PART B
STUDY DESCRIPTION

TITLE OF PROTOCOL	Delirium P01 Renewal Project
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B1. PURPOSE OF PROTOCOL

The Delirium P01 Renewal Project study builds on the Successful Aging after Elective Surgery(SAGES, Protocol: 2009P-000262) study utilizing an innovative, interdisciplinary team to examine the role of inflammation in delirium with state-of-the-art approaches including Alzheimer's disease (AD) biomarkers (cerebrospinal fluid, CSF), neuroimaging markers, and measures of brain plasticity/connectivity (transcranial magnetic stimulation and evoked potentials).

Delirium is well recognized as a common and serious problem for older persons, yet its pathophysiology remains poorly understood. The development of delirium has long been considered to be a marker of brain vulnerability. However, it remains unclear whether delirium itself leads to permanent cognitive impairment or dementia, and if so, what the pathways are by which this might occur. During the original SAGES P01, we successfully assembled and followed a large cohort, of >560 surgical patients and documented that the development of delirium was associated with an accelerated trajectory of long-term cognitive decline (LTCD). In addition, important delirium risk markers were defined related to inflammation, structural disconnectivity as measured by diffusion tensor imaging, and a new measure of global cognitive performance. These important findings have paved the way for us to move forward and pursue the next steps to extend our pathophysiologic understanding of delirium. Furthermore, the relatively large and well-defined cohort created in the first cycle presents an unprecedented opportunity to explore the long-term relationship of delirium, cognitive decline, and Alzheimer's disease.

The Delirium P01 Renewal Project includes a series of 5 interlinked Projects applying innovative approaches to deepen our exploration of some of the fundamental pathophysiologic pathways potentially contributing to delirium and its associated long-term cognitive decline. We will examine the role of inflammation (utilizing state-of-the-art approaches), preclinical Alzheimer's disease (AD) (utilizing cerebrospinal fluid [CSF] and neuroimaging biomarkers), and measures of brain plasticity/connectivity (utilizing transcranial magnetic stimulation and evoked potentials). These approaches were chosen based on their innovation, potential to probe vulnerability, and ability to advance our mechanistic understanding of delirium, cognitive decline and Alzheimer's disease. We will apply these approaches in both 1) a probability sample (N=148) from the original SAGES cohort (SAGES I, Protocol: 2009P-000262), and 2) a newly enrolled, prospective cohort (SAGES II, N=460) which will include CSF sampling obtained prior to spinal anesthesia. Table 1 outlines each Project's specific aims for this protocol and identifies the Project leader.

Project	**Table 1. Program Project Renewal Aims---Specific Aims**
1	**Delirium, Alzheimer's Disease Biomarkers, and Long-Term Cognitive Decline (S. Inouye, M.D.)**
	Aim 1. To prospectively examine in a new cohort of 460 patients (SAGES II) enriched with MCI, the relationship between baseline CSF AD biomarkers (i.e., CSF $A\beta_{42}$; total tau; phospho-tau, tau/$A\beta_{42}$ ratios, and NFL), sampled prior to spinal anesthesia and (1) development of post-operative delirium; and (2) cognitive decline over 18-36 months following delirium. **Aim 2** To evaluate associations of delirium and CSF AD biomarkers (sampled near 4 year follow-up) in a probability sample from SAGES I: (1) 74 patients who developed delirium during the initial hospitalization; (2) 74 who did not develop delirium during the initial or subsequent hospitalizations. Aim 3 (Exploratory)**.** To examine the relationship of novel Single Molecule Array (SiMoA) assays of t-tau and NFL in plasma obtained prior to surgery in SAGES I (n=148) with delirium incidence/severity and LTCD following delirium.

2	**The Role of Inflammation in the Pathophysiology of Delirium and its Associated Long-Term Cognitive Decline, (E. Marcantonio, M.D. and T. Libermann, Ph.D)**
	Aim 1. To use SOMAscan proteomics to discover new inflammatory proteins associated with delirium and LTCD in both plasma and CSF, and then validate these proteins in an independent sample. **Aim 2**. To use mass cytometry (CyTOF) to characterize circulating immune cells associated with delirium and LTCD, and then validate these findings in an independent sample. **Aim 3** (P1 Crosslinking Aim): To measure CRP and the Walston inflammatory index in banked plasma, and freshly collected plasma and CSF, from a probability sample of 148 SAGES I participants
3	**Neuroimaging of the Vulnerable Brain in Delirium: Alzheimer's and Aging Imaging Markers (B. Dickerson, M.D.)**
	Aim 1. To determine in people with evidence of preclinical AD whether AD-related brain atrophy or network dysfunction are associated with delirium, delirium severity, or LTCD **Aim 2**. To determine in people without evidence of preclinical AD whether vulnerable aging-related brain atrophy or network dysfunction are associated with delirium, delirium severity, or LTCD **Aim 3**. To investigate whether cortical atrophy due to preclinical AD or vulnerable aging is associated with postoperative LTCD or delirium with LTCD.
4	**Defining the Phenotype of Complicated Delirium Associated with Long-Term Cognitive Decline (R. Jones, Sc.D.)**
	Aim 1. To develop and evaluate predictors for early identification of complicated delirium using an expert panel. **Aim 2**. To derive predictors from empiric data for the early identification of complicated delirium, using data collected in the SAGES I cohort (N=560). We will integrate and harmonize the expert panel-derived predictive models from aim 1 with the empirically derived model from aim 2. **Aim 3**. To validate the predictive models, we will evaluate associations found in SAGES I in the newly acquired SAGES II sample (N=460) (external validation), and will also examine prediction of adverse clinical outcomes (nursing home placement, death, health care costs) in the SAGES I and II cohorts (predictive validity).
5	**Characterizing the Relationship between Brain Electrophysiology, Delirium, and Cognitive Decline, (A. Pascual-Leone M.D.)**
	Aim 1. To examine whether abnormal network connectivity and cortical plasticity are associated with the risk of developing post-surgical delirium in a cohort of 180 SAGES II patients. **Aim 2**. To correlate neurophysiology measures 2 months and one year after hospitalization with changes in cognitive performance and subsequent cognitive decline in SAGES II patients with versus without delirium during the preceding hospitalization **Aim 3**. To identify differences in neurophysiology as a function of history of delirium and cognitive decline in SAGES I patients, and to correlate these measures with long term cognitive outcomes.

B2. SIGNIFICANCE AND BACKGROUND FOR THE STUDY

Delirium and Brain Vulnerability. Delirium, an acute confusional state, is a clinical syndrome characterized by symptoms of inattention, cognitive dysfunction, and altered level of consciousness, that have an acute onset and fluctuating course.[1] Brain vulnerability is defined as "susceptibility to physical harm or damage,"[2] and our overall Program Project will focus on advancing understanding of brain vulnerability. For this project, brain vulnerability is defined as present when, "systems of resilience do not function adequately, or in which the challenge is experienced in an amplified way."[3] Thus, brain vulnerability occurs either when systems designed to maintain resilience fail or when the challenges are overwhelming.

Delirium has long been considered as the "barometer" for brain health and resilience in older people.[4,5] In fact, its development has been considered to be pathognomonic for brain vulnerability. Previous studies provide strong evidence that persons with underlying cognitive impairment or dementia are at markedly increased risk for delirium. Based on a review of the literature encompassing multiple studies and involving over 6,700 patients, in the face of baseline cognitive impairment or dementia, the relative

risk for development of delirium increases from 1.3 to 15.9. All studies reviewed measured baseline cognitive functioning and adjusted for important confounders (See Table 2).[6] Thus, there is little doubt that occurrence of an episode of delirium can signal underlying vulnerability of the brain with decreased cognitive reserve. Delirium appears to reflect a decompensated cognitive state under stress conditions, and its presence implies diminished neurocognitive resilience. In some cases, delirium may also serve to bring previously unrecognized cognitive impairment to medical attention. Despite some overlap, the risk factors or biomarkers signaling increased risk for delirium may be distinct from those for dementia or AD.[7]

Table 2. Baseline Cognitive Impairment as a Risk Factor for Delirium*

Study (year)	Sample	Sample size	Cognitive baseline	Delirium measure	Mean age at baseline (years)	Patients with delirium	Adjusted effect size (95% CI)
Cunningham (2017)[8]	Scheduled for THR or TKR, age ≥ 65years	315	Neuropsychological testing	Incident delirium by CAM or chart	74	13%	OR 3.2-15.9
SAGES (2016)[9]	Patients age ≥ 70 years scheduled for major surgery	566	GCP composite score	Incident delirium by CAM	77	24%	RR 2.0 (1.5-2.5)
Heng (2016)[10]	Orthopedic trauma and geriatrics service	739	Mini-Cog (n=513)	Incident delirium by CAM	83	11%	OR 3.2 (1.6 to 6.8)
Kennedy (2014)[11]	Emergency department, age ≥ 65 years	700	Documented dementia by chart	Prevalent delirium by CAM	77	9%	OR 4.3 (2.2 to 8.5)
Koster (2013)[12]	Elective cardiac surgery, age ≥ 70 years	300	MMSE < 23	DOSS	74	17%	OR 4.5 (1.9 to 13)
Moerman (2012)[13]	Acute hip fracture, age ≥ 65 years	378	Clinical diagnosis of dementia	Prevalent delirium by DSM-IV	84	27%	OR 2.8 (1.7 to 4.6)
Bo (2009)[14]	Patients age ≥ 70 years, medicine or geriatric wards	252	SPMSQ	Incident delirium by CAM	82	11%	RR 2.1 (1.6 to 2.6)
Rudolph (2009)[15]	Planned cardiac surgery, age ≥60 years	122 initial; 109 validation	MMSE ≤ 23	Incident delirium by CAM	75	44%	RR 1.3 (1.0 to 1.7)
Kalisvaart (2006)[16]	Elective hip surgery, age ≥ 70 years	603	MMSE <24	DSM-IV and CAM	78	12%	RR 5.5 (3.6 to 8.6)
Wilson (2005)[17]	Patients aged ≥ 75 years, medicine wards	100	IQCODE	Incident delirium by DSM-III	85	12%	OR 3.2 (1.2 to 9.0)

*BDRS Blessed Dementia Rating Scale; CAM Confusion Assessment Method; DOSS Delirium Observation Screening Scale; DSM Diagnostic and Statistical Manual of the American Psychiatric Association; IQCODE Informant Questionnaire for Cognitive Decline in the Elderly; MMSE Mini-Mental State Examination; OR odds ratio; RR relative risk; SPMSQ Short Portable Mental Status Questionnaire; TICS Telephone Interview for Cognitive Status.
Four older studies [O'Keeffe (1996)[18], Marcantonio (1994)[19], Pompei (1994)[20], Inouye (1993)[21]] demonstrating cognitive impairment as a risk factor for delirium were reviewed but are not included in the table due to space constraints

In addition, delirium is an independent risk factor for long-term cognitive decline and dementia. In a review of the literature, delirium is associated with odds ratios of 2.4-8.8 for cognitive decline or dementia in eight long-term follow-up studies involving over 5,200 patients (Table 3).[6] Delirium may heighten the impact of noxious insults or precipitating factors on the vulnerable brain, such as surgery, anesthesia, or severe infections. There is also mounting evidence that delirium itself can lead to neuronal death with permanent cognitive impairment or dementia.[6]

Complicated Delirium. Complicated delirium, defined as *delirium that is associated with a higher rate of long-term cognitive decline,* is a main focus of this project. The ability to identify and predict which cases of delirium are likely to lead to long-term cognitive impairment will represent a major advance. In addition, identification of factors associated with complicated delirium will heighten our understanding of pathophysiologic pathways and speed development of more effective interventions.

Probes of Vulnerability. For this study, we have chosen 4 major approaches from 3 conceptual areas (see Figure 1) as our probes of brain vulnerability. These approaches represent logical extensions of our previous work and offer broad frameworks that: (1) are innovative; (2) provide great potential to probe vulnerability; (3) will help to advance our understanding of pathophysiology of delirium in new directions; and (4) are supported by a substantial body of existing evidence (in delirium or related areas, such as dementia). Each approach will be utilized to examine brain vulnerability, and its

contribution to delirium, complicated delirium, and long-term cognitive decline (with and without delirium). We will apply these approaches to both the SAGES I and the new SAGES II cohort, which will achieve at least 18-36 months follow-up.

Table 3. Delirium as a Risk Factor for Long-Term Cognitive Decline and Dementia*

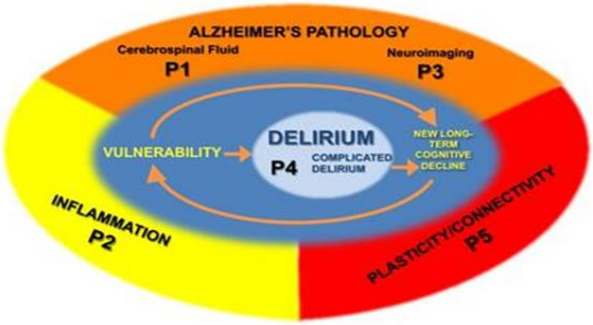
Study (year)	Sample	Sample size	Delirium measure	Cognitive outcome	Mean age (years)	Delirium Rate	Effect size (95% CI)
SAGES 2016[22]	Scheduled for major surgery, age ≥ 70 years	566	Incident delirium by CAM	GCP composite score	77	24%	−1.3 pts/36 mos (−2.1 to −0.5)
CFAS (2014)[23]	Population-based; multi-center sampling	2197	Operationalization of DSM-IV	AGECAT-defined dementia at 2 years	77	6%	OR 8.8 (2.8 to 28)
BRAIN-ICU (2014)[24]	Multi-center ICU admissions	821	CAM-ICU	RBANS score at 1 year	61	74%	-5.6 points per day of delirium
Gross (2012)[25]	Memory clinic patients with AD	263	Retrospective diagnosis of delirium from case notes	Worsening on Blessed IMC score	78	56%	Additional 1.2 points per year
Saczynski (2012)[26]	Elective CABG or valve surgery, age ≥60 years	225	CAM	Trajectory of MMSE change over 1 year	73	46%	Prolonged impairment
Vantaa 85+ (2012)[27]	Population-based; all residents age ≥85	553	Participant and informant interview, chart review	Dementia (DSM-III-R) at 2.5 years	89	13%	OR 8.7 (2.1 to 35)
Fong (2009)[28]	Memory clinic patients with AD	408	Retrospective diagnosis of delirium from case notes	Worsening on Blessed IMC score	74	18%	Additional 2.4 points
Bickel (2008)[29]	Elective hip surgery, age ≥60 years	200	CAM	Cognitive impairment and/or dementia	74	21%	OR 41 (4.3 to 396)
Lundstrom (2003)[30]	Hip fracture patients, age ≥ 65 years	78	DSM-IV	Consensus diagnosis	79	38%	OR 5.7 (1.3 to 24)

* Related analyses with some overlap of data. AGECAT Automated Geriatric Examination for Computer Assisted Taxonomy; Blessed IMC Blessed Information-Memory-Concentration scale; BRAIN-ICU Bringing to Light the Risk Factors and Incidence of Neuropsychological Dysfunction in ICU Survivors; CABG Coronary artery bypass grafting; CAM Confusion Assessment Method; CAM-ICU Confusion Assessment Method-ICU; CFAS Cognitive Function and Ageing Study; DSM Diagnostic and Statistical Manual of the American Psychiatric Association; OR odds ratio; RBANS Repeatable Battery for the Assessment of Neuropsychological Status.

Inflammation: Inflammation has been widely recognized as an important contributor to biological aging,[31-33] dementia,[34] and delirium.[35-37] Major surgery often induces an inflammatory state,[37-39] and dysregulated inflammation has emerged as a major pathophysiologic contributor to delirium.[35-37,40] The onset of peripheral inflammation with release of cytokines and other inflammatory mediators may result in a breakdown of the blood-brain barrier and development of activated microglia. In turn, these activated microglia may induce central nervous system (CNS) inflammation leading to delirium and potentially to neuronal injury and more permanent cognitive impairment.[35,37] Since chronic CNS inflammation is known to be an important contributor to AD, [34,41] this hypothesis provides an intriguing pathophysiologic link between delirium and dementia.

Biomarkers of AD Pathology: As previously noted, AD and dementia represent important risk factors for delirium. Previous studies have documented the high risk for delirium and attendant severe adverse outcomes in persons with dementia and AD.[25,28,42-45] However, little is known about whether pre-clinical stages of AD pose heightened risks of delirium and adverse outcomes. Thus, examination of AD biomarkers, such as CSF markers, MRI or PET neuroimaging markers, and electro-physiological markers may provide important early indicators of brain vulnerability. The proposed studies will allow us to examine: (a) whether those who develop cognitive decline following delirium have higher baseline rates of abnormal cerebrospinal fluid (CSF) AD biomarkers; and (b) whether brain structural or

Figure 1. Conceptual Framework for Program



functional abnormalities identified by magnetic resonance imaging (MRI) or electroencephalo-graphy (EEG) associated with delirium are also predictive of long-term cognitive decline. If such early risk markers are identified, they may provide the opportunity for institution of preventive interventions for delirium and its adverse outcomes.

Plasticity/Connectivity: Some of the earliest physiologic studies of delirium utilized EEG to document classic slow-wave changes (decreased normal alpha wave activity with increased theta- and delta-slow wave frequency activity) associated with delirium, which resolved once the delirium cleared.[46] More recent EEG studies have documented increased spectral variability, decreased EEG complexity, and decreased alpha-band EEG functional connectivity.[47,48] When combined with transcranial magnetic stimulation (TMS), evoked potentials can be used to assess cortical networks, including reactivity, connectivity, and plasticity.[49-52] Thus, TMS-evoked potentials provide a powerful means to examine brain functioning and integrity of neural networks. These are innovative approaches for delirium research.

Pilot and Feasibility Study 1: To demonstrate feasibility and assure tolerability of all proposed study procedures, 4 SAGES I participants (3 female/1 male; mean age 78 years) came to the Clinical Research Center (CRC) for the full study protocol, which included phlebotomy, LP, TMS, and a 45-minute study interview (IRB protocol number 2015P000273). All tolerated the procedures well without major complaints; all said they would participate again. The total duration was 6-6.5 hours in the CRC. Some patients preferred to have all procedures completed in one day (such as MRI and TMS) and others prefer the procedures on separate days. In addition, participants suggested to improve the comfort and convenience of the procedures, including provide padding of chairs, meals, and inform participants about possible breaks during the TMS procedures at the start of the procedures. We will schedule to accommodate the patients' preferences, provide padding, meals and alert patients about possible breaks before the start of the TMS procedure. In addition, patients will be able to opt out on procedures. While the MRI before the TMS is necessary, there will be the possibility to participate in the lumbar puncture (LP) but not MRI/TMS, or MRI/TMS but no LP.

Pilot and Feasibility Study 2: The RISE Study also served to pilot-test some of the procedures proposed for PPG II. This study included interview, phlebotomy, and MRI-PET preoperatively and interview, phlebotomy, LP and MRI-PET at one month post-operatively. No TMS was conducted in this study. As of January 16, 2019, we have successfully completed 38 post-operative LPs and 77 MRIs for this study, without any complications or adverse effects.

B3. DESCRIPTION OF RESEARCH PROTOCOL

A. Study Design – Overview, Methods, Procedures

A.1. Brief overview of the study*:*

This study is based on our previous Program Project (PPG) that created the original SAGES I cohort (SAGES, Protocol: 2009P-000262). During the first PPG, we enrolled a cohort of >560 older surgical patients (SAGES I) and documented: an accelerated trajectory of long-term cognitive decline following delirium and important risk markers for delirium related to inflammation, structural disconnectivity, and impairment in global cognitive performance. These important findings have paved the way for us to move forward to extend our pathophysiologic understanding through innovative probes of brain vulnerability. This project will deepen our exploration of pathophysiologic pathways potentially contributing to delirium and its associated cognitive decline. We will examine the role of *inflammation*; *Alzheimer's disease (AD) biomarkers* (cerebrospinal fluid, CSF, and neuroimaging markers); and measures of *brain plasticity/connectivity* (transcranial magnetic stimulation and evoked potentials). These approaches were chosen based on their innovation, potential to probe vulnerability, and ability to advance our mechanistic understanding. We will also identify and validate predictors of *complicated delirium*, i.e., delirium associated with long-term cognitive decline. All of these studies will utilize both the original SAGES I cohort, and a new prospectively enrolled cohort, SAGES II, which will include CSF sampling obtained prior to spinal anesthesia if possible (some patients will get general anesthesia and for some with spinal anesthesia we may not get CSF).

A.2. Overall Study Design.

This observational study seeks to better describe the complex relationship of *delirium, dementia, and brain vulnerability* by exploring a different aspect of vulnerability involving two different prospective observational cohorts: Successful Aging after Elective Surgery I (SAGES I) and SAGES II.

The SAGES I cohort will be followed with neuropsychological testing to achieve a minimum of 8 years of follow-up under a standing IRB protocol (SAGES I Protocol: 2009P-000262). From the SAGES I cohort, we will invite a subgroup (N=148) of participants who previously agreed to be contacted for other studies to undergo phlebotomy, lumbar puncture (LP), non-invasive transcranial magnetic stimulation (EEG/TMS) and magnetic resonance imaging (MRI) procedures. The SAGES I Subgroup will be called SAGES Select. SAGES Select participants who are not eligible to undergo LP to obtain CSF, will be invited instead to undergo an amyloid-PET scan. We will also continue long-term follow-up for the entire sample (N=560) over 8-12 years of follow-up under IRB protocol 2009P-000262.

SAGES II (n=460) will be newly enrolled and followed at 1, 2, 6, 12, 18, 24, and 36 months after enrollment with neuropsychological testing and will be invited to undergo the same procedures as SAGES I SELECT, with the exception that CSF will be collected prior to spinal anesthesia if possible (rather than a separate LP procedure). SAGES II participants for whom we could not get CSF at anesthesia induction will be invited to undergo an amyloid-PET scan after the one month follow-up visit. The 5 interlinked projects utilize these cohorts as described in Table 4 and below.

Table 4: Field Core Samples for the delirium P01 Renewal Project				
Cohorts	New for Renewal?	Sample Size	Projects	Purpose
SAGES I Cohorts				
Total SAGES I (dementia-free) sample	No	560	1, 4	Continued follow-up (2 waves, minimum 5 & 8 yrs post-op), prediction of LTCD under the protocol (Protocol: 2009P-000262)
SAGES I Select nested cohort (probability sampling based on frequency matching)	Yes	148 (subset of 560)	ALL	CSF (or amyloid-PET) AD, Inflammatory, MRI, TMS/EEG biomarkers of delirium (obtained ~4 yrs after index admission) & LTCD (8 yrs post-op)
SAGES II Cohorts				
Total SAGES II Sample (MCI enriched-15%)	Yes	460	1,2,4	Prediction of delirium and 18-36 mo CD—CSF AD(or amyloid-PET) & inflammatory biomarkers
SAGES II Neuroimaging and Neurophysiology Cohort (MCI enriched-25%)	Yes	180 (subset of 460)	3,4,5	MRI and TMS/EEG biomarkers of delirium, &18-36 mo CD
Table Abbreviations: LTCD-long term cognitive decline, 18-36 mo CD-18 to 36 month cognitive decline, MRI-magnetic resonance imaging, TMS/EEG-transcranial magnetic stimulation-electroencephalography, MCI-mild cognitive impairment, CSF-cerebrospinal fluid, AD-Alzheimer's Disease				

A.2.1 Project 1

Project 1 will investigate the inter-relationship of delirium and long-term cognitive decline (LTCD) with molecular biomarkers of AD pathology according to 3 general components of the new "ATN" (Amyloid–β [Aβ], Tau, Neurodegeneration) descriptive classification scheme for AD biomarkers with the following specific aims: (1) to examine the relationship between baseline cerebrospinal fluid (CSF) AD biomarkers (CSF $A\beta_{42}$, total tau [t-tau], phospho-tau tau/$A\beta_{42}$ ratios, and neurofilament light [NFL]), sampled prior to spinal anesthesia, and development of post-operative delirium and cognitive decline over 18-36 months in a new cohort of 460 older persons undergoing joint replacement surgery (SAGES II); (2) to evaluate associations of history of delirium and AD biomarkers (CSF biomarkers or amyloid PET imaging, sampled near 4 year follow-up) with LTCD (over a minimum of 8 years) in a probability sample from SAGES I (N=148, SAGES I SELECT): 74 patients who developed delirium during the initial hospitalization and 74 who did not develop delirium during the initial or subsequent hospitalizations; and (3) after correlating CSF (or amyloid PET imaging) and plasma levels of novel SiMoA assays of t-tau and NFL obtained at follow-up in the SAGES I SELECT probability sample (N=148), to examine the relationship of pre-operative levels of these markers obtained from stored plasma with delirium incidence/severity and LTCD following delirium.

This project will probe whether fluid biomarkers identify patients who are more vulnerable to delirium, and are most likely to have cognitive decline following delirium. By probing the relationship of delirium and AD biomarkers, we will be well positioned to advance our mechanistic understanding and to develop more effective intervention strategies to forestall LTCD associated with delirium and AD. Moreover, this study may lay the groundwork for identification of potential plasma biomarkers for AD and related dementias (ADRD). If our hypotheses are confirmed, this study will offer compelling support for the importance of prevention of delirium to forestall the progression of cognitive decline in AD/ADRD.

A.2.2 Project 2

Project 2 will leverage banked specimens from the Program Project: Successful Aging after Elective Surgery study (SAGES I), which enrolled and followed 560 participants undergoing major scheduled surgery, and collected plasma at 4 time points relative to surgery. Further, we will collect new blood and CSF samples from a probability sample of 148 SAGES I participants (SAGES I SELECT), and from a new cohort of 460 older patients undergoing total joint replacement under spinal anesthesia (SAGES II). We will use two state-of-the-art approaches, SOMAscan, a next generation proteomics platform, to

discover new inflammatory proteins (Aim 1), and CyTOF, a single-cell mass cytometry platform, to characterize circulating immune cells that regulate inflammation (Aim 2). We will also extend our prior work by examining CSF in addition to plasma, and by quantifying a novel inflammatory index (Aim 3). Using these techniques, we will compare inflammatory proteins and cells in patients who do and do not develop delirium, and in those who have slower and faster rates of LTCD following delirium. Importantly, we will also independently validate all SOMAscan and CyTOF results using standard laboratory methods. This project will lead to more detailed understanding of the full inflammatory protein profile associated with delirium and LTCD, including markers in the CSF, plus origins of the inflammatory response from immune cells. The Aims also represent an initial step toward development of blood and CSF protein, and cytometry-based biomarker panels to refine prediction of delirium and LTCD. Importantly, the proposed work will improve our understanding of the pathophysiology of delirium and its association with ADRD, ultimately leading to targeted interventions to improve outcomes of hospitalized older adults with vulnerable brains.

A.2.3 Project 3

In the prior cycle of this Program Project, a number of findings from diffusion tensor imaging and fluid biomarkers associated with inflammation were identified as predisposing to delirium. However, it is not clear whether those biomarkers might have been associated with preclinical AD pathology, vulnerable aging, or other known pathologies. Using modern magnetic resonance imaging (MRI) techniques, we have identified an "AD-Signature" of cortical atrophy and hippocampal hyperactivation as biomarkers of prodromal or preclinical AD. In addition, measures of changes in the brain that are associated with age-related cognitive decline in the absence of neurodegenerative or cerebrovascular disease can be detected as a "Vulnerable Aging-Signature" of cortical atrophy and reduced frontoparietal functional connectivity. Therefore, the overarching goal of Project 3 is to examine whether neuroimaging biomarkers of preclinical AD or vulnerable aging are predictive of delirium, delirium severity, and complicated delirium (i.e., delirium with LTCD), and to link these neuroimaging biomarkers to molecular biomarkers of pathology measured in the cerebrospinal fluid (CSF) and plasma. The Specific Aims are: 1) to determine in people with evidence of preclinical AD (i.e. patients with *abnormal* CSF AD biomarker levels of tau and beta-amyloid or classified as amyloid-positive in PET imaging) whether AD-related brain atrophy or network dysfunction are associated with delirium, delirium severity, or LTCD; 2) to determine in people *without* evidence of preclinical AD (i.e. patients with *normal* AD biomarkers) whether vulnerable aging-related brain atrophy or network dysfunction are associated with delirium, delirium severity, or LTCD; and 3) to investigate whether longitudinal cortical atrophy (measured pre-operatively and one year after surgery) due to preclinical AD or vulnerable aging is associated with postoperative LTCD or complicated delirium. To test these hypotheses, we will leverage the existing SAGES I cohort (N=560 total, n=126 with longitudinal MRI), augmented by new CSF (or PET) and MRI data collected in 148 SAGES I probability sampled patients (SAGES I SELECT) (74 with delirium, 74 without) about four years after their surgery, and a new SAGES II cohort (N=460), of which 180 patients will have both CSF (or PET) and MRI collected pre-operatively. The long-term objective of this Project is to improve the pathophysiological understanding of brain vulnerability to delirium in order to inform prevention strategies and, ultimately, pathophysiological-based treatments.

A.2.4 Project 4

In the previous Program Project, we have demonstrated that about half of people who develop postoperative delirium return to preoperative baseline cognitive performance levels within about 8 weeks of surgery, but about a third of those who develop postoperative delirium show accelerated cognitive decline out to 36 months following surgery. Our working definition of complicated delirium is delirium associated with a higher degree (or pace) of cognitive decline in long-term follow-up (i.e., ≥ 2-3 years). In this sub-group, the pace is similar to that observed among persons with mild cognitive impairment. Defining complicated delirium in terms of long-term cognitive decline is problematic since the outcome cannot be detected for years. Therefore, this Project will help identify predictors of complicated delirium to assist with early identification. Our aims are: (1) to identify predictors for early identification of complicated delirium using an expert panel, (2) identify predictors for early identification

of complicated delirium using empiric data, and (3) to validate the predictive models in an independent sample (external validity) and against clinical outcomes (predictive validity). We will use information that is potentially available before, during and immediately following surgery. We will also evaluate models using biomarkers derived from cerebrospinal fluid, serum, neurophysiologic measures, and neuroimaging obtained before surgery. We will accomplish our aims with (a) the insights of experts in delirium in a modified Delphi process; (b) secondary data analysis of the rich data already collected in the SAGES I cohort, and (c) validation with new observational and clinical data collected within the context of the new SAGES II cohort study. We will develop multiple models including preoperative, perioperative, and postoperative predictor variable sets, and their combination. The ultimate goal of this work is to improve delirium recognition and treatment by clinicians, and heighten the prognostic importance of delirium among clinicians, their patients, and policy makers.

A.2.5 Project 5

Our understanding of the neurological basis of the risk for and effects of delirium in a given individual remains very limited. This project seeks to address this important knowledge gap by utilizing magnetic resonance imaging (MRI)-guided (neuronavigated) transcranial magnetic stimulation (TMS) with simultaneous electroencephalography (EEG) and electromyography (EMG) to evaluate cortical function in patients undergoing elective surgery. In a prospective cohort of 180 patients (subcohort of SAGES II) we will examine whether decreased brain network connectivity and altered mechanisms of cortical plasticity as characterized by TMS-EEG-EMG are associated with the risk of developing post-operative delirium. We will record TMS-evoked potentials (TEP) from different brain regions (e.g. dorsolateral prefrontal cortex, inferior parietal lobule, and primary motor cortex) before and after intermittent theta burst stimulation (iTBS). Each subject will be assessed with TMS-EEG pre- and post- operatively utilizing the same TMS-EEG procedures. We hypothesize that baseline EEG spectral power and connectivity, TMS-based measures of cortical reactivity and connectivity, and iTBS measures of cortical plasticity will be decreased in patients who subsequently develop delirium, and that patients with greater abnormalities in EEG features and TMS measures at baseline will have greater delirium severity and greater short-term cognitive decline after an episode of delirium. We will correlate neurophysiologic measures with changes in cognitive performance and subsequent cognitive decline in patients with versus without delirium. We hypothesize that EEG alpha power and connectivity, TMS reactivity, TEP cortical connectivity, and efficacy of the mechanisms of cortical plasticity will show greater decreases in patients with delirium than in those without, and will correlate with the magnitude of cognitive decline. Finally, in patients with a previously observed episode of delirium (in SAGES I) or delirium post-surgery (in SAGES II) we will compare those with and without a history of delirium, and hypothesize that cortical physiology abnormalities will correlate with long-term cognitive decline after delirium (complicated delirium). Ultimately, our results will define neurophysiologic characteristics that can identify individuals with a vulnerable brain susceptible to delirium and subsequent cognitive decline, will provide novel tools to efficiently assess the effectiveness of interventions to help increase individual cerebral resilience and reduce the risk of delirium, and will guide development of therapeutic interventions to help normalize cerebral dysfunction and minimize long-term cognitive decline after delirium.

A.2.6. Project 6 Harmonization Project

The goal of the Harmonization Project is to develop and expand innovative measurement methods related to harmonization of delirium measures, outcomes, and predictors for clinical studies and treatment trials, and to refine measures of delirium diagnosis and severity in patients with all stages of dementia. For this project, we will receive de-identified data and will share de-identified SAGES data for harmonization purposes. No HIPAA data will be shared or received. We will only share de-identified data of participants who indicated in the consent form that they agree to the data sharing. This project is part of the NIA R33 grant: “Advanced-Stage Development and Utilization of the NIDUS Research Infrastructure to Advance Interdisciplinary Aging Research in Delirium.” NIDUS (Network for Investigation of Delirium: Unifying Scientists) is a collaborative delirium network which proposes data harmonization across datasets to advance research. We have not yet identified the studies from which

de-identified data will be received nor with whom we will share the de-identified SAGES data. We will inform the IRB as soon as we know with whom we will share data and from whom we will receive data. Since no HIPAA data will be shared, we will be requesting IRB waivers for these harmonization studies.

A.2.7. Natural language processing (NLP) Project
Using deep natural language processing (NLP), we will review SAGES I and SAGES II medical charts from patients' index hospitalization from both BIDMC and BWH, to determine the rate of delirium. We will first conduct a pilot study with N=32 pilot delirium positive cases (by CAM assessment) to test potential delirium terms and narrow the listing to gain high sensitivity (true positive), that is, terms that help us identify who truly has delirium with high frequency. Once we identified the search terms with the best sensitivity, we will use NLP with all medical charts of the SAGES I and II cohorts. This NLP method will support our chart abstraction procedures described under A.4.10.5. Only participants who provided written consent to abstract their medical charts will be included.
Drs. Brandon Westover (BIDMC) and Drs. Kendrick Shaw and Wendong Ge (MGH) will support this effort. Dr. Shaw will be the Site-PI for MGH. Drs. Shaw and Ge will not have access to the BIDMC OMR. Using secure file transfer, they will obtain a spreadsheet containing inpatients notes that were extracted from the BIDMC OMR. These notes will include identifiable data. For BWH and BWH-Faulkner data they will utilize the Research Patient Data Registry (RPDR), a centralized clinical data registry/warehouse. The RPDR gathers data from hospital systems and stores it in one place, bringing clinical information to a researcher's fingertips and ensuring the security of patient information. Dr. Shaw will store the file with the data in a secure folder behind the MGB firewall. After analysis he will delete the data

A.3. The SAGES I cohort and procedures specific to the SAGES I cohort (SAGES I SELECT)

SAGES I (N=560) is an already existing cohort and will be followed with annual neuropsychological testing. A sub-group of SAGES I (N=148, SAGES I SELECT) will be invited back to the Beth Israel Deaconess Medical Center (BIDMC) for additional procedures (e.g., MRI, TMS, LP or PET).

A.3.1. SAGES I Cohort (N=560): Eligibility criteria included for initial enrollment: 1) age 70 years and older; 2) English speaking; 3) scheduled to undergo elective surgery; 4) scheduled at least 6 days prior to surgery to allow adequate time for the baseline assessment; 5) anticipated length of stay of at least 2 days; 6) planned general or regional anesthesia; and 7) living within 40 miles from study site. Eligible surgical procedures were: total hip or knee replacement, lumbar, cervical, or sacral laminectomy, lower extremity arterial bypass surgery, open abdominal aortic aneurysm repair, or colectomy. Exclusion criteria included: 1) evidence of dementia (dementia diagnosis, use of dementia drugs, or score <69 or education-adjusted equivalent on baseline Modified Mini-Mental State Test;[53] 2) active delirium on initial cognitive testing; 3) hospitalization within 3 months prior to enrollment to minimize risk of recent delirium; 4) terminal condition with life expectancy < 6 months including terminal diagnoses such as metastatic cancer, pancreatic cancer, or receiving palliative care; 5) inability to perform cognitive tests due to legal blindness or severe deafness; 6) history of schizophrenia or psychosis; 7) current chemotherapy due to patient time burden; 8) documented history of alcohol abuse or withdrawal within last 6 months, and/or reporting more than 5 (4) drinks per day for men (women); 9) and inability to pass an assessment for capacity to provide informed consent. Initially 560 patients were enrolled; 6 patients were excluded due to baseline dementia. We will continue follow-up interviews with this cohort under the existing IRB protocol (2009P-000262).

A.3.2. SAGES I SELECT Sample Sub-group (N=148). A subset of selected participants from SAGES I will be invited to participate in lumbar puncture or amyloid-PET, MRI and Transcranial Magnetic Stimulation (TMS) procedures based on their delirium status. Delirium is defined by fulfillment of the Confusion Assessment Method[1] or chart criteria.[54] We will invite all SAGES I participants who developed delirium during the index hospitalization to participate, and we will enroll 74 participants into this subgroup. Next, we will enroll a sample of 74 patients who did not develop delirium or sub-

syndromal delirium in the index or subsequent hospitalizations, using a probability sampling approach to generate a frequency-matched comparison group with a comparable distribution of baseline General Cognitive Performance (GCP)[55], age, and their interaction. This SAGES I SELECT subgroup (N=148) will undergo additional procedures, including a lumbar puncture (LP), phlebotomy, PET, MRI and Transcranial Magnetic Stimulation (TMS) described in section C below. All procedures will take place one time only except for TMS which will be offered up to 3 times in selected patients.

A.3.2.1 Setting and Methods: The MRI procedures will be performed on one of the whole body scanners located on the East Campus of the Beth Israel Deaconess Medical Center or at the 1.5 Tesla or 3.0 Tesla GE scanners at the BIDMC East and West Campus. This study will use a 3.0 Tesla scanner that has dedicated time for research-related imaging under the direction of Dr. David Alsop and his study team. The PET procedure will also be performed at the BIDMC Division of Nuclear Medicine and Molecular Imaging (NMMI) on a Siemens Biograph m64 time-of-flight PET/CT scanner by the NMMI team. PET and MRI data will be analyzed at BIDMC, BWH, and at MGH under the direction of Dr. Bradford Dickerson. The EEG, TMS procedures, LP/CSF collection, and phlebotomy of this study will be conducted at the Clinical Research Center (CRC) at BIDMC. TMS/EEG procedures will be conducted under the direction of Dr. Alvaro Pascual-Leone and his study team. Blood will be collected in patients' homes or at the CRC. The LP will be conducted at the BIDMC CRC under the direction of Dr. Tamara Fong, a BIDMC neurologist. The laboratory testing for inflammatory biomarkers, will be conducted in the laboratory of Drs. Towia Libermann/Simon Dillon at BIDMC and Dr. Steven Arnold at MGH.

SAGES I full cohort participants, who are eligible and who indicated interest in continued study participation during a follow-up interview of the initial SAGES I study, will be called by a coordinator to schedule a visit to the BIDMC Research MRI and Clinical Research Center (CRC) for LP, phlebotomy, MRI, and TMS-EEG. SAGES I Select participants who are ineligible for LP will be invited to participate in amyloid-PET imaging instead. Only patients who provide written or verbal consent and who demonstrated capacity to consent by SAGES procedures will be enrolled into this study. The consent will be administered by study staff. All participants will undergo separate eligibility assessments for each procedure (section C).

A.3.2.2. Phlebotomy: During phlebotomy, in total less than 40mL will be collected at two different occasions. To assess LP eligibility (platelet count and INR), <10 mL blood will be drawn in 2 different tubes (~4mL in lavender top and ~3mL in light blue top) in participants' home. An additional 30 ml (three 10 mL heparin tubes) will be collected at the CRC, centrifuged to separate plasma from cellular material, divided into small samples, and frozen at -80C for future use. Blood will be stored in the SAGES freezer. Blood samples will be collected via peripheral venipuncture into sterile vacuum tubes using either a vacutainer or butterfly system. The first draw of blood will be collected in patients' homes by trained and experienced research staff or by a phlebotomy service affiliated with Hebrew SeniorLife. The second draw will be collected in the BIDMC CRC (at the same day as the LP or MRI) by nursing staff. Each procedure will last about 10 minutes. For the first blood draw hemolysis is not a concern and therefore the blood can be drawn in participant's homes. For the second blood draw immediate processing after the draw is essential and therefor the blood needs to be drawn at the CRC.

A.3.2.3. Lumbar Puncture (LP): During LP, 10-15 cc of CSF will be collected and centrifuged at low speed to remove any cellular contaminants, divided into aliquots and frozen in 0.5 ml polypropylene cryotubes for inflammatory and AD biomarkers. CSF will be stored at -80^{o}C. The LP procedure, including consent, will take about 1-2 hours, and will be performed by one of the study Neurologists under supervision of Dr. Tamara Fong and with assistance from the CRC nurses as needed. During the LP, participants are placed in a left lateral position, with the back flexed, and knees are drawn up towards the chest, or sitting up and bent forward, whichever is easier for the participant. The lumbar region of the back will be cleaned with betadine, twice. Lidocaine 1% will be injected into the subcutaneous area between the L3-4 or L4-5 spinous process. Once the area is numb, a 24G or 25G atraumatic Sprotte LP needle will be placed and CSF will be collected. To clear any blood from minor

trauma associated with needle insertion, the first 1-2 ml of CSF are discarded (or more if needed) to eliminate blood. Participants will remain in the clinic for about 15-30 minutes for monitoring after the procedure. They will be given the option to have something to eat or drink before they leave. They will be advised to not do any strenuous physical activity for the next 24 hours, including lifting, bending, doing housework and gardening, or doing exercise such as jogging or bicycle riding. Before participants leave, they will be asked about how they are feeling and given a short questionnaire asking about their experience with the LP. One day after the LP, a member of the research team will call the participant to ask about how s/he is feeling.

A.3.2.4. MRI: Participants will complete a structural Magnetic Resonance Imaging (MRI) scan in the 1.5T or 3T scanner. Upon screening with the MRI team, if a participant indicates they had an injury to the eye involving metal or has potentially contraindicated metallic implants without previous x-ray exams, an orbital or body x-ray may be indicated prior to the MRI to rule out metal objects in the body. The MRI scanning procedures will take approximately 1-1½ hours. During the scan the participant will lie on his/her back in the scanner with their head inside an imaging coil. Special pillows will be used to hold their head securely in place. Ear plugs will be provided to the subject and a pneumatic squeeze ball that triggers an alert at the scanner console when squeezed will be provided. The patient will be able to communicate with the MRI technologists at all times during the exam.

The structural MRI images will be obtained; this takes about 20-30 minutes.
The structural MRI images will be used to accurately identify the regions of interest (ROI) for the TMS-EEG procedures.

A.3.2.5. PET: PET scans will take place under supervision of the NMMI faculty and staff. At the beginning of the session, an intravenous catheter will be placed in the antecubital vein of the left arm to participants prepositioned in the bed of the PET scanner or in an adjacent room prior to the scan. This will be used to inject the F^{18} Florbetapir radiotracer as a slow intravenous bolus (up to 15 mCi will be administered). The catheter will be removed after the injection. The catheter will be placed by and accessed by a certified nuclear medicine technician. F^{18} Florbetapir PET will be acquired with an 370 MBq (10.0 mCi) ± 10% bolus injection followed by 20 min (4X5min frames) acquisition at 50-70 min post-injection as in the acquisition protocols used in ADNI (ADNI 3). Participants are scanned on a Siemens Biograph m64 time-of-flight PET/CT. Subjects will be instructed to remain still, with eyes open or closed depending on the scan sequence, for the total duration of the scan. The duration of the visit itself will be approximately 2-3 hours (consent, preparation, injection, and 20 min of scanning starting at 50 min after injection.

If a participant wants results of their PET scan released to their physician, they may request it. Dr. Tamara Fong will communicate with the BIDMC Department of Nuclear Medicine who will access the database and release an unblinded report to Dr. Fong and to the OMR. Dr. Fong will relay the report and information to the patient's physician, and alert them also that the report and scan can be obtained through the BIDMC Medical Records department. Thus, the report and scan should be accessible through regular channels by all physicians both within and outside the BIDMC system.

A.3.2.6. TMS-EEG: These procedures will take about 4-5 hours. All SAGES I SELECT participants will undergo the first TMS-EEG visit with theta burst stimulation targeted at one of the brain regions identified above (section A.2.5 Project 5) and following procedures as described above. We will then invite all participants back for a second and third time to target the theta burst at the other 2 brain regions within a 6-week timespan after the initial TMS-EEG session.

TMS-EEG Procedures (SAGES I SELECT and SAGES II cohorts):
EEG: A special cap with electrodes is placed on the participant's head. Saline gel will be used to increase electrical conductivity between the electrodes and the scalp. These electrodes will measure electrical activity in the brain; the cap will remain on the patient's head for the duration of the visit. We

will utilize the structural MRI previously acquired in the original SAGES study or will use a standard 10-20 or 10-5 EEG set-up and a standard brain model to ensure correct placement of the TMS coil and EEG electrodes.

TMS: The participant will be seated in a chair and the research team will place the TMS coil against the head. The coil produces a magnetic field that will briefly affect brain function.
Target Regions: All subjects will receive single-pulse TMS to up to three cortical regions. The target regions will be the left dorsolateral prefrontal cortex (DLPFC), left inferior parietal lobe (IPL), and left primary motor cortex (M1).

- Determination of Resting Motor Threshold: Resting motor threshold (RMT) will be determined by applying single pulses to the motor region (M1). RMT will be defined as the minimum stimulus intensity that produced a small motor evoked potential (MEP) in the hand muscles- about 50 μV in 50% of 10 trials - as measured by electromyography (EMG) during relaxation of the tested muscles. RMT will be used to guide intensity to be used for single pulse stimulations.

- Baseline Single Pulse TMS: Single-pulse TMS will be applied to the target regions at 110% - 130% motor threshold and with an inter-pulse interval between 2-6 seconds to establish the baseline TMS-evoked EEG response and to assess cortical excitability.
  Baseline Paired-Pulse TMS: Pairs of TMS pulses with an inter-pulse interval of 100 ms at 110% - 130% motor threshold will be applied to the target regions, and with 2-6 seconds between pulse pairs, to obtain baseline TMS-EEG measures of cortical inhibitory control.

- Determination of Active Motor Threshold (AMT): AMT will be defined as the minimum stimulus intensity that produces a small MEP (about 200 μV in 50% of 10 trials) during isometric contraction of the tested muscles, at about 20% of maximum voluntary contraction while applying single TMS pulses over M1. AMT will be used to determine the intensity for TBS.

- Theta Burst Stimulation (TBS): SAGES II Subjects will receive TBS to M1 (all 3 visits) and SAGES I SELECT subjects will receive TBS to one of the three defined regions (left dorsolateral prefrontal cortex (DLPFC), left inferior parietal lobe (IPL), or left primary motor cortex (M1)), one at each visit. TBS will consist of bursts of 3 pulses at 50 Hz repeated at intervals of 200 ms. Stimulation intensity will be delivered at 80% of active MT (AMT). Corticospinal excitability will be assessed prior to and following theta burst stimulation by measuring peak-to-peak amplitude of MEPs in response to single pulse TMS. MEPs will be recorded prior to TBS and used as a baseline. Following TBS, batches of MEPs will be measured at five, ten, or 15-minute intervals for up to 2 hours to track changes in amplitude over time.

A.4. The SAGES II cohort and procedures specific to the SAGES II cohort

SAGES II Cohort (N=460), will be newly enrolled surgical patients undergoing scheduled eligible surgeries with spinal or general anesthesia at two academic medical centers, Beth Israel Deaconess Medical Center (BIDMC) and Brigham and Women's Hospital (BWH and BWH Faulkner, the same centers where SAGES I and RISE occurred) and followed prospectively at 1, 2, 6, 12, 18, 24 and 36 months. The enrollment age for this cohort will be 65 years and above. Except for age at enrollment, inclusion and exclusion criteria are the same as for the original SAGES I cohort (N=560) outlined above except SAGES II will be enriched with 15% patients with Mild Cognitive Impairment-Amnestic type (MCI-A) identified at baseline in order to examine the impact of delirium in this high-risk group. In a addition to the surgery types listed under SAGES I we will also enroll patients with the following planned surgeries: POPLITEAL, AMPUTATION BELOW AND ABOVE KNEE, PERIPHERAL ARTERY REPAIR OPEN, COLECTOMY LAPAROSCOPIC AND OPEN, BOWEL RESECTION, LUMBAR LAMINECTOMY/FUSION, PANCREATECTOMY, RESECTION

GASTROINTESTINAL MASS, HEPATECTOMY, GASTRECTOMY LAPAROSCOPIC, BOWEL RESECTION LAPAROSCOPIC, ROUX-EN-Y HEPATICOJEJUNOSTOMY, NEPHRECTOMY, laparoscopic, PROSTATECTOMY, laparoscopic, CYSTECTOMY, laparoscopic, NEPHRECTOMY, open. Most PIs and staff members were part of the SAGES I cohort creation and are very familiar with all procedures. We have already successfully pilot-tested all study procedures that we will use for SAGES II (BIDMC IRB protocol #2015 P000273 and PARTNERS IRB protocol # 2017P000064).

SAGES II Sub-group (N=180). This subgroup will meet the same general inclusion and exclusion criteria as all SAGES II patients, will be selected based on their baseline neuropsychological testing and undergo the same specialized procedures as the SAGES I SELECT cohort (except excluding lumbar puncture), including, magnetic resonance imaging (MRI) scans and electroencephalogram (EEG) with non-invasive transcranial magnetic stimulation (TMS). Prior to the procedures subjects must complete a screening form designed to identify any contraindications.

A.4.1. Setting and Methods: The study procedures, including screening, baseline, hospital, and follow-up assessments are detailed below. PET, MRI and TMS-EEG procedures will take place at the same locations under the supervision of the same persons outlined above (Section A.3.2.1). All face-to-face assessments will be conducted either in the subject's place of residence (in-person or remotely via telephone or videoconference using an IRB approved platform like zoom or Starleaf---once proper agreements specific to Zoom are in place), at Hebrew SeniorLife, or in the out-patient clinics according to the convenience and preference of the subject. Patients will be identified from the BIDMC or BWH (including BWH Faulkner) operating room advanced booking schedule and will be approached and enrolled (after permission from their surgeons) in either the subject's choice of their home or during an office visit. We plan to enroll 230 subjects per year. Based on our previous studies, we anticipate that about 500 surgical patients per year will meet our eligibility criteria across the 2 sites and will be available for the study, yielding an eligible-to-enroll ratio of 2.2:1. Thus, we anticipate that we will have adequate availability of patients to conduct the study.

A.4.2. Research staff: All research staff, comprised of experienced clinical research interviewers, will undergo intensive training, following standardized procedures, in all questionnaires and research methods. They will be carefully trained to handle emergency issues in the home setting, and the Project and Core leaders will be available to provide back-up at all times. Baseline standardization and inter-rater reliability assessments will be conducted to verify consistency of all staff on the primary outcomes (including the neuropsychological test battery and functional outcomes), as well as key risk factor variables (including the delirium assessment). Interviewer quality checks with inter-rater reliability assessments on all key study variables will be performed every 6 months for the duration of the study. All staff will undergo training on infection control and proper use of personal protective equipment (PPE). All staff will continue to be compliant with BIDMC, BWH/BWFH, and Hebrew SeniorLife guidance on interacting with study participants and patients, and will undergo daily symptom screening during the COVID19 crisis.

For the in-person assessments, both the study team member and the study participant will wear face masks and face shields at all times. For the timed walk, to ensure participant safety, study staff will walk behind participant during assessment. For the grip strength test, participant will be handed a sanitized dynamometer. Both the study team member and the study participant will perform hand hygiene and wear gloves before handling the dynamometer. After the assessment, both will remove their gloves and perform hand hygiene again.
For all procedures conducted at the BIDMC, staff will follow standard BIDMC COVID-19 guidelines.

A.4.3. Screening assessment (n=460 entire SAGES II cohort): The purpose of the screening assessment is to verify subject eligibility. Based on a 10-minute patient interview along with medical record review, information will be collected to establish eligibility criteria and to rule out the presence of

delirium at baseline. The interview will include: digit span, Confusion Assessment Method (CAM), Delirium Symptom Interview (DSI), the Montreal Cognitive Assessment Test (MoCa). Patients with a positive CAM and/or a MoCA score under 20 will be adjudicated by Dr. Tamara Fong. The screening will also complete the first stage of our two-stage process to identify patients' cognitive status. Patients with Mild Cognitive Impairment-Amnestic type (MCI-A) will be eligible but patients with dementia will be excluded. All screening information will be entered into an iPad or tablet computer by the interviewer, and immediate subject eligibility will be determined based on internal algorithms to determine all inclusion and exclusion criteria and to rule out delirium and dementia. The baseline assessment will take place based on participant's preference in their home, at HSL or BIDMC or any other place determined by the participant.

A.4.4. Baseline assessment interviews (n=460 entire SAGES II cohort): The purposes of the baseline assessment are to characterize the subjects, to document baseline neurocognitive function, to characterize baseline risk factors for delirium and cognitive decline, and to measure potentially confounding factors. This assessment will be conducted immediately following the screening assessment in eligible patients. The 80-minute baseline interview will collect information on cognitive functioning (neuropsychological test battery, Table 5), demographics, education, occupation, medical diagnoses, comorbidity, medications, health habits (e.g., smoking, alcohol), hearing, mobility, basic and instrumental activities of daily living, physical functioning, depression, and anxiety. A family or caregiver interview will be conducted (in-person or over the phone, or if both is not possible we will mail the questionnaire with a pre-stamped return envelope) to establish the subject's baseline cognitive functioning, to assess for evidence of dementia, and to determine any recent changes in mental status. Based on our previous studies, about 3-4% of participants will not have a family member or caregiver available to participate in these interviews; in these cases, we will approach (with the patient's consent) friends, neighbors, visiting nurses, or other reporters about the patient's baseline functioning. All enrollment and baseline assessments will be completed prior to the scheduled surgical admission.

Table 5. Description of the SAGES Neuropsychological Battery to assess cognitive functioning

Test	Description	Domain(s) tested
Trail-making Tests A and B	Participant must connect a sequence of alternating numbers and letters	Executive function, visual spatial function, processing speed
Phonemic F-A-S Fluency	Participant must generate as many words in one minute as possible beginning with a given letter over three trials	Executive function, semantic memory, language
Category Fluency	Participant must generate as many words in one minute as possible from a semantic category (e.g., "animals")	Executive function, semantic memory, language
Visual Search and Attention Test	Four timed visual cancellation tasks where participant must cross out letters and symbols identical to a target	Executive, visual spatial function
Hopkins Verbal Learning Test - Revised (HVLT-R)	A list of words is read to the participant, who is asked to repeat the list back over multiple learning and delayed recall trials	Verbal episodic memory
Digit Span Forward/Backward	Participant is asked to repeat a string of digits forward and in reverse order	Sustained attention
Boston Naming Test	Participant is presented with drawings of common objects, which then must be named correctly	Confrontation naming, language
RBANS Digit Symbol Substitution	Using a key provided, the participant matches symbols to numbers as quickly as possible while being timed	Executive function, visual spatial function, processing speed
AmNART	Participant is asked to pronounced words aloud presented to them on a piece of paper	Verbal IQ

RBANS = Repeatable Battery for the Assessment of Neuropsychological Status
AmNART = American National Adult Reading Test

A.4.5. Blood: (n=460 entire SAGES II cohort): We will collect blood from the entire SAGES II cohort participants at 4 time points: at the time of the baseline study assessment (baseline), on postoperative days 1, and 2 (POD1, POD2) and 1 month after surgery (100 ml total over 6 weeks). If a patient is discharged on the day of surgery, we will obtain blood on POD 1 or POD 2 in patient's discharge location. During the baseline and POD1 (or POD 0) assessments, three green top tubes (30 cc) will be

collected. At subsequent follow-up time points (POD2 and 1 month after surgery), two green tubes (20 cc) will be collected. Blood samples will be collected via peripheral venipuncture or central venous line (if available on PODs 1 and 2). When possible, blood will be obtained simultaneously with phlebotomy for routine clinical laboratory work, thereby eliminating the additional risk imposed by a separate phlebotomy for study purposes. Otherwise, an experienced staff member or phlebotomy services affiliated with Hebrew SeniorLife will collect the blood. Blood samples will be collected into sterile vacuum tubes using either a vacutainer or butterfly system, at the preference of the phlebotomist. All collected blood with be centrifuged to separate plasma from cellular products, divided into small samples, and frozen at -80C for future use.

A.4.6. MRI (n=180 SAGES II Subgroup): The SAGES II subgroup will undergo the MRI scan before hospitalization using the same procedure as outlined above for the SAGES I SELECT subgroup.

A.4.7. TMS-EEG: (n=180 SAGES II Subgroup): The SAGES II subgroup will undergo only one TMS-EEG session, as outlined above for the SAGES I SELECT subgroup, prior to surgery. A subgroup (n=102) will be invited for a second TMS-EEG 2 months after discharge from the index hospitalization and a third TMS-EEG 12-18 months after discharge.

Patients who are not able or decline to participate in the TMS-EEG session will be offered the option to participate in only collection of their resting-state EEG. They will be invited for a second and third EEG 2 months and 12 months after discharge from the index hospitalization. Verbal consent will be obtained from these patients.

A.4.8. Cerebrospinal fluid (CSF, up to n=460 entire SAGES II cohort): CSF collection will occur during spinal anesthesia for elective surgery. The procedure will be performed by the assigned anesthesia team led by experienced anesthesiologists in the operating room under standard monitoring, immediately before the planned operation. Using standard sterile technique and local anesthesia to the skin, the dural sac will be entered with an appropriately chosen Sprotte, Whitacre or Quincke needle 25-22G by the anesthesia team, under supervision of Drs. Kamen V. Vlassakov (BWH), David A. Shaff (BWH-Faulkner), or Lisa J. Kunze (BIDMC). After confirming intrathecal placement and adequate CSF flow, 2.0 ml of CSF will be collected; this amount is limited by feasibility and safety concerns of the anesthesiologists but is sufficient to accomplish the aims. Next, the anesthesiology team will inject local anesthetic for the surgical procedure through the same needle as per usual care. CSF will be centrifuged and managed in the same way as outlined above for the SAGES I SELECT cohort.

A.4.8.1. SAGES II subgroup participants for whom we could not collect CSF will be offered PET scans and using the same procedure as outlined above for the SAGES I SELECT subgroup

A.4.9. Hospital assessments: The purpose of the hospital assessment is to monitor daily for development of delirium and to assess precipitating factors. We anticipate that all patients in the surgical group will be hospitalized. Automatic systems will be in place to notify the principal investigator immediately whenever an enrolled study patient is admitted to the BIDMC or BWH. Upon admission, the patient will be seen by the study team in the hospital (in-person or remotely via telephone or videoconference using an IRB approved platform like zoom or Starleaf), and will undergo daily 10-15 minute interviews including a cognitive screen, digit span test, CAM, DSI, MDAS ratings, and new delirium severity measures. Precipitating factors (e.g., infections, immobilization, surgical procedures, post-surgical complications) will be assessed by interview and review of the medical record using standardized, validated methods applied in our previous studies. The initial hospitalization during the study period will be considered the index hospitalization. The timing of follow-up will begin from the point of discharge. For study purposes readmission within 24 hours is considered a continuation of the hospital stay. Our study team is highly experienced in conducting such interviews in hospitalized persons, which will assist in assuring retention, as well as avoiding any interference with ongoing

medical care. If a patient is discharged on the day of surgery, we will conduct the POD 1 and/or POD 2 hospital interview in patient's discharge location or on the phone.

A.4.10. Post-hospitalization follow-up assessments: Several types of follow-up assessments will be conducted, as detailed below. These are all timed in relationship to the discharge date of the index hospitalization. Intercurrent illnesses and re-hospitalizations will be assessed at each follow-up time-point. If patients are hospitalized at the time of a scheduled follow-up assessment, we will wait for one month from hospital discharge to complete the follow-up assessment, to minimize the effects of acute illness on cognitive functioning. Some attrition is anticipated at each follow-up time point (due to mortality and losses to follow-up in this older population). These attrition rates are accounted for in all power calculations below.

A.4.10.1. Face-to-face interview or phone interviews at two weeks (Delirium group only): The purpose of this 10-15 minute face-to-face or phone interview is to repeat the delirium assessment to better assess the duration and persistence of the index delirium episode. This interview will be conducted at 2 weeks after hospital admission in patients who had delirium at any time during the index hospitalization. This interview will be conducted in all settings of care, including hospital, home, assisted living, post-acute, or nursing home settings. We have extensive experience conducting interviews in all these settings, and the initial informed consent process will include these follow-up interviews. The two-week time period was selected for this interview, because previous work by our group has demonstrated the prognostic importance of the two week period.

A.4.10.2. Retest face-to-face interviews at one and two months follow-up (Retest 1 and 2): The purpose of these 45 minute face-to-face interviews is to repeat our delirium assessments and neuro-psychological test battery to test for both the persistence of delirium and to evaluate retest (learning) effects. All subjects will be interviewed, although only a subset will have developed delirium while hospitalized. The Retest 1 interview (at 1 month) will assess for immediate learning or retest effects, particularly in the patients with normal cognitive functioning. The Retest 2 interview (at 2 months) will assess for delayed learning effects, which our preliminary results suggest are likely to be present in the delirious patients. Patients will also be asked about any rehospitalizations or intercurrent illnesses. We will also ask participant and caregiver about their burden (distress) related to delirium.

A.4.10.3. Telephone follow-up interviews (2 weeks, 4, 9, 15, 21 and 27 months): The purpose of these 10-15 minute telephone interviews is to assess delirium status, functional status, intercurrent illnesses, rehospitalizations, or death. These interim telephone interviews are considered essential to maximize retention, and to conduct brief cognitive testing, discover unreported hospitalizations, and track deaths. As described above, the 2 week phone call will only be placed to patients who developed delirium in the hospital to assess duration of delirium.

A.4.10.4. Face-to-face follow-up interviews (6, 12, 18, then every 6 months up 36 months): The major purpose of these 45 minute interviews is to obtain comprehensive neuropsychological and functional measures to rate our primary outcomes. These interviews will include the neuropsychological test battery, as well as the MOCA, digit span test, CAM, DSI, and MDAS ratings, ADLs, IADLs, SF36, intercurrent illnesses, and information on re-hospitalization, institutionalization, and death. If the subject is hospitalized at the time of the scheduled follow-up, then this interview will be postponed until one month after hospitalization to minimize the effects of the acute illness/hospitalization on the neuropsychological testing results. At each of these follow-up interviews, a surrogate will also be interviewed to complete the Informant Questionnaire on Cognitive Decline in the Elderly (IQCODE) and proxy ratings of delirium and functional status (ADL, IADL) either face-to-face or by telephone.

A.4.10.5. Medical record, health care utilization and vital status reviews (after index hospitalization, each hospitalization after the index hospitalization, and after final interview): Medical records will be obtained from the index hospitalization, and later from all subsequent hospitalizations, and reviewed for

information on development of delirium, intercurrent illnesses, new diagnoses, new surgical procedures, and deaths. Information on hospitalizations is obtained at each of the follow up interviews (telephone and face-to- face). We anticipate that patients may be hospitalized at a large number of different hospitals from which we plan to request medical records. Our informed consent process will include permission to obtain these outside medical records. A standardized medical record abstraction form has already been developed to systematically collect this information. Based on our previous work, these abstractions will take approximately 60 minutes per hospitalization. The final medical record review will be conducted after the patients have their final study interview (18months -12 years).

A.5. Measurement of the Apo E allele

Using genetic material obtained from the baseline blood samples, we will perform Apo-E genotyping for all participants to be used as a covariable in analyses examining long-term cognitive decline.

A.6. Bio-repository

Using all blood and CSF samples from the SAGES I SELECT cohort and the SAGES II cohort, we will create a Study Specimen Bank of plasma, CSF and genetic material for the purpose of completing the aims for the present study. At the end of this study, in about 5 years, after the analyses for our aims are completed, we will seek funding from the NIA to create a bio-repository for future biomarker discovery studies and to share data. The banked specimens will be labeled only with a study number and stored in a -80C freezer at BIDMC. A list linking study numbers to participants will be kept in a secure computer database. Once the bio-repository is created, specimens may be shared with other scientists after a formal application procedure and scientific review. All such studies will be carried out under the close supervision of the study team. In these cases, the study investigators will maintain confidentiality of the specimens and provide no identifying information to the other scientists. We plan to store these specimens indefinitely. None of the results from these studies will be shared with participants or their family members, and none will become part of the medical record.

A.7. Incidental Findings

MRI: In the event of an incidental finding a specifically designed MRI Safety Protocol will be followed to ensure standardized handling of incidental findings. The purpose of the MRI Safety protocol is to provide clinical backup to MRI research staff for any unexpected medical issues that arise in the course of participants undergoing MRI scans and to manage incidental findings on MRI, including maintenance of a database of such findings. If a suspicious finding is observed by the study staff, the finding will be reported to the study PI, the MRI on-call research physician, and to the MRI Research Medical Officer. If the finding is confirmed as suspicious and requiring a follow up, the PI or an MD coinvestigator will contact the patient and explain the recommendation for a full clinical imaging evaluation. The investigator will communicate the recommendation to the surgeon and primary care provider with a medical explanation of the finding. All incidental findings are reported to the safety officer (TBD). The SAGES MRI Safety Panel will maintain a list of all incidental findings and all MRI incidental findings will be included in the annual progress reports.

Lab results: we also developed an incidental findings protocol to manage incidental findings on screening labs obtained for Lumbar Puncture (LP) eligibility. In general, the approach must be undertaken on a case-by-case basis. Incidental findings that could be life threatening, such platelet count less than 50K or INR greater than >5 (with symptoms), may be considered critical and necessitate referral to the Emergency Department or urgent call to PCP. Other IFs will be classified as (1) Urgent; (2) Routine; or (3) No Clinical Relevance based on the estimated risk of harm to the patient. Patients requiring urgent follow-up will not proceed with any study procedures. Study physician must make contact with PCP within 24 hours of abnormal test results, or sooner if patient is symptomatic (hemorrhage or thrombosis). Patients requiring Urgent Follow-up are temporarily excluded from the study; they may be reconsidered in six months time if clinical status has stabilized. Once the platelet or INR value is confirmed to be consistent and stable over time, he/she may proceed to RN adjudication (to confirm absence of bleeding, and thrombocytopenia-associated disorders, e.g. liver disease). If

confirmed by RN, patient may then proceed to LP. For patients requiring Routine Follow-up, a letter will be sent (as attached). Once the platelet or INR value is confirmed to be consistent and stable over time, he/she may proceed to RN adjudication (to confirm absence of bleeding, and thrombocytopenia-associated disorders, e.g. liver disease). If confirmed by RN, patient may then proceed to LP.
All incidental findings are reported to the safety officer (TBD).

PET: Incidental findings on the amyloid PET (e.g., a structural abnormality) will be reviewed by a clinician who will follow our MRI incidental findings protocol, which has already been approved by the Institutional Review Board for our current scans (MRI), and our study physicians will inform the participant and their Primary Care doctor to arrange follow-up screening.

A.8. Information of other IRBs
The IRB of record for this multicenter study will be the BIDMC IRB. All other sites will have reliance agreements using the Online Reliance System SMART IRB: https://smartirb.org
All PIs and field team members across sites meet at least weekly in person and have ad hoc phone call and meetings if needed for unforeseen emergencies or questions that need immediate attention. More information can be found in section B9.

B. Statistical Considerations

B.1. Sample Size Justification:
Our sample sizes were chosen based on an iterative process involving determining the size of the target population eligible, feasibility constraints, and then verifying adequate power to test the hypotheses across all of our proposed studies for Projects 1-5. Through these detailed considerations, and input from our expert statisticians, we have been able to verify adequate power to address all of our study aims with the proposed sample sizes for the SAGES I SELECT subsample (N=148), the SAGES II cohort (N=460) and the SAGES II subsample (N=180). Power has similarly been verified for all aims of each project. For SAGES II, approximately 50% of the cohort will be enrolled at BIDMC and 50% at BWH. The data, however, will be analyzed together. Computations allow for two-sided type-I error probability 0.05 and to take into account cumulative attrition consistent with our experience in this population. We use multiple imputation to account for missing data where appropriate.

B.1.2. Experimental design and sampling: general considerations
This study uses observational, prospectively collected cohort data for SAGES I and II. In specific cases, participants experiencing delirium during the index hospitalization will be matched to those without delirium on the basis of age and other factors. To obtain the probability sample of 148 existing SAGES I participants, a matched sampling procedure was piloted. This approach selects all SAGES I participants who had delirium during their index hospitalization and a sample of the non-delirious frequency-matched to the delirious on general cognitive performance (GCP) and age, both key predictors of long term cognitive decline (LTCD). We anticipate enrolling 74 participants with delirium during index hospitalization, and a corresponding 74 participants without. The nondelirious set will be chosen to have a similar distribution to those with delirium on a propensity score derived from a predictive model for delirium, taking into account SAGES I baseline GCP, age, and their interaction. To demonstrate the feasibility and validity of this approach, we conducted a pilot study that constructed 1001 such matched samples, and estimated the standardized mean difference (delirious minus nondelirious) on GCP, age and Charlson comorbidity index for each. While delirious patients in SAGES I were older, had lower GCP, and greater comorbidity than the nondelirious, the frequency matching effectively eliminated these imbalances. Pre-operative standardized mean difference in GCP score was -0.58; after matched selection, the difference decreased to -0.06 (95% CI: -0.13 to 0.011). For age, the pre-operative difference decreased from 0.29 to -0.04 (-0.16, 0.09) after matching. Likewise for the Charlson, the difference was reduced from 0.18 to 0.09 (-0.05, 0.26). These pilot data demonstrate the feasibility and effectiveness of this approach in reducing potential confounding bias, even for unmatched variables. Of

note, we originally powered analyses for a SAGES I probability sample size of 128 (64 per group) and SAGES II sample size of 400. Because we have expanded the sample sizes (to 148 and 460, respectively) and because power increases with sample size, we expect to exceed the estimated power described in the following sections.

B.1.3. Power by Project:

Project 1

Aim 1. To prospectively examine in a new cohort of 400 patients (SAGES II) enriched with MCI, the relationship between baseline CSF AD biomarkers (i.e., CSF Aβ42, t-tau, p-tau, tau/Aβ42 ratios, and neurofilament light (NFL)), sampled prior to spinal anesthesia and (1) development of post-operative delirium; and (2) cognitive decline over 18-36 months following delirium. *Sample size and statistical power considerations*. Hypothesis 1A: The first part of this compound hypothesis statement can be simplified to detecting a mean difference on the random effect (individually-varying slope) across biomarker groups. A two-group mean comparison will allow for the detection of effect sizes from d = 0.28 to 0.33 standardized units (i.e., standardized mean difference between groups) when biomarker positivity ranges from 25% to 50% (as above). These minimum detectable effect sizes are smaller than those demonstrated in SAGES I for the effect of delirium in the long-term cognitive slope, which was d = 0.48 standardized units,[22] and feasible based on effect sizes from our MSD approach.[56] Hypothesis 1B: The second component requires only that the effect of delirium and biomarker positivity not be completely confounded in predicting cognitive slope, which is highly unlikely. Thus, we are confident that we have adequate power to test this Aim.

Aim 2. To evaluate associations of delirium and CSF AD biomarkers (sampled near 4 year follow-up) in a probability sample from SAGES I (N=128): (1) 64 patients who developed delirium during the initial hospitalization; (2) 64 who did not develop delirium during initial or subsequent hospitalizations. *Statistical power and sample size considerations.* Hypothesis 2a: The analytic question can be simplified as detecting a difference on a continuous dependent variable--the individually varying time slope--across delirium and non-delirium groups. With two groups of size 64, Lehr's equation[57] informs that with assumptions of 5% and 20% type-I and type-II error rates, the minimum detectable effect size is d = 0.5 standardized units. This is close to the observed effect we have reported out to 3 years in our parent study cohort (d = 0.48),[22] and feasible to detect with our MSD approach.[56] Hypothesis 2b: Since we expect to find stronger effects with longer follow-up from this analysis, we anticipate adequate power to address this hypothesis. Utilizing a two-sample t-test for the difference in slopes across biomarker groups results in a minimal detectable effect size of d= 0.53, with an overall positivity rate of 34% (30% in 109 cognitively normal and 60% in 19 with MCI), which should be feasible to detect based on our prior studies. Hypothesis 2c: Since purely descriptive, power is not calculated.

Aim 3. To examine the relationship of novel SiMoA assays of t-tau and NFL in plasma obtained prior to surgery in SAGES I (n=148) with delirium incidence/severity and LTCD following delirium. *Sample size and statistical power considerations.* Hypothesis 3a: With a sample size of 148, the minimum detectable correlation coefficient is r = .25 (using single sample two-sided type-I error level of 5% and type-II error level of 20%). A single sample, one-sided test that the observed correlation is greater than r = .50 is associated with 5% type-I error and 80% power. Given our pilot yielding r=.61 and the literature r=0.52-0.89,[58,59] we anticipate adequate power. Hypothesis 3b: Here we assume similar rates of biomarker positivity as in Aim 2. Given a minimum detectable rate ratio of exp(4/sqrt(n)), where n is the harmonic mean of the sample with and without abnormal levels of t-tau and NFL[60] and levels of type-I and type-II error of 5% and 20%, respectively, as biomarker positivity ranges from 30 to 60%, our minimum detectable odds ratio for the association of biomarker positivity and delirium will range from 2.8 to 3.7, describing areas under the ROC curve (AUC) of 0.62 to 0.66, which are medium to large effects.[61] For delirium severity, the minimum detectable effect size varies from d = 0.50 to 0.55 as biomarker positivity ranges from 30 to 60%. These effects are feasible to detect given prior work showing $A\beta_{42}$ and tau positivity rates of 70-80% in delirium vs. 40-50% in non-delirium, and odds ratios for delirium given biomarker positivity of 2.1-3.2.[62] Hypothesis 3c: The analytic question can be simplified as detecting a difference on a continuous dependent variable--the individually varying time slope--across biomarker abnormal vs. normal groups. With a balanced

distribution of biomarker abnormality and with two groups of size 64, Lehr's equation[57] assuming 5% and 20% type-I and type-II error rates, yields a minimum detectable effect size ranging from d = 0.50 to 0.55 standardized units as biomarker positivity ranges from 30% and 60%. These effects appear feasible to detect given our prior studies and the published literature.[22,62]

Project 2

Aim 1: To use SOMAscan proteomics to discover new inflammatory proteins associated with delirium and LTCD in both plasma and CSF, and then validate these proteins in an independent sample. Sample Size Justification: In our SOMAscan pilot study of 18 matched pairs, we identified YKL40 as one of several strong biomarkers. We computed the estimate of power based upon the observed effect size of YKL40. The mean of paired differences was 125252 RFU (relative fluorescence unit), and standard deviation 64071. For clinical significance, we would like to detect an effect size as small as 1/3 this observed difference. For H1A plasma, with 60 matched pairs, we will have a power of 0.98 to observe this effect size with type-I error of 0.05. For H1A CSF, with 40 matched pairs and similar effect size threshold, the power will be 0.93. For H1B plasma, we will dichotomize the 60 delirium cases into those experiencing faster and slower rates of LTCD. Based on our published finding that patients with faster LTCD tend to have more severe delirium [63], we expect that the effect size will be larger for this outcome, and set the clinically significant threshold at one-half of the observed difference with YKL40. With these assumptions, the calculated power will be 0.99. For H1B CSF, with 20 matched pairs and similar assumptions, the power will be 0.94. Finally, for H1C, we plan to measure selected proteins in plasma and CSF across the SAGES II cohort of 460 patients. We estimate that 24%, or 96 patients will develop delirium. Based on our previous CRP POD2 findings, we assume an estimated relative risk of 2.0 for a biomarker effect for delirium incidence [64]. To account for the impact of confounders in the multivariable model, we conservatively reduce this expected odds ratio effect size to 1.6. With type-I error at 0.05, we obtain a power estimate of 0.8. Thus, we anticipate good power for the H1C validation analyses.

Aim 2: To use mass cytometry (CyTOF) to characterize circulating immune cells associated with delirium and LTCD, and then validate these findings in an independent sample. Sample Size Justification: We base the power analysis for this aim on the pilot study of 7 non-delirious patients with percent of cell types CD45+CD11c+CD14+monocytes to total number of cells, measured at PREOP and POD1. The median of the paired differences was 10.15 with range 10.07-15.19, and the mean was 10.62 with SD of 6.55. We plan to use 40 matched pairs. Since this observed mean paired difference is for the surgery effect and not the delirium effect, we use 50% of this difference as the clinically significant delirium effect. We also assume that the signal to noise ratio is similar for the delirium effect, thus SD is expected to be 6.55. With these assumptions, we obtain a power of 0.98 for H2A. For H2B, focused on LTCD, assuming a larger effect size of 60% of the observed surgical effect, we obtain a power estimate of 0.94. For H2C, the validation sample with 40 matched pairs from SAGES II, we consider a cell surface marker with a relative risk of 1.6 with delirium compared to no delirium. With type-I error at 0.05, we obtain a power estimate of 0.82.

Aim 3: To measure CRP and the Walston inflammatory index in banked plasma, and freshly collected plasma and CSF, from a probability sample of 148 SAGES I participants (SAGES I SELECT). Sample Size Justification: For Aim 3, we have 148 subjects, probability sampled for delirium (74 with and 74 without) and frequency matched for age and baseline GCP. The predictors are the measured levels of plasma inflammatory markers, and CSF inflammatory and AD biomarkers. The outcomes are delirium and LTCD. We use a relative risk of 1.5 as the threshold of clinical significance, and a type-I error of 0.05, and calculate 0.84 power for delirium and 0.80 for LTCD. Thus, we have very good power for this Aim.

Project 3

Aim 1: Determine in people with evidence of preclinical AD whether AD-related brain atrophy or network dysfunction are associated with delirium, delirium severity, or LTCD. Power Analysis: For H1a, the SAGES II MRI sample has 180 subjects. We expect 32% to have abnormal CSF AD biomarkers[65] (n=57). Based on published rates[66], we expect 36% of the AD biomarker positive sample to develop delirium. From our previous studies, the mean of AD-signature cortical thickness is 2.49mm (SD 0.14). We assume this distribution for the non-delirium patients. A conservative effect size of 5%

decrease in cortical thickness for the delirium patients yields a mean of 2.37mm. With type-I error of 0.05, we obtained a power estimate of 0.87. For H1b, the SAGES I SELECT cohort has 148 patients. With 34% assumed to have abnormal CSF AD biomarkers, this yields 44 subjects. We assume that for those without delirium and no LTCD, cortical thickness has the same distribution as stated above and that those with both delirium and LTCD would have even greater atrophy than delirium alone; thus a slightly increased but still conservative threshold of 7% decrease yields 2.37mm. The power estimate is 0.97 given this sample and relative difference of the two cortical thickness means. For H1c, the combined sample size would be 48+44=92. The estimated pairwise Spearman correlation coefficient among the MRI and CSF biomarker variables would be statistically significantly detected at the level of correlation equal to at least 0.4 from the null estimate of 0.1, and power of 0.87.

Aim 2. Determine in people without evidence of preclinical AD whether vulnerable aging-related brain atrophy or network dysfunction are associated with delirium, delirium severity, or LTCD. *Power Analysis:* We expect 68% of the 180 SAGES II subjects for H2a will have normal CSF AD biomarkers, yielding a sample size of 123. Among these, we expect 24% with delirium (30 with delirium, 93 without). From prior work,[67] we assume that the Aging-signature cortical thickness for the power analysis is 1.94mm (SD 0.18) for the non-delirium patients. A conservative effect size of 6% decrease in cortical thickness for the delirium patients yields a mean of 1.82 mm. With type-I error of 0.05, we obtained a power estimate of 0.88. For H2b, the SAGES I SELECT cohort has 148 patients. With 66% assumed to have normal CSF AD biomarkers, this yields 88 subjects. We assume that for those without delirium and no LTCD, vulnerable aging cortical thickness has the same distribution as stated above and that those with both delirium and LTCD would have even greater atrophy than delirium alone; thus a slightly increased but still conservative threshold of 7% decrease yields 1.80mm. The power estimate is 0.94 given this sample and the effect size of 7% in relative difference of the two cortical thickness means. For H2c, the combined sample size would be 123+84=207. The estimated pairwise Spearman correlation coefficient among the inflammatory biomarkers from Project 2 and the vulnerable aging MRI variables would be statistically significantly detected at the level of correlation equal to at least 0.3 from the null estimate of 0.1, and the power of 0.85. At the correlation level of 0.3, power is 0.85.

Aim 3. Investigate whether cortical atrophy due to preclinical AD or vulnerable aging is associated with postoperative LTCD or delirium with LTCD. *Power Analysis:* For H3a, using estimates from prior work,[67] the estimated mean prevalence of cortical atrophy of either preclinical AD or aging is 19.3%. We assume this estimate for the no delirium group, and a relative risk of 2.5 of having cortical atrophy for the delirium group. Of the 126 subjects, 22% (n=28) subjects had delirium, and 98 did not. Given these estimates, the power is 0.82. For H3b, among those with delirium and LTCD relative to those with no delirium, and no LTCD, we assume that the conditional probability of having LTCD given that one has delirium is 70%, and the conditional probability of having LTCD given that one does not have delirium is 20%. Given these estimates, the joint frequency distribution is N=20 for those with both conditions of delirium and LTCD, and N=78 for those with neither condition. The expected relative risk would be higher in this comparison than 2.5 in H3a. With a power of 0.81, and the given sample size of 126, we could detect a statistically significant relative risk of at least 2.75 at type-I error of 0.05.

Project 4

Aim 1a: Mount a Modified Delphi Process Involving Delirium Experts to Identify Predictors of Delirium and Cognitive Decline Following Delirium (Complicated Delirium) for Early Identification. Statistical Power Considerations*:* N/A

Aim 1b. Operationalize and Evaluate the Accuracy of the Predictors for Complicated Delirium. Statistical Power Considerations. Our pilot data suggest we might expect a correlation between model-implied cognitive slope and observed cognitive slope of about r = .54. With 375 patients in the model development sample and 185 in the model testing sample, we used Monte Carlo procedures to determine that we will have an 80% probability of obtaining a correlation between .48 and .60 in both the model development and model testing samples. Therefore, we believe this aim is adequately powered to generate a reasonable probability of identifying a replicable model.

Aim 2a: Identify and Cross-Validate Predictors of Complicated Delirium. Statistical Power

Considerations. Similar to Aim 1b, with 375 patients in the model development sample and 185 in the model testing sample, we will have an 80% probability of obtaining a correlation between 0.48 and 0.60 in both the model development and model testing samples. Therefore, we believe this aim is adequately powered to generate a reasonable probability of identifying a replicable model.
Aim 2b. Harmonize the Expert Panel-Derived Predictive Models from Aim 1 with Empirically Derived Predictors Models from Aim 2a. Statistical Power Considerations: N/A
Aim 2c. Evaluate the Correlation of the Harmonized Models with Observed Long-Term Cognitive Decline Following Delirium. Statistical Power and Sample Size Considerations. If we assume that any of the complicated delirium definitions identifies 33% of the SAGES I delirious sample (N=134) as probable members of complicated delirium group, we have an estimated prevalence of complicated delirium of (0.33 ×134/560 = 44/560 = 8%). We will be able to detect predictors where the underlying correlation (tetrachoric correlation, r_{tet}) between the predictor and complicated delirium assignment is between a small and moderate effect (minimal detectable correlation, r_{tet} = .28), a medium effect size in Cohen's effect size taxonomy. Thus, this aim is adequately powered to replicate predictors that have at least medium effect size, which should be clinically relevant and achievable.
Aim 3a. Replicate SAGES I Findings with Predictive Model in Newly Acquired SAGES II Sample (External Validation). Statistical Power and Sample Size Considerations. With 400 participants, it might be expected that power will be lower than when the sample size is 560 (Aim 2c). However, the SAGES II sample will be enriched for baseline cognitive impairment and as a consequence we can expect a higher prevalence of delirium in this subgroup (because, as described in our preliminary studies, baseline cognitive level is the most powerful and dominant risk factor for delirium). Assuming a prevalence of delirium of 24% and a prevalence of complicated delirium among the delirious of 33%, the minimum detectable association for prognostic factors is r_{tet} = .28. Thus, this aim is adequately powered to replicate predictors that have a medium to large effect size.
Aim 3b. Replicate Associations between Observed Complicated Delirium and Adverse Clinical Outcomes with Predicted Complicated Delirium in the SAGES I & II Cohorts. Statistical Power and Sample Size Considerations. This is a descriptive aim, and one that matches more closely study designs for equivalence testing rather than difference testing. We are not proposing formal equivalence testing, however, and will simply be concerned with *describing* the correspondence of parameter estimates using observed and predicted complicated delirium.

Project 5
Aim 1 To examine whether decreased network connectivity and cortical plasticity are associated with the risk of developing post-surgical delirium in a cohort of 180 SAGES II patients. Power Calculations: Below we present power and sample size assessments in standardized (SD) units as presentation of standardized effect sizes typically used in this field. In prior studies,[61] the magnitude of the difference in TMS-evoked currents between patients with AD and healthy controls was 1.5 SD. In our preliminary work supporting this application,[3] the effect size for the magnitude of differences in the iTBS plasticity measure between controls and patients with diabetes, a group with subclinical cognitive deficits who may be considered comparable in vulnerability to the patients who develop delirium in the current study, was 1.0. In our pilot study, the effect size for the main outcome measures ranged from 0.58 to 1.24. The analyses proposed here are concerned with estimating the risk of incident delirium as a function of cortical physiology markers. Within our proposed sample size of 180 patients, we anticipate that 50 patients will develop delirium and 130 will not, yielding sample cumulative incidence of 28% by the end of hospitalization. This will provide 80% power to detect an odds ratio of 1.26 or greater per half-standard deviation between-person difference in baseline markers of cortical physiology. Expressed as a mean standardized difference between delirium and no-delirium groups on a given marker, this is equivalent to an effect size of 0.47, assuming 50 participants with delirium, an effect that is both clinically meaningful and more subtle than those described above, and consistent with the range of effect sizes demonstrated in our pilot studies. We therefore anticipate adequate power for this aim.
Aim 2. To relate neurophysiology measures 2 months and 12 months after hospitalization with changes in cognitive performance and cognitive decline in SAGES II patients with versus without delirium. Power calculations: We estimate that 50 patients (25 normal, 25 MCI) will develop delirium; the comparison

sample will also contain 50 patients, an anticipated 20 with MCI. We assume a dropout rate of 5% (based on prior work), leading to a total of 95 participants with evaluable data; this will provide 80% power to detect a standardized difference of 0.62 SD between the delirious and non-delirious samples, which is consistent with the minimum observed in our pilot study. For the regression of changes in cortical physiology measures with change in the GCP, we would have greater than 80% power to detect an R^2 of 0.10 or greater. For the long-term follow-up measure, assuming cumulative loss to follow-up of 10% over one year, we anticipate observing the 18-month trajectory of change for 83 patients, providing 80% power to detect partial R^2 of 0.12 or greater in a multivariate model with adjustment for up to 12 covariates. In prior work by our group, the R^2 between TMS plasticity measures and cognition in patients with Diabetes was 0.30.[3] In other pilot work, we found that the R^2 between EEG relative power and measures of attention and working memory in patients with AD was 0.42. Together, these results suggest we will have adequate power for our Aims.
Aim 3. To characterize differences in neurophysiology metrics as a function of history of delirium and cognitive decline, and to correlate these measures with long term cognitive outcomes. Power Calculations: The sample (N=128) is sufficient to provide 80% power to detect a between-group difference of 0.53 standard deviation (SD) units. Thus, we will have sufficient power to detect effects that are more subtle than observed in our pilot analyses, and that have been associated with clinically meaningful relationships with cognition in our preliminary work and in work by other groups.[2,3,14,98]

B.2. Data Analysis

A brief overview of analytic techniques and expertise relevant for each Project's Specific Aims is provided in Table 6. The primary statistical packages used will be Stata (StataCorp. College Station, TX: StataCorp LLC) and R (R Core Team. 2013. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org).

The Data Management and Statistical Analysis (DMSA) Core will provide data management services, statistical analyses and collaboration to the Projects. The primary responsibilities of the DMSA Core will be to develop information systems and software for tracking participants, receiving, management and cleaning of data, generation of data sets, and performing statistical analyses tied to the specific aims of the projects. The specific tasks for the Core are: 1) To generate information systems and software for tracking participants and flow of data; 2) To assemble and manage a database of longitudinal information collected from participants during screening, follow up and the conduct of individual Projects; 3) To collaborate with the Project Leaders in all phases of study design including determining the sampling scheme for the study, addressing non-response and providing real-time selection, enrollment, and matching as needed for substudies; 4) To provide methodologic and analytic expertise to Project Investigators including study design and conduct, development and implementation of data analytical plans, interpretation of statistical results and manuscript preparation; 5) To lead analyses of variables collected by the Epidemiology Core and individual research projects; and 6) To develop composites for cognition and functional outcomes.

Day-to-day and operational leadership will be provided by Thomas Travison, PhD, located at the Marcus Institute for Aging Research at Hebrew SeniorLife (HSL). Other Co-Investigators include Richard Jones, Departments of Psychiatry and Human Behavior and Neurology, Brown University Warren Alpert Medical, and Long Ngo, Department of Medicine, Beth Israel Deaconess Medical Center, Boston, MA. Dr. Dickerson, located in the Massachusetts General Hospital (MGH) Martinos Center for Biomedical Imaging in Charlestown, MA (MGH-East), will lead the analysis of all MRI and PET scans; no patient enrollment or acquisition of MRI or PET scans will be conducted at MGH for this study.

Biomarker data (Blood, CSF, MRI, PET and TMS) will be stored at BIDMC, all other data will be stored and at the Hebrew Rehabilitation Center. MRI and PET data will also be securely shared between Dr. Alsop's lab at BIDMC, Michele Cavallari at BWH, and Dr. Dickerson's lab at MGH as they have done previously for MRI data collected in the original SAGES P01. Drs. Dickerson and Cavallari will perform

MRI and PET analysis at BWH and MGH. Blood and CSF will be securely shared between Dr. Libermann's lab at BIDMC and Dr. Steven Arnold at MGH who will performs some of the Project 1 analysis.

B.2.1. Methodologic concerns

Retest effects. We have developed estimates of learning effects for composite outcomes including measures of General Cognitive Performance in this population, used to measure changes in cognitive status with time, which are crucial to determining the influence of delirium and magnitude of within-person changes in cognitive functioning.[68,69] Models will acknowledge the potential for retest effects and control for these effects in analyses.

Covariates will be chosen for the combination of clinical or theoretical significance and potential to act as confounders or effect modifiers. Specific attention will be given to factors likely to affect the influence of delirium on downstream outcomes in longitudinal analyses, such as anesthesia and surgery types or treatment with antipsychotic medications. We will examine these factors with particular attention to the temporal relation between their introduction during or after the index hospitalization. Following exploratory assessments, they will be included in models either as time-independent or time-dependent covariates or interaction terms as appropriate. In addition, we will follow our routine practice of conducting sensitivity analyses to establish the practical robustness of conclusions; for instance, excluding individuals with specific risk factors to assess robustness of results in specific populations and health conditions.

Missing data. We will take a multi-pronged approach to ensuring that the potential for bias due to missingness is minimized to the greatest degree possible. First, we will utilize the cohort maintenance, data completeness and quality control procedures refined during the planning and execution of SAGES I. These design features have proven highly successful, such that repeated measurements on outcomes and covariates achieved in excess of 99% completeness on nearly all important factors. It is inevitable, nevertheless, that some portion of proposed measurements will go missing. To accommodate attrition and other sources of loss, where appropriate, we will utilize the method of multiple imputation by chained equations to generate plausible values for both outcomes and covariates simultaneously. Importantly, this method is flexible in that it allows for plausible association between covariates of various distributions – i.e. binomial or multinomial distributions for discrete variables – and also can accommodate the clustering of measurements that will manifest through repeated measures at the level of the participant. At least 50 replicates of the analysis file will be generated for inclusion in all analyses conducted for publication. Assurance of convergence will be obtained using graphical methods and the number of imputations and iterations increased as necessary. The starting seed for this procedure will be generated at random and recorded, so that the data generation procedure is fully replicable and consistent with our reproducible approach. We have previously used these methods successfully in SAGES I. In our experience these procedures are generally sufficient to achieve consistency with the assumption that missingness has occurred at random. As a check on our assumptions, we will employ multiple other methods to challenge these assumptions, including multiple sensitivity analyses; list-wise complete analyses; and pattern mixture models of outcomes and the missing data process simultaneously.

B.2.2 Attention to age and sex as biological variables

The analytic approaches derived for each project's aims and hypotheses are described in detail within each project proposal. The outcomes considered are intrinsically aging-related, and our samples are of older individuals; age is also explicitly considered in the construction of matched cohorts and in analytic plans. For each project, the potential influence of sex will be taken into account in stratified analysis and statistical modeling, and results reported by sex in accordance with NIH guidance (NOT-OD-15-102).

Table 6. Planned analyses and design considerations by Specific Aim, with preliminary information supporting hypotheses and power computations.

Project	Specific aim	Analytic approach
1	1: CSF AD biomarkers and incident delirium 2: Biomarkers and history of delirium 3: Plasma t-tau & NFL, incident delirium, and LTCD	Predictive modeling; logistic regression; Receiver-operator curve (ROC) analysis; piecewise linear mixed effect modeling
2	1: Inflammatory proteins (by SOMAscan), delirium, and LTCD 2: Circulating immune cells (by CyTOF), delirium, and LTCD 3: Inflammatory index and incident delirium	Nonparametric inference; support vector machines; ROC; conditional logistic and log-logistic regression; supervised and unsupervised learning; CyTOF; SOMAscan; model selection Log-logistic regression
3	1: Preclinical AD brain vulnerability, delirium, and LTCD 2: Non-AD aging brain vulnerability, delirium and LTCD 3: Longitudinal cortical atrophy, delirium, and LTCD	Logistic and Log-logistic regression. Distributed lag models, repeated measures analysis, generalized linear mixed effects models
4	1: Predictors of delirium and long-term cognitive decline (complicated delirium) 2: Development of predictive models for delirium and cognitive decline (complicated delirium) 3: Validation of prediction model	Delphi process Machine learning, lasso Linear mixed effects modeling
5	1: Cortical plasticity, network connectivity, and delirium 2: Delirium, cortical physiology and cognitive decline (18 months) 3: Delirium, neurophysiology, and long term cognitive decline	Predictive modeling; logistic regression; ROC analysis; model selection by penalized likelihood Mixed effects regression; hierarchical modeling

B.2.3. Data Analysis by Project

Project 1

Aim 1 Analyses: Aim 1 proposes to evaluate the relationship between baseline CSF AD biomarkers (CSF $A\beta_{42/40}$; t-tau; p-tau, tau/Aβ ratios, and NFL) and (1) the development of post-operative delirium; and (2) the pace of cognitive decline over 18-36 months following delirium. Hypothesis 1a posits that patients with abnormal CSF AD biomarker levels at baseline will have an increased risk of delirium following surgery, compared to patients with normal biomarker levels. This aim will be tested in the SAGES II cohort (N=460) with logistic regression models, with the occurrence of delirium as the outcome and biomarker positivity (abnormal level) as the main exposure variable. Control variables will include age, gender, nonwhite race, education, Charlson score, depression, impairment in any instrumental activity of daily living, surgery type, IQCODE, and baseline GCP score. To avoid over-controlling for variables that might be intermediaries between delirium and its effect on cognitive trajectory,[70,71] we will not adjust for hospital-related factors. Missing data will be addressed using multiple imputation (50 imputations) and the chained equations approach.[72] Hypothesis 1b is a compound hypothesis statement that requires testing of 2 sub-hypotheses: (1) patients with abnormal CSF AD biomarker levels at baseline will have an increased risk for cognitive decline compared to those with normal biomarker levels, (2) the greatest decline will be observed in those with delirium and abnormal biomarker levels. This hypothesis will be tested with a generalized linear mixed effect modeling framework with a piecewise time basis describing the course of cognitive change by 3 time-periods (acute period: pre-operative to 1 month; recovery period: 1-2 months; and long-term trajectory: >2-36 months), as we have done previously.[22,26,63] The dependent variable is the GCP composite.[55,73] The first part of this hypothesis, that biomarker positivity is related to cognitive decline, will be tested by regressing repeatedly observed cognition on time, biomarker positivity, and their interaction (with pre-specified control variables as described above). The hypothesis that biomarker positivity is associated with faster long-term decline is evaluated with the interaction of biomarker positivity and the long-term time trend. The second part of this hypothesis (the group with delirium and biomarker

positivity has the steepest slope) requires that in addition to biomarker positivity being related to slope, delirium must be related to slope (as shown previously)[22] and that the two effects are at least partially independent (i.e., the effect of biomarker positivity is only partially mediated by delirium).
Aim 2 Analyses: The goal of Aim 2 is to evaluate associations of delirium and CSF or PET AD biomarkers sampled near 4 year follow-up among delirium cases and controls (n=74 each group). Control variables will include age, gender, race, education, Charlson, depression, functional impairment, surgery type, IQCODE, and baseline GCP score. Missing data will be handled using multiple imputation (50 imputations) and the chained equations approach.[72] Hypothesis 2a. This hypothesis will be evaluated using generalized linear mixed effect modeling as described above for H1b, applying an approach similar to what we have used in our prior publications.[22] The outcome is the composite measure, GCP,[55,73] and the main exposures are delirium group, time, and their interaction. A faster rate of LTCD among those with delirium is demonstrated by a significant interaction term for delirium group by long-term time trend. Hypothesis 2b. This hypothesis will be tested with generalized linear effects models as in H2a with biomarker positivity as the primary exposure, rather than delirium. We assume higher rates of biomarker positivity than in Aim 1, since 50% will have delirium and the overall group will have higher rates of cognitive impairment by frequency matching.
Aim 3 Analyses: Hypothesis 3a posits that CSF and plasma t-tau and NFL will have Pearson correlation of at least r = .50. This analysis will be approached with correlational methods. Variables will be evaluated and transformed as appropriate prior to estimating a correlation coefficient. Hypothesis 3b seeks to test if abnormal levels of t-tau and NFL from stored plasma at baseline will be associated with a higher risk of developing delirium and greater delirium severity. This will be tested with logistic regression (delirium occurrence) and linear regression (delirium severity). The control variables will be the same as above. Missing data will be addressed using multiple imputation (50 imputations) and the chained equations approach.[72] Hypothesis 3c seeks to test if patients with abnormal levels of t-tau and NFL from stored plasma at baseline will have a faster rate of LTCD (over a minimum of 8 years) compared to those without abnormal biomarker levels. This hypothesis will be evaluated using generalized linear mixed effect modeling as described above for H1b and as previously conducted in our prior publications.[22] The outcome is a composite measure of cognitive functioning[55,73] and the main exposures are biomarker abnormality, time and their interaction. A faster rate of LTCD among those with delirium is revealed by a significant interaction between delirium group and the long-term time trend. Hypothesis 2c. The analytic approach is similar to H1b, and posits that the two effects are at least partially independent in the generalized linear effects models (i.e., the effect of biomarker positivity is only partially mediated by delirium). Since purely descriptive, power is not calculated.

Project 2

Aim 1 Analysis: We will perform SOMAscan analysis on 50μl of plasma or CSF according to the standard protocol from SomaLogic at our laboratory. Due to the tight CV of ~5%, samples are run as singlets as is standard for SOMAscan. Calibration is accomplished using 7 replicates of a pooled plasma or CSF sample per run of 24 test samples. The final readout is directly proportional to the amount of target protein in the initial sample. Protein data from the SOMAscan analysis will be normalized using a singular value decomposition based method [74,75]. Proteins that are differentially expressed between those with and without delirium and between those slower and faster rates of LTCD will be identified. Bioinformatics and systems biology analyses will be led by Drs. Libermann and Otu, who have published many papers on this subject [76-96].
Aim 2 Analysis: CyTOF single cell analysis of cryopreserved PBMCs will be performed at the Harvard/Dana Farber Cancer Center (HDFCC) Flow Cytometry core on the Helios instrument, according to established protocols. Each sample will be barcoded for multiplexing using Cell-ID 20-Plex Pd Barcoding Kit (Fluidigm) according to the manufacturer's protocol [97-99] enabling simultaneous CyTOF analysis of up to 20 samples in a single run. We will use a panel of metal-tagged antibodies targeting 29 cell surface proteins for all major immune cell types [100] and up to 16 phosphoepitopes associated with key intracellular signaling proteins described by Gaudilliere et al. [101] for clinical

recovery from surgery. Like in our pilot data, we will define major immune cell subpopulations (e.g. neutrophils, monocytes, CD4 T cells, CD8 T cells, NK, DCs) and their activation/differentiation status using manual gating and unsupervised learning tools available through Cytobank (SPADE, viSNE, CITRUS [102,103]). Major PBMC subsets will be identified by 2-dimensional gating as described in our pilot followed by viSNE analysis to visualize high-dimensional cytometry data on a 2-dimensional map at single-cell resolution. Based on expression of well-defined cell surface markers, immune cells can neatly be subdivided into distinct subsets as demonstrated in our pilot. To further characterize these subsets, we will analyze expression of several important predefined functional proteins in each major immune cell population, particularly proteins linked to signaling pathways important in activation of inflammatory cells (NF-κB, phosphoStat3, etc.). CyTOF combined with machine learning techniques such as viSNE allows a comprehensive and simultaneous assessment of expression of up to 45 phenotypic and functional markers in all major immune cell subsets at single-cell resolution, and can describe the diversity of immune cell subsets as well as define their state of activation, frequency, abundance and ratios with precision. We also plan to correlate CyTOF data with SOMAscan data (Aim 1) by evaluating whether our delirium or LTCD SOMAscan protein signature is enriched for protein expression modules in immune cell subtypes identified by CyTOF using tools such as CIBERSORT or CellMix [104,105].

Aim 3 Analysis: For H3A, the analytic strategy will be similar to that described in Aim 1 with measured levels of inflammatory proteins (and the inflammatory index) being the independent variables, and delirium and LTCD being the outcomes. We will examine plasma markers at PREOP, POD2, and LTFU, and CSF at one time point, LTFU. We anticipate seeing the lowest levels of inflammatory markers in the group without delirium and slow rates of LTCD, and the highest levels in those with delirium and faster rates of LTCD. We will perform multivariable analyses using generalized linear models with a log link for delirium incidence, and identity link for continuous LTCD. In H3B, to examine interactions with CSF AD biomarkers, we will select the most predictive inflammatory (IL-6, CRP, sTNFR1, or the inflammatory index) and AD biomarker ($A\beta_{42}$, t-tau, p-tau, or p-tau/$A\beta_{42}$ ratio) and put both into the generalized linear model together. We expect significant independent effects of both inflammatory markers and CSF AD biomarkers on delirium and LTCD.

Project 3

Aim 1 Analysis: For this aim, we will restrict the sample to only those subjects who have abnormal CSF or PET AD biomarkers. For H1a, we will use 2 GLM models, one for delirium incidence (log link), and one for delirium severity (identity link). The independent variables for these models are the variables from domain 2 (AD cortical thickness, memory network connectivity, hippocampal hyperactivity). Included in these GLM models are also potential confounders of demographic and baseline clinical variables. The estimated coefficients and 95% confidence intervals will allow us to assess the independent adjusted effects of this domain on delirium outcomes in this cohort of patients with abnormal preclinical AD biomarkers. We will estimate goodness-of-fit of these models by checking model residuals, estimates of the area under the ROC curve, and adjusted model R^2 values. For H1b, the four variables from domain 2 will be treated as independent variables in a Generalized Linear Mixed model with identity link, and the dependent variables in this model will be the longitudinal GCP values. In this model, delirium status, and the interaction between GCP and delirium status are also included. This model allows the estimation of the association (slope) between domain 2 variables, and GCP-based long term cognitive decline modified by delirium status. For H1c, we will report the correlation matrix using the nonparametric Spearman correlation coefficients for pairwise correlation among the variables of domains 2 and 4 (Aβ, p-tau, t-tau, NFL).

Aim 2 Analysis and Statistical Power: For this aim, we will restrict the sample to only those subjects who have normal CSF or PET AD biomarkers. The statistical approach for H2a and H2b is the same as described for Aim 1 but the independent variables for these models are the variables from domain 4 (vulnerable aging cortical thickness, frontoparietal functional and structural connectivity). For H2c, we will report the correlation matrix using the nonparametric Spearman correlation coefficients for pairwise correlation among domain 4 variables and the inflammatory biomarker variables found in Project 2.

Aim 3 Analysis and Statistical Power: We will estimate the association between cortical atrophy due to either preclinical AD or vulnerable aging and LTCD (i.e. longitudinally measured GCP). We will treat atrophy as a binary outcome variable in the multivariable GLM model with log link, and repeated GCP measurements as independent variables. We will estimate the slope of GCP at each follow-up time point. In the event that we have high correlation among the GCP measurements, we will make use of the distributed lag model to mitigate the collinearity problem. For H3b, we will add to this GLM model from H3a, delirium status (binary), and the interaction between GCP and delirium status, and will obtain GCP slope conditioning on delirium status.

Project 4

Aim 1a. Analysis Approach. Data to address the Specific Aim 1 qualitative analyses will derive from a modified Delphi process, executed using the Delphi Decision Aid platform developed at the University of Pennsylvania, and will follow the RAND ExpertLens protocol for modified Delphi process, which includes 4 rounds (rounds 0-3) based on a Grounded Theory[106] approach to theme elicitation. The web-based system minimizes demand on participant experts and the research project.

Aim 1b. Analysis Approach. We will use a variety of different modeling approaches with (i) the occurrence of delirium, and (ii) the slope of long-term cognitive performance observed in the SAGES I sample as the outcome (N=560), and expert-panel identified predictors of complicated delirium as predictors. We will use a machine learning engine[107] available in the R environment (R Foundation, Vienna Austria) as well as the lasso.[108] Machine learning describes a variety of approaches to derive predictive models, and specific frameworks we will use include random forests, support vector machines, neural networks, and generalized linear regression. The usual practice in machine learning is to evaluate multiple platforms in a training or derivation portion of the data, and then validate the optimal prediction algorithm in validation partition. Each platform also incorporates random internal cross-validation, drawing randomly from among the observations and the predictors in the model to derive estimates of variable importance (based on predictive or explanatory power when a variable is not included in a prediction model). The lasso invokes a constrained linear regression model that shrinks estimates of coefficients for unimportant variables towards the null.[108]

The rationale for using machine learning, and the lasso, is that the most commonly used alternative -- stepwise variable selection using regression models[109] -- have long been recognized to have validity issues, including that the models are overly optimistic, predictors do not replicate in other data sets, and the interpretation of effects is clouded due to multicollinearity.[110] Machine learning attempts to overcome this through the use of resampling strategies (analogous to the bootstrap, but including random sampling among predictors and observations over an arbitrarily large number of resampling replicates) and flexibility in functional forms and inclusions of higher order interactions. The lasso is motivated by a similar goal but invokes a fundamentally different strategy. The lasso works within a generalized linear regression framework by applying a constraint to the value of the sum of the absolute value of the coefficients, and effectively shrinks estimates of unimportant predictors towards 0. The lasso is well suited to identifying important predictors when a small number of truly important predictors are available within a large sample of noisy predictors. Both machine learning and the lasso are suited for situations where the ratio of predictors is high relative to the number of observations, as is the case in our study. We will use a 67% sample of our observations for model development and the set-aside 33% sample will be used for model testing. The model development sample will include 90 delirious and 285 non-delirious, and the remaining 44 delirious and 141 non-delirious SAGES I participants will constitute the model testing sample. Models will be characterized using data from the testing sample in terms of accuracy, focusing on the root mean squared error (RMSE), but also looking at the overall (pseudo) *r*-squared. The RMSE, a measure of predictive accuracy, is the square root of the mean difference between model-implied (given predictors) and slope. Model fit and replicability are our measures of success for this Aim.

Aim 2a. Analysis Approach. As with Aim 1b we will use machine learning and lasso-constrained linear regression models as described for Aim 1a with (i) delirium and (ii) the slope of long-term cognitive decline observed in the SAGES I sample as the outcome, and proposed factors that predict complicated delirium as independent variables. Models will be characterized in terms of accuracy

(RMSE), and *r*-squared.

Aim 2b. Analysis Approach. Aim 2b is a task that will involve the local investigator committee. This investigator group will review the expert panel-derived predictors of *complicated delirium*, and the Aim 2 empirically-derived predictors of *complicated delirium.* The main goal of this aim is to derive a list of predictors that are harmonized, highly overlapping concepts that can be integrated into a single predictive model yet maintains the intent of the expert panel and the accuracy of the empirically derived measure. The investigator group will review predictors identified by the expert panel, and assign them to our grid that distinguishes domain and time period relative to surgery; expand the domains as necessary; identify identical or overlapping concepts in our locally defined predictors (Aim 1b); and define new predictors or modify existing predictors to match concepts suggested by the expert panel. The Project Leader (Jones) will prepare a draft document that cross walks the expert panel (Aim 1a) and locally defined (Aim 1b) predictors, and presents harmonization suggestions to the local investigator group. Feedback will then be incorporated into a revision. The harmonization, presentation and review, and revision process will continue until an acceptable cross walk and new predictor definition document has been developed

Aim 2c. Analytic Approach*.* This aim has three analytic goals. The first is defining a threshold using our long-term follow-up data from SAGES I that separates *accelerated* decline from decline that is not accelerated. The second analytic goal is to identify the predictors of membership in the group that is experiencing accelerated cognitive decline. The predictors in this model will include the most important (in terms of accounting for adjusted *r*-squared of optimal predictive model) predictors identified in Aim 2b. Post-operative delirium will be included as an intermediate outcome, which is regressed on the predictors but also itself predicts cognitive decline. The third analytic goal is the investigation of any statistical interaction between the predictors and post-operative delirium in predicting membership in the accelerated decline group. We will address all three analytic goals with growth mixture modeling, a statistical approach to longitudinal data analysis with which we have experience.[111] The model can be thought of as a generalized linear mixed effects model for repeated measures data (GCP score) upon which a latent class analysis model is superimposed. The latent classes are composed of individuals who belong to modeled population sub-groups with fundamentally different underlying rates of cognitive decline (e.g., one accelerated, the other not). We will separately consider predictors drawn from preoperative, perioperative, and postoperative time periods, and we will subject our inferences to bootstrap replication to protect against making inferences based on chance.

Aim 3a: Analysis Approach*.* The analysis approach to the replication analyses in the SAGES II cohort will follow exactly as described above for Aim 2c: growth mixture modeling of the joint distribution of postoperative delirium and long-term cognitive decline. We will compare results to logistic regression models with the binary complicated delirium used as the outcome. After a final fitted model is derived, we will use the predictors and produce for each person an “indicator” if he/she is or is not expected to experience complicated delirium. We will compare assignment based on the modeled-implied complicated delirium to observed complicated delirium and summarize in terms of sensitivity, specificity, predictive values, and overall agreement. We believe a good model, in general, will have at least 90% sensitivity and 90% specificity. Post hoc, we will explore ways to improve the scoring (assignment to complicated delirium or no) groups to maximize sensitivity or maximize specificity, and include these improvement options as features in a web-based tool.

Aim 3b: Analysis Approach. The approach to analysis will be to conduct bi-variable regression models (parametric survival models for death, general linear models with a gamma error distribution for costs) with a single outcome (e.g., death or healthcare costs) and a single predictor (observed complicated delirium or predicted complicated delirium). We will not adjust for covariates, as these covariates may completely represent the predicted complicated delirium indicator. If we obtain exactly the same parameter estimate, then we have evidence that we have perfectly represented observed complicated delirium with predicted complicated delirium. We do not expect this to be the case, however. As a means of more fully describing the extent to which the observed associations are reproduced by predicted complicated delirium, we will draw 1,001 bootstrap samples and estimate the model parameters describing the association of the predictor (observed or predicted complicated delirium)

and the outcome (death, healthcare costs). We have set as a threshold for success observing a correlation of 0.86 between parameter estimates for observed and predicted complicated delirium. This implies that the two share 75% of their variance. As with our other aims, we will separately characterize the predictive validity (correlation of effects with those of "true" complicated delirium) for models derived from pre-, peri- and postoperative variables, and their combination.

Project 5

Aim 1 Analyses: H1A asserts that individuals with abnormal cortical physiology are at elevated risk of incident delirium during hospitalization. To examine this, we will develop a predictive model for postoperative delirium taking into account TMS-EEG-EMG measures and important covariates. Multiple logistic regression will be used to establish the independent contribution of each factor. We will also utilize ROC analysis to assess the predictive validity of these measures for delirium across the spectrum of possible TMS-EEG-EMG measures. We will use a combination of clinical judgment, analysis of deviance and likelihood ratio statistics to develop a parsimonious model for delirium risk incorporating the TMS-EEG-EMG measures, while removing those effects that appear redundant. We will use 10-fold cross validation and ridge regression to protect against overfitting in the course of model selection. To address hypothesis H1B (patients with greater baseline abnormalities have more severe delirium episodes and greater cognitive decline), we will take a parallel approach examining the association between baseline cortical physiology (measured using TMS-EEG), delirium severity (as measured via the peak CAM-S),[96] and cognitive status one month after the delirium episode (as measured via the General Cognitive Performance Score)[87,88] using multiple regression methods. We will fit an overall model to data from the entire sample, and stratify by MCI vs. normal baseline cognition. We will use linear models to describe baseline associations between iTBS modulation and AD biomarkers and inflammation (H1C), and between TMS-EEG connectivity measures and MRI connectivity profiles (H1D). Variables chosen for control in multivariable models will be chosen for the combination of clinical significance and the potential to act as statistical confounders; these include measures such as age, baseline cognitive function and scalp-cortex distance. To accommodate missing values, which we anticipate to be minimal, we will utilize the method of multiple imputation by chained equations[97] to generate plausible values for both outcomes and covariates simultaneously.

Aim 2 Analyses: The primary goal of this analysis is to assess the degree to which patients with delirium (approximately 50 anticipated) have a decrease in rs-EEG high-frequency power and abnormal TMS measures of reactivity, connectivity and plasticity. We further seek to quantify the degree to which these cortical physiology changes are associated with the magnitude of perturbations in cognitive functioning attending the delirium episode (as indexed by the GCP score 1 month after surgery), as well as subsequent cognitive decline 18 months after hospitalization. In order to establish that changes in cortical physiology may be associated with delirium rather than hospitalization or surgery, we will assess post-operative changes in the same measures in a comparison sample of 50 participants without delirium. We will identify changes in cortical physiology measures using a mixed effects linear regression model, with stimulation session as the within-subject factor and occurrence of delirium as the second factor. Subsequently, to evaluate whether patients with greater short-term cognitive decline have more severe abnormalities in cortical physiology, cognitive status one month after the delirium episode (as measured via the GCP Score) will be correlated with the changes in cortical physiology measures via multiple regression methods. In conjunction with data core statisticians, we will then apply mixed effects regression models to characterize the adjusted mean GCP scores and the trajectory of scores over time, with the prediction that cortical physiology measured at 2 months post-hospitalization will predict cognitive outcomes 18 months after hospitalization.

Aim 3 Analyses: The primary goal of this analysis will be to examine how the presence or absence of a history of delirium relates to subsequent cortical physiology measures at around 4 years after the index hospitalization, and the degree to which these factors predict subsequent long-term cognitive decline. We hypothesize that SAGES I patients with a history of delirium will have abnormalities on the cortical physiology measures (baseline power and EEG connectivity, TMS reactivity, connectivity AND plasticity) relative to those without. We also hypothesize that cortical physiology measures will be correlated with the magnitude of cognitive decline since the index hospitalization. These hypotheses

will be tested with general linear mixed-effects models controlling for differences in cortical physiology markers (transformed to approximate normality, if indicated). Because patients in SAGES I will have their physiology measurements measured at different timepoints following delirium, we will incorporate time since the index hospitalization as a covariate in the model in order to assess dynamic aspects of the association between delirium and downstream changes in cortical physiology. Participants will be matched by gender and age between the groups with and without delirium. Additional statistical adjustment will be made for index surgical procedure, baseline cognitive function, comorbidity, and vascular risk. Patients will be followed for a minimum of 8 years after initial enrollment in SAGES I (and 4 years after the TMS sessions), and cognitive function and progression to dementia tracked by the Core B team. We will use the same modeling approach to identify the degree of association between neurophysiology metrics and the magnitude of subsequent cognitive decline.

C. Subject Selection

C.1.This study involves two different prospective observational cohorts: Successful Aging after Elective Surgery I (SAGES I) and SAGES II.

C.1.1. SAGES I cohorts

SAGES I Cohort (N=560). Eligibility criteria for this cohort, assembled during the first cycle of the Program Project grant (PPG) is described under A.3.1.

SAGES I SELECT Sub-group (N=148). General eligibility criteria for this subset of selected participants from SAGES I are described under A.3.2. Eligible participants from this subgroup will undergo specialized procedures including, lumbar puncture (LP), magnetic resonance imaging (MRI) scans and electroencephalogram (EEG) with non-invasive transcranial magnetic stimulation **(**TMS**).** Each of these procedures has specialized exclusion criteria and prior to each of these procedures subjects must complete a screening form designed to identify any contraindications and to assure safety. Before each procedure the respective teams and study doctors will review a safety protocol that includes the results of the screening procedures. MRI and TMS screening takes place during the in-person visit, in case of questions during a phone call of the MRI and TMS teams with the potential participant, and then again at the MRI and TMS visits. LP eligibility screening occurs during medical chart review, telephone screening, in-person visit, during potential adjudication through a study clinician for unclear eligibility, and during the LP visit through the study doctor. The SAGES I Select study screening forms details the timepoints of these exclusions. If a participant does not seem to remember her/his medical history well, we will ask for permission to reach out to a proxy using the Telephone Proxy Screener.

Additional exclusion criteria for Lumbar Puncture for the SAGES I SELECT subgroup: The SAGES I SELECT subgroup will undergo additional procedures, including a lumbar puncture. To verify eligibility, participants will undergo a medical history and medication review initially conducted by a research assistant, followed by adjudication by a clinician for unclear exclusion criteria. Finally, the study Neurologist will verify eligibility at the time of the LP visit.
The additional hard exclusion criteria for lumbar puncture include:

- Most recent platelet count within past 6 months < 50,000/μL
- Most recent INR within past 6 months > 1.4
- Lumbar surgery with hardware
- Acute myocardial infarction or heart attack within 6 months
- New or unstable angina (chest pain) within 6 months
- Stroke or intracranial bleed within 6 months
- Ventriculo-peritoneal (VP) shunt for normal pressure hydrocephalus
- Currently taking anticoagulants and antiplatelet medication
- Severe shortness of breath at rest or lying down
- Continuous use of oxygen at home

- Totally blind
- Mass in the brain

Soft exclusion criteria will be adjudicated by a clinician and include:

- Evidence of terminal illness (survival < 6 months)
- New onset of headaches within 6 months that awaken patient from sleep, or that are worse with coughing, sneezing, or bending over
- New onset of altered mental status within 6 months
- New onset of seizures within 6 months
- Evidence of focal neurological deficits (e.g. limb weakness hemiparesis, gaze palsy, visual field cut, pronator drift)
- Evidence of immunocompromised state (e.g. neutropenia/agranulocytosis, current chemotherapy, current immunosuppressive therapy or prednisone treatment, HIV/AIDS)
- Prior Lumbar Puncture within 12 months
- Hospitalization for possible acute myocardial infarction or heart attack within 6 months but no MD diagnosis confirmation
- Hospitalization for possible new or unstable angina (chest pain) within 6 months but no MD diagnosis confirmation
- Hospitalization for possible stroke of intracranial bleed within 6 months but no MD diagnosis confirmation
- Easy bruising or bleeding
- Active skin infections of lower back
- Taking anti-inflammatories (not including baby aspirin (81mg per day)
- Allergies to latex, tape, novocaine, lidocaine, or any other contact allergies
- Medication allergies
- BMI >>35
- History of back pain
- History of leg pain

Additional exclusion criteria for MRI and TMS-EEG include:
Hard exclusion criteria for MRI and TMS-EEG include:

- History of prior neurosurgery
- History of seizures or diagnosis of epilepsy, with the exception of a single seizure of benign etiology (e.g. febrile seizure) in the judgment of the investigator;
- Metal implants or devices such as a cardiac pacemaker or intracardiac lines, medication pump, any bio or neurostimulator, external fixation devices or bone growth stimulators
- Presence of any shrapnel or any metal fragment including bullet fragments anywhere in the body

Exclusion criteria for EEG only

- None

Soft exclusion criteria will be adjudicated by MRI and TMS teams and include:

- Colonoscopy, endoscopy or interventional procedure within the past 30 days
- Ingestion of core temperature sensor or small pill camera within the past 30 days
- Metallic heart valves or any stents
- History of ear surgery or ear implant or prosthesis; cochlear implant
- History of eye surgery (cataracts, etc.) if before 1994 or eye implant or prosthesis (eye springs or wire, etc.)
- Metal injury to the eye
- Penile implant
- Any aneurysm clips

- History of gastric bypass surgery after 2015
- Presence of any shunts (spinal, ventricular, peritoneal, subgaleal, etc.)
- Neurological implants
- Implantable defibrillator or implanted monitoring device
- Indwelling port, catheter, or feeding tube
- Tissue expanders or implants
- Implanted pump (insulin, pain medicine, chemotherapy, etc.)
- Dental work
- History of a fainting spell
- History of a head trauma that was diagnosed as a concussion or was associated with loss of consciousness
- Currently has hearing problems or ringing in the ears
- Problems with previous TMS procedures
- Patient is left-handed

Additional exclusion criteria for PET include:

- Prior scan with radioactive agents either for clinical or research purposes within 12 months, such that the total research-related radiation dose to the participant would exceed the limits set forth in the US Code of Federal Regulations (CFR) 21, Section 361.1.

C.1.2. SAGES II cohorts

SAGES II Cohort (N=460): The inclusion criteria for this newly enrolled surgical patients are outlined in section A.4 above. This group will undergo lumbar puncture during anesthesia if scheduled for spinal anesthesia, which has the same exclusion criteria as for lumbar puncture for the SAGES I SELECT subsample described in section C.1.1 above. If they receive PET because we could not obtain CSF, the same exclusion criteria for PET scans as outlined above in section C.1.1. will apply.

SAGES II Sub-group (N=180). This subgroup will undergo MRI and TMS-EEG with the same exclusion criteria and screening procedures as describe for the SAGES II specialized procedures described in section C.1.1. above

C.3. Gender and minority representation

In the SAGES I cohort, participants are on average 77 years old, 58% are female and 92% are white, 6% African American, 1% Asian, and 1% other. Nine participants (2%) are Hispanic. For the SAGES II study (N=460), we present our targeted enrollment inclusion in the table 7 below. We estimate proportionate representation for the SAGES II cohort. However, given our increased diversity enrollment efforts described below, we anticipate we will be able to increase enrollment for African Americans to at least 9.5%, Asian to at least 4% to 5%, and Native Hawaiian/Other Pacific Islander to at least 0.5% for SAGES II, for an overall minority enrollment rate of 15%. It is important to note that the diversity characteristics of our SAGES I sample (92% white) are representative of the greater Boston metropolitan area in the over 65 age group during the enrollment period (U.S. Census Bureau, 2009-2013 5-year American Community Survey).

We will make every effort to maximize the inclusion in our sample of subjects who are members of one or more minority groups. We will use several successful strategies for the recruitment and retention of minority subjects, including enlisting minority research staff to work with minority subjects and their families, and will preferentially enroll minority participants wherever possible. We will train our staff to be sensitive to issues in minority recruitment using videotapes and materials developed for the *Program for Cultural Competence in Research* by the Harvard Catalyst Program and Dr. Joan Reede, Dean for Diversity and Community Partnership, as well as a video developed by Dr. Ann Kolanowski

(Penn State) and Dr. Keith Whitfield (Duke University) on "How to recruit minority subjects." Strategies to successfully engage and enroll minority populations will be discussed at the weekly field team meetings. Importantly, minority representation will be monitored throughout the study. If early enrollment falls below targets, we will implement corrective action. With these strategies, we anticipate minority representation in the Program Project renewal will be greater than the minority representation in the relevant age groups in the greater Boston metropolitan area.

Children will not be included in the sample, since the study is designed to examine cognitive outcomes in older adults. In addition, because of our targeted elderly age group, no women of childbearing age will be included in the sample

Although we have no gender specific hypothesis, we plan to conduct gender stratified analysis in all projects per NIA guidelines.

Table 7: SAGES II Gender and minority representation

Racial Categories	Ethnic Categories									
	Not Hispanic or Latino			Hispanic or Latino			Unknown/Not Reported Ethnicity			**Total**
	Female	Male	Unknown/ Not Reported	Female	Male	Unknown/ Not Reported	Female	Male	Unknown/ Not Reported	
American Indian/ Alaska Native	0	0	0	0	0	0	0	0	0	0
Asian	10	13	0	0	0	0	0	0	0	25
Native Hawaiian or Other Pacific Islander	1	1	0	0	0	0	0	0	0	2
Black or African American	33	11	0	2	1	0	0	0	0	47
White	198	165	0	16	6	0	0	0	0	385
More than One Race	1	2	0	0	0	0	0	0	0	3
Unknown or Not Reported	0	0	0	0	0	0	0	0	0	0
Total	243	192	0	18	7	0	0	0	0	460

B4. POSSIBLE BENEFITS

B.4.1. **Potential Benefits of the Proposed Research to Human Subjects and Others**

This study is being performed to advance medical knowledge. Although participants in previous similar research studies performed by the study investigators have generally enjoyed their interactions with study personnel, we cannot guarantee any direct benefit will accrue to study participants. Given the burden of data collection required and modest risks, we have made provision for modest subject incentives for participation in the study. For SAGES I SELECT, we will reimburse participants with $30 for completion of each face-to-face follow-up assessment, $20 for each proxy interview, and $20 for phlebotomy. For the LP procedure in SAGES I SELECT, we will reimburse participants with $230. For the PET scan in SAGES I Select and SAGES II, we will reimburse participants with $175. For SAGES II, participants will be reimbursed as follows: $60 for completion of the baseline assessment with phlebotomy and caregiver interview, $80 for completion of all in-hospital assessments including blood and CSF collection, and $30 for completion of each face-to-face follow-up assessment. For both SAGES I SELECT and II, MRI participation will be reimbursed with $100 per session and TMS/EEG with $100 visit 1, and $75 each Visit 2 and 3. In addition, we will provide meals and reimburse costs for travel including mileage/parking or taxi related to the study procedures.

Based on the participant's decision, we will share the results of the amyloid PET with the participants' doctor up to the end of the study (5/31/2023). However, the results may not be meaningful for the participant depending on their current health status.

B.4.2. **Importance of the Knowledge to be Gained**

Delirium remains a common, morbid, and costly problem among hospitalized elders. Yet, our understanding of the complex relationship of delirium, dementia, and the vulnerable brain remains limited. The proposed research will help to elucidate the interrelationship of delirium and dementia, through an integrated series of 5 cross-linking studies to estimate the rate of occurrence of cognitive decline and dementia following delirium, to probe novel risk factors, to develop predictive models, and to elucidate potential explanatory pathways (e.g., inflammation, cortical loss, decreased plasticity). This knowledge holds tremendous potential to advance our fundamental understanding of the inter-relationship of delirium and Alzheimer's disease/related dementias. Through a better understanding of this relationship, we will be better positioned to advance our mechanistic understanding, and to develop and target more effective interventions for delirium. Ultimately, the goal of this work is to identify whether delirium may be an important contributor to long-term cognitive decline associated with dementia and Alzheimer's disease. On balance, the anticipated benefits to society from the knowledge to be gained outweigh the risks presented to the study subjects. Thus, the risk-benefit ratio appears to be quite favorable for proceeding with the proposed Program Project renewal application.

B5. POSSIBLE RISKS AND ANALYSIS OF RISK/BENEFIT RATIO

B.5.1. Possible Risks of the Study

Overall, this study is rated as greater than minimal risk by NIH criteria[112]. All of our procedures are utilized in standard clinical care and are considered low-risk procedures clinically. The sources of risk are of 7 types in this study, specifically risks related to: 1. study interviews, 2. breach of confidential information, 3. phlebotomy, 4. MRI, 5. EEG/ TMS, 6. lumbar puncture (LP) to obtain CSF and 7. Amyloid PET imaging.

1. Risk of participation in interviews: The first risk of the study is the time necessary to participate in the study interviews and assessments. In our prior experience during the first cycle of the PPG (P01AG031720), patients viewed interactions with the research staff as very positive and enjoyable; in fact, we have a very high rate of continued participation in long-term follow-up. However, we acknowledge that the interviews may pose the risk of fatigue or emotional stress. Should a subject become tired or distraught, the interview will be halted. All data collection procedures will be scheduled according to patient's preference during one day or over the course of 2-3 days. According to our experience during the pilot studies, some patients prefer to have all procedures completed in one day (such as MRI and TMS) and others prefer the procedures on separate days. We will schedule to accommodate the patients' preferences.

2. Risk for potential for breach of confidentiality and privacy of Protected Health Information: The study data includes Protected Health Information and information that participants may consider confidential. Moreover, knowledge of a patient's genetic status (ApoE-ε4 allele, Project 1) and AD biomarker status may be a source of emotional stress to the patient or their family. Therefore, the informed consent will state that these results will not be shared with the patients and/or his/her family, and will not become part of the medical record. In addition, the results of these tests will not be included in the main SAGES study database and will be kept behind an additional firewall. These data will be shared with IRB-approved study investigators strictly on a "need to know" basis. All interviewers will undergo extensive training in the principles of informed consent, confidentiality of all study information, and careful handling of Protected Health Information and all study data. In addition, in the consent form we indicate that subjects can withdraw their permission to use their genetic material for the study at any time.

3. Risk related to phlebotomy: SAGES I SELECT blood (40 ml total) will be collected at patient's home (10 ml) by our study phlebotomist Dr. Guoquan Xu who is a surgeon from China or trained interviewers, and at the Clinical Research Center (30 ml) by nursing staff. SAGES II blood will be collected at 4 time-points for each participant (100mL total): preoperatively (PREOP), on postoperative days 1 and 2 (POD1, POD2) and 1 month after surgery.When possible, blood will be obtained simultaneously with phlebotomy for routine clinical laboratory work, thereby eliminating the additional risk imposed by a separate phlebotomy for study purposes. Otherwise, an experienced staff member will collect the blood. The risks of the phlebotomy procedure itself are minimal, and are primarily related to pain or bruising at the needle puncture site. Since the amount of blood required at each time point is small, the risks from anemia or blood loss are negligible (<1%).

4. Risk related to participation in MRI: MRI is a painless and safe technique that can be used to investigate brain structure and functioning. The risks of MRI scanning are minimal. The most common discomforts associated with MRI are due to either symptoms of claustrophobia or the loud sounds generated in the MRI environment. Participants will be able to contact the investigator at any time during the scan via a squeeze ball and intercom system, and can be taken out of the scanner at any stage of the imaging procedure immediately upon their request. Participants will be required to wear ear plugs while in the MRI scanner to minimize risks due to loud sounds. It is possible that in the process of taking part in the study, a significant clinical assessment or brain imaging abnormality will

be found. In this case, we will follow our MRI safety protocol for incidental findings (approved by our IRB) developed by Dr. Tamara Fong for SAGES I and described below. Because an MRI machine uses powerful magnets, a person could be harmed if they entered the MRI scanner with metal in or on their body. To avoid any risks related to metal, patients will undergo a detailed safety screener and will be asked to change into scrubs prior to entering the scanner. Patients with certain implanted materials (e.g. cardiac pacemaker or pacemaker wires, implantable defibrillators, metallic particles in the body, vascular clips in the head or previous neurosurgery, prosthetic heart valves) will not be permitted to participate in the MRI substudy. Due to physical limitations of the MRI scanner, for safety reasons, participants whose abdomen, shoulder, or hip circumference is greater than 180 cm may not be able to undergo the MRI. This could make participants feel uncomfortable. If a participant indicates they have had an injury to the eye involving metal or an implant which may be contraindicated for MRI, an x-ray exam will be completed prior to the MRI. The risks of the x-ray involve minimal radiation exposure (approximately 0.84 milliSieverts total). A possible effect of this radiation could be a slight increase in the risk of cancer, from about 25% to 25.01% chance. Very high speed imaging methods can induce stimulation of peripheral nerves that may result in muscle twitches. This stimulation is due to electric fields induced by rapid magnetic field switching. FDA guidelines restrict the switching rate to a level that only rarely induces stimulation in subjects and our studies comply with these guidelines. While this sensation may be uncomfortable, it is not dangerous (see e.g. Schaefer DJ et al J. Magn Reson Imaging 12:20-29, 2000) and therefore does not affect the risk/benefit ratio. However, mentioned the occurrence of twitching in the consent form.

5. Risk related to participation in the EEG/TMS substudy (Project 5). TMS produces a loud clicking noise that could potentially result in ringing in the ear and temporary shifts in the ability to determine the pitch and loudness of sounds, if no protection is used. Participants will be required to wear some form of ear protection during the procedure to prevent this. This may include ear plugs, stereo headphones, or other devices; the forms of TMS that we will use in this study have never caused hearing problems with the use of hearing protection. Up to 20%-40% of subjects undergoing TMS experience headaches or neck pain, which are believed to be due to muscle tension. All prior cases of headaches induced by TMS have promptly resolved with a single dose of acetaminophen. In some cases, TMS may cause facial, scalp, or dental discomfort on the same side of stimulation. Subjects may experience temporary redness on their head from the band that holds the tracker for the TMS system. Occasional episodes of transient dizziness, syncope or short term, transient, cognitive changes have been reported (1-4%). Seizures have been reported in <1/1000 cases. Risks of EEG alone include scalp irritation from the electrodes and redness from the cap, both of which are temporary.

6. Risk of lumbar puncture (LP): For this study, lumbar puncture will be used to obtain CSF: 10-15 ml in SAGES I SELECT subgroup (via lumbar puncture) and 1.5-2.0 ml for SAGES II (during anesthesia induction; this is the amount that can be readily aspirated by the anesthesiologists). LP can be associated with pain during the procedure, but this is usually temporary and limited to the lower back. In <5% of older adults who undergo LP, severe headache can occur but this is typically mild and will resolve with over-the-counter analgesics. Less commonly (additional 1-4%), a persistent low-pressure headache (or headache only on standing) may develop. Potential but rare risks (less than 1%) of lumbar puncture include infection, bleeding into the CSF space, and damage to nerves in the back. For SAGES I SELECT participants, the risks will be related to the LP itself (described above), plus removal of 10-15 ml CSF. For SAGES II participants, the LP is being performed as part of routine care, so the relevant study risks are only related to removal of 1.5-2.0 ml of CSF; these risks are considered minimal.

7. Risk of PET: The primary risk related to PET is that of radiation exposure associated with the injected radiotracers and accompanying CT. The effective dose resulting from a 370 MBq (10 mCi) dose of Amyvid is 7.0 mSv in an adult (19 x 370 = 7030 µSv = 7.030 mSv). The use of a CT scan to calculate attenuation correction for reconstruction of Amyvid images (as done in PET/CT

imaging) will add radiation exposure. Diagnostic head CT scans using helical scanners administer an average of 2.2 ± 1.3 mSv effective dose (CRCPD Publication E-07-2, 2007). Given that the actual radiation dose is operator and scanner dependent, the total radiation exposure from Amyvid administration and subsequent scan on a PET/CT scanner is estimated to be 9 mSv over a single year of the study. The organ that receives the maximum exposure is the gallbladder. The overall effective and maximum organ-specific doses are well below the 21 CFR 361.1 guidelines for RDRC approved studies. The radiation doses for each PET scan are not themselves expected to produce any harmful effects, although there is no known minimum level of radiation exposure considered to be totally free of the risk of causing genetic defects or cancer. The risk associated with the amount of radiation exposure participants receive in this study is considered low and comparable to everyday risks. Given our minimum enrollment age of 70 years old for SAGES I participants, the issue of risk of pregnancy and inadvertent radiation exposure to a fetus will not be an issue for this study, and no pregnancy tests will be required.

There is a minimal risk associated with a potential idiosyncratic or allergic reaction to the tracer or any element contained in the injection. While these are so rare their frequency is not well-reported, but we have protocols in place to address any potential adverse reactions to the procedure or tracer. There is a minor risk associated with the venipuncture, placement of an intravenous catheter, and radioisotope injection. These risks include pain, bruising, or painful infiltration of a failed injection. Given our highly trained staff, we anticipate that these complications will occur in less than 1:20 cases.

Disclosure of PET results has the following risks: 1. A copy of the consent form, results of the PET scan, and other information collected during this research may become part of the participant's medical record, both at Beth Israel Deaconess Medical Center (BIDMC), if the information is relevant to the care that the participant receives at BIDMC, and with their regular doctor. Medical records are considered permanent records; therefore, information cannot be deleted from the record and may be reviewed by staff when carrying out their responsibilities, as well as by external parties such as health care insurers and others in certain circumstances. 2. The participant chooses to receive the results and they will be sent to their regular doctor, it could create a problem or hardship for the participant depending upon the type of information disclosed.
This information may influence insurance companies and/or employers, resulting in discrimination. We cannot predict how this information will impact participants' employment or insurance status. The study and BIDMC will not compensate for any negative impact this information may have on the participants' insurance or job status.

B.5.2.Protection Against Risk*:*
All procedures for the SAGES I SELECT and II cohorts have already been approved by the Institutional Review Board of Beth Israel Deaconess Medical Center under the SAGES primary protocol number 2009P-000262 with ceded review from BWH and the Hebrew Rehabilitation Center (HRC) and for IRB approval for pilot studies (protocol number 2015P000273).

We will implement safety monitoring procedures, including weekly meetings with the data collection team, weekly meetings with the operations team, monthly meetings with the P01 working group, and semiannual meetings with our Safety Officer, to monitor and enhance the safety of all subjects in this study. All reports of adverse events will be directed immediately to the Principal Investigator (Dr. Inouye) and the leader of the data collection team (Dr. Marcantonio), and will be attended to within 24 hours. Dr. Marcantonio will work closely to coordinate activities at the Brigham and Women's Hospital, and will oversee safety issues there. Serious adverse events will be reported to the IRB, Safety Officer and the NIH immediately. Although our study is not an interventional trial, it does involve substantial data collection burden. Therefore, an independent Safety Officer will be appointed for the duration of the study. S/he will review any adverse events related to subject participation, and make suggestions for corrective action, if necessary.

1. <u>Protection against risk of participation in interviews</u>: All study procedures will be conducted by

trained personnel, who will halt all interviews or procedures at the earliest sign of patient fatigue or distress, or at direct patient request. All interviews and procedures will be streamlined to minimize inconvenience, fatigue and emotional distress, and will be carefully timed to minimize interference with other activities, such as clinic appointments or hospital activities. In addition, ongoing training will be provided to the research staff throughout the study time course in order to minimize adverse events and risks.

2. Protection against risk of breach of confidentiality and privacy of Protected Health Information: To safeguard confidentiality and privacy of Protected Health Information, each study subject will be assigned a unique code number for the study, and the subject's name or identifiers will never be attached to any form, plasma sample, or genetic material. Hard copy forms (e.g., for neuropsychological testing) and electronic data capture (EDC) will be used. EDC devices will be safeguarded with passwords and encryption, and data will be collected using the web-based Research Electronic Data Capture (RedCap). The EDC devices are used only to transmit interview data to the secure server. No research data is ever stored on the EDC devices (tablets) themselves. Files linking the patient's name with study number and identifiers will be kept in password-protected data files, accessible only by trained, HIPAA-certified research staff and investigators who have IRB-approval. Data files will be stored on a password-protected server. All study forms will be kept in secure, locked file cabinets. The study investigators will assume full responsibility to maintain the confidentiality of all data. All study results will be presented only as statistical aggregates that will neither identify nor permit identification of individual subjects. The Data Management and Statistical Analysis Core under leadership of Dr. Thomas Travison will be instrumental in establishing and maintaining the security of all the data collected. RedCap is a secure web-based application designed to support electronic data capture for research studies. The Hebrew Rehabilitation Center (HRC) will host the RedCAP database in a secure manner that is consistent with all IRB policies and regulation at HRC, BIDMC and BWH.

3. Protection against risk of phlebotomy: SAGES I SELECT phlebotomy will be performed at the BIDMC Clinical Research Center or in the patient's home. For SAGES II, wherever possible, the blood will be obtained along with other laboratories ordered for clinical care to minimize any extra phlebotomies. Because of the timing of the specimens, at baseline (clinic visit), postoperative day 1 and 2 during hospitalization, and at one-month follow-up, the vast majority of blood work for SAGES II will not require an extra phlebotomy. If required, this will be conducted in the patient's choice of the hospital or their home. All phlebotomy will be conducted by trained, experienced personnel.

4. Protection against risk related to MRI: The major health risks from MRI scanning arise from the presence of implanted ferromagnetic objects. Subjects will be thoroughly screened to ensure that these are not present and will be tested with a portable metal detector prior to entering the MRI scanning bay. If a subject reports having a history of injury or surgery to their eye involving metal, an orbital x ray will be completed prior to the MRI. The x ray will be reviewed by MRI safety technician in order to determine if the subject is MRI safe. With respect to discomforts arising during scanning (e.g. claustrophobia, loud noises), participants will be able to contact the investigator at anytime during the scan via a squeeze ball and intercom system, and can be taken out of the scanner at any stage of the imaging procedure immediately upon their request. Participants will be given earplugs to reduce scanner noise. No radiation or contrast (i.e., no gadolinium) will be used for these scans. All imaging sequences will be within the FDA guidelines for radiofrequency (RF) power deposition and magnetic field switching. During the first cycle of the PPG, Dr. Tamara Fong developed a MRI safety protocol and led a panel to provide clinical backup to MRI research staff for any unexpected medical issues that arise in the course of participants undergoing MRI scans and to manage incidental findings on MRI, including maintenance of database of such findings, and this procedure will continue with the SAGES II cohort. The Division of MRI at BIDMC follows all recommended safety guidelines and has extensive experience conducting MRI studies in older adults both with and without cognitive problems.

5. Protection against risk related to EEG/TMS. TMS has been used in a growing number of laboratories worldwide since 1984 and safety guidelines have been developed and updated in 2008[113] and 2020[114x]. These updated safety guidelines will be carefully followed in the present study. Implementing the study in the CRC at BIDMC is one way that risks will be reduced because of the highly trained research staff equipped with emergency personnel and equipment. To minimize risk associated with TMS, TMS sessions will be conducted by properly accredited and trained co-investigators, who (1) have been trained in the safe and efficient administration of TMS (through a course offered at the Berenson-Allen Center for Non-Invasive Brain Stimulation at BIDMC) and (2) are certified in basic life support and in the recognition and treatment of seizures, syncope and other medical/neurological emergencies. At each site, there will be a medically responsible physician for the study who will assess all participants prior to entry into the study. In addition, a fully equipped and regularly checked emergency cart is available at all sites where TMS stimulation will occur. This emergency equipment includes oxygen supply, intravenous supplies, and emergency medications (e.g. benzodiazepines) in the event of a seizure. A licensed physician-member of Dr. Pascual-Leone's team will be readily available on-site in the event of an emergency. Participants will be carefully observed for seizures or seizure-like activity during TMS and for 30 minutes after stimulation in accordance with the suggested guidelines.

6. Protection against risk related to lumbar puncture (LP): For SAGES I SELECT, LP will be performed by an experienced neurologist in the BIDMC Clinical Research Center, using standard sterile procedures and local anesthesia. To minimize the risk of post-LP headache, small gauge atraumatic (Sprotte) needles will be used. We will perform pre-LP interviews to screen for safety, and offer patients an overnight stay if requested. We will also follow-up with patients by telephone one day after the procedure. If a post-LP headache develops, additional treatment, e.g. with fluids and analgesics will be administered. If post-LP headache persists, the patient will be referred for appropriate follow-up clinical care by our study Neurologist. For SAGES II, CSF will be obtained as part of the lumbar puncture done prior to administration of spinal anesthesia by an experienced anesthesiologist, and potential complications described above will be treated during hospitalization as part of routine care. Our study team will also follow-up with the patient 24 hours after the procedure to assess for any adverse events.

7. Protection against risk related to PET: For SAGES I SELECT and SAGES II, all PET scans will be performed by experienced, certified nuclear medicine technologists, who have been well-trained to perform PET imaging studies. To minimize risks of radiation exposure, the lowest possible dose of radioactivity compatible with good image quality will be used. The radiation exposure for amyloid (annual effective dose exposure of 7 mSv (without CT) and 9mSV (with CT) over a single year of the study) is very low and well within FDA guidelines for research. These doses are similar to those used in standard medical tests, and have risks that are so low they are difficult to estimate, and are considered to be comparable to everyday risks of exposure. We will also assure that participants have not exceeded recommended radiation exposure risks overall). To be eligible for any PET scan, participants must not have received any prior scan with radioactive agents either for clinical or research purposes within 12 months, such that the total research-related radiation dose to the participant in any given year should not exceed the limits set forth in the US Code of Federal Regulations (CFR) 21, Section 361.1. There is also risk of Idiosyncratic or allergic reaction to injection of tracer. The amount of tracer injected is so small that participants should not experience any side effects. However, if any of these should occur, participants are instructed to inform research staff immediately. We will have clinicians on-call to address any reported adverse reactions following the procedures, and already have standard protocols in place to address any untoward reactions.

The updated consent form for the PET scan informs the participants about the benefits and risks of disclosure of results. We will send a letter to participants who already consented to the PET scan also outlining the benefits and risks. If these participants want to share the PET scan results with their

doctor, they would need to review and sign the updated consent form.

B.5.3. Analysis of Risk/Benefit Ratio

Delirium remains a common, morbid, and costly problem among hospitalized elders. Yet, our understanding of its pathophysiology remains limited, and there are no targeted treatments other than good general medical care. The proposed research will extend our pathophysiologic understanding through innovative probes of brain vulnerability and deepen our exploration of pathophysiologic pathways potentially contributing to delirium and its associated cognitive decline potentially leading to dementia. On balance, the anticipated benefits to society from the knowledge to be gained far outweigh the risks presented to the study subjects. Thus, the risks to subjects are reasonable in relationship to the anticipated benefits to future patients and to society; the risk-benefit ratio appears to be quite favorable for proceeding with the proposed Program Project.

B6. RECRUITMENT AND CONSENT PROCEDURES

Recruitment

For both SAGES I and II, we have obtained the support of the overall hospital leadership of the hospitals from which eligible patients will be screened and enrolled.

Recruitment calls will be placed between 10am-9pm Monday thru Friday and 10am- 5pm Saturday and Sunday. No more than 10 phone calls will be attempted. Research assistants will be provided a phone script which will make sure that patient's privacy will be preserved. If individuals are distressed in any way by the contact, research assistants are instructed to validate the individual's feeling and stop the recruitment call immediately.

B.6.1. Recruitment for SAGES I sub-cohort

SAGES I sub-cohort participants will be identified based on their delirium and cognitive status. If during a SAGES I full cohort follow-up interview they indicated interest in continued study participation, they will be called by a coordinator to ask them if they want to participate in the sub-study at the BIDMC including LP, phlebotomy, MRI and TMS-EEG. Depending on participants' preferences, the procedures will be scheduled on 2 or more different days. Only patients who provide written consent will be enrolled. The consent will be administered by study staff. During SAGES I, we contacted family caregivers or proxies who were referred to us by the study participant. If, for the SAGES I cohort enrollment, we suspect that a participant has cognitive decline (e.g., evidenced during the screening phone call), we will arrange for a proxy to be present during the study visit. If the capacity assessment confirms lack of capacity to consent, we will ask the potential study participant if they are interested in study participation. If we receive assent from the participant, we will ask the proxy or a legal guardian for written consent. Note: This dual assent-consent procedure will be used in any circumstance (SAGES I or II) where concern about lack of capacity to consent arises during follow-up.

B.6.2. Recruitment for SAGES II cohort

Permission to approach patients will be obtained from the attending surgeon of each patient. As with the original SAGES I cohort, for SAGES II, we have obtained the support of the BIDMC, BWH, and BWH Faulkner leadership from which eligible patients will be screened and enrolled. Prior to study initiation, we will send a letter to all attending physicians who admit patients to these services requesting permission to enroll their patients in this study. For the previous PPG, 98% of physicians gave their blanket permission to enroll their patients. Since we have already obtained support of key leaders of the orthopedic services at each hospital (Drs. Ayesha Abdeen, Brandon Earp, and Jeffrey

Lange), we anticipate similarly high participation rate in the current study. If a particular physician refuses participation, his or her patients will be excluded from enrollment into the study. For those surgeons who do not provide blanket consent (2%), we will seek their permission to approach their patients on a case-by-case basis, as successfully done in our initial cycle.

SAGES II: Surgical patients will be identified from the operating room advanced booking schedule of Beth Israel Deaconess Medical Center (BIDMC), Brigham and Women's Hospital (BWH) and BWH-Faulkner according to procedures used successfully in SAGES I and recent pilot studies. Initial screening will be conducted using data from the electronic medical record.

The patient's surgeons will give the potential subjects general information about the research and we will send recruitment letter signed by the surgeon and study PI to potential participants. We will include an OPT OUT possibility. The address of each letter will be double checked before sending to ensure that the letter is properly addressed.

If no telephone call to opt out of the study is received, a study staff member will call the potential participant for a brief telephone screening assessment, with scheduling of a face-to-face visit in their choice of either their home or at the hospital (clinic visit or CRC), according to the patient's preference.

B.6.3. Consent and Capacity Assessment

For both SAGES I and II, patients will be asked to provide informed consent for participation in this study using a written form approved by the BIDMC and BWH and BWH Faulkner Institutional Review Boards. Informed consent will be obtained by trained study personnel following standard protocols. The consent will include a Health Insurance Portability and Accountability Act (HIPAA)-compliant consent form to obtain medical records from outside hospitals. This request for outside medical records is a procedure that has been approved previously for the first cycle of the PPG. For remote visits, a copy of the consent form will also be dropped-off in a contactless manner and a member of the research team will consent participants remotely (by phone or videoconference using an IRB approved platform like zoom or Starleaf). The signed consent form will be picked up in a contactless manner and signed by a member of the research team prior to starting the baseline interview. If possible we may conduct the consent procedures electronically, using the hospital- approved secure REDCAP (Research Electronic Data Capture). Participants may also choose to sign the consent form and mail it back with a stamped and pre-addressed envelope or scan/fax it back with wet signature before the interview. The study investigators and project coordinators will be available to the participants to answer any questions.

The SAGES I full cohort consent form is reviewed under protocol number 2009P-000262.

For the SAGES I SELECT cohort, six separate consent forms will be used. One consent form for the LP, one for the PET, one for phlebotomy procedures, one for the MRI procedures, one for TMS/EEG procedures and one for the amyloid PET.

For SAGES II, the overall consent form will include a request for permission for face-to-face and phone interviews, phlebotomy, daily hospital interviews, chart abstraction of the index hospitalization and re-hospitalizations, proxy interviews, as well as CSF collection at anesthesia induction before surgery. Three separate consent forms will be used in addition. Each one for the TMS/EEG PET, and one for the MRI procedures.

Participants who do not agree to the TMS portion of the study will be asked if they are willing to undergo an EEG recording alone. Participants will be verbally consented by study staff over the phone or in-person prior to the EEG.

For the standard consent procedures for the duration when home visits are not allowed, we will drop-off/pick-up the procedure consent (LP, PET, phlebotomy, MRI, and TMS/EEG) in a contact-free manner or mail or email the consent form with a stamped and pre-addressed envelope. A member of the research team will then consent participants by phone. Participants may sign the consent form and mail it back, or scan/fax it back with wet signature, or provide a wet signature in person at the procedure visits. If possible we may conduct the consent procedures electronically, using the hospital- approved secure REDCAP (Research Electronic Data Capture).

Capacity Assessment: All patients will be assessed for their ability to provide consent with a capacity questionnaire that is already being used for the SAGES I cohort. The capacity assessment takes place immediately after the consent document has been reviewed with the subject and before we ask the subject to sign the consent form.

The research assistant (RA) will tell the potential participant that they would like to verify their understanding of what the study is about and about their rights as a study participant. The RA then asks in total 5 questions about the ability to evidence choice, to understand relevant information and risk factors, and to appreciate the situation and its likely consequences. Answers are coded as either correct or incorrect. If two or more answers are incorrect, the potential participant is not eligible. If the RA thinks a potential participant does not have capacity or is unsure how to code the answers, they will write down the answers of the potential participant verbatim. For SAGES I SELECT, as described above, if a participant *is not able to give consent, we will ask the participant for assent and ask a proxy to provide the written consent.* For SAGES II, all subjects with unclear capacity will be adjudicated immediately via a conference phone call with the study director and a physician investigator on call. Eligibility decisions will be conservative to ensure that all enrolled participants have full capacity to consent.

After capacity to consent has been verified, we will ask the subject to sign the consent form.

Capacity to give consent will be re-assessed during the follow-up period, if the

a. participant cannot remember ever enrolling in the study or if the
b. participant cannot remember previous study visits or if the
c. the participant is not aware that study participation is voluntary.

If there is any concern, we will conduct a verbal follow-up consent and re-assess participant's capacity with our capacity form. If the participant has impaired capacity to give consent, a legally authorized representative (spouse, adult child, parent, sibling, and other relative or close friend) or a legal guardian will be asked for consent. In this case, the patient will still be asked for assent to participate.

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B.6.4.Subject Protection

Our study may involve the vulnerable population of cognitively impaired older persons. At baseline, due to our strict eligibility criteria excluding dementia patients, we anticipate that all of our study patients will be cognitively intact and able to give informed consent. At the time of each subsequent study assessment, we will seek assent from each patient for continued participation in the study. The patient may refuse continued participation at any time. If significant cognitive impairment develops during the course of this study, then assent for continued participation will also be sought from a family member or legal guardian. This dual procedure has been previously approved and successfully applied in our previous studies involving similar study populations.

B7. STUDY LOCATION

Privacy

The comfort and privacy of patients will be protected during every phase of the study by carefully trained research staff. The informed consent, eligibility assessments and clinical procedure will occur in a private room at the BIDMC CRC (LP and TMS-EEG), in the MRI research area (MRI), in patients' homes (baseline and follow-up assessments), or remotely via telephone or videoconference.

All subjects will have the chance to have their questions answered by one of the study's staff or investigators during this visit.

Physical Setting

Baseline and follow-up assessments will occur in the patient's choice of their homes (in-person or remotely via telephone or videoconference using an IRB approved platform like zoom or Starleaf), at Hebrew Senior life, or at a clinic visit. Space will be provided for interviewing in the BIDMC Clinical Research Center (CRC) if needed. A "SAGES Screening and Safety Procedures During COVID19" information sheet will be provided to participants prior to the baseline visit. Baseline and 1 month follow-up phlebotomy will occur in patient's homes (in-person or remotely via telephone or videoconference) or at the CRC. The research team will follow current BIDMC and Hebrew SeniorLife guidance to determine if a visit should be conducted in-person or remotely. As part of a remote visit, sanitized equipment and materials (iPad, iPad stand and set-up instructions, face mask and sanitizer bottle or wipes) for the visit may be dropped off/picked up in a contactless manner by a member of the study team. For remote visits, a copy of the consent form will also be dropped-off in a contactless manner and a member of the research team will consent participants remotely (by phone or videoconference). The signed consent form will be picked up in a contactless manner and signed by a member of the research team prior to the baseline interview starting. The contactless drop-off, study visit, and pick-up may occur on separate days so as not to be too overwhelming for participants during a single visit.

We will retain all options for mode of administration due to the uncertainties with the COVID-19 situation--with clearance for remote or telephone during the time of COVID-19, and for any mode once we are allowed back in the homes. We will also discuss possible visit modes with the participants and follow their preference for the mode of the visits.

<u>BIDMC:</u>

- Patient enrollment, daily interviews and POD1 and 2 phlebotomy will occur on the surgical units of BIDMC and BWH. Daily interviews may be conducted in-person or remotely via telephone or videoconference. The research team will follow current hospital guidance to determine if a visit should be conducted in-person or remotely.
- LPs,TMS-EEG, and phlebotomy will be obtained at the CRC
- Specimen will be processed at the CRC or at the Boston Children's Hospital IDDRC Molecular Genetics Core Facility,
- Blood and CSF analyses will occur at BIDMC under the leadership of Dr. Libermann. Dr. Libermann's laboratory is housed within Beth Israel Deaconess Medical Center (BIDMC) located in the first and third floor of the Research North building. The general laboratory will provide all the resources for proposed assays. Dr. Marcantonio maintains 3 -80C freezers, which house over 25,000 samples of the SAGES Study Specimen Bank. One freezer is located in the Clinical Research Center, while 2 other freezers are maintained in the Libermann laboratory.
- X-ray scans (when applicable) will be performed in the Rabb Building in the Department of Radiology prior to MRI scans
- MRI scans will be performed in the BIDMC East and West Campus Clinical MRI scans or the Outpatient Radiology Department, Shapiro 4th floor under leadership of Dr. David Alsop's Magnetic Resonance Imaging (MRI) Laboratory

- PET scans will be performed in the BIDMC Division of Nuclear Medicine and Molecular Imaging
- TMS-EEG analysis will occur under the leadership of Drs. Pascual-Leone and Shafi at BIDMC. Dr. Pascual Leone's lab is at the Berenson-Allen Center for Noninvasive Brain Stimulation at Beth Israel Deaconess Medical Center and Harvard Medical School (http://tmslab.org/)
- Data analysis will occur under leadership of Dr. Long Ngo

BWH and BWH-Faulkner

- Patient enrollment, daily interviews and POD1 and 2 phlebotomy procedures will occur on the surgical units of BWH and BWH-Faulkner. During the COVID-19 period, daily interviews may be conducted in-person or remotely via telephone or videoconference. The research team will follow current hospital guidance to determine if a visit should be conducted in-person or remotely.
- MRI and PET scan data analysis

Hebrew Rehabilitation Center (HRC): (Marcus Institute for Aging Research):

- HRC will be the Coordinating Center for the entire project, under the leadership of Dr. Sharon Inouye. General data analysis will occur under leadership of Dr. Thomas Travison, leader of the data core of this program project.

Children's Hospital IDDRC Molecular Genetics Core Facility,

- Specimen will be processed at the CRC or at the Boston Children's Hospital IDDRC Molecular Genetics Core Facility,

MGH:

- MRI and PET analysis will occur in under the leadership of Dr. Bradford Dickerson
- Blood and CSF analyses will occur under the leadership of Dr. Steven Arnold
- The MGH labs of Drs. Dickerson and Arnold are located at Massachusetts General Hospital/Harvard Martinos Center for Biomedical Imaging in Charlestown, MA (MGH-East), (http://www.nmr.mgh.harvard.edu/martinos/) No patient enrollment or acquisition of MRI scans, blood or CSF will be conducted at MGH for this project.
- Drs. Westover, Shaw, and Ge will work on the NLP project.

Brown:

- Dr. Richard Jones will conduct all Project 4 analyses at the Alpert Medical School of Brown University.

B8. DATA SECURITY

We will continue our secure data management systems and computing environments that are designed to prevent unauthorized access to data or data loss. Data are maintained on encrypted drives behind institutional firewalls that are managed via password protection to prevent unauthorized access. Participants' personal identifying information is stored in a database with access restricted to study staff. To safeguard confidentiality and privacy of protected health information (PHI), each study subject will be assigned a unique code number for the study, and the subject's name or identifiers will never be attached to any hard copy form, plasma sample, or genetic material. All hard copy study forms will be de-identified kept in secure, locked file cabinets, and will be shredded upon completion of the study analyses. All study results will be presented only as statistical aggregates that will neither identify nor permit identification of individual subjects.

For the NLP project, secure file transfer, MGH (Dr. Shaw) will obtain a spreadsheet containing inpatients notes extracted from the BIDMC OMR. These notes will include identifiable data. For

BWH and BWH-Faulkner, data they will utilize the Research Patient Data Registry (RPDR), a centralized clinical data registry/warehouse. The RPDR gathers data from hospital systems and stores it in one place, bringing clinical information to a researcher's fingertips and ensuring the security of patient information. Dr. Shaw will store the file with the data in a secure folder behind the MGB firewall. After analysis he will delete the data

MRI data: All hard copies of data acquired from the MRI will be kept in locked cabinets to which only Dr. Alsop and the research team will have access. All electronic records of the MRI will be stored on a secure server behind the BIDMC firewall. A copy of the electronic records will also be provided to co-investigators in charge of data analysis at the Hebrew Rehabilitation Center, BWH, and at MGH. Access to the database will require a login name and password. Patient identifiers will be kept separately and will be linked to the study data by a unique study ID. All patient identifiers will be maintained for the duration of the study. After the required time period following the completion of the study, all paper-based documents will be destroyed using a shredder. All electronic documents bearing patient identifiers will be deleted.

PET Scans
All hard copies of data acquired from the PET scan will be kept in locked cabinets to which only Dr. Fong and the research team will have access. A copy of the DICOM images will also be saved on Dr. Fong's laptop which is encrypted and password protected. All electronic records of the PET will be stored on a secure server behind the BIDMC firewall. A copy of the electronic records will also be provided to co-investigators in charge of data analysis at the Hebrew Rehabilitation Center, BWH, and at MGH. Access to the database will require a login name and password. Patient identifiers will be kept separately and will be linked to the study data by a unique study ID. All patient identifiers will be maintained for the duration of the study. After the required time period following the completion of the study, all paper-based documents will be destroyed using a shredder. All electronic documents bearing patient identifiers will be deleted.

Our data monitoring procedures address four dimensions including completeness, accuracy, consistency, and timeliness. Interview staff members undergo intensive training and standardization procedures, including didactics, group trainings, reliability assessment, practice sessions with patients, and paired interviews with experienced personnel. Completeness is assured through cross-checks which are completed by another interviewer within four weeks; data consistency is assured through database programming (e.g., programming to prevent out-of-range or erroneous entries, and regular missing data reports), and regular data quality assessments. Weekly team meetings assure consistent coding, data capture, and quality across all study measures. Ongoing training and inter-rater reliability assessments occur regularly (2 times per year). These measures will continue to allow us to pick up inconsistent data very quickly.

To facilitate inter-rater assessments we will ask participants during the main consent, if they give us permission to record the session for quality assurance and or for training purposes. We will only record the session with participants' permission.
The recordings will be sent securely to a cognition specialists who are part of the study team at Beth Israel Deaconess Medical Center or Hebrew SeniorLife in Boston, MA. The specialists will review the recording for quality control purposes or be used for training. The video will be destroyed at the end of the study. This review is essentially to making sure that the research data from this study are clear and correct and would help with training. The video will be labeled with the participant's study ID number only without any identifiers.

B9 Multi-Site Studies

Is the BIDMC the coordinating site? ☒ Yes ☐ No

Is the BIDMC PI the lead investigator of the multi-site study? ☒ Yes ☐ No

Information on other IRBs
The IRB of record for this multicenter study will be the BIDMC IRB and will have reliance agreements using the Online Reliance System SMART IRB: https://smartirb.org
The following sites are involved in this study:

- Marcus Institute for Aging Research, Hebrew SeniorLife (HSL), Boston MA with the main tasks: administration, coordination, data collection, analysis,
- Beth Israel Deaconess Medical Center, Boston, MA with the main tasks: patient identification, enrollment and daily hospital interviews, phlebotomy, CSF collection, TMS-EEG, and MRI
- Brigham and Women's Hospital (BWH) and BWH-Faulkner, Boston, MA with the main tasks: patient enrollment
- Brown University Warren Alpert Medical School Departments of Psychiatry and Human Behavior and Neurology, Providence RI with the main tasks: data analysis
- Massachusetts General Hospital (MGH), Boston, MA with main tasks: MRI, PET, blood, and CSF analysis. MGH will also help with the NLP project.

Dr. Inouye is the Overall PI of this study and has joint appointments at BIDMC and HSL. All Co-PIs, meet regularly during the formal meetings listed below in person and discuss issues related to the study, including unanticipated problems that would impact the IRBs oversight and decision making. For unforeseen issues needing immediate attention, ad-hoc phone meetings will be arranged

- Executive Committee (monthly), consisting of leaders of all projects and cores, study director, and key interdisciplinary co-investigators. This is the central decision-making and policy development group for the Program Project.
- Operations Committee (weekly), consisting of all core and Project leaders, overall study director, and key project coordinators, which will meet weekly to manage the day-to-day operations of the Program Project cohorts, data collection and management.
- Scientific Working Group (weekly), to ensure scientific advancement and productivity, through collaborative writing of abstracts and papers. Scientific results will be shared and discussed. The working group will include leaders of all Projects and Cores, pertinent co-investigators and trainees.
- Study Specimen Bank/Database Committee (as needed), providing oversight, quality control, volume and use of samples for the different study aims.

B10 Dissemination of Research Results

Please explain whether you will be able to thank subjects and provide research results and, if so, how this will be accomplished. If you do not think this is feasible, appropriate or applicable to this research, please specify why.

We are planning to send out newsletters quarterly thanking participants for their participation and will include progress and results of the study.

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