronic AE Report Form per the parent study instructions. AEs reported after informed consent must be recorded on an AE Report Form and entered in the central database accessed through the study website. All completed AE Report forms

will be reviewed for completeness by the parent trial Safety Officer to ensure that documentation of each event is adequate to permit accurate inferences regarding causation (e.g., temporal associations, onset, course, response to patient or physician intervention, alternative etiologies) and severity.

SAEs must be entered in the central database within 72 hours of learning of the event, and reported to the sIRB within 7 days of learning of the event. SAEs may also be reported to the local IRB as per local institution requirements. SAEs will be reported as required to the NIA and DSMB. If a participant develops an SAE, the participant's primary care provider and the Site PI and/or Study Clinician will collaborate to ascertain the safety of continuing the intervention.

11.3 Data and Safety Monitoring Board

Data and safety monitoring will be provided by the independent, external DSMB for the parent trial. The DSMB will review all AEs and SAEs and will provide a report to the Steering Committee and to the sIRB.

12 DATA MANAGEMENT

The POINTER DCC is responsible for overseeing research clinic data collection and standardization, data management, data transfer and QC analyses of all assessment data.

12.1 Data Collection and Management Responsibilities

The ACOCC is responsible for overseeing data collection across all clinical sites.

12.2 Clinical Data Management

All clinical sites use a secure website for data entry. Access to the data portal by staff is password-protected, and access to certain types of information on the website is restricted depending on the staff member's role in the study. Site-specific and study-wide dynamic reports will be available.

12.2.1 Randomization

POINTER-Microbiome will rely on the parent trial randomization schedule to provide approximately equal numbers of POINTER-Microbiome participants per intervention arm at each of the participating clinical sites.

12.2.2 Participant Tracking

The participant tracking system for the parent trial will be expanded to accommodate POINTER-Microbiome. This system permits dynamic tracking of recruitment, enrollment, participant contact, staff training and certification, completed assessments, data and safety monitoring, and regulatory activities. The system includes a notification system that alerts clinic staff about upcoming appointments and various deadlines.

12.2.3 Security and Data Protection

Procedures are in place to provide disaster recovery of the database if needed using prior nightly back-ups.

12.2.3.1 Database Security

Tracking data (i.e., consent status, sample collection and shipment) will be identified only by participant identification (PID) number and stored in a central U.S. POINTER database, with access only via a secure and encrypted website. Website access and priviledges are managed by the parent trial DCC.

Confidentiality of information within the U.S. POINTER database is protected through a variety of procedures and facilities:

1. The confidential nature of the data collected, processed, and stored is explained to all new personnel.
2. All access to data management team office space is controlled through a single door, which is locked and only accessible by key or security badge.
3. All participant data uploaded to the central database is encrypted as described above.
4. All participant data stored on local servers at the clinical site are encrypted and password-protected.

Protected Health Information such as participant name, address and other identifiers collected and data-entered are securely stored separately from de-identified data. Access to PHI is limited to a few members of the clinical site and the Coordinating Center.

Documents pertaining to the study are to be retained for a minimum of 2 years after discontinuation of the parent trial. These documents should be retained for a longer period if required by local regulations. It is the responsibility of study leadership to inform the Site PI when these documents no longer need to be retained.

All data generated from POINTER-Microbiome specimens will be stored at Duke University and shared with the parent U.S. POINTER trial. Deidentified data will be made public through Sage and the AMPAD Knowledge Portal, an NIH-funded resource for accelerating treatments for AD.

12.2.3.2 Research Clinic Data Security

Hardcopy and/or local server electronic records are stored at the clinical sites. Access to these data is controlled by locked storage or password-protected access to electronic records by authorized study personnel only.

13 ETHICS AND REGULATORY CONSIDERATIONS

13.1 Good Clinical Practice

POINTER-Microbiome, consistent with the parent trial, will be conducted in accordance with Good Clinical Practice (GCP) guidelines, as defined by the International Conference on Harmonization (ICH) Guideline, Topic E6, the United States Code of Federal Regulations, Title 21, Part 50 (21CFR50) – Protection of Human Subjects and Part 56 – IRBs, HIPAA, State and Federal regulations and all other applicable local regulatory requirements and laws.

Study personnel involved in conducting this study will be qualified by education, training and experience to perform their respective task(s) in accordance with GCP.

No study document shall be destroyed without prior written agreement between the U.S. POINTER Coordinating Center and the Site PI. Should the Site PI wish to assign study records to another party or move them to another location, he/she may do so only with the prior written consent of the Coordinating Center.

13.2 Institutional Review Board

The Wake Forest IRB serves as the single IRB (sIRB) of record and is responsible for the review, approval and regulatory oversight of U.S. POINTER and its ancillary studies.

Each participating institution must provide all required documents for the review and approval of this protocol and associated informed consent documents and recruitment materials to the Wake Forest sIRB.

Any amendments to the protocol or consent materials must be approved before they are placed into use. In the United States, only institutions holding a current US Federal-wide Assurance issued by OHRP may participate (http://www.hhs.gov/ohrp/assurances).

Subsequent protocol amendments and, when warranted, changes to the informed consent document must be approved by the sIRB. Protocol and informed consent form amendments can be made only with the prior approval of the U.S. POINTER Coordinating Center. The investigator may not implement any protocol deviation without prior notification to the Coordinating Center and prior review and documented approval of the sIRB, except where necessary to eliminate an immediate hazard to study participants, or when change(s) involve only logistical or administrative aspects of the study (ICH 4.5.4). The Site PI shall notify the Coordinating Center and sIRB of deviations from the protocol or severe adverse events (SAEs) occurring at the site, in accordance with local procedures. SAEs will be reported to the DSMB and to NIA. Per NIA policy, SAEs that are unanticipated will be reported to the NIA and DSMB within 48 hours of site PI notification.

13.3 Informed Consent and HIPAA Compliance

In obtaining and documenting informed consent, the investigator should comply with the applicable regulatory requirement(s) and should adhere to GCP and to the ethical principles that have their origin in the Declaration of Helsinki. Prior to the beginning of the trial, the investigator should have IRB approval of the written ICF and any other written information to be provided to participants. Participants and their family members will be given ample opportunity to inquire about the details of the study. Prior to enrollment in the study, the written ICF must be signed and personally dated by the participant and by the person who conducted the informed consent discussion. Participants will be provided a copy of the signed ICF.

Consent forms will be developed by the U.S. POINTER Coordinating Center. The sample consent form provided to sites will include all of the required elements of informed consent required by the sIRB. The sample consent form will be sent to participating sites where it should be tailored to include site-specific information to meet local IRB regulations. Each Site PI, under the guidance of the local IRB, is responsible for ensuring that all applicable state laws are met with regards to judgment of competency and the consent form process. Each participating site must maintain IRB-approval, along with other required regulatory records and essential documents.

Information about study participants will be kept confidential and managed according to the requirements of HIPAA. HIPAA regulations require a signed HIPAA Authorization or informed consent form that includes HIPAA language informing the participant of the following:

- What PHI will be collected from participants in this study
- Who will have access to that information and why
- Who will use or disclose that information
- The rights of research participants to revoke their authorization for use of their PHI

In the event that a participant revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of participant authorization. Each Site PI, under the guidance of the local IRB, is responsible for ensuring that all applicable HIPAA regulations and State laws are met.

13.4 Study Monitoring

Data collection forms and source documents will be reviewed at regular intervals by the Coordinating Center throughout the study to verify adherence to the protocol, completeness and accuracy of the data, and adherence to local regulations regarding conduct of clinical research.

The monitoring visits will be conducted according to the applicable ICH and GCP guidelines to ensure protocol adherence, quality of data, compliance with regulatory requirements and continued adequacy of the clinical site and its facilities. The Site PI will cooperate in the monitoring process by ensuring the availability of source and other necessary documents/records when requested and will promptly address any matters brought to his/her attention by the monitor.

13.5 Duality of Interest

In an environment where scientists may benefit directly or indirectly from commercial ties, the potential to influence scientific objectivity exists. Clinical trials are especially vulnerable to such influences, whether perceived or real. In these circumstances, a potential conflict of interest occurs when there is a divergence between an individual's private interests and his or her professional obligations to the research project, such that an independent observer might reasonably question whether the individual's professional actions or decisions, including the design, conduct, or reporting of the research are influenced or determined by considerations of personal gain, financial or otherwise. Allegations of conflicts of interest threaten the integrity of the scientific community. Policies that promote disclosure of potential conflicts of interest and propose means to manage those conflicts aim to protect the credibility and integrity of research investigators so public trust and confidence in the research results is preserved. The U.S. POINTER study team has developed a policy to address potential duality of interest and protect the integrity of decision-making within the trial. This document has been approved by the Executive Leadership and is available on the U.S. POINTER website. POINTER-Microbiome will abide by this policy.

14 AUDIT

In accordance with ICH GCP, representatives of Wake Forest University Health Sciences, the Alzheimer's Association, and/or the National Institute on Aging may select this study for audit. The Site PI and study staff are responsible for maintaining the site master file containing all study-related regulatory documentation that will be suitable for inspection at any time by the Coordinating Center, sponsor, its designees, and/or regulatory agencies. Inspection of site facilities (e.g., clinic and community facilities) to evaluate the integrity of trial conduct and compliance with the protocol may also occur.

15 PUBLICATION POLICY

The U.S. POINTER Emerging Science, Publications and Presentations Core will coordinate dissemination of data from this study. The Core, whose membership will be approved by the U.S. POINTER Executive Leadership, includes representation from the study team. The Core will solicit input and assistance from other investigators as appropriate and adhere to documented U.S. POINTER publication policies. A separate document outlining policies regarding publications and access to study data has been approved by the Executive Leadership and is available on the U.S. POINTER website.

16 SHARING POINTER-MICROBIOME STUDY DATA

Data from POINTER-Microbiome will be shared with other researchers pursuant to the 02/26/2003 "Final NIH Statement on Sharing Research Data" (http://grants.nih.gov/grants/guide/notice-files/NOT-OD-03-032.html). The NIH and the Alzheimer's Association endorse the sharing of final research data to serve these and other important scientific goals. To protect participants' rights and confidentiality, identifiers will be removed from the data before they are shared.

17 STUDY GUIDANCE

The Steering Committee is the governing body that provides the leadership for U.S. POINTER and establishes scientific and administrative policy for the study. It holds the primary responsibility for developing the trial design and common clinical protocols, recommending appropriate procedures to manage the conduct and monitoring of study operations, review of ancillary studies, preparation of

publications, and site-related conflicts that cannot be resolved locally or with Coordinating Center guidance, and reporting the study results. The Steering Committee is comprised of the Site PIs, Ancillary Study PIs and Leads, Core workgroup chairs, Coordinating Center PIs and Leads, representatives from the sponsors of the parent trial (Alzheimer's Association) and funding agencies of the ancillary study (NIH), and representatives from the Executive Leadership. Executive level trial oversight by the Steering Committee is outlined in the Steering Committee Charter. POINTER-Microbiome will rely on executive level leadership input from the parent trial Steering Committee.

18 LITERATURE CITED

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