An Open Label Trial to Evaluate the Effects of BCG Immunization on Biomarkers of Inflammation/Immune Response and Alzheimer's Disease in Adults with Mild Cognitive Impairment and Mild-to-Moderate Dementia due to Alzheimer's Disease

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An Open Label Trial to Evaluate the Effects of BCG Immunization on Biomarkers of Inflammation/Immune Response and Alzheimer's Disease in Adults with Mild Cognitive Impairment and Mild-to-Moderate Dementia due to Alzheimer's Disease

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STATEMENT OF COMPLIANCE

This study will be conducted in compliance with the protocol, International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH), Good Clinical Practice (GCP), and the applicable regulatory requirements, United States Code of Federal Regulations (CFR) Title 45 CFR Part 46 and Title 21 CFR Parts 50, 56, and 312.

LIST OF ABBREVIATIONS

AD	Alzheimer's Disease
ADC	Alzheimer's Disease Center
ADRD	Alzheimer's Disease and Related Dementias
BCG	Bacillus Calmette-Guérin
CDR	Clinical Dementia Rating
CFR	Code of Federal Regulations
CN	Normal cognition
C-SSRS	Columbia Suicide Severity Rating Scale
CRF	Case Report Form
CSF	Cerebrospinal Fluid
CMP	Comprehensive Metabolic Panel
CNS	Central Nervous System
CRP	C-Reactive Protein
eCRF	Electronic Case Report Form
EDC	Electronic Data Capture
ET	Early Termination
ESR	Erythroid Sedimentation Rate
FAQ	Functional Activities Questionnaire
FDA	Food and Drug Administration
GCP	Good Clinical Practice
HIPAA	Health Insurance Portability and Accountability Act
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
IIR	Inflammation and Immune Response
IRB	Internal Review Board
LP	Lumbar Puncture
MADRC	Massachusetts Alzheimer's Disease Research Center
MCI	Mild Cognitive Impairment
MDU	Memory Disorders Unit
MoCA	Montreal Cognitive Assessment
mTB	Mycobacterium Tuberculosis
NACC	National Alzheimer's Coordinating Center
NIA	National Institute on Aging
NPIQ	Neuropsychiatric Inventory Questionnaire
PCP	Primary Care Physician
PI	Principal Instigator
PPD	Purified Protein Derivative
RBANS	Repeatable Battery for the Assessment of Neuropsychological Status
SAE	Serious Adverse Event/Serious Adverse Experience
SOB	Sum of Boxes
SOP	Standard Operating Procedure
TSH	Thyroid Stimulating Hormone
Treg	Regulatory T-cells
UDS	Uniform Data Sat
US	United States
WOCBP	Women of Child Bearing Potential

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1 ETHICS/PROTECTION OF HUMAN SUBJECTS

1.1 Institutional Review Board (IRB)

This study will be conducted in compliance with current Good Clinical Practices (GCP) and Title 21 Part 56 of the United States of America Code of Federal Regulations (CFR) relating to IRBs.

1.2 Ethical Conduct of Study

The study will be conducted in accordance with GCP defined by the International Conference on Harmonisation (ICH) and the ethical principles of the Declaration of Helsinki.

1.3 Subject Information and Consent

This study will be conducted in compliance with Title 21 Part 50 of the United States of America Code of Federal Regulations (CFR), Federal Regulations, and ICH Guidance Documents pertaining to informed consent. At the first visit, prior to initiation of any study-related procedures, subjects will be informed about the nature and purpose of the study, participation/termination conditions, and risks and benefits. Subjects will be given adequate time to ask questions and become familiar with the study prior to providing consent to participate. Subjects will give their written consent to participate in the study and will be provided with a copy of the fully executed consent form for their records.

Some of the subjects in this study may be unable to provide informed consent due to cognitive impairment. The capacity to consent is gauged by an experienced licensed clinician investigator during the consent process through an evaluation of the participant's understanding of their condition, their options for treatment, their understanding of the potential benefits and risks of participating in the clinical trial and of their understanding regarding the voluntary nature of their participation in the trial. In addition, participants are evaluated for their level of orientation and understanding of what will happen during the trial. This process is recorded as part of the consent discussion documentation.

Subjects who are deemed unable to provide consent will be given the opportunity to assent, and consent will be obtained from a legally authorized representative. Subjects who are unable to assent or who object to participation in the study will not be enrolled.

2 INTRODUCTION: BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

2.1 Background Information

2.1.1 Alzheimer's Disease and Mild Cognitive Impairment

Alzheimer's disease (AD) is the most prevalent form of dementia affecting more than 5,000,000 people in the US and an estimated 20,000,000 people worldwide, according to the Alzheimer's Association [1]. AD causes progressive neuronal degeneration, resulting in progressive memory loss, dementia and death. AD has no known cure or preventative as yet; however, treatments exist that can temporarily and modestly at best ameliorate dementia symptoms.

Mild cognitive impairment (MCI) is the term used when there is clinical concern about cognitive decline and evidence of impairment(s) in cognitive testing, more than expected for the patient's age and educational background, but not so severe that the person cannot manage their daily functioning without more than minimal assistance. MCI is frequently, though not always, a transitional, prodromal phase of dementia due to Alzheimer's disease. "Dementia" is present when independent daily functioning is affected.

AD is characterized by the presence of amyloid-β (Aβ) plaques, tau neurofibrillary tangles and loss of neurons and synapses in the cerebral cortex, particularly in association areas that subserve cognition. Aβ has been the target of many recent clinical trials; however, these studies have failed to find that reducing amyloid meaningfully slows progression of dementia. It is increasingly evident that AD is a complex disorder with multiple metabolic, inflammatory, vascular and other pathophysiological processes beyond amyloid and tau proteinopathies contributing to neurodegeneration. There is now increasing attention to intervening in these non-amyloid pathways to stem the tide of neurodegeneration in AD.

2.1.2 Alzheimer's Disease and the Immune Response

Inflammation and immune responses (IIR) have major roles in the pathophysiology of Alzheimer's disease (AD). IIR encompasses many complex and highly regulated biological processes with which immune local tissue cells respond to tissue injury and maintain organ function and organismal homeostasis. AD's pathognomonic amyloid-β (Aβ) peptide itself is hypothesized to be a dysregulated ancient innate immune response to allo- or auto-stimuli in the brain.[2] Aβ oligomers and aggregates in AD are thought to elicit or exacerbate IIR,[3] promoting vicious cycles of protein misfolding, endoplasmic reticulum stress, deranged metabolic signaling and mitochondrial function, oxidative/nitrosative stress, and cell death. There is tremendous interest and rapidly growing academic, biotech and pharma research on IIR therapeutic approaches in AD and AD-related disorders as similar vicious cycles have been proposed for a-synuclein pathologies in LBDs, [4,5] tau pathologies in FTD-tau and other tauopathies,[6,7] and TDP-43 pathology in FTD-TDP and ALS.[8,9]

Peripheral inflammatory markers may serve as biomarkers of AD disease state and distinguish healthy older adults from middle-aged adults, and healthy older adults from those with mild cognitive impairment (MCI) or Alzheimer's Disease[10]. Inflammatory biomarkers may even predict those who will convert from MCI to AD over a two-year period [11-13]. Innate immune function is affected by progression of AD; Monocytes of subjects with MCI or AD induce a greater NLRP3 and NLRP1 inflammasome response to extrinsically applied amyloid beta than do healthy controls [14]. The adaptive immune system is also involved in AD progression: T-cell reactivity to amyloid beta increases with age as well as progression of AD [10], and with worsening disease, clonal populations of CD4+ and CD8+ T-cells can be found in the cerebrospinal fluid[15,16]. Regulatory T cells (Tregs) are of particularly significance to AD. While augmenting Tregs increases the number and function of microglia associated with amyloid beta plaques, and improves cognitive functions[12,17-19], they are fewer in number over the course of disease[20].

There is tremendous and rapidly growing academic, biotech and pharma research on IIR therapeutic approaches in AD and AD-related disorders as similar vicious cycles have been proposed for α-synuclein pathologies in LBDs, [5,21] tau pathologies in FTD-tau and other tauopathies,[22,23] and TDP-43 pathology in FTD-TDP and ALS.[24,25]

2.1.3 BCG Rationale

A potentially transformative IIR approach to AD prevention and disease modification that is safe, cheap and ready for testing *now* is Bacillus Calmette-Guérin (BCG) vaccination. Live BCG vaccination has been the mainstay of prevention of tuberculosis for almost 100 years, administered in billions of people in more than 180 countries, from infants to the elderly. Its only significant contraindication is immunocompromise. "Off-target" benefits of BCG vaccination were recognized early in its 40% reduction of all-cause mortality in children and its prevention of non-mycobacterial infections,[26,27] and BCG is now being explored in clinical trials as a prophylaxis against COVID-19.[28] BCG has also demonstrated utility in treating non-infectious diseases. One major use is as a treatment for non-muscle-invasive bladder cancer, where high-dose intravesicular infusion is a treatment of choice,[29] and there has been interest in BCG's beneficial associations and effects in other cancers,[30-32] multiple sclerosis[33-35] and type 1[36,37] and type 2 diabetes.[38-41]

In the CNS, 'sterile inflammation' resulting from autoimmunity, misfolded proteins, or cell damage or death can result in a neurotoxic environment if not adequately kept in balance. T-regulatory cells, or Tregs can regulate immunologic balance by controlling microglial and antigen presenting cell (APC) activation, inhibiting effector T (Teff) cells, and inducing astrocytic release of neurotrophins, thus effectively transforming a neurotoxic environment to a neurotrophic state. For example, two studies noted decreased neuronal cell death in a MPTP model of Parkinson's Disease, finding that the magnitude of dopaminergic preservation correlated with the magnitude of Treg response to BCG treatment.[42,43] BCG's ability to induce and/or restore Treg immune balance is of great interest for treatment in AD,[44,45] where preclinical models find that augmenting Tregs increases the number and function of microglia associated with plaques, and improves cognitive functions.[18,46,47] In AD, Tregs may be overwhelmed due to their low numbers or dysfunction;[48] BCG may increase Treg number and function to rebalance the neurotoxic state to one of neuroprotection.[5]

Limited but provocative epidemiological analyses and *in silico* trial data support a benefit of BCG for AD. A large retrospective analysis found that older, non-demented adults treated with intravesicular BCG for bladder cancer had a significant 4-fold lower risk of subsequent AD over a median 8-year follow-up period than those treated with other means.[45]

BCG, a highly safe, live, attenuated vaccine used throughout the world to prevent tuberculosis and confers many health-promoting non-specific effects, from preventing infectious disease to treating cancers. These effects rely on its induction of trained and heterologous immune responses in the host. Such immune effects directly overlap and influence immune responses known to occur in CNS disease; non-human studies have found benefit to BCG vaccination on models of Parkinson's and Alzheimer's diseases, including cognitive improvement. These studies provide the impetus for our initiating clinical trials with BCG oriented towards primary prevention of AD in older adults or disease modification and secondary or tertiary prevention in adults whom AD pathological changes are already in motion. This pilot study initiates this program, with focus on the immune consequences of treatment and potential changes in AD-specific biomarkers and cognition after vaccination. The BCG strain used in this study is not approved in the US for immunization against tuberculosis or for the treatment of AD.

A pilot, open-label study of BCG immunization (Protocol#2020P002042) in both healthy older adults and adults with Mild Cognitive Impairment is currently underway with a focus on the immune consequences of BCG vaccine and potential changes in AD-specific biomarkers and cognition after vaccination. The 3-month, open-label study has so far demonstrated that repeated administration of BCG vaccine is safe and tolerable in healthy adults and adults with mild cognitive impairment between the ages of 55 and 80 years old. Following two intradermal vaccinations to participants in the unblinded study, there have been no serious adverse events, no clinically significant abnormalities detected in safety lab (eg. metabolic, hematologic, and immune labs) or vital sign measurements, and only mild-to-moderate, expected adverse events related to the lumbar puncture and blood draw procedures in the protocol. This small-scale study has informed the trial design and validated the assays included in the proposed in this protocol.

3 STUDY DESIGN

3.1 Overall Study Design and Plan

This single-site, open-label trial will investigate the effects of BCG vaccination on IIR and AD biofluidbiomarkers, magnetic resonance imaging (MRI) biomarkers, and neurocognitive/behavioral functioning over a oneyear period in older adults with mild cognitive impairment (MCI) to mild-to-moderate dementia due to AD. This study will also gather data on tolerability and safety. Safety will continue to be followed in the second year by phone.

Up to twenty five participants meeting eligibility criteria will be enrolled. Each participant will receive two intradermal BCG vaccinations spaced 4-6 weeks apart and will be evaluated 12, 26, and 52 weeks after the initial immunization. Cerebrospinal fluid (CSF), blood, MRI (in a subset of patients), and neurocognitive/behavioral assessments will be collected at Screening (CSF, blood, and cognitive/behavioral), Week 1 (MRI and cognitive/behavioral), Week 4 (blood), Week 12 (CSF, blood, and cognitive/behavioral), Week 26 (Blood and cognitive/behavioral) and Week 52 (all) to assess biomarkers and safety.Safety measures, including updated medical history and adverse events, will be collected at Week 78 and Week 104.

3.2 Study Objectives

The primary objectives of this study are:

1. To assess the safety and tolerability of BCG vaccination in older adults with MCI and mild to moderate dementia due to AD.
2. To investigate the potential of BCG vaccination as an immunomodulatory and CNS-protective agent for AD by evaluating changes from baseline in IIR and AD biomarkers in blood and CSF following BCG vaccination;
3. To measure changes in neurocognitive/behavioral functioning following BCG vaccination (MoCA, RBANS, CDR-SB, NPI-Q, HADS, FAQ, DCT Clock).

The secondary objective of the study is:

1. To measure changes in structural and functional neuroimaging following BCG vaccination;

3.3 Study Outcome Measures

3.3.1 Safety Measure Outcomes

Medical history, adverse events, and classic metabolic, hematologic, coagulation, and immune blood labs will be collected at Screening to determine eligibility. Adverse events will be solicited at every visit. Hematologic and coagulation blood labs will be repeated at Week 12 and Week 52 visits for LP safety. Vital signs will be measured at all in-person visits. Additionally, four weeks after the first injection, we will assess the injection site and evaluate for any signs of systemic infection prior to second BCG vaccination in the other arm. Vaccination sites will also be assessed at Weeks 12, 26, and 52. We will use the FDA Toxicity Grading Scale, as performed in vaccinetrials, to denote changes from baseline. Safety measures, including updated medical history and adverse events, willalso be collected at Week 78 and Week 104.

3.3.2 **CSF and Blood Biomarker Outcomes**

CSF and blood will be collected at Screening, Week 4 (blood only), Week 12, Week 26 (blood only), and Week 52 visits to examine biochemical biomarkers of:

1. Target engagement: BCG, purified protein derivative (PPD) and / or mycobacterium tuberculosis (heat-killed, mTB) – induced cytokine response in peripheral blood cells will be used to evaluate for BCG vaccine-induced immunity.
2. Pharmacodynamic response: a) Circulating cytokines and other related blood- and CSF-based IIR biomarkers relating to innate and adaptive immune responses; b) Epigenetic changes in peripheral blood cells and / or CSF- derived cell populations as indicators that BCG can lead to long term changes in peripheral and central nervous system immune cells.
3. AD pathophysiology: Amyloid-β42/40, phospho-tau, total tau and / or neurofilament light protein biomarkers (ATN) and other exploratory AD-relevant synaptic and IIR biomarkers may be compared within subjects over time and between subjects and groups.
4. BCG effects on the interactions of IIR and amyloid: We will perform PBMC stimulation with Aβ42 and positive-control antigens (e.g. lipopolysaccharide) and measure the molecular response in the supernatant at multiple timepoints after BCG /treatment. Aβ42 has immunostimulatory / inflammatory effects that increase with AD progression and may be modulated by an immune-provoking stimulus such as BCG.
5. Additional related Alzheimer's Disease and immune measures in plasma, CSF, and their cell populations at the transcriptional, protein, or epigenetic levels.

We have several robust platforms in the Arnold lab (which serves as the Biomarker Core of the Massachusetts Alzheimer's Disease Research Center) to conduct these assays, including highly validated Euroimmun assays, Fujirebio Lumipulse assays, Meso Scale Discovery with plates run on a new Tecan EV0200 multi-functional robotic liquid handling platform, and Quanterix SR-X for ultrasensitive Simoa assays. We have exhaustively validated many assays specifically for use in short-term clinical trials[49], and all assays that we propose demonstrate good precision and biotemporal stability. Change in a composite score from Baseline will be used as an outcome of response to BCG.

3.3.3 **Neurocognitive/Behavioral Measure Outcomes**

We will apply several robust neurocognitive tests to measure the effects of BCG vaccination on mentation, including the MoCA, RBANS, CDR-SB, NPI-Q, HADS, DCT Clock and FAQ.

3.3.4 **Structural and Functional MRI Measure Outcomes**

Up to 12 participants over 60 years old will be invited to undergo 3T structural and functional MRI at Week 1 and Week 52 visits to assess the effect of the study vaccine on regional brain volumes, cerebral perfusion, brain connectivity, and safety. Each scan will include high-resolution T1-weighted multi-echo MPRAGE for volumetric analysis, task-free blood oxygen level dependent (BOLD) sequence to measure resting neural connectivity, diffusion tensor imaging (DTI) to quantify white matter structural connectivity, T2-weighted fluid attenuated inversion recovery (FLAIR) sequence, and susceptibility- weighted imaging (SWI).

4 SUBJECT SELECTION

Each Investigator must adhere to the protocol detailed in this document and agree that any changes to the protocol must be approved by Institutional Review Board (IRB). Each investigator will be responsible for enrolling only those study subjects who have met protocol eligibility criteria.

4.1 Inclusion/exclusion Criteria

Investigators will use their best clinical judgment when selecting potential research subjects for this study and will not enroll any individuals who are frail or in questionable health, even if they meet all inclusion/exclusion criteria.

4.1.1 Inclusion Criteria

Study subjects meeting all of the following criteria will be allowed to enroll in the study:

1. Individuals between the ages of 55-85;
2. MCI or moderate dementia due to AD as defined by the 2011 NIA-AA Workgroup recommendations;
3. MoCA $\geq$ 8 at screening;
4. Global CDR between 0.5-2 (inclusive) at screening;
5. Amyloid and/or tau biomarkers indicative of AD pathology;
6. Education level, English language skills and literacy indicates subject will be able to complete all assessments;
7. Has a study partner who, in the investigator's judgement, has frequent, direct contact with the participant at least several days a week, can accompany the participant to all visits, and is also able to provide information to study investigator/staff;
8. Willing and able to complete all assessment and study procedures, including blood and lumbar punctures, and clinical assessments;
9. If on cholinesterase inhibitor and/or memantine, doses are stable for 3 months prior to baseline;
10. Negative test results for HIV antibody and Tuberculosis (QuantiFERON or T-spot TB test) at screening;
11. No prior BCG exposure either through birth vaccinations (born in North American) or BCG bladder cancer treatment.

4.1.2 Exclusion Criteria

Subjects meeting any of the following criteria during the screening evaluation will be excluded:

1. History of chronic infectious disease, such as HIV or untreated or active hepatitis;
2. History of tuberculosis, positive interferon-gamma release assay (IGRA, also known as the QuantiFERON-TB test) or T-spot TB test, including a test with a high reactivity to mycobacteria of non-tuberculosis variety;
3. Prior BCG vaccination, positive T-spot tuberculosis test or a T-spot test showing significant Mycobacteria exposure;
4. A positive SARS-CoV-2 PCR result within 3 months of screening, or known close contact with a confirmed COVID-19 positive person or symptoms highly suspicious for COVID-19 (per CDC guidelines) within 1 month of screening, including fever, cough, shortness of breath, chills, muscle pain, new loss of taste or smell, vomiting or diarrhea, and/or sore throat, based on clinician's judgment;
5. History of treatment with metformin within the past one year;
6. Treatment with other investigational agents which, at the discretion of the investigator, interfere with safety and/or study outcomes;
7. Current treatment with immunosuppressants (calcineurin inhibitors, corticosteroids, or biological or cytotoxic immunosuppressants, or disease or condition likely to require high dose steroid or

immunosuppressive therapy);

8. Other conditions or treatments associated with increased risk of infections or treatment with immunosuppressive medications for any reason;
9. Current treatment with aspirin > 160 mg/day or chronic, daily NSAIDs;
10. Current (as of time of study screening) or chronic use of antibiotics;
11. History of keloid formation;
12. Living with someone who is immunosuppressed and/or at high risk for infectious diseases (for example, HIV+ or taking immunosuppressive medications for any reason), or in a job (e.g. healthcare) in which the subject works with immunosuppressed populations;
13. Other/confounding neurological or psychiatric condition, unstable medical or psychiatric conditions, contraindications to BCG use and lab abnormalities or concurrent medication use posing risk for BCG or study procedures;
14. Laboratory abnormalities in B12, Folate, TSH, or other common laboratory parameters that may contribute to cognitive dysfunction per clinician judgment;
15. Laboratory abnormalities in CBC, electrolytes, LFTs, BUN, Cr, total serum immunoglobulins, ESR, CRP, or urinalysis posing risk to treatment with BCG per clinician judgment;
16. Laboratory abnormalities in PT-INR, which would pose a risk to performing the lumbar puncture procedure;
17. Discontinuation of cholinesterase inhibitor or memantine within one month (28 days) prior to baseline visit;
18. Females who are pregnant, lactating or of child-bearing potential;
19. If male with female partner(s) of childbearing potential, unwilling or unable to adhere to contraception requirements specified in the protocol.
20. Increased intracranial pressure as determined on a fundoscopy/neurological examination performed within 30 days of LP;
21. Administration of live vaccine within 30 days of screening visit or BCG immunizations
22. Administration of non-live vaccine within 14 days of screening visit or BCG immunizations
23. If participating in optional MRI: Existing contraindication to MRI per MGH Athinoula A. Martinos Center research guidelines

4.1.2.1 Women of Childbearing Potential (WOCBP)

For the purposes of this study, women of childbearing potential are defined as all women who are capable of becoming pregnant, unless they meet one of the following criteria:

1. 12-months post-menopausal.
2. Post-hysterectomy.
3. Surgically sterile.

If a female subject does not meet these criteria and is considered of childbearing potential, they will be excluded from the clinical trial.

Male subjects with female partners of child-bearing potential must use at least 1 of the following contraceptive methods: hormonal contraceptives (oral, injectable, patch, intrauterine devices), a barrier method (such as condoms or diaphragm) used with a spermicide (a foam, cream, or gel that kills sperm), male sterilization, or total abstinence from heterosexual intercourse, when this is the preferred and usual lifestyle of the subject. Abstinence is defined as refraining from heterosexual intercourse during the entire period of risk associated with the study. Note that periodic abstinence (calendar, symptothermal, post-ovulation methods), withdrawal (coitus interruptus), spermicides only,

and lactational amenorrhoea method are not acceptable methods of contraception.

4.2 Recruitment

Up to twenty five subjects will be screened and enrolled in the study, and after screening we expect 12 subjects to complete the study.

4.2.1 Recruitment of Subjects through Advertising

Advertisement flyers will be posted on bulletin boards around MGB to advertise for the study as well as an advertisement on Partners Rally for Research. A phone number will be provided that will ring directly to the research coordinator, and voice messages can be left for the coordinator on a password-protected voice mailbox. The study coordinator will contact the subject and explain study in further detail and if the subject is interested, potentially complete a telephone prescreening. A listing of the study will also be posted on the MGH ACTRU website.

Subjects may also be recruited through the distribution of IRB approved recruitment materials at community outreach initiatives including informational sessions to various community partners such as city-based and non-profit organizations.

4.2.2 Recruitment of Subjects from the Massachusetts Alzheimer's Disease Research Center

Subjects will also be recruited from an observational study that follows a longitudinal research cohort (LC) of approximately 400 active research participants in the Massachusetts Alzheimer's Disease Research Center (MADRC) recruited from the MGH's Memory Disorders Unit clinic and other diverse sources. LC subjects are followed-up on an approximately annual basis, either in-person at the MGH or by means of a telephone follow-up 'visit'. Study staff will only contact subjects that have indicated to the MADRC that they are interested in hearing about/participating in other studies.

4.2.3 Recruitment of Subjects from among MGB Providers

Subjects will be recruited from the outpatient clinical practices of the principal investigator (Dr. Steven Arnold). If the potential subject is the investigator's patient, another member of the study or clinic staff will introduce the study to the patient and determine if they are interested in learning more about the study. An NP investigator will be made available to explain the study to the subject at their request. If the potential subject is not the investigator's patient, the investigators will not directly approach the patient regarding possible participation in the study.

Other providers across MGB will also be made aware of the study (including key eligibility criteria) via an IRB-approved email and a link to the Rally ad. These providers (not participating directly in the study) may discuss the studydirectly with their patients, if they choose to do so. If the prospective participant is interested, their provider will provide them with a link to the Rally ad, which contains the research coordinator's contact information.

4.2.4 Recruitment of Subjects from the CTRU Registry

A research volunteer registry (P2021000916) was established in 2021 to maintain a list of people interested in participating in clinical research with the MGH Clinical and Translational Research Unit (CTRU). The goal of the CTRU, located in CNY 149, is to serve the clinical and research departments, institutes, centers, programs and labs that comprise the MGH Neuroscience community and to be a foundational component and research core facility within MGH's emerging translational programs. Eligible participants who have consented to be contacted for future research may be identified using data stored within the volunteer registry. These participants will be contacted by phone or email and evaluated for interest and eligibility using the study-specific phone screen.

5 SUBJECT ENROLLMENT

5.1 Informed Consent Process

This study will be conducted in compliance with Title 21 Part 50 of the United States of America Code of Federal Regulations (CFR), Federal Regulations, and ICH Guidance Documents pertaining to informed consent.

Potential subjects will be given general information about the research (e.g., through informational sheets, email, online Rally ad, or discussion with their treating physicians). If they are interested in learning more about the study, they will then contact the research coordinator. The research coordinator will obtain verbal consent in accordance with Mass General Brigham's Prescreening Guidelines prior to performing a telephone prescreening interview. Information collected during the telephone prescreen will be entered directly into a REDCap survey for review by clinicians to determine eligibility for the study. Ifthe subject meets pre-screening criteria and wishes to continue the screening process, an in-person screening visit will be scheduled.

At the screening visit, prior to starting the consenting process, one of our licensed Nurse Practitioners, Dr. Alison McManus, DNP, Kelly Devitte-McKee, NP, Amanda DeSenna, NP or one of our physicians Dr. Steven Arnold, MD and Dr. Marc Weinberg, MD will evaluate capacity to consent. These clinicians are experienced in evaluating capacity to consent in cognitively impaired patient populations and perform this assessment regularly. Like informed consent, evaluation of consent capacity is a process. The consenting clinician begins with an informal conversation prior to the start of the consent discussion with a prospective subject and the study partner, including simple questions to determine if the prospective subject may have problems that could affect decision making.

Following the initial informal discussion, an investigator will discuss with the potential subject and their study partner the details of the study using the informed consent document as a guide. This discussion will include all the required elements of informed consent, including the purpose of the research, the procedures to be followed, the risks and discomforts, as well as potential benefits associated with participation, and alternative procedures to study participation. Their questions will be answered to their satisfaction. The subject will be provided with adequate time to reflect on the potential benefits and risks and possible discomforts of participation, and to make an informed decision. The capacity to consent again is gauged by an experienced licensed clinician investigator during the consent process through an evaluation of the participant's understanding of their condition, their options for treatment, their understanding of the potential benefits and risks of participating in the clinical trial and of their understanding regarding the voluntary nature of their participation in the trial. In addition, participants are evaluated for their level of orientationand understanding of what will happen during the trial. Following this process, a discussion with the participant's study partner will also inform the determination of the patient's capacity to consent. All study staff will report any changes in function of the participant to the investigators,who will then reassess and document any changes in the patient's capacity to consent.

If the clinician determines that the subject has adequate consent capacity, the participant will be able to consent for themselves and may enroll into the study. If it is determined by the clinician that the participant is unable to consent, but is willing and able to assent, then the LAR will provide surrogate consent to enroll the participant in the study. In either case, a study partner is required for participation in the study and they will be required to sign the consent form as such.

Prior to proceeding with surrogate consent for assenting subjects, the clinician will also ensure that the surrogate LAR understands that his or her decisions should be based on "substituted judgment," which means that the decision reflects a potential subject's own views when s/he had the capacity to express them. If a potential subject did not

previously express a view on the matter, the surrogate should make the decision based on the potential subject's best interests.

A licensed Nurse Practitioner Investigator or Physician Investigator will then obtain written informed consent or surrogate consent from each participant prior to the initiation of any study procedures. We have included Dr. Alison McManus, DNP, Kelli Devitte-McKee, NP, and Amanda DeSenna, NP as study staff. All are experienced research nurse practitioners who will be consenting participants for this study in addition to the physician investigators. They currently consent on several other IRB-approved interventional trials run by the group, both industry and investigator sponsored. All potential subjects will be given the opportunity to speak with the Physician Investigator should a licensed Nurse Practitioner be involved in obtaining informed consent.

If a participant is deemed unable to consent, either at the start of the extension study or while participating in the study, then consent will be obtained from a legally authorized representative. The relationship of the representative to the subject will be documented and kept for our records. The list of eligible legally authorized representatives, in order of preference, is as follows:

1. Court appointed guardian with specific authority to consent to participation in research or authority to make health care decisions for a class of diagnostic and therapeutic decisions inclusive of the proposed research
2. Health care proxy/person with durable power of attorney with specific authority for making health care decisions inclusive of the proposed research
3. Spouse, adult child, or adult sibling.

Subjects who cannot give informed consent must be able to give assent. If the individual objects to participation, they will not be enrolled. Investigators will document capacity to give assent during the consenting process.

5.2 Remuneration

All subjects will receive:

- Parking fees paid for all in-person visits
- Lunch voucher provided for subject at each study visit that exceeds two hours, which can be used at the cafeteria at MGH CNY 149
- If appropriate, travel expenses will be reimbursed, including mileage at the current IRS travel rate for medical purposes, up to $150 per visit
- Honoraria: Up to $540 if subject completes the study (up to $740 including optional MRIs):
    - $15 for screening (SVa and SVb, inclusive)
    - $25 for each subsequent visit to the screening visit ($125 total)
    - $100 for each lumbar puncture ($300 total)
    - $100 upon study completion

    Optional study activities:
    - $100 for each optional MRI ($200 total)

If for any reason the subject stops the study, they will be compensated for only the visits that theyhave completed.

Payment will be made in the form of a check mailed to the subject's home address. They should receive the check within 2-3 weeks from the date of the study visit or study completion. Their social security number (SSN) will be required to process the payments.

5.3 Discontinuation

5.3.1 Study Discontinuation

A study subject will be discontinued from participation in the study if:

1. Any clinical adverse event (AE), concurrent illness, or other medical condition or situation occurs such that continued participation in the study would not be in the best interest of the subject.
2. The subject meets any exclusion criteria (either newly developed or not previously recognized) and/or fails to continue to meet inclusion criteria which the study physician deems to be a risk to continued participation.

5.3.2 Discontinuation of Study Intervention

Individual subjects will be allowed to continue in the study, but administration of the second immunization will be withheld if, after the first injection, any of the following criteria are met:

1. Any of the following serious adverse events attributable to BCG:
    - Life-threatening adverse event
    - Permanent or severe disability
    - Important BCG-specific medical events that do not result in death, are not life-threatening, and/or do not require hospitalization will be considered as serious if, based on appropriate medical judgment, they jeopardize the participant and would require medical or surgical intervention to prevent a serious adverse event.
2. Presentations of symptoms of disseminated BCG infection including persistent fevers, night sweats, weight loss. These symptoms may not occur within the timeline of the current study (they usually present 4 months to 2 years after vaccination), however should such symptoms occur prior to the second immunization, the study vaccine would be stopped.
3. The occurrence of any serious local skin reactions (≥ grade 3 ulceration, abscess formation, skin necrosis at the site of injection, or the development of suppurative lymphadenitis) as characterized by the standard FDA Toxicity grading scale (https://www.fda.gov/regulatory-information/search-fda-guidance-documents/toxicity-grading-scale-healthy-adult-and-adolescent-volunteers-enrolled-preventive-vaccine-clinical).

5.3.3 Voluntary Withdrawals

A participant may choose to discontinue participation in the study at any time. Participants may be asked to complete an Early Termination Visit when they withdraw consent, i.e. withdrawing consent for participation in future study procedures.

5.3.4 Lost to follow-up

The following actions must be taken in relation to a subject who fails to attend the clinic for a required study visit:

- The site must attempt to contact the subject and re-schedule the missed visit as soon as possible.
- The site must counsel the subject on the importance of maintaining the assigned visit schedule and ascertain whether or not the subject wishes to and/or should continue in the study.
- In cases where the subject is deemed ‘lost to follow up’, the Investigator or designee must make every effort to regain contact with the subject (where possible, 3 telephone calls and, if necessary an email with read receipt). These contact attempts should be documented in the subject’s study record.
- Should the subject continue to be unreachable, only then will he/she be considered to have

withdrawn from the study with a primary reason of "Lost to Follow-up".

5.4 Early Termination Visit (ET)

Subjects who decide to withdraw from the study at any point after receiving the initial BCG injection may be invited to come in for an Early Termination Visit.

5.5 Termination of Study

This study may be prematurely terminated if, in the opinion of the investigator, there is sufficient reasonable cause.

Circumstances that may warrant termination include, but are not limited to:

1. Determination of unexpected, significant, or unacceptable risk to subjects;
2. Unsatisfactory enrollment;
3. Insufficient adherence to protocol requirements;
4. Data that are not sufficiently complete and/or evaluable;
5. If any grade 3 "severe" or higher adverse event occurs in seven or more participants, or if any participants have severe systemic reactions to the antigenicity of the vaccination with respiratory problems, the study protocol will be paused for review. The FDA, and all other relevant oversight bodies will be notified in a timely fashion if a batch of BCG is problematic.
6. If during the study any subject develops a systemic BCG infection in the absence of an underlying immunocompromised state, the study will be stopped for a study of this side effect.

If the study is prematurely terminated or suspended, the investigators will promptly inform the institution and regulatory authorities of the termination or suspension and the reason(s) for the termination or suspension. The IRB will also be informed promptly and provided the reason(s) for the termination or suspension by the investigator, as specified by applicable regulatory requirement(s).

6 STUDY PROCEDURES

6.1 Schedule of Events

Activity	Phone Screen	Visit 1 Screen Visit		Visit 2 Baseline Visit	Visit 3	Visit 4	Visit 5	Phone Check 1	Visit 6/ET	Phone Check 2	Phone Check 3
		Day ≥ -45		Week 1	Week 4	Week 12	Week 26	Week 39	Week 52	Week 78	Week 104
		SVa	SVb	Day 1	Day 28 + 14	Day 84 ±5	Day 182 ±14	Day 273 ±5	Day 364 ± 28	Day 546 ± 28	Day 728 ± 28
Informed Consent		x									
Inclusion/ExclusionReview	x	x		x							
Demographics	x	x									
Medical HistoryReview	x	x	x	x	x	x	x	x	x	x	x
Concomitant Medication Review	x	x	x	x	x	x	x	x	x	x	x
Family HistoryReview		x									
Vital Signs, Height,and Weight [1]		x	x	x	x	x	x		x		
Physical & Neurological Exam [2]		x									
Fundoscopy						x			x		
Screening Labs [3]		x									
BCG Vaccination				x	x						
Injection Site Monitoring [4]					x	x	x		x		
LP Safety Labs [5]		x				x			x		
Blood Draw for Biomarkers			x	x	x	x	x		x		
CSF Draw for Eligibility & Biomarkers			x			x			x		
Optional MRI [6]				x					x		
RBANS, NPI-Q, HADS				**x**		x	x		x		
MoCA, FAQ		x				x	x		x		
CDR				x					x		
DCT Clock				x			x		x		
Adverse Event Review		x	x	x	x	x	x	x	x	x	x

[1] Height measured at screening only. Weight measured at screening, week 26, and week 52

[2] Physical and neurological examinations will be performed at screening and at any other time if indicated per clinicianinvestigator judgement

[3] Eligibility lab tests include CBC w/ diff, CMP, TSH, Folate, B12, HIV antibodies, serum immunoglobulins, ESR, CRP, QuantiFERON-TB Gold Test/T-spot TB test, urinalysis, PT/INR

[4] Vaccination site monitoring may include photographs of the injection site if indicated per clinician investigator judgement

[5] LP safety labs include PT/INR and CBC w/ diff

[6] Multi-sequence MRI for structural and functional measures include T1 MP-RAGE, T2 FLAIR, DWI, DTI, BOLD, ASL

6.1.1 RCT roll over participant schedule

Participants enrolled in the original randomized-controlled BCG trial will be invited to roll into the open-label trial at their scheduled Visit 3 using a Consent addendum. After unblinding the randomization schedule, participants will be told which study vaccine they received at their Baseline Visit.

If at Baseline, the participant received BCG, they will proceed in the study along the same schedule outlined in the Schedule of Events, receiving their second BCG vaccine at Visit 3/Day 28.

If at Baseline, the participant received placebo, their Day 28 visit will be become their new Day 1 visit. At this Day 1 visit we will collect vitals and administer the first BCG vaccine. No additional blood or CSF will be collected, nor will any cognitive testing be performed. The participant will receive their second BCG vaccine approximately 1 month after the first BCG vaccine, and continue on the Schedule of Events outlined after the Day 1 visit in Section 6.1. The total time in the study will be extended by 1 month for participants originally assigned to the placebo arm of the randomized controlled trial.

6.2 Study Visits

6.2.1 Screening

6.2.1.1 Telephone Pre-Screening

Subjects will be screened over the phone by study staff to determine eligibility and to ensure that the subject is safe to undergo all study assessments. These procedures include:

- Obtain verbal pre-screening informed consent from subject
- Assess inclusion and exclusion criteria
- Collect brief demographics
- Obtain neurocognitive disease diagnosis history and medical history, including COVID-19 diagnosis and previous exposure risk
- Review and document concomitant medications and therapies

During this call, study procedures will be discussed in detail and the subject will be given an opportunity to ask questions about the study. All other study procedures will take place after signing an IRB-approved consent form in-person. For those that have impaired decision making, the phone screening process will be coordinated with the study partner and will be conducted in the presence of both the study partner and the potential participant.

6.2.1.2 Screening Visits (Visit 1; Day ≥-45)

All subjects who are eligible after a telephone pre-screening will be invited to MGH CNY for Screening that will include 2 separate visits.

The first visit (SVa) will take approximately 2 hours and following procedures will be performed:

- Obtain written informed consent from subject
- Review inclusion and exclusion criteria
- Obtain medical and family history including COVID-19 history, concomitant medications and therapies
- Vital signs, height, and weight
- Obtain demographic information
- Neurocognitive and behavioral assessments: MoCA (version 8.1), CDR, FAQ
- Perform a physical, including inspection for BCG scar on either deltoid
- Perform a neurological examination
- Blood draw for screening labs

- CBC with differential
- Comprehensive metabolic panel (CMP)
- Thyroid stimulating hormone (TSH)
- B12
- Folate
- HIV antibodies
- Tuberculosis (QuantiFERON-TB Gold Test/T-Spot TB Test)
- C-Reactive Protein (CRP)
- Erythrocyte sedimentation rate (ESR)
- Serum immunoglobins (IgA, IgG, IgM)
- PT/INR

- Urinalysis
- Assess and document adverse events (AEs) after subject signs ICF

If the participant is still eligible after the first Screening visit (SVa), they will be invited to come back for a second Screening visit (SVb) which will take approximately 1 hour. The following procedures will be performed:

- Vital signs
- Blood draw for biomarkers
- Fasting CSF draw for screening (if required) and baseline biomarkers
- Assess for changes to medical history, concomitant medications and/or adverse events

6.2.1.3 Screen Failures

Any subject who signs consent will be considered enrolled in the study. If a subject fails screening, *at a minimum,* the following information should be captured and entered into the Electronic Data System (EDC).

- Inclusion/Exclusion Criteria
- Demographics
- Reason for screen failure

6.2.1.4 Re-screening

One re-screen is allowed ≥30 days after the initial screening. All screening procedures will be repeated at the re-screen visit.

6.2.2 **Baseline Visit/Visit 2 (Day 1)**

All subjects who pass screening will return to MGH CNY for the Baseline visit. At this visit, baseline clinical measures will be collected, and the first BCG injection will be administered. The Baseline visit will occur within 45 days of the screening visit. Baseline measures may be collected up to 3 days before the first BCG dose is administered. The date of the first BCG injection will be considered "Day 1". The visit willtake approximately 3-4 hours and will include:

- Vital signs
- Review inclusion and exclusion criteria
- Assess for changes to medical history, concomitant medications and/or adverse events
- Neurocognitive and behavioral assessments: RBANS (form a), NPI-Q, HADS, DCT Clock
- Blood draw for biomarkers
- MRI (optional)
- First BCG administration followed by 30 minutes of monitoring

6.2.3 Visit 3 (Day 28 + 14 days)

Subjects will be asked to return to MGH CNY for a second BCG injection visit 28 (+14) days after thefirst vaccination. This visit will take approximately one hour and will include:

- Vital signs
- Assess any changes to medical history, concomitant medications and/or adverse events
- Blood draw for biomarkers
- Injection site monitoring
- Second BCG dose administered followed by 30 minutes of monitoring

6.2.4 **Visit 4 (Day 84 ± 5 days)**

Subjects will be asked to return to MGH CNY 84 (± 5) days after the first injection. This visit willtake approximately three hours and will include:

- Vital signs
- Assess any changes to medical history, concomitant medications and/or adverse events
- Blood draw for safety
- Blood draw for biomarkers
- Focused Exam: Fundoscopy
- Neurocognitive and behavioral assessments: RBANS (form b), NPI-Q, HADS, MoCA (version 8.2), FAQ
- Blood draw for LP safety labs
- Blood draw for biomarkers
- Fasting CSF draw for biomarkers
- Injection site monitoring

6.2.5 Visit 5 (Day 182 ± 14 days)

Subjects will be asked to return to MGH CNY 182 (± 14) days after the first injection. This visit willtake approximately two hours and will include:

- Vital signs
- Assess any changes to medical history, concomitant medications and/or adverse events
- Blood draw for biomarkers
- Neurocognitive and behavioral assessments: MoCA (version 8.3), FAQ, RBANS (form c),NPI-Q, HADS, DCT Clock
- Injection site monitoring

6.2.6 Phone Check 1 (Day 273 ± 5 days)

Subjects will be asked to complete a check-in phone call 273 (± 5) days after the first injection. This will take approximately fifteen minutes and will include:

- Assess any changes to medical history, concomitant medications and/or adverse events

6.2.7 Visit 6 (Day 364 ± 28 days)

Subjects will be asked to return to MGH CNY for the final in-person visit at Day 364 (± 14 days) to assess study outcomes. This visit will take approximately four hours and will include:

- Vital signs
- Assess any changes to medical history, concomitant medications and/or adverse events
- Neurocognitive and behavioral assessments: FAQ, MoCA (version 8.1), CDR, RBANS (form a), NPI-Q, HADS, DCT Clock
- Blood draw for biomarkers
- Blood draw for LP safety
- Focused Exam: Fundoscopy
- Fasting CSF draw for biomarkers
- MRI (optional)
- Injection site monitoring

6.2.8 **Phone Check 2 & 3 (Day 546 ± 28 days, Day 728 ± 28 days)**

Subjects will be asked to complete a check-in phone call approximately 18 months and 2 years after the first injection to monitor safety. This will take about fifteen minutes and will:

- Collect any changes to medical history, concomitant medications, and/or adverse events

If the subject reports any new adverse events at the Phone Check that may require follow up, they will be referred to their primary care provider for further evaluation.

6.2.9 **Early Termination Visit**

If the subject withdraws from the study before completion of all study visits, they may be invited to return to MGH CNY for a final visit. This visit will take approximately four hours and will include the following measures:

- Vital signs
- Assess any changes to medical history, concomitant medications and/or adverse events
- Neurocognitive and behavioral assessments: FAQ, MoCA (version 8.1), CDR, RBANS (form a), NPI-Q, HADS, DCT Clock
- Blood draw for biomarkers
- Blood draw for LP safety

- Fasting CSF draw for biomarkers
- MRI (optional)
- Injection site monitoring

The option to decline any of the early termination procedures or the entire visit will be given to all participants withdrawing from the study.

6.2.10 **Protocol Deviations**

A protocol deviation is any noncompliance with the current clinical trial protocol. The noncompliance may be on the part of the subject, the PI or Co-Is, or the study staff. As a result of deviations, corrective actions will be developed by the PI and implemented promptly. All deviations from the protocol must be addressed in the subject's documents. Protocol deviations will be sent to the IRB per their guidelines and entered in the Protocol Deviations Log.

6.2.11 **Missed Visits and Procedures**

Missed visits and any procedures not performed (not attempted) for reasons other than illness, injury, or progressive disability (i.e.: a subject is physically unable to perform them) will be reported as protocol deviations.
Procedures or visits not performed due to illness, injury, or disability, including procedures that were attempted but failed (i.e.: blood samples unable to be drawn after multiple attempts or weight unable to be obtained due to subject immobility) will not be reported as protocol deviations.

6.3 Clinical Assessments

Assessments will be performed at designated time-points throughout the study for clinical evaluation. In addition to the assessments evaluated below, subjects will provide information on their demographics, past medical and AD history, family history, medication usage, and vaccination history.

6.3.1 **Safety Measures**

6.3.1.1 Vital Signs, Height, Weight

Vital signs, including systolic and diastolic blood pressure, pulse rate (radial artery)/minute, respiratory rate/minute, and temperature will be assessed at all in-person visits. Height will be measured at the screening visit only. Weight will be measured at screening, week 26, and week 52 visits. Verbal weight may be documented for those subjects utilizing a wheelchair.

6.3.1.2 Clinical Laboratory Assessments

Study participants will be asked to provide the volumes of blood and urine detailed in Table 1 for eligibility (at screening) and safety lab analyses. The participant will have their whole blood collected by either a nurse or phlebotomist from a peripheral vein (or finger prick for point of care coagulation test). Blood will be handled, processed, and analyzed in accordance with regulations set forth by the American Society for Clinical Pathology and the College of American Pathologists.

Table 1. Safety Laboratory Tests

Visit	Labs	Volume required
Screening (SV1a)	PT/INR, Complete Blood Count w/ differential, Complete Metabolic Panel, C-Reactive Protein, Erythrocyte Sedimentation Rate, HIV Antibody, QuantiFERON-TB Gold test/T-spot TB test, Folate, B12, Serum immunoglobins (IgG, IgA, IgM), TSH, Urinalysis	33 mL blood, 10 mL urine
Screening (SV1b)	N/A	N/A
Week 1	N/A	N/A
Week 4	N/A	N/A
Week 12	Complete Blood Count w/ differential and PT/INR	6 mL
Week 26	N/A	N/A
Week 52	Complete Blood Count w/ differential and PT/INR	6 mL

All subjects will have safety laboratory tests at the designated visits outlined in the protocol. These samples will be analyzed at MGH Core Laboratory. The SI may order additional testing, if thought to be necessary, to further assess an adverse event (AE), or if there is any suspicion that a subject may be pregnant, throughout the course of the study.

Tests for Complete Blood Count (CBC), Comprehensive Metabolic Panel (CMP), and International Normalized Ratio (INR) may be performed with CLIA-waived point of care devices: Sysmex XW-100 CBC Analyzer, Piccolo Xpress Chemistry Analyzer, and the Roche CoaguChek XS Plus PT/INR Analyzer, respectively.

6.3.2 **Physical and Neurological Examination**

A physical and neurological examination will be performed at the Screening visit. The following systems will be examined: general appearance, head, eyes, ears, nose, throat, neck, chest, heart, abdomen, extremities, edema, peripheral vascular, skin and appendages, musculoskeletal, central nervous system and back. A physical and/or neurological exam may be repeated at any visit if indicated per clinician judgement.

A focused fundoscopy examination will be repeated before the second and third lumbar puncture**s** to assess for increased intracranial pressure.

6.3.3 **Phlebotomy for Research: Target Engagement, Cytokine Levels and Biomarker Analysis**

Subjects will provide additional blood samples for biomarker analysis at all in person visits. Target engagement will determine whether the subject's immune system has responded to BCG. In general, whole blood will be either collected and utilized for biochemical / metabolic assays, or spun in EDTA-containing tubes with plasma being frozen for cytokine analysis of IIR and / or AD biomarkers. Remaining plasma may be used for other biochemical analyses. Peripheral blood mononuclear cells (PBMCs) will be isolated from the cell pellets, some of which will be frozen for later culturing and cell-stimulation studies. Other PBMCs will be further isolated into subpopulations of cells and frozen for epigenetic analyses (e.g. ATACseq) and possibly subject to other immunostaining or quantitative biochemical assays.

In addition to these analyses, blood will also be collected for routine blood tests that will be used for research purposes including Complete Blood Count with differential (CBC w/ diff), C-Reactive Protein (CRP), and Erythrocyte Sedimentation Rate (ESR). CRP and ESR tests will be performed at a central laboratory. CBC w/ diff may be performed either on-site using a CLIA-waived point-of-care device (Sysmex XW-100 CBC Analyzer) or at a central laboratory. Blood volumes collected at each visit are detailed in Table 2.

Table 2. Blood Collection for Research

Visit	Blood volume required for biomarker analyses (mL)	Volume required for routine tests(mL)	TOTAL (mL)
Screening (SV1a)	0	0	0
Screening (SV1b)	75	0	75
Week 1	50	0	50
Week 4	75	3 for CBC w/ diff	78
Week 12	125	9 for CBC w/ diff, ESR, CRP	134
Week 26	125	9 for CBC w/ diff, ESR, CRP	134
Week 52	125	6 for ESR, CRP	131
Total	575	27	602

Blood processing will occur on site at MGH. Blood samples will be de-identified, codified, and stored on-site. Blood samples will be stored for research until they are used, damaged, or otherwise unfit for analysis. Some biomarker analyses may be done at Harvard-affiliated core labs.

Samples drawn for biomarker research may also be used for genome analyses in the future. Subjects will not receive the results of these studies if they are done and genetic research results will not be placed in the subjects' medical records.

6.3.3.1 Sending Blood Samples to Research Collaborators Outside Mass General Brigham

Blood samples may be sent to Merck & Co. and Active Motif, Inc. for analyses. Samples may include isolated PBMCs or their derivatives. Samples will be de-identified with a study code and visit date.

6.3.4 **Lumbar Puncture for Immune and Biomarker Analysis**

Lumbar punctures (LP) will occur at Screening, Week 12, and Week 52. Lumbar punctures will be performed after a minimum of an 8 hour fast. The subject will be positioned seated or lying on their side on the examination table. Standard protocols will be used employing palpation to identify the L3-4, L4-5 or L5-S1 vertebral interspaces, sterile conditions, local lidocaine anesthesia, and use of standard of care spinal needles. Clinical judgement will be used to determine the optimal spinal needle type/size for each participant. Lumbar punctures will be performed by qualified, experienced practitioners. Approximately 25 mL of CSF at each visit will be collected. A small portion of this volume will be used for analysis of cell count with differential, total protein, and glucose. The remainder will be spun and cells isolated from supernatant. Supernatant will be frozen for later immunoassays. Cells will be frozen for later biochemical analyses. Subjects will also complete a brief REDCap survey after the LP in which they will answer questions about the tolerability of the procedure. After the LP is complete, subjects will be given time to eat breakfast or have a snack before continuing with the visit.

If a subject has difficult back anatomy or is particularly overweight, and the physician or nurse practitioner does not feel comfortable performing the LP because they are not able to locate the proper lumbar landmarks, the subject will be scheduled to undergo the LP under fluoroscopy at MGH Interventional Radiology. Fluoroscopy time will vary person to person. The average radiation dose is 0.18 millisieverts (mSv), or the equivalent of 21 days of natural background radiation, for each lumbar puncture done under fluoroscopy.

CSF samples will be de-identified, codified, and stored on-site at the MADRC/MIND biorepository at the Arnold Lab. CSF samples will be stored for research until they are used, damaged, or otherwise unfit for analysis. Some biomarker analyses may be done at Harvard-affiliated core labs.

6.3.4.1 Sending CSF Samples to Research Collaborators Outside Mass General Brigham

CSF cell samples may be sent to Merck & Co. and Active Motif, Inc. for analyses. Samples will be de-identified with a study code and visit date.

6.3.5 Injection Site Monitoring

Prior to the first injection, both deltoids will be inspected to confirm there is no evidence of previous BCG vaccination and no obvious symptoms that would contraindicate vaccine injection. At the Week 4 visit, prior to the second injection, the vaccination site will be inspected for any signs of local infection. At Week 12, Week 26, and Week 52 and if indicated by subject-reported AEs, the site(s) of vaccination will be examined, and any visible reactivity will be recorded. In addition, photographs of the injection site may be taken using an MGB-approved and encrypted device. Photographs will be stored on the MGB Dropbox.

6.3.6 Magnetic Resonance Imaging (MRI)

Based on investigator discretion and cognitive ability, up to 12 participants over 60 years old may be invited to participate in an optional 3T structural and functional MRI at Week 1 and Week 52 to assess the effect of the study vaccine on regional brain volumes, cerebral perfusion, and structural and functional brain connectivity. Each scan will include high resolution T1- weighted multi-echo MPRAGE for volumetric analysis, task-free blood oxygen level dependent (BOLD) sequenceto measure resting neural connectivity, diffusion tensor imaging (DTI) to quantify white matter structural connectivity, T2-weighted fluid attenuated inversion recovery (FLAIR) sequence to identify brain lesions, includingcortical and periventricular lesions, and susceptibility-weighted imaging (SWI) to identify micro-hemorrhages andvenous connectivity in lesions, and pseudo-continuous arterial spin labeling (pCASL) to quantify regional cerebral blood flow (a marker of resting cerebral perfusion).

6.4 Neurocognitive Assessments

6.4.1 Montreal Cognitive Assessment (MoCA)

The Montreal Cognitive Assessment (MoCA) is a commonly utilized questionnaire in clinical trials and research settings to measure levels of cognitive impairment.[50] The MoCA measures five areas of cognitive function: orientation, visuospatial, attention and calculation, recall, and language. The MoCA (version 8.1) will take approximately 15 minutes to complete. The MoCA will be administered at the Screening visit (version 8.1) to determine subject eligibility (MoCA $\geq$8) and repeated at Week 12 (version 8.2), Week 26 (version 8.3), and Week 52 (version 8.1).

6.4.2 Repeatable Battery for the Assessment of Neuropsychological Status Update (RBANS)

The RBANS[51] is a commonly used 25-minute, standardized neurocognitive battery. The RBANS measures five neurocognitive domains, with twelve subtests measuring cognitive decline or improvement with immediate memory (List Learning and Story Memory), visuospatial/constructional (Figure Copy and Line Orientation), language (Picture naming and Semantic Fluency), attention (Digit Span and Coding), and delayed memory (List Recall, List Recognition, Story Memory, and Figure Recall). The RBANS has been shown to be effective at both detecting and characterizing forms of dementia. The Delayed Memory domain has been shown to be particularly sensitive to discriminating mild cognitive impairment (MCI) due to Alzheimer's disease from controls, and also is predictive of cerebral amyloid burden. The RBANS will be administered at Week 1 (form a), Week 12 (form b), Week 26 (form c), and Week 52 (form a).

6.4.3 Clinical Dementia Rating (CDR) Scale

The CDR is a 5-point scale used to characterize six domains of cognitive and functional performance applicable to Alzheimer disease and related dementias using a 30-minute semi-structured interview: Memory, Orientation, Judgment & Problem Solving, Community Affairs, Home & Hobbies, and Personal Care. The necessary information to make each rating is obtained through a semi-structured interview of the patient and an informant (study partner). The CDR table provides descriptive anchors that guide the clinician in making appropriate ratings based on interview data and clinical judgment. In addition to ratings for each domain, an overall CDR score may be calculated using an algorithm. This score is useful for characterizing and tracking a patient's level of impairment/dementia: 0 = Normal; 0.5 = Very Mild Dementia; 1 = Mild Dementia; 2 = Moderate Dementia; 3 = Severe Dementia. The CDR scores will be converted into a 'sum of boxes (CDR-SOB)' score, which is more sensitive in distinguishing between individuals with milder declines in functioning as it increases the range of possible scores [52]. The CDR will be administered at Screening to determine subject eligibility (CDR between 0.5-2 inclusive) and will be repeated at Week 52. The portion of the CDR completed with the study partner may be completed over the phone at Week 52.

6.4.4 DCT Clock

DCT clock, a digitized version of the standard pen and paper neuropsychological clock drawing test, is a non-invasive, computer-based cognitive assay. The DCT clock application is preloaded onto the iPad. The test involves participants drawing two clock faces on an iPad, with a digital pen that precisely tracks and records drawing behavior. The positional data generated during this assessment is then analyzed by DCT's proprietary algorithms that evaluate hundreds of features captured in the pen stroke information. By comparing test results to normative data, the system then determines whether the test was within normal limits and provides a detailed breakdown of performance on the various cognitive tasks evaluated during the test.

6.5 Neuropsychiatric Assessments

6.5.1 Neuropsychiatric Inventory Questionnaire (NPI-Q)

The NPI-Q[53] will rate symptoms in 12 sub-domains of behavioral functioning including: hallucinations, delusions, agitation, dysphoria, anxiety, euphoria, apathy, disinhibition, irritability, aberrant motor activity, eating abnormalities, and night-time behavioral alternations. The NPI-Q is administered to an informant (study partner) and will be done at Week 1, Week 12, Week 26, and Week 52. The NPIQ may be completed over the phone at Week 12, 26, and 52.

6.5.2 Functional Activities Questionnaire (FAQ)

The FAQ[12,54]is a brief rating scale used to determine a subjects' level of functional independence when performinga range of instrumental activities of daily living (IADLs), with repeat assessments useful for monitoring performance in these areas over time. The FAQ total score (ranging from 0-30) reflects the sum of ordinal ratings (0 = fully independent, 1 = has difficulty but does by self, 2 = requires assistance, and 3 = dependent) across ten items assessing a variety of functional activities (i.e., preparing a balanced meal, financial management skills, and shopping), with higher scores indicating increasing levels of dependence. For activities not normally undertaken by a person, a score of 1 is assigned if the subject would be unable to complete the task if required, or a score of 0 is assigned if the subject believes they could successfully carry out the task if needed. Overall, the FAQ is a sensitive marker of functional impairment among individuals with varying dementia severity and has been shown to differentiate mild cognitive impairment from early dementia due to Alzheimer's Disease with 80% sensitivity and 87% specificity. The FAQ demonstrates high reliability (exceeding 0.90), takes about 5 minutes to complete, and requires limited rater training to administer. The FAQ is administered to an informant (study partner) and will be done at Screening, Week 12, Week 26, and Week 52.

6.5.3 Hospital Anxiety and Depression Scale (HADS)

The HADS (Hospital Anxiety and Depression Scale) aims to measure symptoms of anxiety and depression. HADS is a 14-item scale with seven items each for anxiety and depression subscales. Scoring for each item ranges from zero to three. A subscale score >8 denotes anxiety or depression. HADS will be administered at Week 1, Week 12, Week 26, and Week 52.

7 STUDY INTERVENTION ADMINISTERED

Table 3. Summary of Investigational Products

	Investigational Products	
Product Name:	Bacillus Calmette-Guérin (BCG) vaccine [Tokyo BCG (JBL, Tokyo, Japan)]	Diluent (0.9% sodium chloride for injection)
Dosage Form:	For intradermal injection	For intradermal injection
Unit Dose	0.36-3.9 x 10^6 colony forming units(CFU)/injection of 0.1ml	0.1 mL
Route of Administration	Intradermal injection	Intradermal injection
Physical Description	sterile, freeze-dried	BCG diluent
Manufacturer	Japan BCG Laboratory (JBL)	Supplied by MGH clinical trials pharmacy (manufacturer varies)

7.1 Study Drug

Japan BCG is an approved drug in Japan and has been sanctioned by UNICEF and WHO as a standard vaccine for the prevention of tuberculosis. BCG has been administered for this purpose for over 100 years on a global basis, with typical distributions of over 50,000,000 doses to newborns per year. The vaccine has also been approved and utilized for treatment of bladder cancer for the past 30 years.

JBL is the manufacturer of BCG products produced from the BCG strain of Tokyo 172-1 and Dr Faustman and MGH oversee and regulate this manufacturing. This protocol will be reviewed by the Japan BCG Laboratory ethics committee prior to commencementof study activities. Drug-related contracting will occur through Dr. Faustman who maintains the Master Drug File,coordinates various IND filing around this vaccine and will interface with regulatory affairs management.

The study drugs (BCG vaccine and diluent) are manufactured by Japan BCG Laboratory and supplied by the MGH Clinical Trials Pharmacy (manufacturers vary), respectively, and will be managed and stored by the study pharmacist in the Faustman lab according to the directions provided by the manufacturer in the approved product labeling. The same study pharmacist and study drug have been used for the past 6 years in Dr. Faustman's protocol #2013P002633 in addition to the pilot protocol #2020P002042.

Japan BCG is a freeze-dried preparation made from the Tokyo 172 strain of *Mycobacterium bovis* Bacillus Calmette and Guérin (BCG). Japan BCG is an attenuated BCG product derived from the original attenuated BCG generated by Albert Calmette and Camille Guerin at the Pasteur Institute.

7.2 Study Drug Packaging and Labeling

The Japan BCG drug product is a sterile, freeze-dried preparation of BCG.

This drug is stored at 2-8ºC.

7.3 Study Drug Preparation

The BCG vaccine is supplied as a 10-dose ampoule. For reconstitution, 1.0 ml of sterile saline is added to the 10-dose ampoule. Each 0.1ml dose is drawn by syringe from the ampoule following reconstitution and administered intradermally. Once reconstituted, the pharmacy provides the study clinic with the pre-filled syringe in a covered surgical tray to prevent light exposure. If the prepared syringe cannot be immediately administered, it will be temporarily stored at 4ºC until administration (< 1 hour from reconstitution). The BCG vaccine will be prepared by the study pharmacist at MGH.

Each ampoule may be used for a maximum of two study participants in this study. The ampoule will be reconstituted, and two syringes may be drawn at the same time. As above, the prepared syringes will be covered and stored at 4ºC until administration within 1 hour of reconstitution. The remaining unused volume in the ampoule will not be used for additional patients in this trial.

7.4 Dosage and Administration

All subjects will be consented by a study clinician before any of the following procedures are done. The skin site (deltoid region, alternating between first and second dose) is first cleansed with an alcohol or acetone sponge and allowed to dry thoroughly. Next, 0.36-3.9 x 10^6 colony forming units (CFU), reconstituted by the study pharmacist in 0.9% saline diluent within 1 hour, will be administered intradermally via a 15mm, 26-gauge needle.

Dose selection is based on published data illustrating that two doses of BCG in autoimmune type 1 diabetes confer the immune regulatory/inflammation modulation properties that are of interest in the present study. Specifically, administration of two doses in type 1 diabetes is associated with short term changes in biomarkers with 22 weeks of observations.[36] Moreover, two doses of BCG resulted in a clinical correction of HbA1c in type 1 diabetes with stable correction of disease process for 8 years.[37]

7.5 Study Drug Accountability

Vaccine accountability will be maintained by the study pharmacist.

7.6 Study Drug Handling and Disposal

Vaccine handling and disposal will be managed by the study pharmacist.

7.7 Contraindications and Warnings

7.7.1 Prohibited Medications

Treatment with the following medications during the study is prohibited:

- Metformin
- Aspirin > 160 mg/day
- Chronic daily NSAIDS
- Immunosuppressants including calcineurin inhibitors, corticosteroids, or biological or cytotoxic immunosuppressants (e.g. imuran, methotrexate, cyclosporine, etanercept, infliximab)
- Antibiotics within 14 days of immunizations
- Administration of live vaccine within 30 days of screening visit or BCG immunizations
- Administration of non-live vaccine within 14 days of screening visit or BCG immunizations

7.7.2 **Warnings**

BCG contains live, attenuated mycobacteria, and carries risk for adverse consequences particularly in immunocompromised individuals for whom the attenuated strain could become pathogenic as described below (section 9.1, BCG vaccine risks).

8 BIOSTATISTICAL ANALYSIS

8.1 Sample Size

A sample size of approximately 15 subjects was chosen based on feasibility and is not based on statistical considerations. The primary objective of this early-phase trial is to measure safety and target engagement. While there is no 'best practice' method of determining target engagement of the BCG vaccine, the method that best takes advantage of the collected biomaterials is the *in-vitro* IFN-gamma release assay. PBMCs are stimulated and after several days the culture media is tested for presence of the IFN-gamma cytokine. In a previously published study, it was demonstrated a significant IFN-gamma release difference associated with BCG treatment in n = 14 subjects [55].

8.2 Safety Endpoints

The primary safety endpoint will be the incidence of treatment emergent Grade II-IV adverse events.
Other safety endpoints are:

- Incidence and severity of treatment emergent adverse events (AEs)
- Clinical laboratory tests
- Vital signs
- Physical examinations
- Use of concomitant medications for treatment of AEs

All concomitant medications will be tabulated according to drug class and preferred term using the WHO dictionary. The safety data will be summarized by treatment group. Treatment AEs will be coded and graded using MedDRA grading criteria. The treatment groups will be compared with respect to occurrence of each adverse event and incidence of Grade II/IV adverse events. Withdrawal, abnormal laboratory tests, vital signs and use of concomitant medications used for treatment of AEs will be assessed to characterize the safety profile of BCG. Descriptive statistics denoting the changes from baseline to the final assessment visit with respect to key laboratory parameters and vital signs will also be provided.

Adverse events (AE) occurring after the start of study drug dosing at Baseline will be summarized descriptively. All AEs will be coded according to system organ class (SOC) and preferred term (PT) using a Medical Dictionary for Regulatory Activities (MedDRA) dictionary. Summary tables showing the number of subjects and percent within each category will be generated for each of the following types of adverse events and its relationship to study treatment (related to study treatment):

- All events
- Serious events
- Deaths
- Events leading to withdrawal
- Severe events

Treatment AEs will be coded and graded using MedDRA grading criteria. and compared with respect to occurrence of each adverse event and incidence of Grade II/IV adverse events. Total number of adverse events will be compared between groups using Fisher's exact test. Any treatment AE still present upon completion of treatment (including early discontinuation) should be monitored until resolution or until the AE is declared a chronic condition. AEs will be monitored until they become chronic or have completely resolved.

Laboratory parameters will be summarized by visit; descriptive statistics denoting the changes from baseline to the final assessment visit with respect to key laboratory parameters and vital signs will also be provided. Frequencies of high and low values with respect to the normal range will be displayed, as will shift tables comparing each treatment visit and Baseline visit by time point.

Vital signs will be summarized across groups by visit using descriptive statistics, and at each outcome visit and at end of study. Physical examination findings and number of subjects will be summarized as the count and percentage of subjects by eCRF pre-defined categories at last visit. Change from baseline at last visit will be summarized in a shift table comparing baseline and last visit results. Concomitant medications will be summarized, drug class and preferred term.

8.3 Biofluid Biomarker, Clinical and Neuroimaging Endpoints

The change in the following assessments from Screening and/or Baseline to each post-baseline visit as described below based on the Schedule of Activities after BCG vaccination:

- Biochemical Biomarkers in Blood (Screening/Baseline, Weeks 4, 12, 26, 52) and CSF (Screening/Baseline, Weeks 12, 52) : a) Target engagement (e.g., PPD-induced PBMC cytokine release); b) Pharmacodynamic response (e.g., circulating cytokines in blood and CSF); c) AD pathophysiology (e.g., amyloid-b, tau and neurofilament light in CSF and blood); d) PBMC stimulation with AD-relevant immunogens (e.g., amyloid-b); e) Other novel biochemical biomarkers
- Clinical Neurocognitive and Behavioral Measures (Baseline, Week 12, 26 and 52): MoCA, RBANS, CDR-SB, NPI-Q, HADS, DCT Clock and FAQ
- Multi-Sequence Structural and Functional MRI (Baseline, Week 52)

Mixed-effects Model for Repeated Measures models will be used for biofluid and clinical endpoints to calculate differences at each post-baseline visit in which the biomarker is collected using baseline assessment as a covariate. Observed Case analysis will be used for differences in change of MRI measures over 52 weeks.

9 RISKS AND DISCOMFORTS

9.1 BCG Vaccine Risks

The BCG injection is associated with minimal, brief discomfort. The following risks may also be associated with BCG vaccination per the manufacturer product insert.

Although BCG vaccination often causes local reactions, serious or long-term complications are rare. Reactions that can be expected after vaccination include moderate axillary or cervical lymphadenopathy and induration and subsequent pustule formation at the injection site; these reactions can persist for as long as 3 months after vaccination. The intensity and duration of the local reaction depends on the depth of penetration of the puncture device and individual variations in patients' tissue reactions. Slight tenderness at the puncture site may be encountered as well as some itching. The initial skin lesions usually appear within 10–14 days and consist of small red papules at the site. The papules reach maximum diameter (about 3 mm) after 4 to 6 weeks, after which they may scale and then slowly subside. More serious local reactions include ulceration at the vaccination site, regional suppurative lymphadenitis with draining sinuses, and caseous lesions or purulent draining at the puncture site. These manifestations might occur up to 5 months after vaccination and could persist for several weeks.

Acute, localized irritative toxicities of BCG may be accompanied by systemic manifestations, consistent with a "flu-like" syndrome. Systemic adverse effects of 1–2 days' duration such as fever, anorexia, myalgia, and neuralgia, often reflect hypersensitivity reactions. However, symptoms such as fever of 103 °F or greater, or acute localized inflammation persisting longer than 2–3 days suggest active infections, and evaluation for serious infectious complication should be considered.

Rare adverse events (mostly suppurative adenopathy) have been reported in Europe and other locales, mostly in the setting of mistakenly subcutaneous, rather than intradermal vaccination. The frequency of more severe cutaneous reactions, such as an ulcer >1 cm, axillary adenopathy which may be suppurative, or other localized skin reactions, is approximately 0.02% for first vaccinations and 0.04% for second vaccinations. Suppurative adenopathy is not treated but observed.

The most serious complication of BCG vaccination is disseminated BCG infection. The most frequent disseminated infection is BCG osteomyelitis (0.01 to 43 cases per million doses of vaccine administered) which usually occurs 4 months to 2 years after vaccination. Fatal disseminated BCG infection has occurred at a rate of 0.06–1.56 cases per million doses; these deaths occurred primarily among immunocompromised persons (tested for and excluded from this study).

9.2 Lumbar puncture

Pain may occur during the procedure. This is usually temporary, confined to the lower back, and minimized with the cutaneous and soft tissue administration of 1% lidocaine as a local anesthetic. Short-lived LP associated headaches occur in <1%-36% of subjects with an incidence that decreases with age. Less commonly, a persistent low-pressure headache may develop as a result of a post-LP CSF leakage. Potentially more serious, but very rare risks, include infection, damage to radicular nerves and bleeding into the lumbar CSF space. The risk of these procedure related complications is much less than 1%. There is also a very rare risk of an allergic reaction to the lidocaine.

9.3 Magnetic Resonance Imaging (MRI)

MRI is a safe procedure for participants who do not have metal implants or other contraindications. Individuals who have electrically, magnetically, or mechanically-activated implants (such as heart pacemakers) or those who have clips on blood vessels in their brain will not be allowed to participate in the study due to risks associated with MRI scanning. The MRI will be operated in a manner accepted by the Food and Drug Administration (FDA). The protocol requires participants to remain still within a relatively confined space during the scanning session, and the scanner makes loud knocking and beeping sounds as it takes images. While participants with severe claustrophobia are excluded from the study, some participants may find the physical confinement or noise uncomfortable. Every effort will be made to enhance each subject's comfort level, and participants will be given earplugs to reduce discomfort due to noise. If a subject notices any discomfort while in the MRI scanner, they should notify the administrator immediately. If the discomfort cannot be stopped, the scanning session will be stopped. The MRI has the potential, during normal routine use, to cause localized warming of the skin and underlying tissues. Participants should immediately inform the study staff if they experience discomfort due to warming of the skin and the procedure will be stopped.

9.4 Phlebotomy

The risks associated with having blood drawn include bruising and local discomfort. Rarely an infection may occur at this site, and if an infection does occur it will be assessed and treated by the study physician.

9.5 Neurocognitive testing

The neurocognitive tests that will be administered to assess mental performance may be stressful and potentially cause anxiety, fatigue, and frustration. In our prior experience with similar protocols, risks have occurred infrequently and very few subjects have terminated testing. However, testing will be discontinued immediately upon any request by the subject to do so.

9.6 Neuropsychiatric and Functional Questionnaires

Questionnaires administered during the protocol may cause participants to feel sad or upset about their diagnosis and daily functioning or how it affects their quality of life. Study staff is experienced with such evaluations and sensitive to these issues. Any question can be omitted per the subject's request.

9.7 Radiation

In general, exposure to ionizing radiation may have health risks. Participants who require fluoroscopy-guided LP will be exposed to a maximum of approximately 0.18 millisieverts (mSv) for each LP. This is the same amount of radiation as a person would normally get in 21 days from natural background radiation and is comparable to the dose from a single chest x-ray.

9.8 Genetic Research Risks

The study includes the potential to generate genetic data from patient samples. Genetic information that results from this study does not have medical or treatment importance at this time. Genetic information obtained in this study will not be placed in the medical record. Taking part in a genetic study may also have a negative impact on family or other relationships. We will inform subjects that there may be new technologies that may be developed in the future that may allow the linking of genetic or medical information back to subjects.

To maximize confidentiality, all samples and the information associated with the samples will be coded to prevent the exposure of the participant's information and identity. In addition, sample information is stored in one secured database while genetic data is stored in an independent secured database.

9.9 Other Risks

Reviewing health-related information might be stressful or make the participant feel uncomfortable. Participants do not have to answer any questions they do not want to. In addition, there may be incidental medical findings as a result of the clinical examinations. Standard lab tests completed through MGH Core Lab will be placed in subjects' medical records but no additional research results will be placed in their medical records. Coded, de-identified data/samples may be provided to authorized federal data repositories for broad-sharing with approved researchers. As with all database systems, there is a slight risk that there could be a breach in the security of these data banks resulting in the access of information. Safeguards are in place to minimize privacy risks.

10 POTENTIAL BENEFITS

The aim of this trial is to further understand the effects of BCG on IIR and AD biofluid and MRI biomarkers and neurocognitive/behavioral functioning over one year. We do not know what the potential benefits may be to participants who choose to participate in this study. There may be no benefit at all. This trial is assessing multiple biomarkers in concert with clinical outcomes, which will provide a detailed understanding of the effect of BCG and provide a well-curated data set for the Alzheimer's research community to improve our understanding of the disease with an eye towards better treatments and prevention.

11 MONITORING AND QUALITY ASSURANCE

11.1 Independent Monitoring of Source Data

The PI will ultimately be responsible for the validity and integrity of the data collected at the MGH site, and for ensuring that the study is conducted in accordance with the IRB-approved protocol. After data is collected and recorded on forms, the study coordinator may input the data into the Partners approved StudyTRAX EDC within 7 days of the study visit. Entries will be reviewed for accuracy and completeness by a second study coordinator. Finally, the study team will conduct quarterly reviews to check that data in StudyTRAX accurately reflects the data collected on the original data capture forms and the PI will maintain oversight of this process. The research team (PI, Co-I, research coordinators) will subsequently meet to discuss the results of this review, as well as case report forms and source documentation.

All electronic documentation will be stored on password-protected devices. Paper forms will be stored in locked cabinets located in secured areas.

11.2 Safety Monitoring

The adverse event (AE) definitions and reporting procedures provided in this protocol comply with all applicable United States Food and Drug Administration (FDA) regulations and International Conference on Harmonization (ICH) guidelines. The PI will carefully monitor each subject throughout the study for possible adverse events. All AEs will be documented on CRFs designed specifically for this purpose. It will be important to report all AEs, whether serious or non-serious.

11.3 Adverse Event Reporting Guidelines

11.3.1 Adverse Event

An adverse event (AE) is any unfavorable and unintended sign (including a clinically significant abnormal laboratory finding, for example), symptom, or disease temporally associated with a study, use of a drug product or device, whether or not considered related to the drug product or device.

Examples of adverse events include: new conditions, worsening of pre-existing conditions, clinically significant abnormal physical examination signs (i.e. skin rash, peripheral edema, etc.), or clinically significant abnormal test results (i.e. lab values or vital signs), with the exception of outcome measure results, which are not being recorded as adverse events in this trial (they are being collected, but analyzed separately). The BCG vaccine causes a circular and contained inflammatory response with scab followed by scar formation. For this study, this expected BCG vaccine site reaction is not considered an adverse event. Stable chronic conditions (e.g. arthritis) that are present prior to the start of the study and do not worsen during the trial are NOT considered adverse events. Chronic conditions that occur more frequently (for intermittent conditions) or with greater severity are considered as worsened and therefore would be recorded as adverse events. Adverse events are generally detected in two ways:

- Clinical ➔ symptoms reported by the subject or signs detected on examination.
- Ancillary Tests ➔ abnormalities of vital signs, laboratory tests, and other diagnostic procedures (other than the outcome measures, the results of which are not being captured as AEs).

If discernible at the time of completing the AE log, a specific disease or syndrome rather than individual associated signs and symptoms should be identified by the PI and recorded on the AE log. However, if an observed or reported sign, symptom, or clinically significant laboratory anomaly is not considered by the PI to be a component of a specific disease or syndrome, then it should be recorded as a separate AE on the AE log. Clinically significant laboratory abnormalities, such as those that require intervention, are those that are identified as such by the PI.

Participants will be monitored for adverse events from the time they sign consent until completion of their participation in the study (defined as death, consent withdrawal, loss to follow up, early study termination for other reasons, or following completion of the entire study). An unexpected adverse event is any adverse event, the specificity or severity of which is not consistent with the current product insert.

The study procedures and the well-being of all participants will be monitored closely by the Principal Investigator, Steven Arnold MD, and the Co-Investigators Alison McManus DNP, FNP-BC, and Marc Weinberg M.D., Ph.D. Throughout the course of the study, constant feedback with the subject is maintained in order to assess comfort and safety and to minimize risks throughout the procedure. The above investigators will be responsible for determining if a subject should be removed from the study. Criteria for removal include the following: 1) if a subject is unwilling or unable to participate in study procedures 2) if the subject refuses to participate and consent, 3) if the subject acquires a medical condition that prohibits further participation, 4) if in the opinion of the MGH principal investigator, Dr. Steven Arnold, it is decided that it is not in the subject's best interest to continue participation.

Unanticipated problems including adverse events will be reported to the PHRC as described in the PHRC policy on Unanticipated Problems Involving Risks to Subjects or Others including adverse events.

All data will be managed in compliance with applicable regulatory requirements. The study coordinator, under the supervision of the PI, will collect, transcribe, correct, and transmit the data onto source documents, Case Report Forms (CRFs), and/or other forms used to report, track, and record clinical research data.

11.3.1.1 Serious Adverse Events

All adverse events will be reviewed by the Principal Investigator, Dr. Steven Arnold, and will be reported to Partners IRB and to the Human Research Committee (HRC) in accordance with HRC Guidelines. A serious adverse event (SAE) is defined as an adverse event that meets any of the following criteria:

1. Results in death.
2. Is life threatening: that is, poses an immediate risk of death as the event occurs.
    - This serious criterion applies if the study subject, in the view of the PI, is at immediate risk of death from the AE as it occurs. It does not apply if an AE hypothetically might have caused death if it were more severe.
3. Requires inpatient hospitalization or prolongation of existing hospitalization.
    - Hospitalization for an elective procedure or a routinely scheduled treatment is not an SAE by this criterion because an elective or scheduled "procedure" or a "treatment" is not an untoward medical occurrence.
4. Results in persistent or significant disability or incapacity.
    - This serious criterion applies if the "disability" caused by the reported AE results in a substantial disruption of the subject's ability to carry out normal life functions.
5. Results in congenital anomaly or birth defect in the offspring of the subject.
6. Necessitates medical or surgical intervention to preclude permanent impairment of a body function or permanent damage to a body structure.
7. Important medical events that may not result in death, are not life-threatening, or do not require hospitalization may also be considered SAEs when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include blood dyscrasias or convulsions that do not result in inpatient hospitalization.

An inpatient hospital admission in the absence of a precipitating, treatment-emergent, clinical adverse event may meet criteria for "seriousness" but is not an adverse experience and will therefore not be considered an SAE. An example of this would include a social admission (subject admitted for reasons other than medical, e.g., lives far from the hospital, has no place to sleep).

The PI is responsible for classifying adverse events as serious or non-serious and determining if there is reasonable possibility that the drug was the cause.

11.3.2 **Assessment and Recording of Adverse Events**

The PI will carefully monitor each subject throughout the study for possible AEs. All AEs will be documented on CRFs designed specifically for this purpose. All AEs will be collected and reported in the EDC system.

11.3.3 **Assessment of Adverse Events**

At each visit (including telephone visits), the subject will be asked if they had any problems or symptoms since their last visit in order to determine the occurrence of adverse events. If the subject reports an adverse event, the Investigator will probe further to determine:

- Type of event
- Date of onset and resolution (duration)
- Severity (mild, moderate, severe)
- Seriousness (does the event meet the above definition for an SAE)
- Causality, relation to investigational protocol
- Outcome

11.3.4 **Relatedness of Adverse Event to Investigational Protocol**

1. Not Related:	Concomitant illness, accident, or event with no reasonable association with protocol.
2. Unlikely:	The reaction has little or no temporal sequence from administration of the investigational protocol, and/or a more likely alternative etiology exists.
3. Possibly Related:	The reaction follows a reasonably temporal sequence from administration of the investigational protocol and follows a known response pattern to the suspected investigational protocol; the reaction could have been produced by the investigational protocol or could have been produced by the subject's clinical state or by other modes of therapy administered to the subject.
4. Probably Related:	The reaction follows a reasonably temporal sequence from administration of investigational protocol; is confirmed by discontinuation of the investigational protocol or by re-challenge; and cannot be reasonably explained by the known characteristics of the subject's clinical state.
5. Definitely Related:	The reaction follows a reasonable temporal sequence from administration of investigational protocol; that follows a known or expected response pattern to the investigational protocol; and that is confirmed by improvement on stopping of the investigational protocol, and reappearance of the reaction on repeated exposure.

11.3.5 **Recording of Adverse Events**

All clinical adverse events are recorded in the Adverse Event (AE) Log in the subject's study binder. Entries on the AE Log (and into the EDC) will include the following: name and severity of the event, the date of onset, the date of resolution, relationship to investigational product, action taken, and primary outcome of event.

11.3.6 **Adverse Events and Serious Adverse Events - Reportable Events**

All events that meet the above criteria for Serious Adverse Events must be reported to the IRB within 24 hours of a study investigator being notified of the event.

Any adverse reaction that is serious, unexpected, and associated with the use of the product will be reported to the FDA in an IND safety report no more than 15 calendar days after the PI determines that the suspected adverse reaction qualifies for reporting, or 7 days for unexpected fatal or life-threatening adverse reaction reports.

12 DATA COLLECTION AND MANAGEMENT

12.1 Purpose of EDC

Study data will be collected and managed using Studytrax electronic data capture software. The purpose of Studytrax is to track subject enrollment and flow through the study (e.g. Scheduling of visits), capture data measures, and facilitate the transfer of data into statistical packages for analysis. In compliance with HIPPA regulations, the database security features of Studytrax target multiple levels including the data element (e.g., restricted access to fields), user (e.g., password authentication access), application (e.g., role-based access to features, access audit trails), and hosting services (e.g., firewall, secure sockets layer). These features ensure access control, audit control, data integrity, user authentication, and transmission security. The research project will be set up in Studytrax to ensure exported datasets are de-identified as defined in the HIPAA privacy regulation [45 C.F.R. §164.514 (b)(2)]. A 21 CFR Part 11 compliance document is available upon request from the creators of the software, a company called ScienceTRAX, LLC.

12.2 Role of Data Management

All data will be managed in compliance with applicable regulatory requirements. Study personnel will collect, transcribe, correct, and transmit the data onto source documents, Case Report Forms (CRFs), and/or other forms used to report, track, and record clinical research data. Data management is responsible for developing, testing, and managing clinical data management activities.

12.3 Data Entry and Checks

The study personnel are instructed to enter information into Studytrax within 7 days. Data capture is the responsibility of the staff under the supervision of the PI. During the study, the PI must maintain complete and accurate documentation for the study.

The Studytrax platform provides password protection. An edit checking and data clarification process will be put in place to ensure accuracy of the data. Logic and range checks as well as more sophisticated rules will be built into the system to provide immediate error checking of the data entered. The system has the capability to automatically create electronic queries for forms that contain data that are out of range, out of window, missing, or not calculated correctly.

12.4 Data Lock Process

The platform will have the ability to lock the project-specific visits to prevent any modification of data once the project is closed. Once this option is activated, every user will have Read-Only access to the data.

12.5 Data Handling and Record Keeping

The PI is responsible for ensuring accuracy, completeness, legibility, and timeliness of the data reported. Data reported in the eCRF derived from source documents should be consistent with the source documents and discrepancies should be explained.

12.6 Confidentiality

Studytrax hosted solutions are fully HIPAA Compliant and ensure access control, audit control, data integrity, user authentication, and transmission security. Studytrax uses the data center services of Rackspace, a premier hosting company. Rackspace offers top of the line hosting facilities. As a summary of the Rackspace facilities: (1) Access to data center is secured by Biometric hand scanners and monitored 24×7 by closed circuit cameras. (2) Public access to data center is strictly forbidden. Only level three technicians are permitted in the data center. (3) HVAC [Heating Ventilation Air Conditioning] systems are used to completely circulate and filter all the air every 90 seconds. (4) Continuous UPS [Uninterrupted Power Supply] systems keep all servers up and running in the event of a total power outage. (5) Diesel engines are located on-site to provide power for extended power outages. (6) Enterprise-class routing equipment used in conjunction with multiple fiber carriers to ensure zero downtime due to network access.

Data is protected from loss by the following: (1) A redundant array of independent disk [RAID] Level 5 is used to ensure that data will not be lost if a hard drive fails, (2) full database backups are done nightly, (3) database log file backups are done every 15 minutes, (3) database integrity checks and index maintenance are performed nightly, (4) the database and log backup files are retained as part of Rackspace's backup process and also transferred every hour to Microsoft's Azure geographically redundant storage.

Data security is assured by the following: (1) All server requests are transmitted over SSL using 256-bit encryption, (2) a dedicated Cisco router firewall only allows requests to Studytrax, (3) the database is stored on a separate server in a private independent subnet with no public IP address, (4) database and log files are encrypted, and (5) database and log backups are encrypted.

12.7 Retention of Records

Research records will be retained in accordance with IRB policies.

12.8 Publications

The Principal Investigator, Steven E. Arnold, will be responsible for publication of results from this study. Dr. Arnold's responsibilities will include the following:

- Analyze and interpret data gathered in this study and write publications from these data
- Submit manuscripts to selected journals and address peer reviewers' comments
- Submit abstracts to selected meetings and present data at the meetings
- Determine authorship on the basis of the Uniform Requirements for Manuscripts

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