COVER PAGE

Official Study Title: **Cognitive and Functional Connectivity Effects of Methylene Blue in Healthy Aging, Mild Cognitive Impairment and Alzheimer's Disease**

NCT number: NCT02380573

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Unique Protocol ID: HSC20150410H

Protocol Template Form

Item 1 UTHSCSA Tracking Number	Cognitive and Functional Connectivity Effects of Methylene Blue in Healthy Aging, Mild Cognitive Impairment and Alzheimer's Disease

Item 2 Abstract / Project Summary	Provide a succinct and accurate description of the proposed research. State the purpose/aims. Describe concisely the research design and methods for achieving the stated goals. This section should be understandable to all members of the IRB, scientific and non-scientific. **DO NOT EXCEED THE SPACE PROVIDED**.

Purpose/Objectives: The purpose of the current proposal is to test the hypothesis whether daily oral MB for two week period (and up to 12 weeks if subject decides to continue participation) will improve memory, attention, cognition and functional connectivity in healthy aging volunteers, mild cognitive impairment (MCI) and Alzheimer's disease (AD) subjects by using objective MRI readouts and standard neuropsychological tests.

Research Design/Plan: Adult volunteers will be studied using a randomized, double-blinded, placebo-controlled design. MB and Placebo arms will be included for healthy aging, MCI and AD groups. MB and placebo pills will be compounded into blue immediate release capsules. Each arm will also receive a pill of Azo (phenazopyridine hydrochloride) with each daily dose as urinary tract analgesic and to maintain coloration of the urine in both groups. Subjects will have MRI scanning on two different days, fourteen days apart. After visit #2 is completed, the subject will be given the option to continue the study for another 10 weeks.

Methods: This study will use a low-dose oral USP MB (282mg) daily over a two-week period and subjects will take drug daily for two weeks combined with an additional daily dose of 97.5mg of Azo. Subjects will take 3 pills of MB/placebo each day (1 with each meal (94mg of MB per pill) and 1 pill of Azo for 14 days.

Daily dose intake may be extended to 12 weeks after the initial follow up visit.

Clinical Relevance: Middle-aged and elderly people with MCI are at high risk for developing Alzheimer's disease, a condition that slowly destroys memory and thinking skills. Approximately 5.2 million Americans are living with AD today and this number is predicted to triple by 2050. There is a critical need for therapies that can arrest the progression from MCI to AD and other dementias, and for objective and sensitive measures of efficacy for interventions.

Item 3 Background	
Describe past experimental and/or clinical findings leading to the formulation of your study. *For research involving unapproved drugs, describe animal and human studies.* *For research that involves approved drugs or devices, describe the FDA approved uses of this drug/device in relation to your protocol.*	Middle-aged and elderly people with mild cognitive impairment (MCI) are at high risk for developing Alzheimer's disease (AD), a condition that slowly destroys memory and thinking skills. Approximately 5.2 million Americans are living with AD today and this number is predicted to triple by 2050[1]. There is a critical need for therapies that can arrest the progression from MCI to AD and other dementias, and for objective and sensitive measures of efficacy for interventions. USP methylene blue (MB) is a FDA-grandfathered drug safely used to treat methemoglobinemia, carbon dioxide and cyanide poisoning in humans[2]. Daily 4 mg/kg oral MB has been used safely for one year in clinical trials. Oral MB readily enters the brain and has unique energy-enhancing and antioxidant properties and acts in the mitochondria to sustain or enhance ATP energy production, thereby promoting neuronal cell survival and cognitive enhancement [3]. MB has recently been shown to reduce behavioral impairments in animal models of Parkinson's disease [4] and Alzheimer's disease [5 6]. Our laboratory has shown with magnetic resonance imaging (MRI) that low-dose MB (intravenous 1 mg/kg) increases glucose uptake, oxygen consumption, blood flow [7] and evoked responses [8] in the rat brain *in vivo*. We also found that MB treatment reduces MRI-defined lesion and behavioral deficits in animal models of traumatic brain injury [9 10] and stroke [11 12]. As the first step to translate these experimental findings to humans, we are carrying out a

	randomized, double-blinded, placebo-controlled, phase II clinical trial (NCT01836094) to evaluate the effects of oral USP MB (280 mg) on memory and cognition by using cerebral blood flow (CBF), evoked functional MRI (fMRI) and resting-state functional connectivity fMRI (fcMRI) on young (30 yo) healthy volunteers. Our findings suggest the MB has favorable cognitive and memory effects. The subjects, 28 subjects to date, did not experience any negative adverse event. There are also a few MB clinical trials in mild to moderate AD and dementia (NCT01689246, NCT01689233, NCT01626378). An abstract of a phase II clinical trial reported that daily oral doses of 300 mg non-reduced form of MB (methylthioninium chloride), under trade name "Rember" (TauRx Therapeutics, Inc.), slowed the progression of AD compared to placebo [13]. Ongoing phase III clinical trials use a proprietary reduced form of methylene blue (leukomethylene blue, LMTX™, TauRx) in mild and moderate AD and variant frontotemporal dementia. Our clinical trial differs from these clinical trials in several aspects: (1) TauRx phase II and phase III trials enrolled subjects with mild to severe Alzheimer's disease and frontotemporal dementia (no MCI). Our study will be the first phase II clinical trial using MB in MCI, where early intervention is likely more effective. (2) TauRx phase II clinical trial used a large number of subjects with mild to moderate Alzheimer's dementia and a larger dose up to 300 mg daily for one year with Rember™ (non-reduced form of MB). Our phase II trial will offer a markedly lower sample size, lower dose, and shorter duration, enabled by objective and powerful fMRI readouts. (3) The TauRx phase III clinical trial uses LMTX™, a new reduced form of MB that is less well studied. Our phase II trial will use the non-proprietary *non-reduced* form of MB (methylthioninium chloride), which has been used in most preclinical studies and clinical trials. (4) Subjects will be recruited from among well-characterized participants and a large cohort in the Texas Alzheimer's Research and Care Consortium (TARCC). These subjects all have longitudinal psychometrics, and serum protein biomarkers on file, and frozen serum samples and DNA stored for future analyses. (4) The TauRx trials target to inhibit tau aggregation in neurofibrillary tangles of AD and demented patients, whereas we target to support healthy cognitive function by increasing mitochondrial oxidative energy metabolism and reducing oxidative stress in aging individuals before onset of AD neuropathology and dementia. Our proposed phase II trial is very similar to our current phase II trial (NCT01836094) except it now includes middle aged, MCI and Alzheimer's disease subjects and daily MB dose for 2 to 12 weeks (as opposed to healthy subjects and single dose) as well as additional neuropsychological tests.
Item 4 Purpose and rationale *Insert purpose, objectives and research questions/hypotheses here. If you cut and paste from another document, make sure the excerpted material answers the question*	The purpose of the current IND proposal is to test the hypothesis whether daily oral MB for 2 to 12 weeks will improve memory, attention, cognition and functional connectivity in *healthy middle aged, and healthy elderly* as well as *MCI* and *AD* subjects by using objective MRI readouts and standard neuropsychological tests. subjects by using objective MRI readouts and standard neuropsychological tests. MRI readouts include evoked functional MRI (fMRI), cerebral blood flow (CBF), and resting-state functional connectivity fMRI (fcMRI). We will utilize a double-blinded, placebo-controlled phase II clinical trial. MRI and neuropsychological tests will be administered over about 1.5 hours before oral MB, and again about 2 weeks after oral MB. The subject will have the option to continue for another 10 weeks after the completion of the first two weeks. The primary outcome measures will include fMRI readouts to assess episodic memory, working memory and sustained attention, respectively, before and two weeks after MB. Secondary outcome measures will include standard neuropsychological tests.

Item 5 Study Population(s) Being Recruited In your recruitment plan, how many different populations of prospective subjects do you plan to target? Provide number: 4	Identify the criteria for **inclusion**:	Identify the criteria for **exclusion**:

e.g., a population can be individuals with type 2 diabetes controlled with diet and/or a population of healthy controls. Or a population can be individuals attending an education program, etc. List each different population on a separate row and provide a short descriptive **label**: *(e.g., normal-healthy, diabetics, parents, children, etc.)* *To add rows use copy & paste*		
healthy middle aged	45 – 64 years old All genders All minorities English, Spanish, or multilingual speakers	Pregnancy or breastfeeding, Contraindication for MRI, such as Claustrophobia and magnetic metal implants, Glucose-6-phosphate deficiency, methemoglobinemia, Anemia, Allergy to MB, Color-blindness, Craniotomy, craniectomy or endovascular neurosurgery, A current diagnosis of stroke, transient ischemic attack (TIA), any primary neurodegenerative disorder, or any other causes of neuropsychologic disturbances or secondary dementia (MCI or AD does not exclude subject), A serious intercurrent illness likely to cause death within the next 5 years, such as terminal cancer, Alcohol and/or drug abuse, Any detection of an unknown disease process (eg. new tumor) on the study's neuroimaging at the discretion of the investigators, A systolic blood pressure ≥180 mmHg and/or a diastolic blood pressure ≥105 mmHg, Severe difficulty or an inability to perform any one of the 6 Katz Activities of Daily Living, Patients who are unlikely to comply with trial visit schedule or with trial medication, On any psychiatric serotonergic antidepressant medication or psychotropic medication within the last 5 weeks, Diagnosis of epilepsy, traumatic brain injury with loss of consciousness, psychosis, panic attacks, Chronic kidney disease or Cirrhosis, Liver or renal transplants. Known hypersensitivity to thiazide diuretics and phenothiazines, Any other condition, which in the opinion of the investigator, would put the participant at risk and warrant exclusion from the study
healthy elderly	65 – 89 years old All genders All minorities English, Spanish, or multilingual speakers	Pregnancy or breastfeeding, Contraindication for MRI, such as Claustrophobia and magnetic metal implants, Glucose-6-phosphate deficiency, methemoglobinemia, Anemia, Allergy to MB, Color-blindness, Craniotomy, craniectomy or endovascular neurosurgery, A current diagnosis of stroke, transient ischemic attack (TIA), any primary neurodegenerative disorder, or any other causes of neuropsychologic disturbances or secondary dementia (MCI or AD does not exclude subject), A serious intercurrent illness likely to cause death within the next 5 years, such as terminal cancer, Alcohol and/or drug abuse, Any detection of an unknown disease process (eg. new tumor) on the study's neuroimaging at the discretion of the investigators, A systolic blood

		pressure ≥180 mmHg and/or a diastolic blood pressure ≥105 mmHg, Severe difficulty or an inability to perform any one of the 6 Katz Activities of Daily Living, Patients who are unlikely to comply with trial visit schedule or with trial medication, On any psychiatric serotonergic antidepressant medication or psychotropic medication within the last 5 weeks, Diagnosis of epilepsy, traumatic brain injury with loss of consciousness, psychosis, panic attacks, Chronic kidney disease or Cirrhosis, Liver or renal transplants. Known hypersensitivity to thiazide diuretics and phenothiazines, Any other condition, which in the opinion of the investigator, would put the participant at risk and warrant exclusion from the study
MCI	45 – 89 years old All genders All minorities English, Spanish, or multilingual speakers Participants will meet the criteria for amnestic and non-amnestic (single or multiple domain) and mild AD such as those currently used by Texas Alzheimer's Research and Care Consortium (TARCC) consensus diagnosis. The AD subjects will only include early stage, late-onset, sporadic-type MB.	Pregnancy or breastfeeding, Contraindication for MRI, such as Claustrophobia and magnetic metal implants, Glucose-6-phosphate deficiency, methemoglobinemia, Anemia, Allergy to MB, Color-blindness, Craniotomy, craniectomy or endovascular neurosurgery, A current diagnosis of stroke, transient ischemic attack (TIA), any primary neurodegenerative disorder, or any other causes of neuropsychologic disturbances or secondary dementia (MCI or AD does not exclude subject), A serious intercurrent illness likely to cause death within the next 5 years, such as terminal cancer, Alcohol and/or drug abuse, Any detection of an unknown disease process (eg. new tumor) on the study's neuroimaging at the discretion of the investigators, A systolic blood pressure ≥180 mmHg and/or a diastolic blood pressure ≥105 mmHg, Severe difficulty or an inability to perform any one of the 6 Katz Activities of Daily Living, Patients who are unlikely to comply with trial visit schedule or with trial medication, On any psychiatric serotonergic antidepressant medication or psychotropic medication within the last 5 weeks, Diagnosis of epilepsy, traumatic brain injury with loss of consciousness, psychosis, panic attacks, Chronic kidney disease or Cirrhosis, Liver or renal transplants. Known hypersensitivity to thiazide diuretics and phenothiazines, Any other condition, which in the opinion of the investigator, would put the participant at risk and warrant exclusion from the study
AD (Alzheimer's Early-stage, late-onset, sporadic-type)	65 – 89 years old All genders All minorities English, Spanish, or multilingual speakers Participants will meet the criteria for amnestic and non-amnestic (single or multiple domain) and mild AD such as those currently used by Texas Alzheimer's Research and Care Consortium (TARCC) consensus diagnosis. The AD subjects will only include early stage, late-onset, sporadic-type MB.	Pregnancy or breastfeeding, Contraindication for MRI, such as Claustrophobia and magnetic metal implants, Glucose-6-phosphate deficiency, methemoglobinemia, Anemia, Allergy to MB, Color-blindness, Craniotomy, craniectomy or endovascular neurosurgery, A current diagnosis of stroke, transient ischemic attack (TIA), any primary neurodegenerative disorder, or any other causes of neuropsychologic disturbances or secondary dementia (MCI or AD does not exclude subject), A serious intercurrent illness likely to cause death within the next 5 years, such as terminal cancer, Alcohol and/or drug abuse, Any detection of an unknown disease process (eg. new tumor) on the study's neuroimaging at the

		discretion of the investigators, A systolic blood pressure ≥180 mmHg and/or a diastolic blood pressure ≥105 mmHg, Severe difficulty or an inability to perform any one of the 6 Katz Activities of Daily Living, Patients who are unlikely to comply with trial visit schedule or with trial medication, On any psychiatric serotonergic antidepressant medication or psychotropic medication within the last 5 weeks, Diagnosis of epilepsy, traumatic brain injury with loss of consciousness, psychosis, panic attacks, Chronic kidney disease or Cirrhosis, Liver or renal transplants. Known hypersensitivity to thiazide diuretics and phenothiazines, Any other condition, which in the opinion of the investigator, would put the participant at risk and warrant exclusion from the study

Item 6
Research Plan / Description of the Research Methods ***a.*** *Provide a* ***comprehensive narrative*** *describing the* ***research methods****.*
Provide the ***plan for data analysis*** *(include as applicable the* ***sample size calculation).***

Step-by-Step Methods:

Adult volunteers will be studied using a randomized, double-blinded, placebo-controlled design. Placebo arms will be included for healthy middle aged, healthy elderly, MCI and AD groups as controls. USP Methylene blue (MB) and placebo pills will be compounded into blue immediate release capsules. The subject will take 3 pills of MB or placebo (94mg per pill) three times a day with each meal. Placebo will be FD&C Blue # 2 which is a powder similar to the powder form of methylene blue. Each arm will also receive a pill of phenazopyridine hydrochloride (97.5 mg, marketed as Azo Standard) with each daily dosage. Phenazopyridine is a safe OTC product used for urinary discomfort that turns urine brownish. This drug will be given to both MB and placebo groups so that both show similar urine discoloration. This choice of administration is justified not only to maintain the blind procedure but because it would prevent any mild discomfort experienced by MB urinary excretion during chronic treatment. The subject, person administering the drug, and data analysts will not know whether the intervention is placebo or MB. The design will only be unblinded at the last steps when necessary. All subjects will be told that the pill they take may or may not cause urine/feces discoloration depending on individual's metabolism.

Step by Step:
The healthy elderly, MCI and AD subjects will be recruited from the Texas Alzheimer's Research and Care Consortium (TARCC). A neuropsychological battery of tests (eg. logical memory I, logical memory II, verbal fluency/COWA, Instrumental Activities of Daily Living (IADL), Global Depressive Scale (GDS), etc.) will be administered for all subjects before MRI study (2 month window).

Baseline MRI scan will be performed during visit 1.

Subject will be given labeled pill organizers with pills to take on days 1 through 14. Subject will be contacted via telephone on days 3, 7 and 11 (± 2 days) to document any adverse event or problem. After 2 weeks ± 3 days of MB (282 mg, oral x 14 days) or placebo, another neuropsychological tests and MRI scan will be obtained. On visit 2, the subject will be given the option to continue for another 10 weeks. During visit 3, after 10 weeks ± 3 days, the subject will complete the subset of neuropsychological tests completed in visit 1, and some of the MRI scans completed in visit 1.

For MRI, task-evoked BOLD fMRI, cerebral blood flow (CBF), resting-state fMRI, and CBF will be performed. For the task-evoked BOLD fMRI, subjects will perform the following scans while in the

scanner:

Delayed match-to-sample memory task: The DMS task measures reaction time in terms of memory retrieval latency. The subject views a 6x6 pattern for 4 seconds. After six seconds, the subject is presented with two patterns and the subject presses the left or right button based on whether the picture on the left or the right is the same as the previous picture. The correct and incorrect answers are recorded. This exercise is repeated with 22 random patterns for approximately 10 mins[18 19].

Face-Name Task: The subject is shown blocks of stimuli where a novel or familiar face is paired with a name. In a later run, the subjects are asked whether the correct name is matched with the correct face. The correct and incorrect answers are recorded. The acquisition of data is divided into encoding and recognition tasks. This exercise is repeated many times lasting about 10 minutes [20 21].

Psychomotor vigilance task: The PVT measures attention and time-on-task effects[22]. The subject receives a visual cue that alerts them to press a button as fast as possible. The reaction time is recorded. A rest period follows for 4-8 seconds. This exercise is repeated 30 times, lasting about 7-10 minutes.

Subject behavioral data (ie. Correct responses, reaction time) will be recorded simultaneously while the MRI data is acquired by a computer.

For CBF, the subject is instructed to close his or her eyes and relax in the scanner without falling asleep for 5-10 minutes.

For resting-state fMRI, the subject is told to close eyes and not to think about a particular topic, lasting about 10 minutes.

In addition, CBF will be measured during inhalation of medical-grade 5% CO2 in air for 3-5 minutes for calibration on all three visits. Standard anatomical MRI will also be acquired for co-registration. Diffusion tensor imaging (DTI) (5-7minutes) will also be acquired at one of the three visits.

Intervention Groups:

Arms	Assigned Interventions
Experimental: Healthy Middle Aged	Drug: Methylene Blue (USP grade, 282 mg oral, up to 12 weeks) Phenazopyridine hydrochloride (97.5 mg oral, up to 12 weeks)
Placebo: Healthy Middle Aged	Drug: FD&C Blue # 2 (USP grade, 282 mg oral, up to 12 weeks) Phenazopyridine hydrochloride (97.5 mg oral, up to 2 weeks)
Experimental: Healthy Elderly MB	Drug: Methylene Blue (USP grade, 282 mg oral, once daily, up to 12 weeks) Phenazopyridine hydrochloride (97.5 mg oral, up to 12 weeks)
Placebo: Healthy Elderly MB	Drug: FD&C Blue # 2 (USP grade, 282 mg oral, up to 12 weeks) Phenazopyridine hydrochloride (97.5 mg oral, up to 12 weeks)
Experimental: MCI MB	Drug: Methylene Blue (USP grade, 282 mg oral, once daily, up to 12 weeks) Phenazopyridine hydrochloride (97.5 mg oral, up to 12 weeks)
Placebo: MCI MB	Drug: FD&C Blue # 2 (USP grade, 282 mg oral, up to 12 weeks) Phenazopyridine hydrochloride (97.5 mg oral, up to 12 weeks)
Experimental: AD MB	Drug: Methylene Blue (USP grade, 282 mg oral, up to 12 weeks) Phenazopyridine hydrochloride (97.5 mg oral, up to 12 weeks)

Placebo: AD MB	Drug: FD&C Blue # 2 (USP grade, 282 mg oral, up to 12 weeks) Phenazopyridine hydrochloride (97.5 mg oral, up to 12 weeks)

The daily 282mg of MB or place will be divided into three doses of 94mg each to minimize the risk for urinary irritation. A second pill of Azo will be taken during one of these doses.

OUTCOMES:

Primary Outcome Measures:

Working memory task fMRI measures
[Time Frame: baseline, 2 weeks ± 3 days, 12 weeks ± 3 days]
Delayed Match to Sample Task behavioral (ie. correct number of responses) and fMRI measures will be acquired simultaneously

Episodic memory task fMRI measures
[Time Frame: baseline, 2 weeks ± 3 days, 12 weeks ± 3 days]
Face-Name Task behavioral (ie. correct recalls) and fMRI measures will be acquired simultaneously

Sustained attention task fMRI measures
[Time Frame: baseline, 2 weeks ± 3 days, 12 weeks ± 3 days]
Psychomotor vigilance task behavioral (ie. reaction time) and fMRI measures will be acquired simultaneously

Neuropsychological Battery
[Time Frame: baseline, 2 weeks ± 3 days, 12 weeks ± 3 days]
TARCC designed psychometric tests

Secondary Outcome Measures:

Cerebral blood flow MRI measures
[Time Frame: baseline, 2 weeks ± 3 days, 12 weeks ± 3 days]
Resting measurements will be used to assess response and CBF

Tertiary Outcome Measures:

Functional Connectivity (resting state) fMRI measures
[Time Frame: baseline, 2 weeks ± 3 days, 12 weeks ± 15 days]
fMRI measurements will be obtained while the subject rests in the scanner

CO2 challenge: Cerebral blood flow measurements will be acquired during a brief (3-5 minutes) inhalation of medical-grade 5% CO2 in air for fMRI signal calibration purposes.
[Time Frame: baseline, 2 weeks ± 3 days, 12 weeks ± 15 days]

Data Analysis Plan: Statistical parametric analysis will be performed to generate activation maps using established fMRI software. Task-evoked changes in brain activities will be analyzed and contrasted between placebo and MB conditions. ANOVAs, paired t and 2-sample t-tests will be used in analyzing the reaction times and success/failure rates in the DMS, FNT and PVT, with blocks (pre-MB vs. post-MB) as a within-subject variable and treatment (MB vs. Placebo) as a between-subject variable. Paired t-test will be used for group comparison with $p < 0.05$ (False Discovery Rate $q < 0.05$) considered statistically significant.

References

1. Alzheimer's A. 2014 Alzheimer's disease facts and figures. Alzheimer's & dementia : the journal of the Alzheimer's Association 2014;**10**(2):e47-92.
2. Bruchey AK, Gonzalez-Lima F. Behavioral, Physiological and Biochemical Hormetic Responses to the Autoxidizable Dye Methylene Blue. American journal of pharmacology and toxicology 2008;**3**(1):72-79.
3. Rojas JC, Bruchey AK, Gonzalez-Lima F. Neurometabolic mechanisms for memory enhancement and neuroprotection of methylene blue. Progress in neurobiology 2012;**96**(1):32-45.
4. Telch MJ, Bruchey AK, Rosenfield D, et al. Effects of post-session administration of methylene blue on fear extinction and contextual memory in adults with claustrophobia. Am J Psychiatry 2014;**171**(10):1091-8.
5. Medina DX, Caccamo A, Oddo S. Methylene blue reduces abeta levels and rescues early cognitive deficit by increasing proteasome activity. Brain pathology (Zurich, Switzerland) 2011;**21**(2):140-49.
6. Wischik CM, Edwards PC, Lai RY, et al. Selective inhibition of Alzheimer disease-like tau aggregation by phenothiazines. Proceedings of the National Academy of Sciences of the United States of America 1996;**93**(20):11213-18.
7. Lin AL, Poteet E, Du F, et al. Methylene blue as a cerebral metabolic and hemodynamic enhancer. PloS one 2012;**7**(10):e46585.
8. Huang S, Du F, Shih YY, et al. Methylene blue potentiates stimulus-evoked fMRI responses and cerebral oxygen consumption during normoxia and hypoxia. NeuroImage 2013;**72**:237-42.
9. Watts LT, Long J, Chemello J, et al. Methylene Blue is Neuroprotective against mild Traumatic Brain Injury. J Neurotrauma 2014;**13**:1063-71.
10. Long JA, Watts LT, Chemello J, et al. Multiparametric and longitudinal MRI characterization of mild Traumatic Brain Injury in rats. J Neurotrauma 2014, in press:10.1089/neu.2014.3563.
11. Rodriguez P, Jiang Z, Huang S, et al. Methylene blue treatment delays progression of perfusion-diffusion mismatch to infarct in permanent ischemic stroke Brain Res 2014;**1588**:144-49.
12. Shen Q, Du F, Huang S, et al. Neuroprotective efficacy of methylene blue in ischemic stroke: an MRI study. PLoS One 2013;**8**:e79833.
13. Wischik CM, Bentham P, Wischik DJ, et al. O3-04-07: Tau aggregation inhibitor (TAI) therapy with rember™ arrests disease progression in mild and moderate Alzheimer's disease over 50 weeks. Alzheimer's & Dementia 2008;**4**:T167.
14. Peter C, Hongwan D, Kupfer A, et al. Pharmacokinetics and organ distribution of intravenous and oral methylene blue. European journal of clinical pharmacology 2000;**56**(3):247-50.
15. Walter-Sack I, Rengelshausen J, Oberwittler H, et al. High absolute bioavailability of methylene blue given as an aqueous oral formulation. European journal of clinical pharmacology 2009;**65**(2):179-89.
16. Gonzalez-Lima F, Bruchey AK. Extinction memory improvement by the metabolic enhancer methylene blue. Learn Mem 2004;**11**(5):633-40.
17. Naylor GJ, Martin B, Hopwood SE, et al. A two-year double-blind crossover trial of the prophylactic effect of methylene blue in manic-depressive psychosis. Biological psychiatry 1986;**21**(10):915-20.
18. Mackworth JF. Vigilance, arousal, and habituation. Psychological review 1968;**75**(4):308-22.
19. Sawaguchi T, Yamane I. Properties of delay period neuronal activity in the monkey dorsolateral prefrontal cortex during a delayed matching-to-sample task. J Neurophysiol 1999;**82**:207-2080.
20. Rombouts SA, Barkhof F, Goekoop R, et al. Altered resting state networks in mild cognitive impairment and mild Alzheimer's disease: an fMRI study. Hum Brain Mapp 2005;**26**(4):231-9.
21. Wang L, Li H, Liang Y, et al. Amnestic mild cognitive impairment: topological reorganization of the default-mode network. Radiology 2013;**268**(2):501-14.
22. Lim J, Wu WC, Wang J, et al. Imaging brain fatigue from sustained mental workload: an ASL perfusion study of the time-on-task effect. Neuroimage 2010;**49**(4):3426-35.
23. Mumford JA, Nichols TE. Power calculation for group fMRI studies accounting for arbitrary design and temporal autocorrelation. NeuroImage 2008;**39**(1):261-68.

Item 7 Risks Section:

Complete the following table to describe the risks of all **research procedures** listed in Step 2, Institutional Form (items 28-34). *Do not list risks of Routine care procedures here.*

☒ N/A, Risks are described in the informed consent document – do not complete this table.

Research procedures	**Risks**
example: • History and physical • Questionnaire • Laboratory tests *Add or delete rows as needed*	List the reasonably expected risks under the following categories as appropriate: