Effects of BCG Immunization on Cerebrospinal Fluid and Blood-Based Biomarkers in Older Adults: A Pilot

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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
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
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
MADRC	Massachusetts Alzheimer's Disease Research Center
MCI	Mild Cognitive Impairment
MDU	Memory Disorders Unit
MoCA	Montreal Cognitive Assessment
NACC	National Alzheimer's Coordinating Center
NIA	National Institute on Aging
NPIQ	Neuropsychiatric Inventory Questionnaire
PCP	Primary Care Physician
PI	Principal Instigator
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.

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.

3 STUDY DESIGN

3.1 Overall Study Design and Plan

This single-site, open-label clinical trial will investigate the immune and neurobiological biomarker effects of BCG vaccination in healthy older adults and older adults with mild cognitive impairment (MCI), and gather data on tolerability, safety and study feasibility. Twenty participants (approx. n=10 cognitively normal, n=10 mild cognitive impairment) will receive two intradermal BCG vaccinations spaced 4 weeks apart and will be evaluated 12 weeks after the initial immunization. Cerebrospinal fluid (CSF) and blood will be collected at Screening (blood only), Baseline, Week 4 (blood only), and Week 12 for to assess safety, IIR and AD biomarkers.

3.2 Study Objectives

The primary objective of this study is:

1. To determine the most promising biomarkers of IIR and neurodegeneration in blood and CSF in terms of feasibility of measurement, technical specifications, and BCG response of assays.

The secondary objectives of the study will be:

1. To evaluate clinical neurobehavior changes from Baseline, including (MoCA, RBANS, NPI-Q and FAQ) following BCG vaccination.
2. To assess the safety and tolerability of BCG vaccination in healthy older adults and older adults with MCI.

3.3 Study Outcome Measures

3.3.1 CSF and Blood Biomarker Outcomes

CSF and blood will be collected at Baseline, Day 28 (blood only) and Day 84 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.

3.3.2 **Cognitive Measure Outcomes**

While we do not necessarily anticipate significant cognitive change within the study's duration, we will apply several robust clinical outcome measures to measure beneficial (or deleterious) effects of treatment, including the MoCA, RBANS, NPI-Q and FAQ.

3.3.3 **Safety Measure Outcomes**

Classic metabolic, hematologic, and immune blood labs will be collected at Screening, Day 28, and Day 84 Visits and monitored for change following BCG vaccination. Four weeks after the first vaccination, we will assess the first vaccination site and evaluate for any signs of systemic infection prior to second BCG vaccination in the other arm. We will use the FDA Toxicity Grading Scale, as performed in vaccine trials, to denote changes from baseline.

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 Internal 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 55-80;
2. MoCA ≥18;
3. Normal cognition as defined by MoCA ≥ 26 or MCI as defined by the NIA-AA Workgroup (2011) and MoCA score between 18 and 25 (inclusive);
4. Education level, English language skills and literacy indicates subject will be able to complete all assessments;
5. Ability to provide informed consent;
6. Willing and able to complete all assessment and study procedures, including blood and lumbar punctures, and clinical assessments;
7. If on cholinesterase inhibitor and/or memantine, doses are stable for 3 months prior to baseline;
8. Negative test results for HIV antibody and Tuberculosis (QuantiFERON) at screening;
9. No prior BCG exposure either through birth vaccinations (born in North American) or BCG bladder cancer treatment.
10. Documentation of current flu season vaccination dated at least 14 days prior to baseline visit.

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), 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. Previous participation (ever) in active immunization research for AD or passive immunotherapy or other disease-modifying treatments for AD within the past three months;
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. 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;
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. Administration of live vaccine < 60 days prior to Baseline;
21. Increased intracranial pressure as determined on a fundoscopy/neurological examination performed within 30 days of LP;
22. COVID-19 vaccination < 14 days prior to baseline or any BCG immunizations.

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-six subjects will be screened and enrolled in the study, and after screening we expect twenty subjects to complete the study.

4.2.1 Recruitment of Subjects through Advertising

Advertisement flyers will be posted on bulletin boards around MGB campuses 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.

4.2.2 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 email and provided with a link to the Rally ad. These providers (not participating directly in the study) may discuss the study directly with their patients, if they choose to do so. If the prospective participant is interested, their provider will give them a copy of the consent form (unstamped) for review as well as a link to the Rally ad, which contains the research coordinator's contact information.

4.2.2.1 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.

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, letters, 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 Partner's Prescreening Guidelines prior to performing a telephone prescreening interview. If the subject meets pre-screening criteria and wishes to continue the screening process, an in-person screening visit will be scheduled.

At the screening visit, an investigator will meet with the potential subject to review and discuss 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. A licensed Nurse Practitioner Investigator or Physician Investigator will then obtain written informed consent from each participant prior to the initiation of any study procedures. 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. While some subjects will have MCI or subjective memory loss, they must be deemed capable of providing informed consent by the Nurse Practitioner or Physician Investigators. Subjects who are not capable of providing informed consent will be excluded from this study.

5.2 Randomization

This is an open-label trial and all participants will receive active treatment.

5.3 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 58 cents per mile, up to $150 per visit
- Honoraria: Up to $315 if subject completes the study:
    - $15 for the screening visit
    - $25 for the Day 1, Day 28, and Day 84 visits ($75 total)
    - $100 for each lumbar puncture (x 2)
    - $25 upon study completion

- If for any reason the subject stops the study, they will be compensated for only the visits that they have 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. Their social security number (SSN) will be required in order to process the payments.

5.4 Discontinuation

5.4.1 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.

5.4.2 Discontinuation of Treatment

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 usually do not occur within the timeline of the current study (usually 4 months to 2 years after vaccination), however should such symptoms occur prior to the second immunization, the treatment would be stopped.
3. The occurrence of any serious local skin reactions (≥ grade 3 ulceration, abscess formation, skin necrosis at the site of vaccination, 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).

5.4.3 Voluntary Withdrawals

A subject may choose to discontinue participation in the study at any time. An Early Termination Visit will occur when a subject withdraws consent, i.e. withdrawing his or her participation in future study procedures.

5.4.4 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 a certified letter to the subject's last known mailing address or local equivalent methods). 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".

5.5 Early Termination Visit (ET)

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

5.6 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 three or more participants, or if 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. If during the study three or more subjects, representing greater than 25% of enrolled subjects, develop 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	Screening Visit	Baseline Visit/ BCG Vaccine 1	BCG Vaccine 2	Outcome Visit/ Early Termination	Check-In Phone
		<-28days	Day 1	Day 28 +14 days	Day 84 ±5 days	Day 98 ±5 days
Informed Consent		x				
Inclusion/Exclusion Review	x	x				
Demographics	x	x				
Create IBCID		x				
Medical History	x	x				
Concomitant Medication Review	x	x	x	x	x	x
Family History		x				
Vital Signs, Height, Weight[1]		x	x	x	x	
Physical & Neurological Exam		x				
Screening Lab Tests for Eligibility[2]		x				
BCG Vaccination			x	x		
Vaccination Site Monitoring			x	x	x	
Fundoscopy					x	
Safety Lab Tests[3]				x	x	
Blood Draw for Biomarkers[4]			x	x	x	
COVID-19 Testing[5]		x				
PT/INR		x			x	
Fasting Lumbar Puncture for Biomarkers[4]			x		x	
MoCA and FAQ		x			x	
Cognitive/Behavioral Assessment[6]			x		x	
Adverse Event Review		x	x	x	x	x

[1] Height and weight will be measured and recorded at the Screening visit only.
[2] Eligibility lab tests include CBC with diff, CMP, TSH, Folate, B12, HIV antibodies, serum immunoglobulins, ESR, CRP, QuantiFERON-TB Gold Test, urinalysis
[3] Safety lab tests include CBC with diff, CMP, CRP and ESR.
[4] Blood biomarkers will include PBMCs for PPD and amyloid-beta stimulation assay / flow cytometry, and serum for IIR and AD biomarkers. CSF biomarkers will be used for cytology, IIR and AD biomarkers tubes for monocyte levels of innate immunity.
[5] COVID-19 Testing includes SARS-CoV-2 PCR (nasal swab)
[6] Cognitive assessments will include RBANS, NPI-Q

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, family 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.

6.2.1.2 Screening Visit

All subjects who pass a telephone pre-screening will be invited to MGH CNY for a study visit. If necessary, some of the following procedures may be performed virtually to minimize the length of the office visit. This screening visit will take approximately two hours. The following procedures will be performed at the visit:

- Obtain written informed consent from subject
- Review inclusion and exclusion criteria, medical history including COVID-19 history and demographics, concomitant medications and therapies
- Vital signs, height, and weight
- Perform a physical, including inspection for BCG scar on either deltoid
- Perform a neurological examination
- Perform nasal swab testing of SARS-CoV-2 PCR
- Urinalysis
- Phlebotomy for screening tests
    - CBC with differential
    - Comprehensive metabolic panel (CMP)
    - Thyroid stimulating hormone (TSH)
    - B12
    - Folate
    - HIV antibodies
    - Tuberculosis (QuantiFERON-TB Gold Test)
    - C-Reactive Protein (CRP)
    - Erythrocyte sedimentation rate (ESR)
    - Serum immunoglobins (IgA, IgG, IgM)
    - PT/INR
- MoCA (version 8.1)
- FAQ
- Assess and document adverse events (AEs) after subject signs ICF

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/BCG Vaccine 1 (Day 1)**

All subjects who pass screening will return to MGH CNY for the Baseline visit. At this visit, all baseline clinical measures will be collected, and the first BCG vaccine injection will be administered. The Baseline visit will occur within 28 days of the Screening visit**.** If necessary, baseline measures may be collected up to 3 days before the Day 1 vaccination visit. The visit will take approximately three hours and will include:

- Vital signs
- Assess any changes to medical history, concomitant medications and/or adverse events
- Neurobehavioral assessments
    - RBANS
    - NPI-Q
- Phlebotomy for biomarker research
- Fasting lumbar puncture for biomarker research
- First BCG vaccine administration followed by 30 minutes of monitoring

6.2.3 **BCG Vaccine 2 (Day 28 + 14 days)**

Subjects will be asked to return to MGH CNY for a second BCG vaccination visit 28 (+ 14) days after the first 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
- Phlebotomy for safety labs
- Phlebotomy for biomarker research
- Vaccine site monitoring
- Second BCG vaccine administered followed by 30 minutes of monitoring

6.2.4 **Outcome Visit (Day 84 ± 5 days)**

Subjects will be asked to return to MGH CNY for the final in-person visit at Day 84 (± 5) days to assess study outcomes. This visit will take approximately three hours and will include:

- Vital signs
- Assess any changes to medical history, concomitant medications and/or adverse events
- Vaccine site monitoring

- Neurobehavioral assessments
    - FAQ
    - MoCA (version 8.1)
    - RBANS
    - NPI-Q
- Phlebotomy for safety labs
- Phlebotomy for biomarker research
- Point of care PT/INR testing for lumbar puncture safety
- Focused Exam: Fundoscopy
- Fasting lumbar puncture for biomarker research

6.2.5 **Follow-up Phone Call (Day 98 ± 5 days)**

All subjects who complete the Outcome visit will be called on Day 98 (± 5) days by the study coordinator or clinician to review changes to concomitant medications and/or adverse events.

6.2.6 **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 2 hours and will include the following measures:

- Vital signs
- Assess any changes to medical history, concomitant medications and/or adverse events
- Neurobehavioral assessments – RBANS, NPI-Q, FAQ, MoCA
- Phlebotomy for biomarker research
- Phlebotomy for safety lab testing
- PT/INR (if opting for lumbar puncture)
- Optional fasting lumbar puncture

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

6.2.7 **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 or Co-Is 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 in the EDC System.

6.2.8 **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, and medication usage.

Subject interviews and the neurocognitive and neuropsychiatric assessments detailed below may be conducted virtually using Zoom videoconferencing platform depending on institutional guidance relating to the COVID-19 pandemic and subject consent. Any visit components conducted virtually will be documented as such and efforts will be made to collect all intrasubject clinical assessments in the same format (virtually or in person).

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 the Screening, Baseline, Day 28, and Day 84 visits. Verbal weight may be documented for those subjects utilizing a wheelchair. Height and weight will be measured and recorded at the Screening visit only.

6.3.1.2 Clinical Laboratory Assessments

Study participants will be asked to provide approximately 25 mL of blood for eligibility and safety lab analysis at the Screening visit, 11 mL for safety lab analysis at Day 28, and 12 mL for safety lab analysis at Day 84, for a total of 48 ml (3.3 tablespoons) throughout the study. The participant will have his or her whole blood collected by either a nurse or phlebotomist from a peripheral vein. 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.

Safety Laboratory Tests

Lab test	Source	Volume required [mL] (total)	Completed at following visits
Coagulation Testing (PT/INR)	Finger prick or intravenously from peripheral vein	1 (2)	Screening, Day 84
Complete Blood Count with Differential Testing	Intravenously from peripheral vein	3 (9)	Screening, Day 28, Day 84
Complete Metabolic Panel Testing		5 (15)	Screening, Day 28, Day 84
C-Reactive Protein Testing (CRP)		1 (3)	Screening, Day 28, Day 84
Erythrocyte Sedimentation Rate (ESR)		2 (6)	Screening, Day 28, Day 84
HIV Antibody Testing		6 (6)	Screening
QuantiFERON-TB Gold Test		4 (4)	Screening
Serum Folate / B12 Testing		1 (1)	Screening
Serum Immunoglobins		1 (1)	Screening
Serum Thyroid Stimulating Hormone Testing		1 (1)	Screening
Urinalysis	Urine cup	10 (urine)	Screening
SARS-CoV-2 PCR	Nasal swab	n/a	Screening

All subjects will have safety laboratory tests at the designated visits outlined in the protocol. These samples will be analyzed at a central 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) and Prothrombin Time and International Normalized Ratio (PT/INR) which are required prior to performing a lumbar puncture may be performed using CLIA waived point of care testing using the Sysmex XW-100 CBC analyzer and the Roche CoaguChek XS Plus PT/INR analyzer.

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 focused fundoscopy examination will be repeated at the outcome visit for assessment of increased intracranial pressure.

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

Subjects will provide additional blood samples for biomarker analysis at Day 1, Day 28, and Day 84 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.

60 ml (3.9 tablespoons) of blood will be collected for these analyses at the Day 1, Day 28, and Day 84 visits for a total of 180 ml (12.2 tablespoons) over the course of the study. Blood processing will occur on site at MGH. Blood samples will be de-identified, codified, and stored on-site at the Arnold Lab biorepository. Blood samples will be stored for research until they are used, damaged, or otherwise unfit for analysis.

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

Lumbar punctures (LP) will occur at Baseline and Day 84 visits. Lumbar punctures will be performed after a minimum of an 8 hour fast. The subject will be positioned seated or lying on his/her 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 designated 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.

CSF samples will be de-identified, codified, and stored on-site at the Arnold Lab biorepository. CSF samples will be stored for research until they are used, damaged, or otherwise unfit for analysis.

6.3.5 Vaccination Site Monitoring

Prior to the first vaccination, 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 Day 28 visit, prior to the second injection, the vaccination site will be inspected for any signs of local infection. At the Day 84 visit and if indicated by subject-reported AEs, the site of vaccination on the arm will be examined and any visible reactivity will be recorded.

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.[54] 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 to determine subject eligibility (MoCA $\geq$18) and at the Outcome visit.

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

The RBANS[55] 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 the Baseline and Outcome visits.

6.5 Neuropsychiatric Assessments

6.5.1 Neuropsychiatric Inventory Questionnaire (NPI-Q)

The NPI-Q[57] 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 will be done at the Baseline and Outcome visits.

6.5.2 Functional Activities Questionnaire (FAQ)

The FAQ[12,53] is a brief rating scale used to determine a subjects' level of functional independence when performing a 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 will be done at the Screening and Outcome visits.

7 TREATMENT ADMINISTERED

Summary of Investigational Products

	Investigational Products	
Product Name:	Tokyo BCG (JBL, Tokyo, Japan)	Diluent (0.9% sodium chloride for injection)
Dosage Form:	For intradermal injection	For intradermal injection
Unit Dose	1.8-3.9 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; packaged with a sterile, normal saline diluent	BCG diluent
Manufacturer	Japan BCG Laboratory (JBL)	Japan BCG Laboratory (JBL)

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.

Dr. Faustman manufactures the only GMP-quality BCG in the world through contract agreements with the Japanese government. This protocol will be reviewed by the Japanese government ethics committee prior to commencement of 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 (Tokyo, Japan) 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.

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. The drug product is packaged with a sterile, normal saline diluent.

7.3 Study Drug Storage

This drug is stored with 4ºC refrigeration.

7.4 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).

Each ampoule may be used for a maximum of two study participants in this pilot study of 12 participants. In this case, the ampoule will be reconstituted, and the syringes 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 pilot trial.

7.5 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, 1.8-3.9 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]

7.6 Study Drug Accountability

Vaccine accountability will be maintained by the study pharmacist.

7.7 Study Drug Handling and Disposal

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

7.8 Contraindications and Warnings

7.8.1 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
- additional live vaccinations within 60 days of BCG immunizations

7.8.2 **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 Statistical methods

This is a single-site, open-label pilot clinical trial in which twelve older adult volunteers with normal cognition or MCI will be recruited for BCG vaccination with the intent of generating preliminary descriptive data on IIR and neurobiological biomarker effects and evaluate tolerability, safety and study feasibility. No sample size calculations are performed as no prespecified comparisons are planned. Statistical analyses will describe baseline characteristics of the sample and change in biomarkers, safety and clinical measures, with pre-post paired t-tests or non-parametric equivalents (Wilcoxon sign-rank) used to evaluate within subject change. All outcomes in this pilot study are considered exploratory and no claims are attached to them, thus multiple comparison correction is not applied. A P value <0.05 will be considered significant. Findings will be used to inform the design of subsequent placebo-controlled trials of BCG vaccination for prevention and/or disease modification of Alzheimer's disease and related disorders.

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.

An allergic reaction can occur including serious reactions such as shock or anaphylaxis. Although anaphylaxis is very rare, the subjects will be observed for an allergic reaction after BCG vaccination.

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. Lower rates of post-LP headache have been noted with the atraumatic needles that will be prioritized for use in this study. 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 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.

9.4 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.

9.5 Neuropsychiatric and Functional Questionnaires

Questionnaires administered during the protocol may cause subjects 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.

9.6 Radiation

In general, exposure to ionizing radiation may have health risks. Subjects who require Fluoroscopy-guided lumbar puncture will be exposed to a maximum of approximately 0.18 millisieverts (mSv) for this research. 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.

9.7 Other Risks

Reviewing health-related information might be stressful or make the subject feel uncomfortable. Subjects 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. No research results will be placed in their medical record. No health or medical information obtained from research on a subject's sample will be returned to the them or their physician. 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 IIR and neurobiological biomarker effects of BCG. We do not know what the potential benefits may be to subjects 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 PI or his designee (Co-I) will conduct monthly reviews to check that data in StudyTRAX accurately reflects the data collected on the original data capture forms. 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 in locked cabinets located in secured areas. 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 a clinician investigator and recorded on the AE log. However, if an observed or reported sign, symptom, or clinically significant laboratory anomaly is not considered 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 a clinician investigator.

Subjects 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 clinician Co-Investigators. 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. Shock or anaphylaxis as a result of BCG vaccination will also be documented as SAEs.

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).

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.

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. If the PI is not available to assess seriousness and relatedness

within 24 hours, a clinician investigator will make the assessment and speak with the PI as soon as they are available.

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. Study staff should fill out the AE Log and enter the AE information into the EDC system within 48 hours of learning of a new AE or receiving an update on an existing AE.

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**

The following are considered reportable events and must be reported via the EDC system within 24 hours of study staff being notified of the event.

- All events that meet the above criteria for Serious Adverse Events

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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