CLINICAL RESEARCH PROTOCOL

ATX-001: A 6-month, Phase II Randomized, Double-Blind, Placebo-Controlled, Flexible Dosing, Crossover Trial of Atomoxetine in Subjects with Mild Cognitive Impairment.

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Anonymous Philanthropic Donor

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1. BACKGROUND AND SIGNIFICANCE

The Alzheimer's Disease (AD) epidemic is a looming crisis, with an urgent need for new therapies to delay or prevent symptom onset and progression. There is growing awareness that clinical trials must target stage-appropriate pathophysiological mechanisms to effectively develop disease-modifying treatments [1]. Advances in AD biomarker research have demonstrated changes in amyloid, brain metabolism, and other pathophysiologies prior to the onset of memory loss, with some markers possibly changing one or two decades earlier [2,3]. These findings suggest that amyloid-based therapies would optimally be targeted long before symptoms first become apparent with mild cognitive impairment (MCI). Alternatively, since MCI coincides with the onset of brain atrophy[4-6], this early stage of AD pathogenesis may offer a critical window of time to initiate novel therapies aimed at the secondary wave of events that lead to progressive neurodegeneration. This proposal seeks to apply insights that have recently emerged from basic research in animal models of AD: loss of norepinephrine (NE) incites a pro-inflammatory condition that is neurotoxic and reduces Aß clearance, and remarkably, rescue of norepinephrine reverses these effects and slows neurodegeneration. This proposal seeks to extend this proof-of-concept to humans for the first time. We propose that atomoxetine, a selective norepinephrine transport inhibitor, is an ideal drug to translate these findings to humans because it is already FDA-approved and safe in the elderly. Here we provide a brief rationale for our proposal to test the proposed mechanism of action of atomoxetine in subjects with MCI using biomarkers of inflammation and neurodegeneration. The study will provide the essential next step to move this idea into an NIH funded multi-center clinical trial.

1.1 Preclinical studies have identified a surprising but powerful role for locus coeruleus (LC) and NE in AD pathogenesis. Extensive LC degeneration is nearly universal in AD[7-11], among the earliest pathologies[12-14], and it has long been known that decreased NE is linked to cognitive, mood and neuropsychiatric symptoms[15-20]. *<u>However, recent studies show that LC dysfunction in AD animal models also plays a direct causal role in pathogenesis, with NE loss accelerating amyloid deposition and neuron death via pleiotropic effects on chronic inflammation, neurotrophin expression, cerebral metabolism, cholinergic neurons, and blood brain barrier permeability, in addition to deleterious effects on cognition – recapitulating key aspects of AD</u>* [20-27]. These effects of decreased NE levels have been confirmed and extended in several independent laboratories and lines of AD mice[21,23-25,28]. Thus, combining LC lesions with expression of familial AD mutations more closely recapitulates the neuropathological and cognitive symptoms of AD than mutant APP expression alone, and implicates LC loss as a crucial component of AD.

1.2 Neuroinflammation is a key mechanism linking loss of LC neurons and NE innervation with AD***.*** Recent studies provide insights into the mechanisms by which LC dysfunction and NE loss contribute directly to AD pathogenesis. Major targets of NE include microglia, astrocytes, and endothelia, which in healthy conditions acts through ß-adrenergic receptors to suppress the expression of multiple pro-inflammatory genes, including major histocompatibility complex class II, tumor necrosis factor-α, interleukin-1β, and the

inducible nitric oxide synthase [29,30]. Furthermore, NE is critical for the expression of anti-inflammatory molecules such as nuclear factor kappa B (NF-kappaB) inhibitory IkappaB proteins, and of heat shock protein (HSP70)[31]. Heneka et al.[27] demonstrated that in APP23 transgenic mice, but not wildtype mice, LC lesion profoundly increased Aß plaque load, brain inflammation, and spatial memory deficits. These effects of diminished NE levels were mediated by impaired microglial phagocytosis and clearance of Aß, and a switch in microglial cytokine expression from a neuroprotective anti-inflammatory profile to a pro-inflammatory and neurotoxic profile [24,26,27].

1.3 Animal Model Experience. Importantly, treatments that increase NE in AD animal models ameliorate AD pathology and cognitive decline. Pharmacological treatments of 5X FAD mice (5 months of age) with the NE transporter (NET) inhibitor atomoxetine and the NE precursor L-threo-3,4-dihydroxyphenylserine (LDOPS) effectively raised brain NE levels, reduced inflammatory changes, increased expression of Aß clearance enzymes, increased BDNF, reduced Aß burden, and improved spatial memory function [25,32]. Moreover, as shown in the preliminary data, our colleague Dr. David Weinshenker has confirmed and extended many of these findings using a genetic strategy to knock out NET. These findings, together with the strong links between LC/NE loss in AD and disease progression in AD animal models, clearly demonstrate the exciting disease-modifying potential of drugs that increase NE levels. *The urgent and essential next step is to translate these discoveries to humans in a clinical investigation of the NET inhibitor atomoxetine, an FDA-approved drug for treatment of ADHD*.

This pilot study in humans will focus on biomarkers and safety. Our central hypothesis is that atomoxetine will increase central NE levels and reduce CSF pro-inflammatory analytes, increase Aß clearance and slow neurodegeneration in subjects with mild cognitive impairment (MCI). The primary outcome will be measures of inflammatory markers in the CSF, to test the proposed mechanism of action of NE. The neuro-inflammatory response observed following LC lesions and its exacerbation of AD pathology and behavior in animals, converge with extensive evidence linking inflammation to AD in humans as summarized in several excellent reviews [28,33-40]. While some evidence comes from epidemiological and pathological studies, recent biomarker studies in living subjects have also directly shown signs of a pro-inflammatory state [38,39,41,42]. Of note, increased pro-inflammatory and decreased anti-inflammatory markers account for the majority of changes detectable in a large panel of CSF analytes in MCI and AD [41,42] (see preliminary data). Because biomarkers in the CSF are responsive to several different types of drug treatments [43,44], including atomoxetine [45], multi-analyte panels will provide an excellent tool to investigate treatment effects on inflammatory biomarkers.

1.4 Previous Human Experience Atomoxetine appears to be safe in the elderly, and has the potential to prevent, slow, or even reverse disease progression. We believe that atomoxetine, an FDA-approved NET inhibitor that is a safe and widely prescribed treatment for children and adults with ADHD, is the best choice among currently available pro-noradrenergic drugs to test in MCI. Atomoxetine has high affinity and selectivity for the NET (Ki for reuptake inhibition *in vitro* = 1.9 nM for NET vs. 750 nM for SERT and 1600 for DAT). It has very weak affinity for adrenergic, dopaminergic, histaminergic, serotonergic,

and cholinergic receptors that are typically blocked by first generation tricyclic reuptake inhibitors (e.g., desipramine) [46].

A few studies have examined atomoxetine in elderly patients with neurodegenerative disease to assess safety, tolerability and symptomatic effects. Marsh et al [47] studied 12 patients with Parkinson's Disease (PD) with doses up to 100 mg daily (mean tolerated dose 89.6 mg), with excellent safety, tolerability, and improved executive function. Weintraub and colleagues [48] found that 80 mg once daily was well tolerated by PD subjects as a treatment for depression; only 4 of 29 withdrew because of adverse effects. A 6-month phase II trial in mild to moderate AD tested up to 80 mg once daily in 47 subjects, and only 5 of them withdrew because of adverse effects [49].

The proposed Phase IIa, double-blind, placebo-controlled, 6 month crossover study will examine the effect of atomoxetine in 40 subjects with MCI. The primary outcome will be CSF biomarkers of inflammation and their relationship to effects on noradrenergic function. We will also explore rates of change in biomarkers of neurodegeneration (Aß, tau, brain atrophy rates), and collect data on safety and symptom progression. Collectively, these results will inform design of a larger phase II multi-center trial application to the NIH within 2 years time.

2. SPECIFIC AIMS

2.1 Aim #1 **To evaluate safety and tolerability of atomoxetine in individuals with MCI and define the dose-response effect on biomarkers of noradrenergic function**. Although atomoxetine is used clinically for ADHD, it is unknown whether atomoxetine administration will measurably impact indices of NE tone in subjects with MCI and be safe and tolerable in these patients. Safety will be assessed by standard adverse event scales, suicidal ideation monitoring, electrocardiograms, and vital signs. We will determine the maximum tolerated doses of atomoxetine (10, 18, 40, 60, 80, 100 mg po daily) and the dose-response effect on NET inhibition. NE metabolites, serum atomoxetine levels, and NET occupancy will be measured in the blood each week after escalating doses of atomoxetine, and in the CSF after stable maximal tolerated doses.

EXPECTED OUTCOMES: Atomoxetine will be safe, well-tolerated with a treatment-associated drop out rate < 15%, and will show a dose-response effect on blood and CSF markers of noradrenergic tone, including NE and its primary neuronal metabolite dihydroxyphenylglycol (DHPG).

2.2 Aim #2 **To assess the effect of chronic atomoxetine treatment (6 months) on biomarkers of inflammation and neurodegeneration in subjects with amnestic MCI.** In rodent models of AD, LC lesions accelerate AD related pathophysiology via dysregulation of microglial function and inflammation, whereas NET inhibition improves microglial function, ameliorates Aβ plaque deposition, and increases spatial and social memory. The effect of NET inhibition on inflammation and AD biomarkers in a clinical population is unknown. This study will examine the effect of atomoxetine on:
Biomarkers of inflammation (including TNF-alpha, IL1, IL-6, IL-17, IL-10, IL-4 and BDNF and VEGF) in blood and CSF using the Rules Based Medicine (RBM) InflammationMAP analyte panel. We predict that atomoxetine treatment will measurably reduce CSF levels of pro-inflammatory biomarkers and increase levels of anti-inflammatory biomarkers.

CSF biomarkers of AD (Aß40, Aß42, tau, phospho-tau) using highly sensitive and standard methods adopted in ADNI. Because these markers are most dynamic during preclinical stages of AD pathogenesis, we predict that 6 months of atomoxetine treatment will not result in any significant change in CSF Aß, tau and phospho-tau levels compared to placebo.

Brain imaging biomarkers (vMRI, ASL-MRI, FDG PET). The annual rate of brain atrophy is among the best biomarkers of neurodegeneration and predictive of the conversion from MCI to AD. The rate of change in medial temporal lobe atrophy in atomoxetine vs. placebo treated subjects will be measured in order to guide statistical projections and power analyses for subsequent clinical trials. However, the 6-month design limitation will make it unlikely to observe significant changes in atrophy rates, given these are slowly evolving processes. We predict that more robust brain imaging markers of function, including FDG PET to measure cerebral metabolism, and arterial spin labeling MRI (ASL-MRI) to measure cerebral blood flow, both of which are dynamic and reduced in early stages of AD, will show significant improvement with atomoxetine treatment compared to placebo.

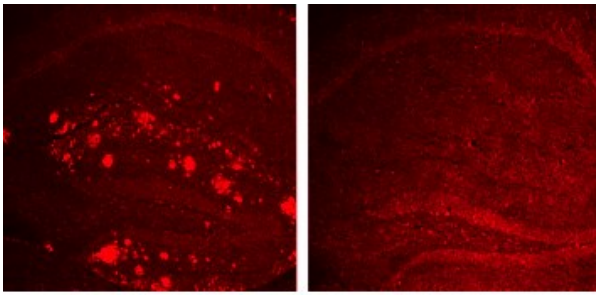
EXPECTED OUTCOMES: Pro-inflammatory analytes, including IL1-alpha, will be reduced by ≥ 25% in the CSF with atomoxetine treatment. We also predict significant increases in FDG-PET and ASL-MRI measures of cerebral metabolism and blood flow, respectively, with atomoxetine treatment.

2.3 Aim #3 **To explore the effect of chronic atomoxetine treatment (6 months) on cognitive and behavioral symptoms in subjects with amnestic MCI.** Although our primary goal is to measure drug effects on biomarkers of inflammation, preclinical studies have demonstrated symptomatic benefits of NE pharmacotherapies. There are no prior studies of atomoxetine effects on symptoms in subjects with MCI. Cognition, function, and neuropsychiatric symptoms will be measured at baseline and after 6 months of treatment using standardized instruments from ADNI and other ADCS trials.

EXPECTED OUTCOMES: Atomoxetine treatment will result in a favorable trend on cognitive assessments without worsening of behavior and neuropsychiatric symptoms.

3. PRELIMINARY DATA

3.1 NET Inhibition reduces amyloid deposition in AD mouse models. As described in the background, LC lesions consistently and dramatically accelerate Aß deposition through the effects of NE loss on microglial function, inflammation, and Aß clearance in a variety of transgenic mouse lines with familial AD mutations [21-24,26-28], while enhancing NE levels pharmacologically with atomoxetine and the NE precursor LDOPS has beneficial effects on AD pathology and behavior [32].



Our collaborator, Dr. David Weinshenker at Emory, has pursued complementary studies in AD mouse models using genetic approaches. Shown here is an example of hippocampal Aß burden as measured with Thioflavin S staining in an AD mouse model expressing APP/PS1 mutations (left), and the same strain of mouse

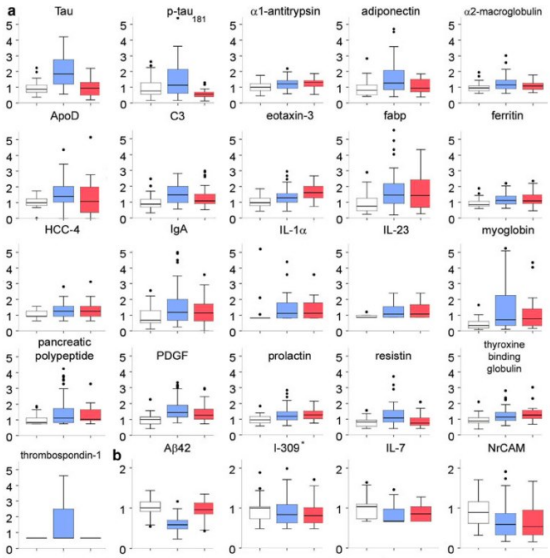
also genetically deficient in the norepinephrine transporter (NET, right panel). Genetic inhibition of NET resulted in a dramatic amelioration of Aß deposition and improved behavior (not shown). Although genetic strategies have significant differences from the proposed pharmacological treatment with atomoxetine, these results are consistent with pharmacological rescue of NE in the AD mouse models, and provide proof-of-principle that NE-based treatment strategies offer considerable promise for AD treatment.

3.2 Measurement of CSF Analytes and Inflammatory Markers in MCI and AD.

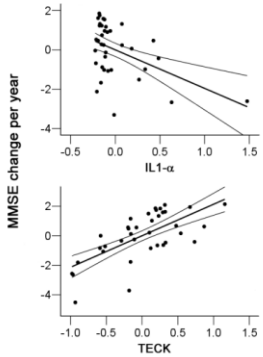
Dr. William Hu has established a CSF biomarker laboratory for the Emory ADRC and will be responsible for the proposed CSF studies. He has established a rigorous process with standard operating procedures for routine lumbar punctures in the memory disorders clinic, using collection and analysis procedures outlined in national recommendations based on ADNI that appear to predict conversion from MCI to AD [50]. An example of our results for soluble Aß42, total tau, and phospho-tau$_{181}$ in Emory control, MCI, and AD cases are shown in the table.

	Control (n=12)	MCI/AD (n=35)
Age (SD), yr	58.4 (11.3)	65.6 (7.6)
Aß42	223.7 (121.4)	110.6 (59.2)
Total Tau	52.1 (26.9)	126.1 (51.7)
p-Tau$_{181}$	32.4 (23.9)	82.7 (36.3)

Preliminary studies of CSF biomarkers have successfully demonstrated altered inflammatory analytes in MCI and AD using Rules Based Medicine (RBM) panels. The figure on the right from Dr. Hu shows only those RBM analytes that are significantly increased or decreased in AD compared to controls and non-AD dementias [41]. Note that many of the altered analytes reflect inflammation (e.g., C3, eotaxin, HCC-4, IgA, IL-1alpha, IL-23, resistin, IL-7). Boxplots show median values and quartiles of those CSF biomarkers that differed in levels between subjects with normal cognition and AD. Values shown are normalized to mean values of cognitively normal subjects. The analytes elevated in AD are shown on the top (a), and analytes decreased in AD on the bottom (b). White bars are cognitively normal subjects (n=33), blue bars autopsy-confirmed cases of AD (n=66), and red bars autopsy-confirmed non-AD neurodegenerative cases (n=25).



Among the inflammatory markers changed in AD, IL1-alpha and TECK appear to best predict subsequent cognitive decline. The figure on the right shows the correlations with the levels of each analyte and annual change in MMSE score. Collectively, these results demonstrate our ability to collect and analyze CSF in study participants, and highlight the early changes in inflammatory biomarkers that occur in MCI and AD. We predict that increasing



NE levels in MCI patients treated with atomoxetine will correlate with reductions in CSF IL1-alpha, TECK, and possibly other inflammatory analytes in the RBM assay.

4. EXPERIMENTAL DESIGN AND METHODS

4.1 Overview: A double-blind placebo-controlled crossover trial will be conducted by recruitment at a single site (Emory). Initially, forty subjects with mild cognitive impairment (amnestic or multi-domain subtypes) will be randomly assigned to treatment with placebo or flexible doses of the NET inhibitor atomoxetine, starting with 10 mg po daily and increasing weekly by increments to a maximum of 100 mg po daily or the maximum tolerated dose. Participants will be treated for 6 months, and will undergo venous blood draws and lumbar puncture for biomarker analyses at baseline and at 6 months. At the six month time point, subjects assigned to active treatment will crossover to placebo and those subjects who were initially randomized to placebo will initiate active treatment. The primary goals are to measure safety and norepinephrine metabolites in order to verify the intended pharmacological response to treatment, and biomarkers of inflammation in order to test the hypothesized mechanism of action of treatment. Secondary objectives will mirror the design of the ADNI to monitor potential effects of treatment on clinical findings and biomarkers of AD progression, including CSF amyloid, tau, and phospho-tau, and volumetric MRI, as well as FDG-PET and arterial spin labeling MRI.

4.2 Sample Size and Power. The study design consists of an atomoxetine group and placebo controlled group of MCI. Approximately 15% of subjects with MCI go on to develop AD annually [51,52], but the ADNI study has demonstrated that biomarkers of AD provide much more robust measures of decline and can dramatically reduce the size of clinical trials[53]. Forty MCI patients will be randomized to placebo or atomoxetine with crossover at the six month timepoint. Assuming 15% (n=6) drop-out rate over the course of the trial, there will be 34 completers. Based on the inflammatory biomarker studies of our colleague Dr. William Hu (see preliminary studies) [41,42,54], and assuming that 80% of the patients taking the placebo experience an overall worsening of the RBM analytes that were significantly altered (increased or decreased) in AD compared to controls, and at least 25% of the patients taking atomoxetine show no worsening profile, would yield 87.7% power. This is a reasonable expectation as we expect some key biomarkers will be more reactive than others.

4.3 Atomoxetine Dosing. Atomoxetine is a selective norepinephrine reuptake inhibitor that is approved by the FDA at a dose range of 10-100 mg daily for the treatment of ADHD in adults. It has well-established efficacy and safety in the treatment of ADHD. The drug is available for purchase (manufacturer Eli Lilly). Our rationale for atomoxetine dosing is based on prior studies.

The trial will consist of a double-blind administration of either atomoxetine, with dose escalation from 10-100 mg po daily, or placebo. The schedule will be 10 mg po once daily (after breakfast to prevent nausea), with weekly increases pending tolerability: 18 mg week 2, 40 week 3, 60 mg week 4, 80 mg week 5, and then 100 mg week 6 unless side effects emerge. If there are any concerns about side effects, the dose will be decreased to the previously tolerated level. Clinician judgment will be used to maintain dose or after one

week increase by 10 mg. Dosage will be increased in the same manner for subjects who crossover from placebo to atomoxetine at the six month timepoint.

A compounding pharmacy approved by Emory's Investigational Drug Service (IDS) will form capsules containing atomoxetine or matching placebo. Study medications will be stored at the Emory IDS and dispensed by study personnel. Subjects will be dispensed the initial 10 mg or placebo capsules after the baseline visit (2b), and will return every 2 weeks for safety assessment and additional medication dispensing. Crossover to either placebo or active treatment will occur at week 29. Participants will be provided sufficient medication for dosing adjustments. Weekly phone visits during this dose escalation phase will provide additional review of safety and tolerability. Dosing instructions will be provided at each phone call or visit.

4.4 Future research samples. Per individual subject consent, blood, cell lines and cerebrospinal fluid samples will be stored indefinitely and may be used for future investigation regarding atomoxetine pharmacology and/or side effects, potential development of new biomarkers, and potential treatments for Alzheimer's disease. Samples may also be used to deal with future safety issues for atomoxetine or related drugs for Mild Cognitive Impairment or Alzheimer's disease that are not known at the present time. These stored samples may also be used for genetic (DNA) research.

5. RESEARCH STUDY PERSONNEL

5.1 Principal Investigator (PI) for the study is Allan I. Levey, MD, PhD., Professor and Chair, Department of Neurology. The PI will delegate authority for all study procedures performed as noted on the Delegation of Authority log.

5.2 Co-Investigators include Drs. Lah, Hu, and Cellar.

5.3 Research Coordinator. Experienced research coordinator(s) will be utilized to coordinate the research study.

5.4 Psychometrician. Psychometric tools will be administered by research staff certified to conduct the specific tests utilized. The psychometrician will be blinded as to subject adverse events.

5.5 Blinded clinical rater. Clinical rating tools will be administered by a rater who is blinded as to subject adverse events.

5.6 Statistician. A statistician employed by the School of Public Health will be employed for the project.

6. SAFETY MONITORING AND ADVERSE EVENT REPORTING

6.1 Internal Safety Monitoring will be performed by the Investigators who will review data from all subject visits and review all aspects of patient safety. Serious and unexpected adverse events and/or unanticipated problems involving risks to participants or others will be reviewed and reported to the Emory IRB, as per the Emory IRB policies and procedures, by the research coordinator in collaboration with the investigators.

All laboratory reports must be reviewed, initialed and dated by the Principal Investigator or Sub-Investigator who is a physician or other qualified health care professional who is

involved in the study conduct. Each abnormal test will be evaluated using the CTCAE Version 4 and will be assessed as clinically significant (CS) or not clinically significant (NCS). All CS abnormal laboratory values that represent an unexpected change from baseline will be assessed as an Adverse Event (AE) and an AE form will be completed.

Blood pressure cut-off for maximum tolerated dose of atomoxetine is systolic blood pressure (SBP) $\geq$ 160; diastolic blood pressure (DBP) $\geq$ 100. Blood pressures will be obtained after patient has been sitting with both feet on the floor x 5 minutes. Three blood pressures will be taken and the average of the three blood pressures will be recorded. In the event that a subject experiences blood pressure elevations as described, the dose of atomoxetine will be decreased to the maximum dosage to maintain SBP <160 and DBP <100.

6.2 Independent Data Safety Monitoring Board has been engaged by the Principal Investigator.

Composition of the DSMB: The committee will consist of permanent members from the Clinical Research Oversight Committee and ad-hoc members from the Department of Psychiatry and Behavioral Sciences as needed to review reports.

Submission of Reports to the DSMB: At least four weeks prior to each DSMB meeting, the study principal investigator (PI), or his or her designee, will prepare a report to be reviewed during that meeting. The report will follow the template available. Data required for the report include the following:

1. The most recent IRB-approved version of the informed consent form, or most recent draft if the study does not yet have IRB approval.
2. A summary of the study protocol.
3. The total number of participants who have signed consent for the study.
4. The total number of participants randomized (if applicable)
5. The number of participants terminating early from the study, with a tally of the reasons for these early terminations.
6. The number of serious adverse events that have occurred, with a summary report of each event and its resolution.

   A serious adverse event is defined as: “Any adverse experience occurring that results in any of the following outcomes: death, a life-threatening adverse experience, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant disability/incapacity, or a congenital anomaly/birth defect.”
7. A summary of all adverse events occurring over the course of the study.
8. Copies of all Reportable Events submitted to the IRB.
   1. The PI will notify the DSMB of any and all actions taken by the IRB in response to reportable events.
9. A summary and explanation of any breaches of participant confidentiality that have occurred during the study.
10. A summary of any new research findings by the PI or others that may alter the safety/adverse event profile of the intervention being studied (e.g. newly published studies).

All data will be presented to the DSMB in a manner to maintain patient confidentiality whenever possible.

At the time of the initial study submission to the IRB, the PI will submit the protocol to the DSMB.

All Serious Adverse Events and Reportable Events will be reported to the DSMB within ten days of their occurrence.

Procedures and Responsibilities of the DSMB: Upon receipt of a report to the DSMB, one of the DSMB co-chairs will assess whether the report requires a full DSMB review, or whether an expedited review can be performed. Expedited reviews will be reviewed only by one of the co-chairs. Criteria for a report to receive expedited review include ALL of the following:

Criteria for Expedited Review:

1. Absence of any Serious Adverse Events (SAEs) or Reportable Events during the most recent reporting period.
2. Absence of any adverse event that was not expected (i.e. explained in the informed consent form or identified in the protocol.
3. Absence of any significant violations of patient confidentiality.

The DSMB will meet monthly. The DSMB will review each research protocol and plans for data and safety monitoring once per year (or every 6 months if the protocol is considered 'high risk' by the IRB). Upon completing the review, the DSMB will issue a report that summarizes the following:

All serious and unexpected adverse events or other unanticipated problems that involve risk to study participants, and whether these appear related to the study-based interventions or research assessment protocols. Reports will not specifically disclose the treatment arm of the study unless this disclosure is required for safety reasons. The DSMB will not receive unblinded data routinely from the PI at the time of review. However, the DSMB at any time may request from the PI the unblinded treatment assignments if the DSMB believes that knowing the treatment assignment could be expected to affect risk/benefit decisions for the trial.

There will be ongoing communication between the PI, the Emory IRB, and the DSMB as required. The effective functioning of the DSMB depends completely upon the full and timely reporting by the PI of safety-related concerns.
Statistical consultation will be provided on an as needed basis by the Division of Biostatistics.

6.3 Emory University Investigational Review Board (IRB) policies and procedures will be employed: **Unanticipated Problems involving Risks to Participants or Others (UPs):** The PI must report to the IRB (using any IRB specified forms, as applicable) any UP in accordance with the timetable set forth in the provision below entitled Timetable for

Reporting. The PI will advise the Emory IRB of: (a) the relationship between the problem and the intervention or study protocol; (b) whether or not a protocol change is necessary to reduce risk; and (c) whether the information about the problem affects the informed consent process.

Serious Adverse Events: At the time of renewal for the study, the PI will report to the Emory IRB a summary of the serious adverse events related to the research that occurred in the previous approval period. If adverse events occur at a greater frequency, severity, or duration than was previously anticipated, those become unanticipated and are promptly reportable.

Protocol Deviations: The PI will report to the Emory IRB any circumstances of which the PI becomes aware per which there has been a substantive deviation from the protocol that adversely affected at least one of the following: 1) the rights, welfare, or safety of subjects; 2) the subjects' willingness to continue participation; or 3) the integrity of the research data. Deviations that are reported as protocol modifications undertaken to eliminate apparent immediate hazards to Human Subjects do not need to be reported twice. The IRB will evaluate reported protocol deviations to determine any action to be taken or other reporting requirements that need to be fulfilled.

Protocol Modifications: The PI will request approval of any changes (including, but not limited to, any changes in research personnel) that the PI plans to make to a Research protocol, which has already been approved by the Emory IRB. Except in cases in which the change must be made to eliminate apparent immediate hazards to Human Subjects, the Emory IRB must approve of the modification **before** it can be initiated.

Non-Compliance: The PI shall notify the IRB of any circumstances of which the PI becomes aware of failure to follow Emory IRB Policies &Procedures, federal regulations, or other applicable laws. This report shall be made to the IRB as soon as possible after the non-compliance occurs. The IRB will work closely with the PI to develop a reasonable corrective and preventive action plan that can be implemented by the study team.

7. PATIENT SELECTION

7.1 Recruitment Plan. Drawing from well over 5 million individuals, the Emory ADRC has a large clinical practice and clinical research registry of subjects interested in participating in research studies. As of September 2011, the ADRC registry has an enrollment of 486 participants, among them 104 with MCI. The MCI participants include 72 subjects with amnestic MCI (single or multiple domain). These ADRC participants all have annual research evaluations that include detailed cognitive assessments, and all have consented to be contacted for opportunities to participate in research studies. In addition to the existing pool of ADRC participants, the Emory memory disorders clinics, staffed by Drs. Levey, Lah, Hu, Cellar and other specialty-trained health professionals provide evaluation and treatment ~2000 patients annually, of whom ~500 are new visits (and ~100 MCI). Potential study subjects will be identified from those subjects being followed by the Emory Alzheimer's Disease Research Center and by the patients of the PI and sub-PI's. Other potential study subjects may be recruited if they contact the Emory ADRC specifically to find out about potential research studies that might be appropriate for either themselves or a family member.

7.2 Inclusion Criteria

1. Subjects must have a subjective memory concern as reported by subject, study partner or clinician.
2. Meets ADNI criteria for diagnosis of MCI [52]. Subjects with amnestic (single or multi-domain) will be eligible, as both subtypes of MCI are at high risk for progression to AD.
3. Abnormal memory function documented by assessment using the Logical Memory subscale (Delayed Paragraph Recall, Paragraph A only) from the Wechsler Memory Scale-Revised (the maximum score is 25):
   (a) <11 for 16 or more years of education
   (b) <9 for 8-15 years of education
   (c) <6 for <7 years of education
4. Mini-Mental State Exam score between 24 and 30 (inclusive). Exceptions may be made for subjects with less than 8 years of education at the discretion of the PI.
5. Clinical Dementia Rating = 0.5. Memory Box score must be at least 0.5.
6. General cognition and functional performance sufficiently preserved such that a diagnosis of AD cannot be made by the physician at the time of the screening visit.
7. Cholinesterase inhibitors and memantine are allowable if stable for 12 weeks prior to screen.
8. Stability of Permitted Medications for 4 weeks. In particular, subjects may washout from excluded medication for at least 4 weeks prior to screening.
9. Geriatric Depression Scale (GDS) ≤ than 6.
10. Male or female outpatients aged 50-90 (inclusive).
11. Study partner has regular contact with the subject adequate to provide a reliable assessment of the subject's level of function, and can be available for all clinic visits, either in person or by telephone, for the duration of the study.
12. Visual and auditory acuity adequate for neuropsychological testing.
13. Good general health with no diseases expected to interfere with the study.
14. For women of child-bearing potential (i.e., one who is biologically capable of becoming pregnant), must be willing to use a medically acceptable form or birth control or practice abstinence for the duration of her participation in the trial. Acceptable methods of birth control include: oral or patch contraception, or medroxyprogesterone (Depo-Provera®) or other intramuscular contraceptive injection, or implantation of levonorgestrel (Norplant®) system, an IUD, a reliably-employed barrier method (e.g. diaphragm, cervical cap or condom), or a male partner who is surgically sterilized.
15. Modified Rosen Hachinski ≤ 4.
16. Completed six grades of education or has a good work history (sufficient to exclude mental retardation).
17. Able to communicate in English with study personnel.
18. Able to understand the nature of the study and must provide written informed consent prior to conduct of any study procedures.
19. Willing to undergo repeated MRIs (3Tesla) and at least three PET scans. No medical contraindications to MRI.
20. Agrees to blood collection for APOE and biomarker testing.
21. Agrees to lumbar puncture over the course of the study for the collection of CSF.

CSF levels of Ab42, total Tau, and Tau phosphorylated at threonine 181 consistent with underlying AD pathology according to established threshold values at Emory and the ADNI Biomarker Core

7.3 Exclusion criteria

1. Any significant neurologic disease other than MCI and suspected incipient AD, such as Parkinson's disease, multi-infarct dementia, Huntington's disease, normal pressure hydrocephalus, brain tumor, progressive supranuclear palsy, poorly controlled seizure disorder, subdural hematoma, multiple sclerosis, or history of significant head trauma followed by persistent neurologic deficits or known structural brain abnormalities.
2. Screening/baseline MRI scan with evidence of infection, large vessel infarction or other focal structural lesions that could account for the memory deficits. Subjects with multiple lacunes or lacunes in a critical memory structure are excluded.
3. Contraindication to MRI due to presence of pacemakers, aneurysm clips, artificial heart valves, ear implants, metal fragments or foreign objects in the eyes, skin or body, or excessive weight.
4. Presence of clinically significant suicide risk, based on the Investigator's judgment informed by a structured clinician interview. Any suicide attempt within the past 1 year of the screening visit is exclusionary.
5. Major depression, bipolar disorder as described in DSM-IV within the past 1 year, or history of schizophrenia (DSM-IV). Psychotic features, agitation or behavioral problems within the last 3 months which could lead to difficulty complying with the protocol.
6. History of alcohol or substance abuse or dependence within the past 2 years (DSM-IV criteria).
7. Allergic to any component of atomoxetine (Strattera).
8. Any uncontrolled medical condition that is expected to preclude completion of the study, or any medical condition which would represent a contraindication to atomoxetine pharmacotherapy (e.g. hepatic insufficiency, untreated hypertension, untreated cardiovascular or cerebrovascular disease).
9. Known serious structural cardiac abnormalities, cardiomyopathy, serious heart rhythm abnormalities, or other serious cardiac problems.
10. History of narrow angle glaucoma.
11. History of pheochromocytoma.
12. Clinically significant abnormal findings on screening laboratory tests or physical exam. Abnormalities in Vitamin B12 level, TFTs or LFTs that might interfere with the study. A low Vitamin B12 level is exclusionary, unless follow-up labs (homocysteine and methymalonic acid indicate that it is not physiologically significant.
13. Women who are pregnant or lactating, or who plan to become pregnant during the study.
14. Current use of warfarin (exclusionary for lumbar puncture).
15. Inability to obtain initial CSF sample.
16. Use within 60 days of a monoamine oxidase inhibitor .
17. Current use of anti-psychotic medication.

18. Current use of the following anti-depressant medications that act on NET: duloxetine, venlafaxine, desvenlafaxine, imipramine, or amitryptiline.
19. Current participation in another clinical trial. Participation in clinical studies involving neuropsychological measures being collected more than one time per year.
20. CSF profile is not consistent with underlying Alzheimer's Disease pathology.
21. Reasonable likelihood for non-compliance with the protocol or any other reason, in the opinion of the investigator, prohibits enrollment of subject into the study.
22. Exceptions to these guidelines may be considered on a case-by-case basis at the discretion of the protocol director.

7.4 Patient Numbering

Patients who have signed the informed consent form to begin screening procedures will be assigned a Subject Identification Number at that time. The Subject Identification Number will consist of the 3-digit study number (001) followed by a 3-digit subject identifying number. The patient will keep the same number throughout the study; a separate randomization number will not be provided at Baseline.

7.5 Randomization

Subjects will be assigned a randomization number as above. To achieve balanced patient groups, subjects will be randomly assigned on a 1:1 basis in a blinded fashion to atomoxetine or placebo treatment during the initial phase, stratified based on APOE4 results (carrier or non-carrier), and CSF biomarkers for high or low. To accomplish this, the biostatistician will use blocks of size 2 and provide a computer generated randomization to the Alzheimer's Disease Cooperative Study group and the Investigational Drug Service.

When ordering study medication, the study coordinator will receive a blinded subject assignment number, via the Alzheimer's Disease Cooperative Study Group. An identical subject assignment legend will be held in safe and confidential custody in a sealed envelope by the Investigational Drug Service.

Manual Randomization Plan

The study biostatistician will keep a copy of randomization scheme and will be on call. The Investigational Drug Service will also have a copy of the randomization scheme and will serve as back-up for the study biostatistician if the biostatistician is not available. When a subject meets the eligibility criteria and informed consent has been signed, the study biostatistician will inform the study coordinator and pharmacist the randomization assignment code for the subject based on the randomization plan.

Mis-Randomization Plan

There are two scenarios in mis-randomization, i.e., the patient is ineligible and randomized, or the patient is assigned to wrong treatment. Considerable care should be taken to avoid mis-randomization. If the participant is ineligible and randomized, we must subsequently withdraw that participant from the study and classify them as a mis-randomization, the patient should be included in the baseline analysis and treated as withdrawn. If the patient

is assigned to wrong treatment group, the patient should remain in the group randomized and be included in the analysis based on the “intent-to-treat” (ITT) principle. Secondary analysis can be performed according to patient’s actual treatment and compared to the ITT analysis.

Unblinding
In the event that it becomes medically imperative to know what the subject is receiving, the investigator will contact the Investigational Drug Service to determine. Date and reason for disclosure must be documented. If possible, the investigator will confer with the safety officer before unblinding.

8. STUDY ASSESSMENTS AND PROCEDURES

The study design and assessment schedule are presented in Table 1.

8.1 Informed Consent. The study will be discussed with the study subject and appropriate others (family, study partner) and if they are interested in learning more about the study they will be provided with a copy of the informed consent form to review in detail. The study subject will be contacted at a later date to determine if they would like to be screened for possible participation in the study. If they are interested, a screening visit will be made. Potential subjects are encouraged to discuss participation in these studies with their family or physician prior to screening for the study. In addition they are encouraged to contact the office with any questions or concerns.

At the time of the screening visit the consent form will again be reviewed with the study subject and appropriate others, questions will be answered and if they are still interested in study participation the consent will be signed. Ample opportunity will be given to respond to any questions and provide requested information regarding the study. After all questions are answered to participant and study partner satisfaction, the consent is signed.

This study includes subjects who must score between a 24 and 30 on the MMSE (Mini Mental Status Examination) and will therefore be experiencing mild cognitive impairment as a result of their Alzheimer's disease. Individuals with mild impairment will likely be able to provide informed consent however it should not be assumed that all subjects have the capacity to consent based only on an MMSE score. Capacity for consent will also be determined by the study subject’s ability to engage in a discussion regarding the study and their ability to understand what will be involved in study participation. The individual obtaining consent will review and discuss the research project and the consent document with the potential study participant and their family/study partner/legally authorized representative and will decide if they are able to understand the nature of the research, appreciate the experimental nature of the study, and understand the potential risks as well as alternatives to study participation.

In the event that the subject is not competent to provide consent, assent is obtained. A study subject’s legally authorized representative will be present during the initial consenting process. Signed consent will be obtained from all study subjects. The same process described previously will be employed in obtaining legally authorized representative’s consent, when deemed necessary. Consent will be obtained prior to conducting any screening assessments or procedures.

8.2 Screening Phase. The following tasks will be performed at the first screening visit (**1a**):

- Subjects will sign a written informed consent prior to the performance of any study-related procedures.
- The eligibility of the subject to participate in the trial will be assessed by a comprehensive evaluation. Patients that appear to be eligible will be administered the
  - Mini Mental Status Exam (MMSE),
  - Logical Memory (LM) and
  - Clinical Dementia Rating-Sum of Boxes (CDR-SB) assessments.
  - Geriatric Depression Scale (GDS)

Those participants who do not meet criteria for amnestic MCI will be excluded from participation. Patients meeting criteria for amnestic MCI (single or multi-domain) without any exclusionary diagnoses will complete:

- Medical history, including review of medications
- Physical and neurologic exams
- Blood pressure, heart rate, respiratory rate, temperature
- Height and weight
- ECG
- Modified Rosen Hachinski
- Suicidality assessment
- Urinalysis
- Blood work:
  - CBC
  - Comprehensive metabolic panel
  - TSH
  - Vitamin B12 level
  - Coagulation panel
  - Blood work for APOE
  - Serum pregnancy test (for women of child-bearing potential).

If safety laboratory tests and ECG are consistent with protocol eligibility at the second screening (**1b**), the tasks below may be performed on the same day, or may be performed on separate days. Screening 1b will occur within one calendar month of screening 1a.

- If pre-enrollment MRI is not available at the time of screening, the baseline MRI will be completed and reviewed prior to screening lumbar puncture (LP) to determine eligibility. Pre-enrollment MRI completed no more than two years prior to screening may be used for review.
- Blood work
  - Inflammatory biomarkers
  - Metabolomics

    - Primary Panel for Inflammation, Oxidative, and Nitrosative Stress
    - Norepinephrine metabolites (catecholamines)
    - Pax Gene
- Salivary amylase
- If the subject has had a clinic LP within 6 months prior to the date of visit 1b and CSF has been banked for research purposes, no screening LP will be performed. The banked CSF will be used for analysis. In all other cases, an LP will be performed by an MD or NP following an overnight fast. CSF will be assessed for the following:
    - Glucose
    - Protein
    - Cell count with differential
    - Aβ42, tau, phospho tau
    - Inflammatory Biomarkers
    - NE Metabolites (catecholamines)
- Contact subject on the day after LP to inquire about new adverse events.

8.3 Baseline Visits. The baseline visit will occur within 1 calendar month of Screening visit **1b**. Patients that continue to meet eligibility requirements will undergo the following test at visit **2A**:

- MRI performed at the Emory Center for Systems Imaging **if not performed as part of the screening visit.**
- FDG PET Scan

Visit 2b will consist of the following tests/procedures:

- Interim history review
- Concomitant medication review
- Adverse event review
- Suicidality assessment
- Confirm continued eligibility
- Blood pressure, heart rate, respiratory rate, temperature
- Weight, physical and neurologic exams
- American National Adult Reading Test (ANART)
- Montreal Cognitive Assessment (MoCA)
- Category Fluency (Animals)
- Trails A & B
- Boston Naming Test (30 item)
- Auditory Verbal Learning Test
- Clock drawing
- Neuropsychiatric Inventory IQ
- ADAS-Cog 13 (with Delayed Word Recall and Number Cancellation)
- Activities of Daily Living (FAQ)
- Blood work

    - Serum atomoxetine level
    - NET occupancy
    - Blood work for future research (per individual subject consent)
- Randomization to drug or placebo
- Study drug dispensed
- Subjects will be directed to take their daily pill in the AM after breakfast to prevent nausea.

8.4 Visit 3/Week 1 (Telephone/Email Visit) ± 2 days

- Interim history review
- Concomitant medication review
- Adverse event review
- Study medication compliance review

8.5 Visit 4/Week 2 (Office Visit) ± 2 days

- Blood pressure, heart rate, respiratory rate, temperature
- Weight
- Blood work
    - CBC
    - Comprehensive metabolic profile
    - Serum atomoxetine level
    - NET occupancy
    - Norepinephrine metabolites (catecholamines)
- Interim history review
- Concomitant medication review
- Adverse event review
- Suicidality assessment
- Study medication accountability
- Study medication dispensed

8.6 *Visit 5/Week 3 (Telephone/Email Visit) ± 2 days*

- Interim history review
- Concomitant medication review
- Adverse event review
- Study medication compliance review

8.7 Visit 6/Week 4 (Office Visit) ± 2 days

- Blood pressure, heart rate, respiratory rate, temperature
- Weight
- Blood work
    - CBC
    - Comprehensive metabolic profile
    - Serum atomoxetine level
    - NET occupancy
    - Norepinephrine metabolites (catecholamines)

- Interim history review
- Concomitant medication review
- Adverse event review
- Suicidality assessment
- Study medication accountability
- Study medication dispensed

8.8 ***Visit 7/Week 5 (Telephone/Email Visit) ± 2 days***

- Interim history review
- Concomitant medication review
- Adverse event review
- Study medication compliance review

8.9 Visit 8/Week 6 (Office Visit) ± 2 days

- Blood pressure, heart rate, respiratory rate, temperature
- Weight
- ECG
- Physical and neurologic exams
- Blood work
    - CBC
    - Comprehensive metabolic profile
    - Serum atomoxetine level
    - NET occupancy
    - Norepinephrine metabolites (catecholamines)
- Interim history review
- Concomitant medication review
- Adverse event review
- Suicidality assessment
- Study medication accountability
- Study medication dispensed

8.10 ***Visit 9/Week 7 (Telephone/Email Visit) ± 2 days***

- Interim history review
- Concomitant medication review
- Adverse event review
- Study medication compliance review

8.11 ***Visit 10/Week 11 (Telephone/Email Visit) ± 2 days***

- Interim history review
- Concomitant medication review
- Adverse event review
- Study medication compliance review

8.12 Visit 11/Week 18 (Office Visit) ± 2 days

- Blood pressure, heart rate, respiratory rate, temperature
- Weight
- Physical and neurologic exams
- Blood work
    - CBC
    - Comprehensive metabolic profile
    - Serum atomoxetine level
    - NET occupancy
    - Norepinephrine metabolites (catecholamines)
- Suicidality assessment
- Study medication accountability
- Study medication dispensed

8.13 *Visit 12/Week 22 (Telephone/Email Visit) ± 2 days*

- Interim history review
- Concomitant medication review
- Adverse event review
- Study medication compliance review

8.14 *Visit 13/Week 26 (Telephone/Email Visit) ± 2 days*

- Interim history review
- Concomitant medication review
- Adverse event review
- Study medication compliance review

8.15 Visit 14/Week 29 (Office Visit) Crossover Visit- May be performed over multiple days.

- Blood pressure, heart rate, respiratory rate, temperature
- Weight
- Physical and neurologic exams
- Cognitive Testing (full battery)
    - MMSE
    - Logical Memory (LM)
    - Clinical Dementia Rating-Sum of Boxes (CDR-SB) assessments
    - Montreal Cognitive Assessment (MoCA)
    - Category Fluency (Animals)
    - Trails A & B
    - Boston Naming Test (30 item)
    - Auditory Verbal Learning Test
    - Clock drawing
    - Neuropsychiatric Inventory IQ
    - ADAS-Cog 13 (with Delayed Word Recall and Number Cancellation)
    - Activities of Daily Living (FAQ)
    - Geriatric Depression Scale (GDS)

- Blood work
    - CBC
    - Comprehensive metabolic profile
    - Serum atomoxetine level
    - NET occupancy
    - Norepinephrine metabolites (catecholamines)
    - Metabolomics
    - Inflammatory biomarkers
    - Primary Panel for Inflammation, Oxidative, and Titrosative Stress
- Salivary amylase
- **Brain MRI**
- **FDG PET[1]**
- **Lumbar puncture [1](if LP attempt is unsuccessful, may send to radiology for LP under fluoroscopy. CSF will be assayed for:**
    - Glucose
    - Protein
    - Cell count with differential
    - Aβ42, tau, phospho tau
    - Inflammatory Biomarkers
    - NE Metabolites (catecholamines)
- Interim history review
- Concomitant medication review
- Adverse event review
- Suicidality assessment
- Study medication accountability
- Study medication dispensed (crossover)
- Contact subject on the day after PET scan and LP to inquire about new adverse events.

  [1]Subject to remain on drug until LP and imaging are completed.

8.16 *Visit 15/Week 30 (Telephone/Email Visit) ± 2 days*

- Interim history review
- Concomitant medication review
- Adverse event review
- Study medication compliance review

8.17 *Visit 16/Week 31 (Office Visit) ± 2 days*

- Blood pressure, heart rate, respiratory rate, temperature
- Weight
- Blood work
    - CBC
    - Comprehensive metabolic profile
    - Serum atomoxetine level
    - NET occupancy

        - Norepinephrine metabolites (catecholamines)
- Interim history review
- Concomitant medication review
- Adverse event review
- Suicidality assessment
- Study medication accountability
- Study medication dispensed

8.18 Visit 17/Week 32 (Telephone/Email Visit) ± 2 days

- Interim history review
- Concomitant medication review
- Adverse event review
- Study medication compliance review

8.19 *Visit 18/Week 33 (Office Visit) ± 2 days*

- Blood pressure, heart rate, respiratory rate, temperature
- Weight
- Blood work
    - CBC
    - Comprehensive metabolic profile
    - Serum atomoxetine level
    - NET occupancy
    - Norepinephrine metabolites (catecholamines)
- Interim history review
- Concomitant medication review
- Adverse event review
- Suicidality assessment
- Study medication accountability
- Study medication dispensed

8.20 Visit 19/Week 34 (Telephone/Email Visit) *± 2 days*

- Concomitant medication review
- Interim history review
- Adverse event review
- Study medication compliance review

8.21 Visit 20/Week 35 (Office Visit) ± 2 days

- Blood pressure, heart rate, respiratory rate, temperature
- Weight
- ECG
- Physical and neurologic exams
- Blood work
    - CBC

        - Comprehensive metabolic profile
        - Serum atomoxetine level
        - NET occupancy
        - Norepinephrine metabolites (catecholamines)
- Interim history review
- Concomitant medication review
- Adverse event review
- Suicidality assessment
- Study medication accountability
- Study medication dispensed

8.22 Visit 21/Week 36 (Telephone/Email Visit) ± 2 days

- Interim history review
- Concomitant medication review
- Adverse event review
- Study medication compliance review

8.23 Visit 22/Week 40 (Telephone/Email Visit) ± 2 days

- Interim history review
- Concomitant medication review
- Adverse event review
- Study medication compliance review

8.24 Visit 23 /Week 47 (Office Visit) ± 2 days

- Blood pressure, heart rate, respiratory rate, temperature
- Weight
- Physical and neurologic exams
- Blood work
        - CBC
        - Comprehensive metabolic profile
        - Serum atomoxetine level
        - NET occupancy
        - Norepinephrine metabolites (catecholamines)
- Interim history review
- Concomitant medication review
- Adverse event review
- Suicidality assessment
- Study medication accountability
- Study medication dispensed

8.25 *Visit 24/Week 51 (Telephone/Email Visit) ± 2 days*

- Interim history review

- Concomitant medication review
- Adverse event review
- Study medication compliance review

8.26 ***Visit 25/Week 55 (Telephone/Email Visit) ± 2 days***

- Interim history review
- Concomitant medication review
- Adverse event review
- Study medication compliance review

8.27 Visit 26/Week 58 End of Study Visit (over 2 days) **± 2 days**

- Blood pressure, heart rate, respiratory rate, temperature
- Weight
- ECG
- Physical and neurologic exams
- Cognitive Testing (full battery)
  - MMSE
  - Logical Memory (LM)
  - Clinical Dementia Rating-Sum of Boxes (CDR-SB) assessments
  - Montreal Cognitive Assessment (MoCA)
  - Category Fluency (Animals)
  - Trails A & B
  - Boston Naming Test (30 item)
  - Auditory Verbal Learning Test
  - Clock drawing
  - Neuropsychiatric Inventory IQ
  - ADAS-Cog 13 (with Delayed Word Recall and Number Cancellation)
  - Activities of Daily Living (FAQ)
- Geriatric Depression Scale (GDS)Blood work
- Blood work
  - CBC
  - Comprehensive metabolic profile
  - Serum atomoxetine level
  - NET occupancy
  - Norepinephrine metabolites (catecholamines)
  - Metabolomics
  - Inflammatory Biomarkers
  - Primary Panel for Inflammation, Oxidative, and Nitrosative Stress
- Salivary amylase
- Brain MRI
- FDG PET[1]
- Lumbar Puncture[1] (if LP attempt is unsuccessful, may send to radiology for LP under fluoroscopy**.** CSF will be assayed for:
  - Glucose
  - Protein

        - Cell count with differential
        - Aβ42, tau, phospho tau
        - Inflammatory Biomarkers
        - NE Metabolites (catecholamines)
    - Interim history review
    - Concomitant medication review
    - Adverse event review
    - Suicidality assessment
    - Study medication accountability
    - Contact subject on the day after LP to inquire about new adverse events.

8.28 Visit 271/Week 62 Termination Visit ± 2 days

- Blood pressure, heart rate, respiratory rate, temperature
- Weight
- ECG
- Physical and neurologic exams
- Blood work
    - CBC
    - Comprehensive metabolic profile
    - Serum atomoxetine level
    - NET occupancy
    - Norepinephrine metabolites (catecholamines)
- Interim history review
- Concomitant medication review
- Adverse event review
- Suicidality assessment

8.29 Assessments: MCI will be assessed using raters blinded to the study design. The battery of tests that is employed for the ADNI2 study (see baseline visit) is used. The screening battery will consist of: MMSE, Logical Memory, CDR-SB and Geriatric Depression Scale as to determine subject eligibility.

8.30.1 Safety Measurements:

a. Suicidality Assesmment – Structured Clinician Interview will be used to assess for suicidality at baseline and throughout the trial.
b. Vital Signs - Systolic blood pressure (SBP), diastolic blood pressure (DBP) and heart rate (HR) will be measured while the patient is in sitting position after five minutes rest at each office visit.
c. Physical Exam – A physician will perform a physical exam on all MCI patients at the screening visit to evaluate for any significant medical conditions. Subsequent physical exams will be performed by an MD or Nurse Practitioner.
d. Electrocardiogram – A 12-lead ECG will be performed at the screening visit, at weeks 6 and 35 (at maximum tolerated dose), week 58 and at the final study visit. ECGs demonstrating patterns consistent with acute or recent myocardial disease or significant conduction defects, including a QTc >480 msec (Barrett's formula), will be excluded.

e. Safety Labs - Pre-enrollment lab work is done to ensure subjects are not at undue risk to participate in the study. Serial CBC and CMP will be performed throughout the course of the study, as well as at the termination visit. CSF cell count, glucose and protein will be assayed at screening, crossover and termination visits. Clinical assessment of laboratory values is described in section 6.1.

8.31.2 Other Assessments:

a. Demographic Data - Demographic data (gender, date of birth, marital status, ethnicity, race) will be collected for all patients.
b. Concomitant Medication - All meds taken by subjects during study will be documented.

8.32 Patient Retention

Subjects who are enrolled in the study will be reimbursed for parking and transportation costs at the amount of $25 for each completed in-office study visit, pro-rated for visits completed. Subjects will also be paid $100 for each MRI, $100 for each LP and $100 for each of the three FDG PET scans completed. If all study visits are completed, subjects will receive a total of $1300 total.

Reimbursement will be made following visits 2b, 11 and 21. Emory University is required to complete form 1099 for any participant payments over $600 in a year. To comply with this federal mandate the researchers are required to obtain subject's social security number to complete the form.

9. DATA RECORDING, RETENTION, AND MONITORING

9.1 Electronic Case Report Forms

Electronic Case Report Forms will be completed for each patient who signs Informed Consent. All clinical information requested in this protocol will be recorded on the eCRFs. If an error is made on documents collected (source documents), a single line will be drawn through the error and the correct response will be written adjacent to the error; the change will be initialed, and dated.

9.2 Retention And Availability Of Records

The Investigator will make study data accessible to Regulatory Agency inspectors upon request. A file for each patient will be maintained that includes the signed ICF and the Investigator's copies of all source documentation related to that patient. The Investigator will ensure the reliability and availability of source documents from which the information on the eCRF was derived.

Study documentation will be maintained, including documents created or modified in electronic format, for at least 15 years following the completion of the study. ICFs, and adequate records for the receipt and disposition of all study drugs will be retained for a period of 2 years following the Food and Drug Administration (FDA) or other regulatory

approval date of the drug or until 2 years after the drug investigational program is discontinued, unless a longer period is required by applicable law or regulation.

10. STATISTICAL ANALYSIS PLAN

Statistical analyses will be performed using SAS 9.3. Given our expected sample size of 34 at the end of study, we will have some analytical limitations. Therefore, at the 6- and 12-month time points, we will assess the overall change (improvement/worsening) among the preselected RBM analytes found to be significantly different in preliminary studies of CSF markers. We will then statistically compare the percentage of overall change between the atomoxetine and placebo groups using Pearson's chi-square test or a Fisher Exact test (if necessary). Because IL1-alpha and TECK appear to best predict subsequent cognitive decline, we will compare the levels of these inflammatory markers in each group using a Wilcoxon Rank-Sum test. We will also use Spearman's correlation to determine an association between NE levels and these two biomarkers.

12. ASSAY MEASURES AND ANALYSES

12.1 Lab Manual is available for details pertaining to specific collection, processing, storage and transporting guidelines.

12.2 Genotyping for apolipoprotein E4 allele status will be performed in the Emory Genetics Core facility. Apolipoprotein E4 allele status will not be disclosed to study subjects.

12.3 CSF and Serum Biomarkers. AD biomarkers including Aß42, tau, and phospho-tau levels in the CSF will be assayed at baseline, 6 months, and 12 months following atomoxetine or placebo treatment. These assays will be performed in Dr. William Hu's biomarker laboratory using standardized measurements of CSF AD biomarkers (Innogenetics, Ghent, Belgium) on a Luminex-200 platform. Specialty assays for additional markers such as eotaxin-3, TECK and others have also been established and validated. RBM is a commercial vendor performing the multi-analyte panel as described previously [41].

12.4 Salivary Amylase. a noradrenergic biomarker**, will be** assayed in the laboratory of Dr. James Ritchie, using a Salivary α-Amylase assay kit (Salimetrics, State College, PA). Approximately 1 ml of saliva will be collected at baseline, 6- and 12 months following atomoxetine or placebo treatment. Saliva samples will be stored at -20°C until assay.

12.5 Norepinephrine and metabolites will be assayed in the laboratory of Dr. David Goldstein, Chief of Neurocardiology at NINDS and one of the leading authorities in the field. The assay will use HPLC with electrochemical detection after batch alumina extraction, a method that was perfected in the lab and routinely used for many years. Analytes to be measured include the catechols norepinephrine (NE), epinephrine, and dopamine, the catecholamine precursor DOPA, the main neuronal metabolite of NE (dihydroxyphenylglycol, DHPG), and the main neuronal metabolite of dopamine, dihydroxyphenylacetic acid (DOPAC).

12.6 Drug levels. Serum atomoxetine levels will be assayed using a highly sensitive liquid chromatography tandem mass spectrometry method[56] established in the Emory clinical laboratories to monitor compliance and quantify dose-response effects.

12.7 NET Occupancy. In the absence of data from PET studies allowing definitive knowledge of NET occupancy as a function of drug dose, an *ex vivo* assay established in the laboratory of Dr. Mike Owens, will use serum to estimate NET occupancy. Serum samples will be used to directly measure inhibition of active [^{3}H]-monoamine transport in cells expressing the human serotonin, norepinephrine or dopamine transporters. Uptake measured in drug-free baseline serum is compared to that observed in the subject's samples while on drug and expressed as both % baseline uptake and % occupancy. These assays are sensitive to free drug concentrations representative of extracellular space within the brain where transporter inhibition is hypothesized to be correlated with therapeutic efficacy.

12.8 MRI. Brain MRI scan will be obtained or reviewed at screening to verify eligibility, at baseline, at 6 months and after 12 months of treatment or placebo, using protocols adapted from the ADNI study as described below.

MRI films performed clinically within the past two years will be reviewed by the investigators for eligibility in the study. If a subject does not have clinical MRI films available, the MRI will be performed and reviewed prior to performance of the LP for safety reasons and to ensure that the subject meets eligibility criteria.

The 3T MR acquisition sequences 1) Structural MRI, 2) FLAIR, 3) T2*GRE; 4) arterial spin labeling (ASL), 5) neuromelanin enhancement; and 6) resting state connectivity. MRI measurements of brain structure have been show to demonstrate brain atrophy (which correlates with neuron loss) in MCI and AD and increasing rates of brain atrophy as subjects become more impaired. Therefore, structural MRI is used as a measure of the rate of disease progression, and possibly as a measure of treatment effect, in AD treatment trials. Structural MRI (MPRAGE//IRSPGR) data will be used both as the rate of change as well as a predictor of future change. Cerebrovascular disease (especially white matter lesions (WMLs) will be assessed with FLAIR. Recently, iron imaging, especially microbleeds (T2* GRE) has been used in anti-amyloid clinical trials, because of the association of microbleeds with anti-amyloid therapy; this will be measured with T2*GRE. Cerebral blood flow, which closely correlates with cerebral hypometabolism as an early biomarker of AD pathogenesis, will be measured by ASL imaging. A novel protocol developed at Emory by Drs. Xiaoping Hu and Dan Huddleston to visualize neuromelanin containing neurons will be used to quantify norepinephrine containing neurons in the locus coeruleus. Finally, resting state fMRI will be used to determine functional connectivity changes that have recently been observed in AD.

12.8.1 Clinical Read of MRIs

All MRIs will be reviewed by the investigators and the investigator will determine and document any clinically significant findings.

12.9 FDG PET Scan. -Cerebral metabolic rate for glucose (as measured by fluorodeoxyglucose [FDG] uptake) will be obtained at baseline and at 6 and 12 month timepoints in the study.

In preparation of the FDG PET Scan, subjects will be required to fast for at least four (4) hours prior to the scanning session. Subjects' blood glucose is checked prior to scanning and must be < 180 mg.dL. After the injection of 5 mCi of tracer, subjects are in a quiet, dimly lit room with eyes and ears unoccluded for 30 minutes, after which they are placed in the scanner. Data are acquired as 6 x 5 minute frames.

13. OPEN-LABEL EXTENSION

Participants who complete study Visit 26 will be eligible to receive open-label Atomoxetine at the maximum-tolerated dose received during the double-blind phase of the trial. Atomoxetine will be dispensed immediately following Visit 26, after obtaining subject consent, in the following manner:

- 40 mg p.o. – for 2 weeks, followed by telephone visit to assess AEs
- 80 mg p.o. – for 4 weeks, followed by a telephone visit to assess AEs
- 100 mg p.o. for 6 months at a time(or maximum tolerated dose based on data from double-blind phase of the study)

Subjects who elect to participate in the open-label phase of the study will forego study Visit 27. Participants and investigators will remain blinded about when the subjects received drug versus placebo during the active phase of the study. Atomoxetine administered during the open-label phase will be identical in appearance to drug received during the double-blind phase of the trial. Participants who wish to receive open-label drug will be asked to sign a new consent form (after the investigator or study coordinator has explained procedures for the open-label phase of the trial and after all participant questions have been addressed).

Participants who elect to receive open-label drug will be required come to the clinic to have safety labs performed every six months, including CBC with differential and chemistry (including liver enzymes). Once the results of safety laboratory studies are received, open-label Atomoxetine in sufficient quantity for 6 months therapy (100 mg/day) will be dispensed at no cost to the participant. For the duration of open-label participation, participants will undergo an annual MRI and PET scan, in addition to CSF collection via lumbar puncture. In addition, a short battery of cognitive tests (MMSE, CDR) will be performed annually. Participants will be asked to report any adverse events that develop during the open-label phase of the trial.

The open-label phase of the study will be terminated after 2 years (i.e., 104 weeks) following successful completion of the active phase of the study. The P.I. reserves the right to stop open-label dispensing at any time.

14. TABLE 1 Schedule of Assessments

Schedule of Assessments

Visit Number	1a	1b	2a	2b	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
Study Week	Screening		Baseline		1	2	3	4	5	6	7	11	18	22	26	29[1]	30	31	32	33	34	35	36	40	47	51	55	58	62
	a	b[1]	a[1]	b																									
Dosing (max tolerated dose)				10 mg	18 mg	40 mg	60 mg	80 mg	100 mg							10 mg	18 mg	40 mg	60 mg	80 mg	100 mg								
Consent	x																												
Confirm eligibility	x			x																									
Medical history and medication review	x																												
Physical and neurological examination	x			x						x			x			x						x			x			x	x
Vital signs + height (screening only) & weight	x			x		x		x		x			x			x		x		x		x			x			x	x
ECG	x									x												x						x	x
Blood and/or urine[7] for clinical labs	x					x		x		x			x			x		x		x		x			x			x	x
Serum pregnancy if indicated				x																									
APOE Genotyping				x																									
Blood for biomarkers, drug levels, NE and metabolites		x		x[6]		x		x		x			x			x		x		x		x			x			x	x
Labs for future research				x																									
Salivary amylase		x														x												x	
MRI		x[3]	x[4,5]													x												x[5]	
FDG PET			x													x												x	
Lumbar puncture		x														x												x[5]	
Study Drug dispensed				x		x		x		x			x[2]			x		x		x		X[2]			x				
Modified Rosen Hachinski	x																												
Telephone Call/Email Contact					x		x		x		x	x		x	x		x		x		x		x	x		x	x		
Cognitive Assessment	x			x												x												x	
Suicidality Assessment	x			x		x		x		x			x			x		x		x		x			x			x	x
Assessment of current medications, interim history, & adverse event review	x			x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Study Drug Compliance					x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	
Randomization/Study Arm Crossover				x												x													

[1]Visit may be performed over 2 days, [2]3 months of study meds dispensed at V11, V23., [3] MRI to be done if no clinical MRI w/l past 2 years for review. [4]If pre-enrollment MRI is not available at the time of screening, the baseline MRI will be completed prior to screening LP to determine eligibility. [5]MRI will be done prior to LP to avoid meningeal reactions for subjects who have not had MRI in past 2 years
Early termination will lead to same visit type as termination week 58 visit. [6] No NE and metabolites at this visit. [7] Urine at Visit 1a only. Areas highlighted in yellow represent office visits. Visit14/Week 29, highlighted in blue is the crossover visit.

15. STUDY GLOSSARY

AD	Alzheimer's Disease
ADAS-Cog	Alzheimer's Disease Assessment Scale – Cognitive
ADCS	Alzheimer's Disease Cooperative Study
ADHD	Attention Deficit Hyperactivity Disorder
ADNI	Alzheimer's Disease Neuroimaging Initiative
ADNI2	NIH grant, Funding began 2011
AE	Adverse Event
ANART	American National Adult Reading Test
APOE/APOE4	Apolipoprotein (APOE) epsilon 4 (APOE4)
ASL	Arterial Spin Labeling
Aß	Beta Amyloid
BDNF	Brain-derived neurotrophic factor
CBC	Complete Blood Count
CDR-SB	Clinical Dementia Rating-Sum of Boxes
CFR	US Code of Federal Regulations
CS	Clinically Significant
CSF	Cerebrospinal Fluid
CSSRS	Columbia Suicide Severity Rating Scale
CT	Computerized Tomography
CTCAE	Common Terminology Criteria for Adverse Events Version 4
DBP	Diastolic Blood Pressure
DHPG	Dihydroxyphenylglycol
DNA	Deoxyribonucleic Acid
DOPAC	Dihydroxyphenylacetic Acid
DSMB	Data Safety Monitoring Board
DSM-IV	Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition
DTI	Diffusion Tensor Imaging
ECG	Electrocardiogram
eCRF	Electronic Case Report Form
EDC	Electronic Data Capture
FAQ	Functional Activities Questionnaire (Activities of Daily Living)
FDA	Food and Drug Administration
FLAIR	Fluid Attenuation Inversion Recovery
fMRI	Functional Magnetic Resonance Imaging
GDS	Geriatric Depression Scale
HIPAA	Health Insurance Portability and Accountability Act
HPLC	High Performance Liquid Chromatography
HR	Heart Rate
HSP70	Heat Shock Protein
IDS	Investigational Drug Service
IL	Interleukin
IRB	Investigational Review Board
IUD	Intrauterine Device

LC	Locus Coeruleus
LDOPS	L-threo-3,4-dihydroxyphenylserine
LFT	Liver Function Tests
LM	Logical Memory
LP	Lumbar Puncture
MCI	Mild Cognitive Impairment
MMSE	Mini Mental State Exam
MoCA	Montreal Cognitive Assessment
MPRAGE	Magnetization Prepared Rapid Gradient Echo
MR/MRI	Magnetic Resonance/Magnetic Resonance Imaging
NCS	Not Clinically Significant
NE	Norepinephrine
NET	Norepinephrine Transporter
NIH	National Institutes of Health
NINDS	National Institute of Neurological Disorders and Stroke
PD	Parkinson's Disease
PET	Positron-Emission Tomography
PI	Principal Investigator
RBM	Rules Based Medicine
SAE	Serious Adverse Event
SBP	Systolic Blood Pressure
SD	Standard Deviation
T	Tesla
T2*GRE	T2 Star-Weighted Gradient-Echo
TECK	Thymus-Expressed Chemokine
TFT	Thyroid Function Tests
UP	Unanticipated Problems
VEGF	Vascular endothelial growth factor
vMRI	Volumetric Magnetic Resonance Imaging
WML	White Matter Lesions
WMS-R	Wechsler Memory Scale – Revised

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