Hyperbaric Oxygen Therapy for Cognition in Diabetic Elderly at High Dementia Risk
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This is a resubmission of an application responding to "PAR-16-365-Pilot Clinical Trials for the Spectrum of Alzheimer's Disease and Age-related Cognitive Decline (R01)". It examines the efficacy of hyperbaric oxygen therapy (HBOT) in improving cognition in cognitively impaired elderly with type 2 diabetes (T2D), who have high dementia risk. It is a collaboration of the Icahn School of Medicine at Mount Sinai, NY, the University of Wisconsin, Madison WI, the Sagol Center for Hyperbaric Medicine and Research at Asaf Harofeh Medical Center, Israel—one of the largest hyperbaric units worldwide, treating over 100 patients daily—and the Sheba Medical Center, Israel (8 miles from Asaf Harofeh).

HBOT is a treatment in which oxygen-enriched air (up to 100%) is administered to patients in a chamber at a pressure above one atmosphere absolute (1ATA), the ambient atmospheric pressure. *The combined action of hyperoxia and hyperbaric pressure leads to significant improvements in tissue oxygenation, resulting in benefits to the cebrovascular milieu, with improvements in ischemic damage and cerebral blood flow (CBF).* Recently, our group published compelling evidence from clinical trials indicating HBOT neurotherapeutic effects in stroke, with improved cognitive function and elevated brain activity in SPECT. Additionally, our preliminary data showing better cognitive performance and brain activity in T2D subjects with mild cognitive impairment (MCI) suggests potential HBOT neurotherapeutic effects,.

Elderly with T2D have higher risk of developing dementia, MCI, and cognitive decline. T2D is a vascular disease culminating in tissue hypoxia, so cerebrovascular disease is the primary contributor to this increased risk. Impairment of cerebrovascular integrity in T2D may lead to reduced CBF on the vessel level, and reduced glucose metabolism on the neuronal level, which may be rectified with HBOT by cerebral vascular repair processes. We thus propose a randomized controlled trial (RCT) of the beneficial effects of HBOT on cognition and on ischemic injury (through assessment of CBF using MRI, and of glucose metabolism using FDG-PET). *We focus on T2D elderly with MCI, who are enriched in cerebrovascular disease and are in the prodromal phase of the dementia process, an ideal opportunity for HBOT to protect and enhance cognition by reversing, preventing, or delaying neuropathologic processes that cause cognitive decline.* We will test the extent to which 60 sessions of 90 minutes of 100% oxygen at 2 ATA—compared with a sham condition—improve cognitive performance in the short term (immediately after treatment) and a longer term (12 months after treatment). Subjects will be recruited through talks in the community, advertisements, and companies which have large lists of elderly interested in receiving emails about health related topics. In addition, we have a large-scale project in Israel (NIA R01AG034087), where 1200 T2D elderly are followed for cognitive decline providing an additional large recruitment source.

We hypothesize that HBOT compared to a sham condition (n=77 in each group) improves cognitive function and favorably alters cerebrovascular pathology (increasing CBF and glucose utilization) in T2D patients with MCI, focusing on the following outcomes:

Aim 1. Cognitive functioning: Aim 1a: *The primary outcome will be a composite measure of cognitive function balancing tests of both executive and memory function.* Domain-specific measures of cognition (executive function and episodic memory) will be secondary outcomes.

Aim 2. Ischemic Injury: CBF at the level of capillaries in gray matter measured by MRI arterial spin labeling, *and in macrovessels, measured by a novel 4D Flow MRI technology developed by our group.*

Aim 3. **Neuronal function**: [F18]FDG-PET measuring cerebral glucose utilization.

Aim 4. **Mediation by brain biomarkers**. To examine whether CBF and glucose utilization mediate HBOT effects on cognitive function. Hypothesis: inclusion of the biomarkers in a mediation model will attenuate the effect of HBOT on cognition, suggesting them as underlying mechanisms.

We propose an RCT examining the short and long-term efficacy of HBOT, a therapy with strong biological plausibility for affecting beneficially impaired cognition *through vascular mechanisms, in elderly with T2D-related MCI, who are enriched in cerebrovascular disease and are at high dementia risk.* We focus on both cognitive and biomarker outcomes, providing context for identifying relevant mechanisms, and explore factors associated with HBOT efficacy, suggesting subgroups who may most benefit from the therapy. The study will be performed in Israel, with optimal infrastructure and expertise for all the study components at significantly lower costs. *HBOT can be widely deployed in the US so if successful, this pilot study will provide the basis for a multi-center large-scale clinical trial for definitive evidence of its benefits to cognition in T2D patients at high dementia risk. With the current lack of effective treatments for dementia while the proportion of elderly increases, the accelerating prevalence of T2D and dementia amplify this application's public health impact.*

B. SIGNIFICANCE

B1. The urgent need for therapies for dementia- An urgent need exists to identify effective interventions to arrest or reverse dementia at its earliest stages. Mild cognitive disturbances and pathophysiologic changes associated with dementia can precede clinical symptoms by more than a decade. Mild cognitive impairment (MCI), characterized by mild memory loss and/or mild executive dysfunction, is the presumed prodrome of clinical dementia. The greatest risk factor for cognitive impairment is age; currently 13% of the US population is over 65, but they are projected to increase by 115% in 40 years[1]. This group has the largest health care expenditures. Recent estimates of MCI prevalence are 10% in 70 year-olds, 20% in 80 year-olds, and as high as 40% in 85 year-olds[2]. Enhancing or preserving cognition in elderly at high risk of cognitive decline and dementia would have enormous benefits for the affected individuals, and their support systems that bear the social and financial burdens of long-term care. However, studies of pharmaceutical interventions to prevent or arrest cognitive decline in MCI—such as cholinesterase inhibitors, vitamins, anti-inflammatories, anti-hypertensives, and statins—have been largely negative[3-7]. A major challenge in understanding and treating cognitive decline and dementia is the lack of a firm relationship between cognitive outcomes and targeted neurobiological mechanisms. For example, clinical trials with amyloid-reducing agents have succeeded at target engagement, but failed to cause clinical benefit for Alzheimer's disease (AD). Thus, novel disease modifying strategies—such as hyperbaric oxygen therapy (HBOT)—with strong biological plausibility to benefit cognition in aging, must be investigated.

B2. The primary underlying mechanism for HBOT benefits on cognition is cerebrovascular- HBOT is a treatment in which oxygen-enriched air (up to 100%) is administered to patients in a chamber at a pressure above one atmosphere absolute (1ATA), which is the ambient atmospheric pressure. For the peripheral vasculature, a well-accepted indication of HBOT is hypoxia-induced foot ulcers; there is broad evidence suggesting significant improvements through stimulation of vessel repair processes and angiogenesis[8, 9]. Similarly, in the brain, based on current evidence in 1) animals, 2) small clinical trials in humans, and 3) our own stroke clinical trial (see D1a), the combined action of hyperoxia and hyperbaric pressure of HBOT improves hypoxia/ischemic injury and consequently cerebral blood flow (CBF) leading to cognitive improvements[10, 11]. Angiogenesis is induced by HBOT through downregulation of hypoxia-inducible factor-1α (HIF-1α)[12, 13] including in the hippocampus[14, 15]. HBOT also increases vascular density in the hippocampus, improves spatial learning in rodents[16], reduces cortical infarct area[17] and improves CBF in the piriform cortex of adult rats with vascular dementia[18]. Small HBOT studies performed on humans also support our hypotheses[19] and proposed mechanism of action. In a randomized clinical trial (RCT), HBOT treatment as an adjuvant to donepezil showed improved cognitive performance compared with donepezil alone in vascular dementia patients[20]. Similarly, HBOT had positive effects compared to hyperbaric air on neurological and cognitive outcomes in a small (n=26) controlled trial of patients with cerebrovascular disease (patients, but not investigators, were blind to the study condition)[21]. More recently, HBOT—compared with normobaric oxygen—was associated with increased CBF in frontal and temporal regions in healthy subjects[22]. Finally, our clinical trial on post-stroke patients showed enhanced CBF and cognition following HBOT. On the neuronal level, it is well established that glucose utilization is impaired in ischemic cortical regions[23-25]. In animal models there is growing evidence for improved neuronal hypometabolism with HBOT[26-28], suggesting that intracellular bioavailability of oxygen attenuates the deleterious effects of ischemia on neuronal glucose utilization[29]. The evidence thus supports investigation of CBF (using MRI) and glucose utilization (using FDG-PET) as biomarker outcomes of HBOT in T2D patients, who are enriched in cerebrovascular disease.

B3. Type 2 diabetes (T2D) patients are at high risk for cognitive compromise- T2D is consistently associated with risk for cognitive decline[30], MCI[31], and dementia[32-34]. Its average onset at 55[35], 15-35 years before dementia onset, provides a window of opportunity for prevention. T2D is diagnosed in 26% of those above age 65 in the US; over half have T2D or "pre-T2D"[36], an enormous number at high risk for cognitive compromise. T2D affects multiple domains of cognitive function, primarily executive functions[37] and episodic memory[38]. The primary outcome of the proposed RCT is a summary measure of these cognitive domains, with the executive function and episodic memory domains as secondary outcomes.

B3a. T2D affects cognition primarily through cerebrovascular mechanisms- Increased risk for vascular pathology in T2D[39] gives strong biological plausibility to involvement of cerebrovascular pathology in the association of T2D with dementia. Brain MRI shows cerebrovascular abnormalities in T2D patients: stroke[40], lacunar infarcts[41], microbleeds[42], white matter hyperintensities[43], reduced CBF[44, 45] and cerebrovascular reactivity[46]. The negative effect of T2D on cognition was largely mediated by cerebrovascular disease in the AGES-Reykjavik Study[47]. Consistent with this, we have shown that both metabolic syndrome and insulin resistance are associated with a significant reduction of CBF. On ASL perfusion MRI, this effect was observed robustly in prefrontal and temporo-parietal areas, and lower CBF mediated the negative effect of metabolic syndrome on memory[48, 49]. Consistent with compromised cerebrovasculature, T2D and elevated HbA1c levels have been associated with brain hypometabolism as measured by [F18]FDG-PET[49]—a proxy for neuronal activity[50]—and our group has found significant and widespread reductions in glucose metabolism with higher levels of insulin resistance among late-middle-aged individuals (see Figure 5).

B3b. T2D patients have smaller brain volume but not more amyloid pathology: Elderly subjects with T2D have smaller total brain[51-53] and gray matter volume[51, 53, 54], as well as larger CSF volume[46, 55]. Smaller hippocampi were found in both middle-aged[56-58] and older T2D patients[59, 60]. In pre-T2D subjects, smaller hippocampi were associated with impaired glucose tolerance[61], insulin resistance[62] or higher fasting glucose levels[63]. Our group has also shown insulin resistance associated with smaller medial temporal lobes volume[64], and volume mediated the its association with cognition, in a group of largely pre-T2D middle- and older-aged adults. In contrast, evidence for a role of amyloid in T2D dementia risk is equivocal, given that T2D has not been associated with amyloid neuropathology in many postmortem studies[65-68] and in recent amyloid PET studies[49]. Overall, the data point toward a primary role of cerebrovascular disease in T2D cognitive compromise. Thus, the proposed study will focus on the biological outcomes with the highest relevance to the potential HBOT beneficial effects to T2D, CBF in small vessels (measured by MRI ASL), and neuronal hypometabolism (measured by FDG-PET).

B4. Rationale for use of HBOT in T2D-related MCI- As described above, current evidence strongly supports a vascular pathology mechanism underlying cognitive compromise in T2D. While the bulk of the evidence fails to support more AD neuropathology in T2D than in non-T2D, AD neuropathology is present in T2D. This is important since HBOT benefits on the brain are primarily through a vascular mechanism and improvement in vascular function may have downstream beneficial effects on tau and amyloid[69, 70]. We focus on T2D patients, who are enriched in cerebrovascular pathology, and thus may be strong candidates for improvements by HBOT. The enriched levels of neuropathology[71]nand the long prodromal period of MCI provide an ideal opportunity for HBOT to protect or enhance cognition by reversing, preventing, or delaying neuropathologic processes that cause cognitive decline. Importantly, this application responds to the National Plan to Address Alzheimer's Disease (NAPA) 2015 update that specifically urges acceleration of treatments for non-AD dementia[72], and may have implications for AD per se.

C. INNOVATION

As the US prevalence above age 65 increases by 2050 to an alarming 28%[36] for T2D and 20% for dementia[73], finding therapies to prevent or delay the development of dementia due to T2D is imperative.

C1. First study of HBOT in MCI at high risk due to T2D-This is the first RCT of HBOT in MCI patients with particularly high dementia risk due to comorbid T2D. In contrast to many unsuccessful pharmacologic approaches, it innovates by examining a relatively safe, non-pharmacologic therapy with strong biological plausibility, and solid preliminary data for positive effects on clinical symptoms, and on vascular markers. The design includes short-term (immediately post-treatment) and longer-term (12 months post-treatment) outcomes, to investigate the legacy effects of HBOT over time.

C2. Innovative design and cognitive outcomes- In the context of cognitive impairment, this will be the first HBOT RCT with a parallel sham condition, rather than a cross-over design (which does not permit evaluating long-term effects of HBOT), or HBOT as an adjuvant to donepezil[20] (without a sham condition). The sham control creates identical conditions to the HBOT treatment (noises, mask on face, nurse in the chamber, etc.), except for hyperbaric pressure. The overall cognition primary outcome includes a balanced combination of four tests of executive functions and four tests of episodic memory, cognitive domains characterizing T2D-related cognitive compromise. Both domains have benefitted from HBOT[74, 75] and will be secondary outcomes. We will also expand the secondary outcomes with executive functions assessments using innovative and sensitive computer-administered tests. Although this is not an ADCS trial, the innovative strategies for cognitive assessment may inform future MCI RCTs, consistent with a key ADCS aim.

C3. Innovative integration of cognitive and biomarker outcomes relevant to T2D and to HBOT beneficial effects- Objective biomarkers can detect progression of dementia pathology long before frank cognitive impairment[76]. Focusing on both cognitive and biomarker outcomes provides the context for identifying underlying mechanisms. We will examine HBOT effects on small and large vessel CBF using MRI, and on cerebral glucose uptake using FDG-PET[77]. Both the cognitive and biomarker outcomes are relevant to T2D-related cognitive impairment. Moreover, there is robust biological plausibility for HBOT beneficial effects on these biomarkers since they are linked to vascular integrity, as shown in our preliminary data.

C4. A unique opportunity to explore contributing factors- The Israeli HMOs have well computerized medical charts. Thus, upon participant's approval, we will request historical information on diagnoses, blood tests, and medications. Exploring factors (e.g. T2D microvascular complications) potentially associated with HBOT efficacy (see D6) is valuable in a pilot RCT, to identify groups that benefit most, refining the design for a large-scale multi-center RCT.

D. APPROACH

D1. Preliminary Studies

Overall- This study will examine the short and long-term effects of HBOT on overall cognition, CBF, and glucose metabolism, in T2D patients with MCI, who are enriched with cerebrovascular pathology and have high dementia risk, making them an optimal population for the potential beneficial effects of HBOT. Our preliminary data show beneficial effects of HBOT on cognition and CBF in post-stroke patients and in a small sample of T2D-MCI patients, feasibility and rationale for the use of FDG-PET as a biomarker outcome in this study, clinical and neuroimaging capabilities at the Sheba Medical Center, available infrastructure of the Israel Diabetes and Cognitive Decline study, and a summary of the study team's expertise and well-coordinated long-term collaboration, fully supporting the successful execution of the proposed RCT.

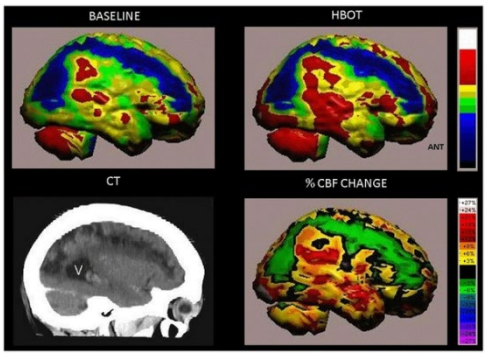

Figure 1- A patient in the treated group with left hemiparesis due to ischemic stroke from 26 months prior to the study. Brain perfusion maps (upper 2 images) show the infarcted brain (deep blue color) involving right antero-postero-lateral frontal, right superior-parietal and right parieto-occipital regions. Curved sagittal view in CT MIP reconstruction shows the anatomical stroke area (left lower image, V= posterior horn of right ventricle). The peri-infarct region show improved perfusion after HBOT (right upper image). Right lower image: Quantitation of cerebral blood flow change (delta between baseline and HBOT).

<u>D1a. HBOT induces neuroplasticity in post stroke patients- Randomized prospective trial</u>[78]- Patients 6-36 months after stroke (n=74) were randomly assigned to a "treated" or "crossed" group. Brain activity (by SPECT imaging), neurologic functions (by the NIH stroke scale), activities of daily living and quality of life were assessed at baseline and after HBOT (40 90-minute sessions, 5 days/week, 100% oxygen at 2ATA). Patients in the crossed group were also assessed after an initial two months control period of no treatment. Neurological functions, ADL, and quality of life improved significantly following HBOT sessions, but with no improvement after the crossed group control period. Elevated brain activity was detected mostly in regions of live cells (as confirmed by CT) with low activity (based on SPECT) (**Figure 1**). This highlights the advantage of our design, examining MCI subjects, who may have compromised neuronal and vascular function due to their T2D but are not demented yet, suggesting substantial brain tissue has the potential to recover.

D1b. Improvement of memory in post stroke by HBOT [75]- This study addressed HBOT effects on memory impairment after stroke at late chronic stages in a retrospective analysis of 91 stroke patients 18 years or older (mean 60 years) who had either ischemic or hemorrhagic stroke 3–180 months (M 30–35 months) before HBOT (40 to 60 90-minute sessions, 5 days/week, 100% oxygen at 2ATA). Memory tests before and after HBOT therapy used the NeuroTrax computerized testing battery (verbal or nonverbal, immediate or delayed memory). In all tests there was a statistically significant improvement after HBOT (all p-values <.0005, effect sizes medium to large), largest in immediate (28% improvement) and delayed (27%) verbal memory, respectively (**Figure 2**). SPECT before and after HBOT (n=56) showed several regions had significantly higher relative change in activation, particularly in temporal lobe (entorhinal cortex, inferior and middle temporal gyri). These results support strongly the potential of HBOT to improve cognition and brain activation even years after the acute event. Of note, Drs. Efrati and Beeri co-authored this paper, illustrating their productive collaboration.

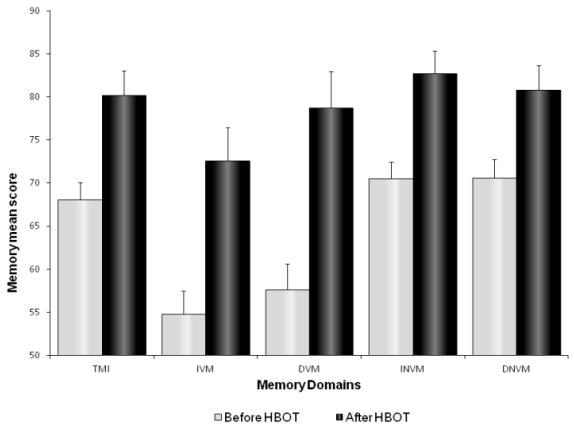

Figure 2- Memory scores (mean+SE) before and after HBOT. All improvements are significant at a level of p<0.0005.

D1c. Improvement of cognitive impairment in MCI by HBOT- In a pilot study for proof of concept, two T2D MCI patients were treated using the proposed HBOT protocol. The overall cognition measure improved by 32.6% (from 0.99 to 1.47) and 30.0% (from 0.81 to 1.15) for subject 1 and 2, respectively; SPECT results showed substantial improvements in CBF in broad cortical regions (see **Figure 3**, subject 1**)**. The provocative results of this very small pilot study support both the cognitive and biomarker hypotheses.

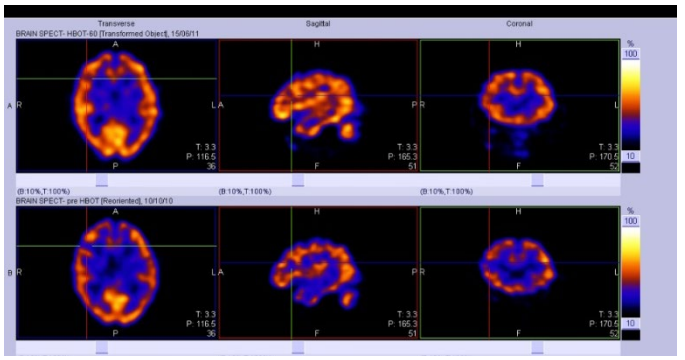

Figure 3- SPECT of a subject with MCI before (lower panel) and after HBOT, showing extensive increases in brain activity by SPECT

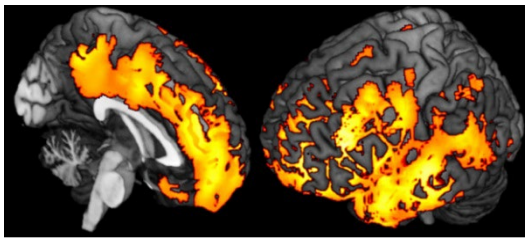

Figure 4- Controlling for age and gender, participants with metabolic syndrome had lower cerebral blood flow (an index of neural function) in parietal, frontal, and temporal lobes (shown in orange overlay, $FWE_{corrected}$, $p < 0.05$). Left panel: medial surface of left hemisphere; Right panel: left lateral cortex.).

D1d. Low cerebral blood flow is associated with lower memory function in metabolic syndrome[48]- Dr. Bendlin, our neuroimaging collaborator, compared CBF using arterial spin labeling perfusion MRI (ASL-MRI) in middle-aged adults (n=69; mean age=60.4) with and without the metabolic syndrome (a pre-T2D condition). Mean gray matter CBF was 15% lower in the metabolic syndrome group (**Figure 4**). Voxel-wise image analysis indicated that this decrease pertained to a large portion of the cortical surface. The poorer memory in the metabolic syndrome group was partially mediated by CBF. These findings demonstrate feasibility of the proposed ASL-MRI and its sensitivity to the pre-

T2D metabolic syndrome supporting Aim 2a.

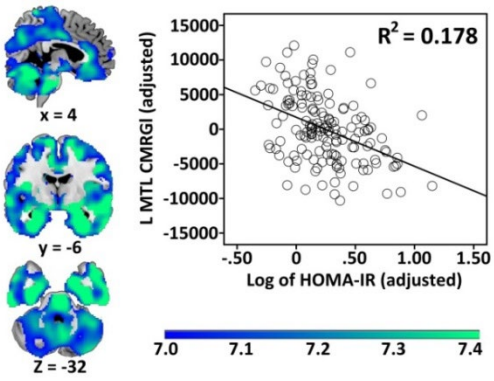

Figure 5- The association between higher HOMA-IR and lower regional FDG-PET uptake in 150 late middle-aged adults (p<.05 FWE). Uptake values were adjusted for age, sex, family history status, APOE4, and glucose metabolism in the reference region. The color bar depicts t-values. Brains are oriented in neurological space. FWE= family-wise error corrected.

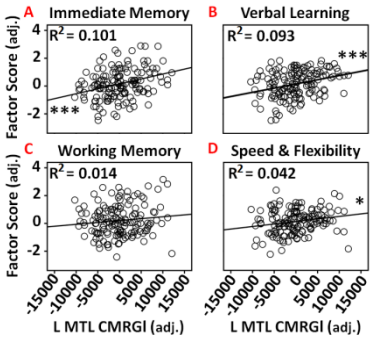

Figure 6- (A-B) Higher FDG-PET in left medial temporal lobe predicted better performance on verbal learning and immediate memory. (C-D) Left medial temporal uptake was not associated with working memory, but was associated with the speed and flexibility factor. Signal was adjusted by the FDG reference region, age, sex, APOE4, and family history status, and HOMA-IR.*=p<.05; **=p<.001.JAMA Neuro

D1e. Insulin resistance is associated with poorer brain glucose uptake- Dr. Bendlin's group examined the association of insulin resistance (using HOMA-IR) with global and regional FDG-PET uptake in 150 middle-aged adults. Higher insulin resistance (a pre-T2D condition) was associated with lower FDG-PET uptake (**Figure 5**); uptake in regions more strongly associated with insulin resistance (such as medial temporal lobe) was associated with cognitive function (**Figure 6**). These results show relationships of a T2D characteristic (insulin resistance) with regional glucose uptake supporting its use as a biological outcome in the proposed study.

D1f. The Israel Diabetes and Cognitive Decline Study (IDCD)[80-85]- This prospective population-based longitudinal study, to identify T2D-related characteristics linked with cognitive decline, has cognitive, functional, demographic, psychiatric, DNA, and inflammatory marker assessments every 18 months. T2D subjects from Maccabi (n=1200) were initially cognitively normal, 65+, averaging 73 years old at baseline, 13 years of education, and 10 years of T2D in the Diabetes Registry; 42% were women. All subjects approved, through their informed consent, to be approached for other relevant studies, providing an opportunity to approach them for this RCT. The IDCD rate of cognitive decline is used to calculate statistical power.

D1g. Neuroimaging at Sheba- We recently received an R21 (AG043878) using MRI in IDCD subjects. Demonstrating willingness to participate, all the 18-months follow up subjects signed the informed consent

amended to include neuroimaging. The Sheba Helsinki Committee requires that all scanned subjects receive a clinical report from a neuroradiologist, providing a great incentive. In the last 10 months, we scanned 150 IDCD subjects using the ASL methods described in D2k; Dr. Bendlin confirmed superb quality. Sheba performs over 4,500 PET-CT exams per year, about 450 in brain, 15% of them for research (see letters from Drs. Ben Chaim and Konen, chairs of Sheba's Nuclear Medicine and Radiology Departments, describing this infrastructure).

D1h. A multidisciplinary team of integrated and productive investigators covering all the necessary expertise for successful implementation of this RCT: Dr. Beeri (PI) is an expert in the neuropsychology of cognitive aging and dementia, with a main focus on the contribution of T2D; Dr. Sano (joint PI) is a neuropsychologist who is a national leader in clinical trials for MCI and dementia; Dr. Efrati (PI Asaf Harofeh) is a world renowned expert of HBOT; Dr. Bendlin (PI: U Wisconsin site) is an expert in neuroimaging of dementia and focuses on metabolic disorders' effects on the brain; Dr. Ravona (PI: Sheba site) is a geriatric psychiatrist, a young, rising physician-investigator; Dr. Leroith is an internationally recognized expert in mechanisms underlying complications of T2D; Dr. Ashery's recent research focuses on the biological mechanisms of HBOT. Dr. Bagiella is a biostatistician with expertise in complex longitudinal analyses of clinical trials. Dr. Beeri has published with all the PIs and has other joint grants with them. Thus, despite the distance, the team is well integrated, with expertise to ensure successful implementation of all components, analyses, and interpretation of results.

Table 1: Eligibility criteria

Inclusion Criteria	Exclusion Criteria
1. Diagnosis of T2D	**1.** Brain disease that affects cognition (e.g. Parkinson's disease, schizophrenia).
2. Diagnosis of MCI	**2.** Stroke
3. ≥ the age of 65	**3**. Epilepsy
4. Hebrew fluency	**4.** Chest pathology incompatible with HBOT
5. An informant	**5.** Inner ear disease
	6. Claustrophobia
	7. Cholinesterase inhibitors
	8. Subjects with an indication for HBOT
	9. Individuals with previous HBOT treatments.
	10. Individuals with cancer and other medical illnesses requiring intensive therapy.
	11. Individuals with proliferative retinopathy.

D2. Methods:

D2a. Participants- Elderly patients (n=250) with T2D and MCI (amnestic or non-amnestic) will be enrolled to examine potential HBOT therapeutic effects on the brain preceding dementia, when neuropathologic processes causing decline still have potential for prevention or delay. MMSE>24 and clinical dementia rating [CDR] = 0.5 will be required. An informant must be available for supplemental information throughout the trial.

Candidates will complete a cognitive assessment, physical and neurological examinations, and a clinical interview with an informant present. Cholinesterase inhibitors are an exclusion since their interaction with HBOT is not known. Subjects with an indication for HBOT (such as diabetic foot) will be excluded, to avoid randomization to the control arm. Israel is a country of immigration, so Hebrew fluency is required for valid neuropsychological testing[84]. **Table 1** summarizes eligibility criteria. Subjects will be preferably from central Israel

since the HBOT treatment will be performed in Asaf Harofeh, located in central Israel. The treatment is very time consuming and far away participants may have a hard time complying to the treatment protocol.

D2b. Recruitment- Subjects will be recruited through talks in the community, advertisements, and companies which have large lists of elderly interested in receiving emails about health related topics. IDCD subjects diagnosed at follow up with MCI (21% at the 36-months follow up) will be offered participation in the proposed trial. Although the IDCD study participation was far less demanding than the proposed RCT, recruiting 1200 subjects within 2 years reinforces our confidence in recruiting 154 subjects for this RCT—including many already in the IDCD—in 3 1/2 years. Additional recruitment will be performed through clinics (e.g. diabetes and executive clinics).

Table 2- Summary of study procedures

	Screen	Baseline/ beginning of intervention	12 weeks /end of intervention	6 months	12 months
Informed consent	X				
History, cognitive assessment (for MCI classification), clinical evaluation	X				
Medication review	X	X	X	X	X
Physical exam	X		X		
Blood test		X	X		X
Follow up information		X	X	X	X
Adverse event monitoring*			X*		
Group assignment: review program and expectations at time of randomization		X			
Cognitive testing (ADAS COG13+executive functions tests)		X	X	X	X
Functional assessment (CDR)	X		X	X	X
ADL, IADL		X	X	X	X
Depression questionnaire	X		X	X	X
MRI ASL		X	X		X
FDG-PET		X	X		X
Blindness testing			X		

The intervention will begin within three weeks after the Baseline assessments and end at 12 weeks after 60 HBOT/Sham treatments. A physician is always present during the HBOT sessions, and a nurse is in the chamber throughout the whole treatment, so adverse events will be de facto closely monitored at each session up to the end of the intervention.

D2c. Procedures- **Table 2** presents study procedures for each subject. HBOT sessions will take place in Asaf Harofeh Medical Center; informed consent and all other assessments, including cognitive, functional, MRI, and FDG-PET will be performed at the Sheba Medical Center. Adverse event monitoring is done at every HBOT and sham session, by a nurse in the chamber throughout the session and a physician always present. Physical exams and blood collection for safety monitoring will occur before randomization, and at 12 weeks. Medication review will occur before randomization, before and after the intervention, and 6 and 12 months later, with cognitive testing at these times except before randomization. The T2D-related biomarkers (Blood test, MRI ASL and FDG-PET) will be assessed before and after the intervention, and 12 months later. After intervention, a questionnaire will examine whether blindness was maintained. At the end of the participant's 12 months' participation in the trial, the participant can choose to receive HBOT treatment for which the costs will be covered by the study team. At the end of the second HBOT treatment course, an additional neuropsychological assessment will be administered to the participants.

D2d. Randomization and Blinding of Intervention-Eligible candidates will be randomized with equal probability to the HBOT and sham interventions. When a cluster of at least 3 subjects from one of the interventions is filled, the intervention for that cluster will begin (77 subjects will be recruited for each treatment). Clusters will be a random effect in the analyses (see D6). Three study technicians who activate HBOT or sham protocol sessions will be the only unblinded staff who have the key for the subjects' group assignments. All subjects and other clinic staff will remain blinded to group assignment. Study nurses will monitor AEs during all sessions and a physician will be present during each session. Importantly, staff from Sheba, who assesses outcomes, will not be exposed to the subjects during their intervention and vice-versa.

Procedures before enrollment will maximize participant adherence to the intervention. Only volunteers who fully understand the expectations regarding consistent participation in the treatment and completion of assessments will be enrolled. The judgment of field site staff will be essential to determine eligibility based on anticipated adherence. During initial telephone screening and again prior to the baseline visit, subjects will be asked to confirm their willingness to be assigned to either intervention. Dr. Efrati's group has long experience with RCTs using HBOT conditions, ensuring subject satisfaction and continued commitment to the program.

D2e. HBOT intervention- The multiplace HBOT unit at Asaf Harofeh is state of the art, the most advanced available in the market. The inside looks like an airplane, with comfortable chairs for 11 subjects and the nurse who stays throughout the session (a significant advantage compared to the monoplace chamber). The HBOT protocol is 90 minutes, 5 times/week, 60 sessions, 100% oxygen at 2 ATA with 5 minute air breaks every 30 minutes. This safe protocol is used worldwide for treatment of ischemic non-healing wounds (diabetic foot or post radiation injury), was used in our stroke clinical trial[78], and in our MCI preliminary data, indicating favorable cognitive outcomes. It improves on a prior study on memory-impaired elderly[89] that was too short to reduce ischemic injury or for angiogenic processes to occur, even in peripheral tissue[90-92].

The team members present at all times during the HBOT/sham sessions are a physician, two nurses (one inside the chamber and one outside), and at least one of the three unblinded technicians. Only study subjects—not clinical patients—are in the chamber during research hours. When subjects arrive at the Hyperbaric Unit, one of the nurses signs them in; measures their glucose, blood pressure, heart beat, and temperature; and asks about any change in their medical condition. Subjects with signs of any infectious disease (such as upper respiratory tract infection), or arriving with systolic blood pressure >170mmHg or <90mmHg, or glucose <75mg/dl do not enter until it improves. While in the chamber, subjects may drink, read, write, sleep, hear music with headphones, or watch TV. Video games, laptops, phones, or other electronic devices are not allowed in the chamber. Subjects have their own masks. The atmospheric pressure goes up to 2.0 ATA during the first five minutes of the session, with a noise of circulating air. Subjects will feel pressure in their ears; the nurse will advise releasing the pressure by pumping the ears (closing their nose with their fingers and pushing air). In the last five minutes of the session, the pressure is slowly decreased to 1ATA.

D2f. Sham intervention- Sham was selected as the control condition rather than "usual care" to equate intervention groups with respect to other variables that could influence cognition and functional status, such as a new challenge (completing an activity program), peer socialization, and attention from staff.

Except for pressure, all the conditions of the HBOT intervention are provided in the sham intervention (nurse measures vitals and asks about health before entering the chamber, time in the chamber, number of sessions per week and overall, nurse in the chamber at all times, mask on the face, etc.). In contrast to 2 ATA for the HBOT, the sham condition pressure goes up to 1.1 ATA during the five first minutes of the session with noise of circulating air. This provides a minimal pressure sensation in the ears, with the same nurse advice on pumping the ears. Pressure then decreases very slowly during the next half hour; in the last 5 minutes of the session, the air is circulated again with its related noise. Sham and HBOT sessions will never be adjacent, so subjects from the two groups cannot meet and compare sessions. This sham model makes the two conditions very comparable, except for the hyperbaric effect, to minimize breaking the subjects' blindness.

Consideration of alternative sham and HBOT conditions: The two clinical trials described in the preliminary data section had a treatment group and a crossover group (assessed at baseline, after two months with no intervention, and again after HBOT intervention). A more effective design is a sham condition that is very similar to the HBOT condition but without known treatment effects (and also permitting the assessment of the long-term effect of HBOT). We considered a sham condition using a slightly higher 1.3 ATA level, but this hyperbaric condition has an increase in tissue oxygenation greater than 50%[92], which may have substantial physiological effects, so 1.1 ATA for sham is preferable. We considered 100% oxygen with 2.5 ATA in the HBOT condition, but in our experience, this protocol induced more adverse events.

D2g.Optimizing adherence to the intervention. One challenge in a study that requires high commitment is achieving high rates of intervention adherence for the duration of the trial, especially in the elderly due to restrictive life events such as illness, caregiving responsibilities, environmental barriers, and disabilities. Nonetheless, several studies indicate that the elderly with cognitive impairment can successfully adhere to intensive programs and benefit from the experience, particularly when these programs provide adequate structure and support to the participant[93, 94]. Our HBOT studies had relatively low dropout rates of 5% (stroke study) to 20% (mTBI study). The great expertise and experience of the team, and the liberty to pass the time in the chamber reading, watching TV, listening to music, etc. facilitate high compliance. At screening, and again at randomization, Drs. Ravona and Efrati will discuss with each subject what is expected, and the importance of strong commitment. We will attempt to assess for outcomes subjects who withdraw from the intervention.

D2h. Outcomes. ***Primary cognitive outcome****. The primary cognitive outcome is a balanced composite sum of z-scores of four executive function tests (Trails B, Mazes, Digit-Symbol, and Category Fluency), and four episodic memory tests (immediate and delayed recall of the word list from the ADAS-Cog, and immediate and delayed*

recall of Logical Memory Story I from the Wechsler Memory Scale-III). These functions are affected by T2D[37] and commonly administered in other MCI trials[95 96, 97]. Z-scores are reversed if necessary so that a positive value refers to good cognition. We have published Hebrew norms for T2D elderly subjects for all the executive functions (except Mazes, which is not language dependent), and the episodic memory tests. [84]

For Trails B, subjects draw lines connecting alternating numbers and letters (1, A, 2, B, etc.); for Mazes, subjects draw lines from start to finish in mazes of increasing complexity, without back-tracking or crossing a boundary; for Digit-Symbol, numbers and abstract symbols are paired in a legend, and subjects fill in the symbols for a series of numbers; and Category Fluency totals words for two categories, animals, and for fruit and vegetables. ADAS word list- Subjects read aloud 10 unrelated words on printed cards, and are asked to immediately recall as many as possible, in three learning trials. Delayed recall tests recall of the 10 words, after 15 minutes. Logical Memory subtest of the Wechsler Memory Scale-III is a paragraph recall test. We will use the 1st story for immediate recall and for delayed recall, 15 minutes later.

***Secondary cognitive outcomes**. These will be the domain-specific composites, i.e. the 4 tests each for executive function and episodic memory, both affected by T2D[37, 38], and benefitted by HBOT[74, 75]. Since there is especially broad evidence for impairment of executive functions in T2D[12, 98], another secondary outcome will sum z-scores* for 3 computer-based tests (not language dependent), developed through NIH initiatives for new sensitive instruments that will ultimately be public domain (NIH Toolbox assessing multiple domains: http://www.nihtoolbox.org; EXAMINER assessing executive abilities: http://examiner.ucsf.edu). For Flanker, subjects make a speeded response—whether the central arrow is pointing left or right, between flanker arrows pointing in the same or opposite direction; for Set-Shifting, subjects match figures by either color or shape depending on a cue on the screen; and for Dot-Counting, subjects count out loud the number of targets displayed among distractors, and recall the number of targets when queried, 1 to 4 screens later.

Secondary functional outcomes- Three measures will be the CDR-SB, which summarizes impairment in 6 domains (memory, orientation, judgment/problem solving, community affairs, home/hobbies, and personal care)[99] based on subject and informant interviews, and the ADL[100] and IADL[101] questionnaires.

Primary imaging outcomes. Given our robust preliminary data in T2D and pre-T2D showing reduced CBF and glucose metabolism, and supported by others[45, 49, 102], and the evidence of effects of HBOT on CBF and glucose metabolism (see B2), our imaging outcomes are CBF (Arterial Spin Labeling MRI for small vessels*)* and [F18]FDG-PET.

D2i. Maintenance of participant blinding- Effectiveness of blinding will be evaluated at the end of the study in two ways: 1) we will ask whether participants received the "real" intervention. A post-intervention questionnaire (CSRQ-64[103]), which typically takes 5-10 minutes, will explore reasons for their answer: in 64 statements on 8 cognitive abilities, (e.g., "Did you improve, remain the same, or worsen, specifically because of being in the study?"), 2) We will compare participants' rates of voluntary withdrawal. A difference between groups' percentages over 15% will be interpreted as perception of, or lack of confidence in, the intervention.

D2j. Neuroimaging- Participants will undergo a full protocol to acquire CBF and ancillary data on a 3 Tesla (3T) GE x750 scanner with echo planar and asset capability using an 8-channel head radio frequency (RF) coil (General Electric, Milwaukee, WI). The MRI protocol will include: ASL-MRI, T2FLAIR, and T1-weighted imaging. **ASL-MRI-** ASL-MRI will be acquired to assess CBF at baseline, 12 weeks (immediately after treatment) and 12 months after treatment. The sequence will be acquired using background-suppressed pseudo-continuous ASL (pcASL), featuring a 3D fast spin echo spiral sequence (TR = 6000 ms; TE = 21 ms; FOV = 240 x 240 x 160 mm; slice thickness = 4 mm no gap; matrix size=128 x 128; NEX=3; and labeling RF amplitude=0.24 mG, with a post-labeling delay of 2025 ms. **3D T2FLAIR** will be acquired to assess WMH burden (TR 6000, TE 120, TI 1870, 256 x 256 matrix, FOV 256 mm acquired over 88 2mm thick slices, no gap, with final voxel resolution 1mm x 1mm x 2mm; 2). **3D T1-weighted imaging** will be acquired to use for registration, atrophy correction, and regional volumes (inversion-recovery prepared fast spin echo, TR 8.2, TE 4.0, TI 450, matrix 256 x 256, FOV 256 mm, acquired over 156 1mm thick, no gap, axial slices with isotropic 1mm voxel resolution) . Participants will be asked to refrain from caffeine and nicotine for 4 hours prior to the study. The pcASL sequence includes 3 averages (NEX=3). The sequence also includes a fluid-suppressed proton density (PD) acquisition, with the same imaging sequence/image slab location as the pcASL but without the RF labeling preparation, for CBF quantitation and image registration. The entire pcASL sequence—all 3 excitations plus PD scan—is acquired in 4.5 minutes and can be inspected for quality on the scanner and repeated if necessary. We have previously reported excellent test-retest reliability (intraclass correlation coefficient >.95) of this pcASL procedure[104].

MRI Quality Control: The quality control of the scanner is done by Sheba Department of Radiology in conjunction with GE field engineers. This is augmented by administration of a MAGPHAN Quantitative Imaging phantom which has been consistently scanned at least monthly since April 2009; the scanner has been highly stable over time. Based on our experience, we anticipate technical problems <2%. There is sufficient time in the session for rescanning if motion or other artifacts are observed. Trained and certified MR technologists operate the console; scans are prescribed in a standardized fashion and assessed at the scanner for acceptable quality. Scans are downloaded to the imaging lab server immediately after the session and the post-processing procedures are implemented including an additional quality check by the study team. If baseline re-imaging is necessary, study intervention will be delayed until satisfactory images are obtained.

MRI Data Backup: Multiple backups are performed to prevent technical loss of data. At the scan console, the exam is backed up to DVD and kept at the MR facility. The exam is also uploaded to a PACs server and permanently archived. A complete copy is downloaded to a 40TB RAID6 server and weekly backups (to a separately located server) are made of processed and incremental data.

MRI Image Processing: Images will be pre-processed prior to statistical analysis using well established tools and pipelines including SPM12 and FSL. The pipelines are scripted and extendable such that improved methods developed in the software or locally can easily be applied to all data in batch. The pipeline steps include: 1) Conversion of images to NIFTI file format for broad compatibility between software packages; 2) Insertion of the

exam into the imaging SQL database (so Drs. Beeri, Livny-Ezer, and Bendlin can view the data online via web tools); 3) coregistration of each ASL CBF map to the baseline map which is in turn coregistered to the T1-weighted 3D SPGR volume at baseline; coregistration uses a mutual information algorithm and linear least squares minimization in SPM12; 4) transformation into MNI canonical atlas space using the ICBM 152 template in SPM12. Finally, data are smoothed with a Gaussian kernel of 8mm FWHM to accommodate individual variability and comply with assumptions of random field theory necessary for statistical analysis of the images. *T2FLAIR images will be segmented according to methods previously used by our group[106]. WMH will be used as covariates in the second-level analysis.* **FDG-PET:** Dr. Simona Ben Chaim, Chair of the Nuclear Medicine Department at Sheba, will oversee acquisition of [F18]FDG on a Philips Gemini GXL + PET scanner in 3D mode. [F18]FDG-PET Acquisition: Participants will be studied after a minimum 4 hour fast (water allowed). Because participants with glucoregulatory dysfunction are being imaged, blood glucose will be closely monitored prior to the injection of [F18]FDG. Blood glucose prior to [F18]FDG administration must be ≤ 180 mg/dL for [F18]FDG to be injected. Following injection with 5.0 ± 0.5 mCi of [18F][F18]FDG participants will remain awake but relaxed in a quiet room. Imaging will begin 45 min. post injection, and the scan acquired as six 5-min. frames. A 5-min. transmission scan will be acquired following the emission scan. The dynamic PET data will be reconstructed using ECAT v7.2.2 software. A filtered back projection algorithm (DIFT) will be used with brain mode sinogram trimming, zoom = 2.8, and a 4mm Gaussian filter to a reconstructed image of 128 x 128 x 63 voxel matrix (voxel size = 1.84mm x 1.84mm x 2.43mm). The PET data will be corrected for the attenuation of annihilation radiation (using segmented attenuation maps), scanner normalization, and scatter radiation. [F18]FDG image processing: Data will be transferred to the ADRC imaging lab at the UW-Madison using a secure protocol our group has in place. The reconstructed images will be used for comparison of regional differences of [F18]FDG uptake in the targeted areas of the brain. The dynamic [F18]FDG will first be corrected for frame to frame motion using SPM12 software. Any frames with excessive motion will be discarded from further processing. The data will then be summed to create a single image representing [F18]FDG uptake in the brain from the period of 45–75 minutes. This image will then be scaled so the mean whole brain activity will be equal for all subject scans*. Images will be corrected for atrophy based on the three-compartment approach (includes GM, WM and CSF) based on the method published by Meltzer et al.[107, 108]. In brief, the MR T1 image is segmented in SPM12 to provide GM, WM & CSF maps. The partial volume corrected gray matter tissue tracer concentration will be used for the second level group analysis, extracted from regions of interest (dorsolateral prefrontal, medial temporal, and lateral parietal cortices).* These regions have been selected based on their vulnerability to T2D and AD. Data will be extracted bilaterally using the Automated Anatomical Labeling (AAL) atlas implemented in WFU PickAtlas, and averaged across hemispheres prior to being entered into statistical models. Secondary analyses will include exploratory whole-brain analyses conducted on a voxel-wise basis.

D2K. **Inflammatory and diabetes and dementia related markers -** Morning bloods will be obtained after a 10-hour fast, then centrifuged. Bloods will be stored at -70C until determination in Sheba Medical center.

We will analyze the bloods to determine the diabetes and dementia related biomarkers in several well established methods, like:

High sensitivity C-reactive protein- CRP levels will be measured with a sandwich-type ELISA in which polyclonal rabbit anti-CRP antibodies are used as catching antibodies and a biotinylated mAb against CRP (CLB anti-CRP-2) is used as the detecting antibody. The inter- and intra-assay coefficients of variation are expected to be less than 5%.
Interleukin-6- IL-6 will be measured using a commercial solid phase immulite (Diagnostic Products Corporation [DPC], Los Angeles, CA, USA). Reported inter-assay coefficient of variation is 6% and intra-assay 4%, with a detection limit of 1.0 pg/ml.
Haptoglobin- Haptoglobin will be typed (Hp 1-1, Hp 2-2 or Hp 2-1) by Dr. Andy Levy, at the Technion Israel Institute of Technology by polyacrylamide gel electrophoresis from 10 ul of plasma according to established methods. Dr. Levy's lab processes about 20 thousand blood draws per year for haptoglobin typing. The haptoglobin gene on chromosome 16 exists as two allelic variants, producing distinct set of polymeric haptoglobin molecules. Distinct signature patterns of polymeric species which are detected by gel electrophoresis are obtained from individuals homozygous for the Hp 1 or Hp 2 alleles (Hp 1-1 or Hp 2-2 respectively), or who are heterozygous (Hp 2-1). Polyacrylamide electrophoresis is the most common method for typing haptoglobin with 100% concordance with genotyping.
Diabetes related markers e.g. Advanced Glycation End products (AGEs) (Hemoglobin A1c, LDL etc.)
Dementia related markers e.g. Plasma Amyloid Beta and Tau- Amyloid Beta and Tau proteins will be analyzed via Immunoprecipitation–mass spectrometry (IP–MS) assay. If in the future new dementia and diabetes related biomarkers will be discovered, we request the IRB's approval to analyze those markers as well.

D2I. Performing the study at the pilot stage in Israel is optimal - Dr. Shai Efrati, the Asaf Harofeh site PI , is the chairman of the Israeli Society for Hyperbaric & Diving Medicine and an internationally renowned leader in HBOT. The Asaf Harofeh Hyperbaric Unit is one of the largest, most active worldwide with about 100 patients treated per day, in a variety of FDA approved indications (e.g. non-healing ischemic wounds, post radiation injury, chronic osteomyelitis). *There is widespread accessibility of HBOT chambers in the US (please see letter of support from Dr. Peters, the American Undersea & Hyperbaric Medical Society Executive Director describing ~1400 US HBOT chambers, with an estimated annual growth of 8-10%). This suggests that finding collaborative HBOT facilities in the US would not be a problem in a large-scale multi-center RCT for cognitively impaired T2D patients based on this pilot study.* Importantly, the typical US cost of HBOT treatment is significantly higher than in Israel, making the proposed pilot study sample size feasible within the current funding mechanism. The Sheba Medical Center is the largest hospital in the Middle East, and is home for the NIH funded IDCD study, so it has all the necessary infrastructure, personnel, experience, and expertise in recruitment of elderly subjects with T2D, and of assessment of the cognitive and biological outcome measures of the proposed study. It is only 8 miles from Asaf Harofeh, facilitating visits by subjects who will be primarily from the Tel-Aviv area encompassing both hospitals. The professional staff is of superb quality but at significantly lower cost than in the US. Finally, subjects will be recruited through talks in the community, advertisements, and companies which have large lists of elderly interested in receiving emails about health related topics. Medical information will be given us by the subject.

This data is very valuable, enabling a pilot study to refine the design for a future large-scale multi-center RCT, by exploring T2D-related characteristics, such as severity of disease, which may identify groups of subjects with more benefit from HBOT. *Finally, generalizability of results to Americans is crucial. Americans and Israelis are similar in prevalence of several cardiovascular risk factors and diseases, specifically T2D. The prevalence of T2D in both the US and in Israel is about 25% for individuals above the age of 65*[109, 110]*, and the mortality rate associated with diabetes is essentially identical in both countries, slightly below 40 per 100,000*[111, 112]*. The distribution of type of medication used (none, oral, or insulin) is also very similar in the US*[110] *and Israel*[80]*.*

D2J. Pitfalls and solutions**-** Potentially larger sham dropout rates- The sham group will feel less ear pressure than the HBOT group. Since the intervention is time consuming, there may be more dropouts among those who think they are in the control group. In the majority of RCTs of behavioral interventions, there are no differences in the dropout rates of control groups[e.g.113-117]. All subjects will be told that they will be randomized to high or low hyperbaric pressure, and that it is unknown yet if either works. At the end of the participant's 12 months participation in the trial, the participant can choose to receive HBOT treatment for which the costs will be covered by the study team. At the end of the second HBOT treatment course, an additional neuropsychological assessment will be administered to participants. The nurse might perceive the difference between HBOT and sham but will be instructed not to discuss this with participants. No non-T2D control arms- inclusion of non-T2D parallel HBOT and sham arms would show whether efficacy is specific to T2D, or generalizes to all MCI patients. However, for this pilot funding mechanism, the aim is proof of concept that HBOT improves cognition, focusing on T2D-MCI patients, who are enriched in cerebrovascular disease—the main target of HBOT—and at high risk for decline, improving statistical power. With ½ of the US elderly suffering from T2D or pre-T2D, this study has potential for broad public health impact. *Collecting AD neuropathology data- Despite associations of insulin with amyloid*[118-120] *and of T2D with tau pathology*[121]*, we decided against measuring them in CSF, due to a focus on vascular mechanisms, incomplete scientific evidence supporting AD neuropathology as an HBOT mechanistic target in a T2D context, and minimizing participants' burden.*

D3. Logistics and communication among the international investigators*- Dr. Beeri has collaborated for many years with all investigators and has numerous joint publications demonstrating her capability to effectively lead international studies with complex logistics. Drs. Beeri and Sano will lead 2 biweekly conference calls. One, with the Sheba and the Asaf Harofeh teams, will discuss day-to-day aspects of the trial (recruitment, refusals and ways to overcome them, data entry and missing data, staffing and coordination, and adverse events). The other, with the senior investigators, Drs. Bendlin, Ravona, Efrati, Ashery, and Leroith, will monitor overall progress toward the trial's goals. Both meetings will have minutes that will be sent to the meeting participants and action items will be reviewed in each meeting. Drs. Bendlin and Livni communicate very often on the neuroimaging aspects of other collaborative studies facilitating continuous communication on the proposed study. Images upload to the warehouse is done at the scan day and Dr. Bendlin has access to them (see D5).*

Funds are allocated for Drs. Beeri and Sano travel to Israel for close study supervision of progress, and for the annual AAIC conference where the senior investigators will meet face-to-face.

D4. Study Timelines- The first three months will be devoted to obtaining IRB approvals from all sites and training study physicians, neuropsychologists, and nurses for data collection. Screening and enrollment will occur from month 4 of the study and until the middle of year 4 (~47 subjects/year); data collection and cleaning will extend for another year. Analyses and preparation of the primary manuscript will be in the final 6 months.

D5. Data handling and transmission- The Mount Sinai Department of Psychiatry data management infrastructure is a web based data warehouse including all its cognition and AD projects. A region specifically designed for the proposed study includes MRI and PET scan data. The original study materials, including the MRI and PET scans, will be transmitted via a secure FTP. Data entry is through a web portal by a double entry system. The warehouse files have built-in range and logical checks to flag or prevent impossible values. Each study team member has identification to download data files via a secure web portal, so analyses do not affect warehoused data. Data transmission has been successful in the IDCD and our other international projects.

D6. Statistical Analysis Plan- Efficacy analyses will be performed for the intent-to-treat (ITT) population, our primary analysis, and for fully and partially compliant per-protocol (PP) populations. The ITT population will include all participants in the group to which they were randomized, regardless of any protocol deviation including non-compliance, adverse events, or loss of follow-up. The PP populations will include participants in the group according to the intervention actually received, but a separate analysis will be performed for those who were fully compliant (at least 80% of sessions completed) and for those who were partially compliant (at least one session completed). A complier-average causal effect (CACE) analysis using a latent class modeling approach will also be performed on both fully and partially compliant populations. Patients missing a baseline value of a continuous efficacy outcome measure will be excluded from all analyses and maximum likelihood estimation methods will be used to handle missing data arising from an unobserved follow-up visit[122].

Aims 1a and 1b: The primary (overall cognition) and secondary (executive functions, episodic memory domains, and computer-based tests) cognitive outcome measures (all summaries of z-scores), will each be analyzed using a mixed model analysis of covariance (ANCOVA) with time of assessment (baseline or 12 weeks) as the within-subjects factor, treatment group (HBOT vs. sham) as the between-subjects factor and baseline value of the outcome measure as the covariate. *Both, subject and 'cluster' (the variable used to identify to which group of at least 3 subjects each individual is assigned) will be modeled as random effects to account for the correlation among repeated measurements made over time within a subject as well as potential 'cluster effects' arising from blocks of at least 3 patients receiving treatment at a particular time after randomization.* An interaction term between time of assessment and treatment group will be included in each model allowing for estimation of the mean change from baseline to 12 weeks in each treatment group as well as a test for difference

in change scores between groups. A test of parallel treatment effects across the range of baseline values will be conducted by including a three-way interaction term among time of assessment, treatment group and baseline value of the outcome variable as well as all lower order two-way interaction terms. If the three-way interaction term is statistically significant it will be retained in the final model and estimates of the difference in mean change scores between treatment groups will be evaluated at selected percentiles of the baseline distribution. Secondary mixed model ANCOVA analyses will be performed for cognitive assessments at 6 and 12 months following treatment. As an exploratory analysis, linear mixed effects models will assess the relationship between treatment group (fixed effect) and the longitudinal trend (random intercept and slope for each subject) in the primary and secondary cognitive outcome measures, assessed at 12-weeks, 6 months and 12 months following treatment. The primary hypothesis will be tested by assessing the interaction between treatment group and time of cognitive follow-up assessment, with a significant interaction indicating a difference in longitudinal cognitive trends between treatment groups. Potential confounders (age, gender, education, *duration of T2D, HbA1c, diabetes medications [insulin vs. no medications and oral agents vs. no medication], levels of atrophy and white matter hyperintensities*) will be included as covariates if there is imbalance at baseline ($p<0.1$) or association between the covariate and the response ($p<0.15$).

To explore whether specific dichotomized T2D characteristics (such as the last HbA1c level (dichotomized at 7) in the Diabetes Registry, the number of T2D complications, and the number of severe hypoglycemic episodes in the year preceding randomization) are associated with greater improvements following treatment with HBOT, mixed effects ANCOVA models similar to those for Aims 1 and 2 will add a main effect for the specific T2D characteristic and an interaction term between treatment group and the characteristic. Using the MHS dataset, we will also explore associations of dosage of the most prevalently used medications with cognitive outcomes. When potential confounders are associated with cognitive outcomes, this also may identify subgroups for which HBOT is particularly effective.

Aims 2a and 2b: CBF and cerebral glucose utilization will be analyzed with a similar strategy to Aims 1a and 1b except that there will be no 6 month assessment. Mixed effects ANCOVA models will be used to compare changes in these outcome measures from baseline to 12 weeks following treatment, and, as exploratory analyses, linear mixed effects models will be used to assess relationships between treatment group (fixed effect) and the longitudinal trend (random intercept and slope for each subject) in MRI-ASL and FDG-PET outcome measures, assessed at 12-weeks and 12 months following treatment. Vascular effects of HBOT vs. Sham: Since CBF derived from ASL perfusion may represent combined effects of neural metabolism and vascular effects, a secondary mixed effects ANCOVA model (similar to Aim 2b) of vascular changes from baseline to 12 weeks as measured by ASL CBF will adjust for neural metabolism derived from FDG-PET.

Aim 3: Outcome measures (changes in CBF and cerebral glucose utilization) identified as having significant associations with treatment group in Aims 2a and 2b will be further explored as mediators of the relationship between treatment group and the change in cognition. To ensure that measurement of mediator

variables precedes measurement of cognition, we will define the outcome as the change in cognition from baseline to 12 months, and the mediators as changes in CBF and cerebral glucose utilization, from baseline to 12 weeks. The product of coefficients approach will be used within a multiple mediator modeling framework to provide point estimates of both the specific indirect effect (i.e. the effect of treatment group on the change in cognition from baseline to 12 months via a mediator among other mediators in the model) and the total indirect effect (i.e. the sum of specific indirect effects for all mediators in the model). The bootstrapping method[93] will be used in each multiple mediator model to compute bias-corrected standard errors (and 95% confidence intervals) for the specific and total indirect effect point estimates.

D7. Power Analysis and sample size justification

Aim 1. Power is presented for detecting the difference in mean change (from baseline to 12 weeks) in overall cognition z-scores between the sham and HBOT treatment groups. Power calculations are based on two-sample t-tests and are conducted with a 2-sided 5% significance level (**Table 3**). A 2-sample t-test provides a more conservative power estimate than the proposed mixed model approach, which is expected to explain more variability and thus have more power to detect the same effect size with the same sample size.

Table 3: Two group t-test of equal means, equal n's)*

	Outcome		
	Overall Cognition z-score	**CBFV†**	**CGU‡**
Sham Mean Change, D_1	-0.020	-0.450	-0.08
HBOT Mean Change, D_2	0.224	2.28	2.065
Difference in means, D_1–D_2	-0.244	-2.73	-2.145
HBOT SD	0.50	5.60	4.40
Minimum N per group	67	67	67
Enrollment N per group**	77	77	77

* Test of significance level=0.05; **assuming a 13% drop-out rate; Two-sided test; Power=80%; Effect size=0.49; † CBF= CBF; ‡ CGU = cerebral glucose utilization

The predicted mean change in the sham group from baseline to 12 weeks is -0.02 (based on the IDCD 12-week change in overall cognition). Assuming an SD of 0.50 in both the sham and HBOT groups, with a minimum sample size of 67 patients per arm, we have 80% power to detect an improvement in the HBOT group of 0.224, a "medium" effect size of 0.49. To account for an anticipated dropout rate of 13% (a reasonable estimate compared to the 5% dropout rate in our stroke study), we plan to enroll 77 patients per group for a total of 154 patients. Our HBOT trial years after mild traumatic brain injury[74] had an effect size of 0.47 for information speed processing which is clinically comparable to our primary outcome measure of overall cognition, and our HBOT trial on stroke patients[78] showed an effect size of 0.49 for the National Institutes of Health stroke scale, suggesting our detectable effect size of 0.49 is feasible.

Aim 2: As for Aim 1, power is presented for detecting the difference in the mean changes in CBF velocity and cerebral glucose utilization between the Sham and HBOT groups. Assuming a mean change in CBF velocity of -0.45 in the Sham group and an SD of 5.6 in both groups, with a minimum sample size of 67 patients per arm we have 80% power to detect an improvement in the HBOT group of 2.28, an effect size of 0.49. *To account for an anticipated dropout rate of 13%, we plan to enroll 77 patients per group for a total of 154 patients.* For cerebral glucose utilization, assuming a mean change in the sham group of -0.08 and an SD of 4.40 in both groups, with

67 patients per group we are powered to detect an improvement in the HBOT group of 2.065, an effect size of 0.49. According to literature sources[48, 123] these are observable effect sizes.

***D8. Future directions**: If this pilot study is successful, we will pursue a multi-center large-scale clinical trial to provide definitive evidence for the benefits of HBOT to cognition in T2D patients at high risk for dementia, taking advantage of the new knowledge acquired in this pilot study regarding effect sizes, design, recruitment strategies, attrition, cognitive outcomes, biomarkers, and subgroups benefitting most. Since HBOT is widely available and well tolerated, the proposed trial's success may suggest testing HBOT efficacy in non-T2D elderly with MCI as well.*

E. HUMAN SUBJECTS RESEARCH

1. Risks to the Subjects - Human Subject Involvement and Characteristics.

a. Involvement and Characteristics:

Involvement. The T2D subjects involved in this RCT will participate in neuropsychological assessments involving a clinical/diagnostic interview and paper and pencil tests. In addition, they will be interviewed in a risk factor inventory in which basic demographic, some medical information, and past behaviors (e.g. smoking, physical activity) will be assessed. In addition, subjects will go through an MRI examination and a PET scan. Subjects will be also asked about their activities of daily living and instrumental activities of daily living. Only subjects who have a diagnosis of T2D and a diagnosis of MCI will participate in the study. Subjects will be randomized to HBOT or sham. Both groups will be treated in the oxygen chamber for 60 sessions, 5 times/week, 90 minutes each session and will receive 100% oxygen. The HBOT group will have 2.0 ATA during the treatment while the sham group will receive 1.1 ATA during the first and last five minutes of the 90-minute sessions. All other conditions and assessments will be identical for the two groups. All assessments will be performed at baseline (before treatment), at 12 weeks (immediately after treatment) and at 12 months (to assess maintenance of HBOT effects in the long term). At 6 months, only the cognitive assessments will be assessed. Upon informed consent, subjects' long term diabetes characteristics (such as HbA1C, duration of disease, anti-diabetic medication status, and hypertension status) will be given by the subject who will provide the investigating team with the computerized medical chart from the HMO.

Characteristics. Eligibility criteria are summarized in **Table 1**, **section D2a** of the application. This study will consist of 154 elderly subjects (>65 years of age) who have T2D and a diagnosis of MCI (both amnestic and non-amnestic). Subjects must have at least one informant and must speak fluent Hebrew for the neuropsychological assessment. Subjects may not have a brain disease that might affect cognition such as schizophrenia or Parkinson's disease, they may not have epilepsy, inner ear disease, or claustrophobia. Subjects cannot be receiving cholinesterase inhibitors and cannot have an indication for HBOT, such as diabetic foot, since then they cannot be randomized to the sham group. There are no inclusion or exclusion criteria on the basis of sex, race, ethnicity, religion, or any other minority affiliations. Within the elderly, Israel is primarily a

country of immigrants and subjects may come from over 60 countries, from all continents.

b. Sources of Material. The sources of the research material will be obtained from individually identified living human subjects in the form of digitalized brain scan images, computerized medical records, and interview data and medical examination. This material will be obtained specifically for research purposes and only research staff will be able to access it.

c. Potential Risks. The potential risks are small. The assessments, tests, and questionnaires are innocuous, though the subjects may experience some boredom in the course of the interviews. It is possible that the perception of a poor performance in the cognitive assessment may lead to some anxiety but not more than is experienced in everyday life. Lying down in an MRI for 60 minutes may cause some discomfort and claustrophobia. The MRI machine is dangerous to approach when carrying metallic or magnetic objects or if subjects have surgical clips, magnetic prostheses, shrapnel or other metallic substances in their body. A study doctor will assess each subject just prior to the procedure to ensure that subjects are free of such materials. In addition to the MRI technicians, a member of the study team will accompany subjects to reassure participants and aid in relaxing them. When subjects finish all MRI procedures, they will lie down for a few minutes; they will be seated for another few minutes, until they feel comfortable and secure to stand up and leave. The FDG-PET exam will take about 50 minutes and will be performed on a separate day from the MRI so not to over-burden the subjects. The HBOT protocol will include 60 sessions, five times/week, for 90 minutes each session, at 2.0 ATA and 100% oxygen. The sham group will receive exactly the same protocol but with 1.1ATA at the first five and last five minutes of the 90-minute sessions. Description of the HBOT, MRI, and FDG-PET risks and the measures the study team will take to minimize them are described in the “Adequacy of Protection Against Risks” below.

2. **Adequacy of Protection Against Risks**

a. Recruitment and Informed Consent. Recruitment will be done through talks in the community, advertisements, and companies which have large lists of elderly interested in receiving emails about health related topics. All IDCD subjects have signed through the IDCD informed consent that they agree to be approached for other research projects. Subjects will have MCI but frank dementia is an exclusion criteria so they will have capacity to understand the informed consent (even mild to moderate cases with frank dementia typically have capacity to sign consent). Dr. Ravona-Springer, the PI of the Sheba site, is a senior geriatric psychiatrist and will perform the diagnostic process to confirm MCI. Cognitive assessments, MRI, and PET scans will be performed in Sheba. The HBOT and sham interventions will be performed at the oxygen multiplace chamber at Asaf Harofeh.

Consent will be obtained from the patient. The IRB-approved consent document will explain the purpose of the study, procedures to be followed, potential risks and benefits to be expected, costs, treatment alternatives, confidentiality statement, an offer to answer questions, a statement regarding the right to refuse to participate or withdraw, and a statement on rights and obligations if injury occurs during the course of the trial.

Subjects will be told that all identifying information will be maintained solely by the research staff and kept strictly confidential. The project will be required to be approved by all three sites IRB committees. The consent form informs the subjects upfront that this is a joint study of the Icahn School of Medicine at Mount Sinai, Asaf Harofeh, Sheba Medical Center, and University of Wisconsin. Informed consent will be obtained by Dr. Ravona, Dr. Efrati, or the other study physicians. All subjects are required to have an informant and the study is fully explained to the subjects before the initial assessment is performed. Dr. Efrati will again explain the study and the commitment required before entering the oxygen chamber for the first time. Signed and witnessed consents are kept in a locked file, in a locked room, with other patient data.

MRI: For the MRI component of the renewal, subjects will be asked to come to the Sheba Medical Center Department of Radiology where the MRI machines are located. Subjects will respond to a detailed questionnaire regarding the presence of body metals and only then entered into the magnet. It will be explained to subjects that lying down in the MRI may provoke claustrophobia and discomfort, because their head will be in a relatively narrow tube and the MRI makes loud sounds. Subjects will be told that the whole MRI session will take approximately 60 minutes; afterwards they will rest for a few minutes and sit for a few more minutes, and can leave when they feel comfortable.

FDG-PET- PET imaging involves exposure to small amounts of ionizing radiation, which has no expected harmful effects. No adverse effects from radiopharmaceuticals used in this study have been reported. However, the possibility exists for a rare reaction to any of the substances or procedures to which the subject is exposed and for that reason a physician will be present at all times in the PET-CT area when participants are in the scanner. The radiation dose received by the subjects from the [F-18]FDG scan will be below the limits established in 21CFR §361.1 for whole body (50 mSv), active blood forming organs, lens of eye and gonads (5 rad) and target organ (15 rad). The effective human radiation dose for a subject undergoing FDG-PET will be less than 15 mSv, based upon the published dosimetry data for FDG. FDG is a commonly used radioligand with no known side-effects. All radioligand samples will meet strict sterility and quality requirements and are closely monitored.

HBOT- Hyperbaric oxygen therapy is generally safe and well tolerated. Most side effects are mild and reversible, although severe consequences can occur in rare cases[89]. Middle ear barotrauma is the most common side effect of hyperbaric oxygen, with an incidence of less than 2%[89]. Middle ear effusions (which may be hemorrhagic) and tympanic membrane rupture occur in less than 1% of patients. Middle ear symptoms may be alleviated by teaching the patient autoinflation techniques which will be done before subjects enter the chamber. Sinus barotrauma (sinus squeeze) is the second most common complication of hyperbaric oxygen and is usually seen in patients with upper respiratory tract infections. Subjects with such conditions will not be allowed to continue the intervention until symptoms are over. Seizures due to central nervous system oxygen toxicity are a rare but a possible dramatic consequence of HBOT; estimates of incidence range from 1 in about 3000 to 2.4 per 100,000 treatments[90, 91]. The risk is increased by HBOT exposure greater than 120 minutes and by pressures greater than 2.8 ATA. In our study, patients will be treated for 90 minutes and at 2.0 ATA. Patients receiving glucocorticoids, insulin, thyroid replacement, and sympathomimetic medications may be at higher risk of central

nervous system oxygen toxicity. HBOT has been associated with hypoglycemia in some patients with diabetes, and hypoglycemia should therefore be considered in the differential diagnosis of HBOT-associated seizures[92]. Seizures due to oxygen toxicity do not typically result in permanent structural brain damage[89]. In the unlikely case of a seizure in this trial, it will be managed acutely by reducing the inspired oxygen concentration to that of air (FIO2 = 0.21), administering anticonvulsant therapy, and terminating hyperbaric treatment. Importantly, a nurse from the Hyperbaric Unit team will be at all times with the subjects inside the chamber, and a physician will be at all times in the Unit providing immediate medical response if any of the adverse events arises. All other potential adverse effects (reversible myopia, pulmonary oxygen toxicity) are very rare and reversible.

b. Protection Against Risk. The overall risks of this proposal are small. Potential risks will be minimized by enrolling only participants who meet eligibility requirements and have undergone screening medical assessment, including laboratory tests, to ensure health status, warning subjects and their caregivers of potential study risks, inquiring about potential adverse reactions at regular intervals and performing screening laboratory tests at regular intervals to help ensure continuing good health. Professional treatment will not be altered or modified for research purposes. Subjects are informed that they may terminate testing at any time should they find any aspect objectionable. The interviewers and research assistants will be clinically trained and sensitive to signs of stress, anxiety and/or fatigue. In the event that study participation results in injury or illness requiring emergency medical treatment, appropriate acute medical care will be provided at no cost to the subject. A Data Safety and Monitoring Board will be established to monitor adverse events and other safety concerns.

Protection of **patient confidentiality** and privacy will be rigorously guarded through several measures: 1) by the assignment of coded numbers to each file in the computer database; 2) storage of written information in locked filing cabinets at all times; 3) storage of electronic information in databases accessible only through a password that is not available to anyone outside of the study staff; and 4) no use of names or other identifying information in publications or other publicly available documents. However, subjects will be told that should confidential information be accidentally disclosed to the wrong party, it could have adverse consequences for the subject in terms of liability in legal proceedings, or discrimination in obtaining life or private health insurance. Again, we note that Israelis have the right for complete health coverage.

3. **Potential Benefits of the Proposed Research to the Subjects and Others**

Risks of this project to the subjects are judged to be small, the discomfort of having an MRI and a PET-scan, and the potential ear pressure during the HBOT sessions. Potential benefits include improved symptoms of cognitive function or slower rate of progression of dementia symptoms. Improved symptoms would be expected to improve patient quality of life. These potential benefits exceed the potential harm by HBOT to study participants. Additional possible direct benefits are those that might arise from a comprehensive cognitive and neuroimaging examination. Such assessments may provide clinically relevant information and lead to referrals for otherwise unrecognized cognitive or brain abnormalities that may require treatment. For this reason, the relatively small risks to subjects are reasonable in relation to the anticipated benefits to subjects and others.

4. **Importance of the Knowledge to be Gained**.

The overriding aim of this project is to examine the potential beneficial effect of HBOT on cognitive function in diabetic elderly who are at high risk of developing dementia since they already have mild cognitive impairment. The study will also examine whether HBOT affects beneficially the brain through improved cerebral blood flow and glucose uptake. Cognitive decline and dementia are major public health concerns today and will only become even greater concern in the future as longevity increases and the age structure of the population shifts toward increasingly older ages. Currently, approved treatment for dementia is only modestly effective, and it would be an important advance if HBOT will show effectiveness. In relation to this potential benefit, potential risks are reasonable.

5. **Data Safety and Monitoring Board**

A Data and Safety Monitoring Board (DSMB) will consist of four members, with expertise in the four areas relevant to this RCT: type 2 diabetes, cognitive aging, HBOT, and neuroimaging. No investigator involved in the trial is a member of the DSMB. The initial task of the DSMB will be to review the protocol, procedures and consent form to identify any necessary modifications. If modifications are necessary, revisions will be reviewed by the DSMB prior to its recommendation on initiation of the project. The modifications will be submitted and approved by the IRBs before study initiation. Throughout the study, any changes to the protocol will be submitted to the DSMB. The DSMB will identify the data parameters and format of the information to be regularly reported. The DSMB will be informed of the occurrence of any serious adverse events and immediately notified of fatal or life-threatening events. The DSMB may at any time request additional information from the PI. The DSMB will initially be provided with data blinded to treatment status, but they may request an unblinding if there is a safety concern. Based on the review of safety data, the DSMB will make recommendations regarding the conduct of the study. These may include amending safety-monitoring procedures, modifying the protocol or consent, terminating the study or continuing the study as designed. The discussions and decisions of the DSMB will be summarized in written reports and provided to the PIs. All DSMB reports will be shared with the Mount Sinai IRB, which is the supervisory IRB for this study and which will transfer these reports to the other 2 IRBs involved (Asaf Harfeh, Sheba) and will become part of the study regulatory binder. The DSMB will meet in person or by conference call on a quarterly basis. The DSMB will also provide advice regarding any discrepancies found by the data auditing system or other sources.

6. **Data and Safety Monitoring Plan.**

a. Data Quality The quality of the data will be monitored by the PIs. The following elements will be monitored: recruitment proceeding as expected; subjects fully match the inclusion/exclusion criteria; deviations

from the protocol; timeliness, accuracy, and confidentiality of all information both in study documents and database.

We are cognizant that overseeing a project that takes place in a geographically distant place and in another country poses challenges with respect to data quality and protocol adherence. The successful implementation of the IDCD study clearly demonstrates the feasibility of this international collaboration. In addition, we will conduct bi-weekly conference calls, one with the data collection team, and one with the senior investigators. Prior to each meeting, Dr. Beeri sends an email compiling the issues that should be discussed. The investigators of the study have maintained, literally, email and phone contact on a daily basis. Drs. Beeri and Sano work closely for over a decade. Dr. Beeri has international collaborations with all other study investigators involving other projects in Israel (IDCD study, IRAP study) and travels every other month to Israel, for face to face meetings and review of data and procedures on site. The PIs of the Asaf Harofeh (Dr. Efrati) and Sheba (Dr. Ravona) sites are both physician scientists. Thus, the investigators have all the necessary expertise relevant for this RCT, have continuous contact, and are well integrated around this (and other) joint dementia-related projects.

b. Safety Monitoring Safety monitoring for adverse events (AEs) will be conducted in real time by the Drs. Beeri and Sano together with Drs. Efrati and Ravona, both of whom are MDs, the Project Director, Research Coordinators, and the IRBs at Mount Sinai, Asaf Harofeh and Sheba and reported to the Data Safety and Monitoring Board. They will determine the severity of the event, will assign attribution to the event, and will monitor the event until its resolution. All adverse events will be indicated on the source documentation for the study, and on the specific adverse event report form. The following information about adverse events will be collected: 1) the onset and resolution of the AE, 2) an assessment of the severity or intensity (use existing grading scales whenever possible), 3) an assessment of the relationship of the event to the study (definitely, probably, possibly or not related), and 4) action taken (e.g., none, referral to MD, start or adjust concomitant medication). All serious adverse events (SAEs) will be reported to the three sites' IRBs within 24 hours of the investigative team learning about them. The initial report will be conducted by phone, email or fax. This initial contact will later be followed by a written report to the IRB (using their SAE reporting form). All serious adverse events (SAEs) will be reported to the DSMB within 48 hours.

F. **INCLUSION OF WOMEN AND MINORITIES-** Since in Israel about half of those above the age of 65 who have type 2 diabetes are women, we anticipate a similar proportion of women in the RCT. A large proportion of the Israeli elderly are immigrants from approximately 60 countries and there is **no** exclusion criteria for any country of origin, ethnic group or religion.

G. **INCLUSION OF CHILDREN-** Children will not participate in this study since it investigates the elderly.

CLINICALTRIALS.GOV REQUIREMENT- This project includes a trial which requires registration in

ClinicalTrials.gov. The project will be registered with ClinicalTrials.gov.

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