Apathy in Dementia Methylphenidate
Trial 2 (ADMET 2)

ADMET 2 Protocol
Version 1.5

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Document history

Version 1.0 (5 Feb 2015); no history. Document developed from grant.

Version 1.1 (3 June 2015)

Numerous editorial and wording changes were made throughout document to improve clarity. Substantive modifications include:

2 Objectives
- Removed Apathy Evaluation Scale

3 Design
- Removed Apathy Evaluation Scale

4 Eligibility

Revised inclusion criteria by:
- Adding the bolded statement to "Provision of informed consent for participation in the study by potential participant or surrogate (with participant assent if the potential participant is unable to provide informed consent) and caregiver"
- Removing primary in "Availability of caregiver, who spends greater than ten hours a week with the potential participant and supervises his/her care, to accompany the participant to study visits and to participate in the study"
- Adding the following criterion: "If female, woman must be post-menopausal for at least 2 years or have had a hysterectomy"

Revised exclusion criteria by:
- Adding the bolded statements to "Use of trazodone > 50mg or lorazepam > 0.5mg or for indications other than sleeping difficulties within the 30 days preceding randomization or a period of time equal to 5 half-lives of drug, whichever period of time is longer. Other benzodiazepines are prohibited in the past 30 days or within 5 half-lives, whichever period of time is longer
- Adding the following criterion: "Women with childbearing potential"

5 Randomization and masking
- Revised emergency unmasking method; clinic needs to request treatment assignment through data system instead of from the medical monitor
- Removed medical monitor from providing treatment assignment to participant and caregiver after 6-month follow-up and all collection is complete

6.3 Psychosocial treatment
- Revised the following to allow for clinic variability: "Psychosocial intervention may consist of three components: a counseling session, the provision of education materials, and availability for crises."
- Added "Dementia Care Guidelines for Families" to list of educational materials

6.3 Choice of methylphenidate formulation
- Added section to address formulation difference

6.5 Management and reporting of adverse events

- Removed "report of protocol violations" to the medical monitor duties
- Specified that investigators will notify the medical monitor and Coordinating Center within one day of learning of death or serious adverse event

7.2 Overview of consent issues

- Defined assent by the Alzheimer's Association and local IRB guidelines

7.4 Baseline visit procedures

- Provided time frame for baseline visit and added that ECG results should be reviewed prior to randomization (indicated by bolding): "The baseline visit may take place across more than one day, but must be completed within the two weeks preceding randomization. The clinical staff should perform and review results of the electrocardiogram (ECG) within the two weeks preceding randomization."
- Removed Apathy Evaluation Scale

8.2 Schedule in-person visits

- Removed Apathy Evaluation Scale

8.6 Data collection by visit

- Removed Apathy Evaluation Scale

9.1 Neuropsychology, neuropsychiatry, and other assessments

- Replaced 'blind to treatment assignment' with the following (indicated by bolding): "A trained clinician, not involved in collecting other assessments or procedures, will complete the CGIC using a 7-point Likert scale to rate each participant……"
- Removed Apathy Evaluation Scale

9.2 Cognitive assessments

- Removed statement that trail making test will be stopped after 5 errors

10.2 Analysis for specific aims

- Added "As required by NIH policy, we will conduct valid subgroup analyses of the primary outcomes by gender and race/ethnicity subgroups to determine if there is possible subgroup differences (i.e., treatment interactions) in treatment effects.
- Added "As required by NIH policy, we will conduct valid subgroup analyses of the of the adverse event outcomes by gender and race/ethnicity subgroups to determine if there is possible subgroup differences (i.e., treatment interactions) in treatment effects."
- Removed Apathy Evaluation Scale
- Added that US and Canada will be analyzed for treatment effect differences

11 Data monitoring

- Added that NIA will approve DSMC member selection

13.3 Consent procedures

- Defined assent by the Alzheimer's Association and local IRB guidelines

13.4 Potential risks and benefits

- Added information on 2013 FDA warning about relationship between priapism and methylphenidate use
- Added information on 2015 FDA warning about relationship between rhabdomyolysis and methylphenidate use

13.5 Safety monitoring
- Removed "report of protocol violations" to the medical monitor duties
- Specified that investigators will notify the medical monitor and Coordinating Center within one day after learning of death or serious adverse event

Appendix: Design summary
- Updated eligibility criteria to match revisions in section 4
- Removed Apathy Evaluation Scale

Version 1.2 (22 August 2016)

6.7 Concomitant medications
- Clarified the procedures for reducing, discontinuing, and restarting the study drug when the participant is experiencing agitation or delusions:
  - Added “delusions” to the type of symptoms that may be present when treating participants with agitation.
  - Added that the study drug may be “temporarily” discontinued.
  - Removed that the study drug may be restarted within a month of stopping.
  - Added that if the participant is prescribed an SSRI/other medication, the SSRI/other medication may only be restarted “once the participant has stopped taking the SSRI/other medication for at least 30 days or 5 half-lives, whichever is longer.”
- Clarified the procedures for discontinuing and restarting the study drug when the participant is experiencing depression:
  - Added that the study drug may be “temporarily” discontinued until “the participant is on a stable dose of the SSRI for at least 30 days or 5 half-lives, whichever is longer.”

7.4 Baseline visit procedures
- Increased time frame for baseline visit (indicated by bolding): "The baseline visit may take place across more than one day, but must be completed within the three weeks preceding randomization. The clinical staff should perform and review results of the electrocardiogram (ECG) within the three weeks preceding randomization."

9.1 Neuropsychology, neuropsychiatry, and other assessments
- Decreased the maximum score for Dependence Scale from 20 to 15.

13.5 Safety monitoring
- Increased time frame for baseline visit (indicated by bolding): “Therefore, an ECG must be performed no more than three weeks prior to randomization to allow the investigator to evaluate the clinical significance of potential findings.”

Version 1.3 (02 May 2017)

13.4 Potential risks and benefits

- Added information from 2017 FDA warning about relationship between serotonin syndrome and methylphenidate use

Version 1.4 (10 Jul 2018)

All sections
- Corrected spacing
- Referenced citations by (Author year)

Document distribution
- Added "20" to the years

Summary
- Added the goals of the blood-based biomarkers substudy

1.7 Study rationale for Apathy in Dementia Methylphenidate Trial 2 (ADMET 2)
- Added the goal of the biomarkers substudy

2 Objectives
- Added the objective of the biomarkers substudy

3.1 Overview
- Added the biomarkers substudy design of blood sample collection at baseline and 6 month follow-up

3.2 Sample size and power
- Added the biomarkers substudy has a convenience sample, based on availability.

3.3 Organization
- Added the Biomarkers executive committee

7.4 Baseline study procedures
- Added the baseline blood-based biomarkers blood sample collection

8.2 Scheduled in-person visits
- Added the month 6 blood-based biomarkers blood sample collection

8.6 Data collection by visit
- Added the baseline and month 6 blood-based biomarkers blood sample collection

10.1 Analysis overview
- Added a short description of the blood-based biomarkers analysis

10.2 Analsysis for specific aims
- Added a description of the blood-based biomarkers analysis

11 Data monitoring

- Added that the CC will track collection, shipment, and receipt of blood-based biomarker samples

13.3 Consent procedures
- Added that newly or currently enrolled participants will also be asked to consent to the blood-based biomarkers substudy

13.4 Potential risks and benefits
- Added a description of the risks to participants if they participate in the biomarks substudy blood draw

13.6 Confidentiality of participant and caregiver data
- Added that blood samples will be de-identified with using only study ID codes

14 Blood-based biomarkers substudy
- Added this chapter

15 Literature cited
- Removed numbers on citations in this section
- Added new citations for blood-based biomarkers substudy
- Moved several citations to the correct alphabetical location

Appendix: Design summary
- Added "(2015 to 2017)" to Banner Alzheimer's Institute
- Added blood-based biomarker substudy objective
- Added blood-based biomarker substudy sample information
- Added blood-based biomarker substudy analysis information

Version 1.5 (25 Feb 2019)

2 Objectives
- Added two secondary apathy outcomes.

10.2 Analysis for specific aims
- Added two secondary apathy outcomes.

Appendix: Design summary
- Added two secondary apathy outcomes.
- Added "(2015 to 2019)" to Wake Forest University

Summary

Apathy in Dementia Methylphenidate Trial 2 (ADMET 2) is a Phase III, placebo-controlled, masked, 6 month, 10-center randomized clinical trial sponsored by National Institute of Aging involving 200 participants with Alzheimer's disease (AD). ADMET 2 is designed to examine the efficacy and safety of methylphenidate as treatment for clinically significant apathy in AD participants. ADMET 2 will enroll participants from real world settings such as outpatient, nursing home, and assisted living facilities and will examine the effects of methylphenidate on apathy and cognition. ADMET 2 will also conduct careful safety monitoring.

A substudy of blood-based biomarkers will also be conducted to understand biomarker correlates of change in apathy over time, and to identify possible predictors of treatment response.

1. Background and significance

1.1. Public health significance

Alzheimer's disease (AD) is a growing public health problem whose global burden is expected to exceed 80 million cases by 2040 (Ferri 2005). An estimated 5.1 million Americans have AD (Brookmeyer 1998). Considering current demographic trends and recent improvements in the treatment of AD, the prevalence of this condition in the United States is expected to quadruple in the next 50 years (Brookmeyer 1998). AD debilitates patients and families both emotionally and economically (Rabins 2006). Societal costs are about $100 billion per year in the US alone (Fillit 2000) and are expected to triple by 2040 (Fox 2001).

1.2. Neuropsychiatric symptoms of Alzheimer's disease

While cognitive and functional decline are the hallmark of AD, neuropsychiatric symptoms (NPS) afflict almost all patients (Lyketsos 2007). NPS are sometimes referred to as "behavioral disturbances" or "non-cognitive mental disturbances" which include agitation, delusions, hallucinations, depression, sleep disturbance, and problem behaviors. NPS add significant disability for patients and caregivers (Rabins 2006). A range of associated adverse consequences have been reported including worse quality of life, greater disability, accelerated cognitive or functional decline, greater burden on caregivers, earlier institutionalization, and accelerated mortality (Rabins 2006). Practice guidelines developed for the treatment of AD consistently refer to management of NPS as central to the treatment of AD (e.g., American Psychological Association Practice Guidelines for the Treatment of AD (revised) (APA 2007); American Association for Geriatric Psychiatry Position Statement on Principles of Dementia Care, 2006) (Lyketsos 2006). The importance of NPS in the context of AD has also been recognized by the Food and Drug Administration (FDA), which is open to considering new indications for treatment of specific NPS in this context (Campbell 1997). Despite this, the empirical evidence supporting pharmacological and non-pharmacological interventions for NPS is sparse (Benoit 2004; Cummings 1994).

1.3. Apathy as a significant neuropsychiatric symptom

Apathy is one of the most prevalent NPS in AD. Clinically significant apathy is defined as a loss of will and initiative, lack of interest in activities, lack of productivity, as well as limited affective response to positive or negative events (Benoit 2003), and is present for at least four weeks. According to some studies, apathy affects approximately 50% of people with dementia (Steinberg 2006). While the prevalence of apathy increases with severity of dementia, it is a chronic and persistent problem at all stages of the illness (Mega 1996). A recent door-to-door epidemiological study conducted in Spain found that apathy was the most prevalent NPS in Alzheimer's patients, affecting 30% of study participants (Hill 1963). More broadly, the study identified apathy as the most common NPS in all dementia types, affecting 54% of study participants (Fernandez 2007). An epidemiological study conducted in Brazil reported a similar prevalence of apathy. The researchers in this study observed apathy in 53% of AD patients (Tatsch 2006). Another study reported a lower, but still significant, prevalence of 36% in those with dementia (Lyketsos 2002). Apathy has devastating effects on the quality of life for both AD patients and their caregivers. Patients suffering from apathy experience decreased motivation, relying heavily on caregivers to initiate and oversee daily activities. One study found apathetic patients 2.8 times more likely than non-apathetic patients to struggle with at least one activity of daily living, such as dressing, bathing, and eating (Freels 1992). Thus, apathy compounds the negative effects of cognitive impairment, further degrading the quality of life of AD patients. In addition, caregivers who lack an understanding of apathy as a syndrome that accompanies AD may misinterpret apathetic patients as insensitive and uncaring

(Campbell 1997). Caregivers of apathetic AD patients report significant levels of distress and fewer positive experiences associated with caregiving than caregivers of non-apathetic AD patients (Greene 1982; Kaufer 1998). Greater caregiver distress is linked with increased service utilization and accelerated institutionalization (Smyth 1997), which, in turn, creates a significant financial burden (Storga 1996; Herrmann 2006). Therefore, the management of apathy is a major priority in caring for patients with AD.

1.4. Etiology of apathy in AD

Post-mortem studies comparing AD patients with controls support the argument that disruptions in the dopaminergic system are common in AD (Nazarali 1992; Storga 1996). These studies reported that disruptions in the dopaminergic transporter and postsynaptic loss of striatal dopamine type 2 (D2) receptors were frequently found in AD patients (Joyce 1998). Furthermore, loss of striatal D2 receptors was prevalent among the 30% of AD patients who also exhibited extrapyramidal features (Joyce 1998; Pizzolato 1996). Moreover, neuroimaging studies suggest that disruptions in D2 receptor levels and metabolism became increasingly severe as cognitive impairment (Itoh 1994; Kemppainen 2000; Pizzolato 1996) and NPS progressed (Tanaka 2003). This study suggests, however, that dopaminergic disruptions may not manifest themselves as general signs of disease progression, but may be specifically related to the onset of apathy.

A recent study showed that apathy in AD correlates with decreased dopamine transporter density in the striatum (David 2007). Furthermore, Benoit et al (2004) reported that higher scores on the Apathy Inventory were associated with decreased orbito-frontal perfusion (using perfusion SPECT imaging) in AD patients. Specifically, they observed that perfusion patterns correlated with specific apathetic symptoms. For example, lack of initiative was associated with decreased right cingulate perfusion, lack of interest was associated with decreased right middle orbitofrontal perfusion, and emotional blunting was associated with decreased left orbitofrontal perfusion. Lanctôt et. al. (2007) reported similar findings. Using SPECT imaging, she found that apathetic AD patients have decreased perfusion in right orbitofrontal and left anterior cingulate cortex compared with non-apathetic AD patients. Marshall et al (2007) reported decreased bilateral anterior cingulate fluoro-D-glucose uptake in apathetic AD patients compared with non-apathetic AD patients. These findings are further supported by a recent neuropathologic report showing that chronic apathy in AD is associated with higher density of neurofibrillary tangles in the anterior cingulate (Marshall 2006).

Further evidence for a relationship between apathy and dopaminergic disruption comes from studies of other neurodegenerative diseases with apathy as a syndrome. For example, a recent MRI study found that unmedicated schizophrenics show decreased activation of the ventral striatum during the presentation of reward-indicating cues. The authors suggest that the increased dopaminergic turnover observed in schizophrenia may increase brain reward system (BRS) "noise," resulting in impaired reward processing and anhedonia (Juckel 2006). Similarly, the high prevalence of apathy among cocaine dependent individuals suffering from withdrawal has been proposed to be a result of subcortical dopamine dysfunction (Kalechstein 2002; Volkow 1997). Degeneration of nigrostriatal dopamine neurons is key in the pathogenesis of Parkinson's disease (PD), leading some authors to suggest that it is an ideal clinical model for the study of the neuropsychiatric consequences of impaired BRS functioning. The prevalence of apathy in PD has been reported to be as high as 54% (Aarsland 2007), and treatment with dopaminergic agents, including L-DOPA (Czernecki 2002) and pramipexole (Lemke 2007), has been shown to improve both motor deficits and apathy in PD. In summary, these studies provide strong support for the hypothesis that apathy has a neurobiological basis and that it is associated with impaired perfusion patterns in the orbitofrontal and anterior cingulate cortices, which in turn results from damage to dopaminergic innervations from the progression of Alzheimer's pathology.

1.5. Treatments for apathy in AD

Despite the high prevalence of apathy in AD and its serious consequences, there are no proven treatments for this condition. Clinicians familiar with AD care always prefer non-pharmacologic treatment strategies. The available data on non-pharmacologic treatments for AD, albeit scarce, suggest reliable but limited effects. Recently, pharmacologic options for the treatment of apathy have been explored. Antidepressant medications have been considered, but some evidence suggests they could be detrimental rather than helpful in the treatment of apathy in AD (Lemke 2007). Cholinesterase inhibitors (ChEIs) have also been studied. While these studies demonstrated a modest improvement in apathy (Cummings 2001; Feldman 2001; Tariot 2001; Tariot 2000) with no clear differentiation between medications (Rosler 2002), about half of the patients showed no relief of apathetic symptoms following ChEI therapy (Cummings 2001). Furthermore, these studies were not tailored specifically for apathy. Generally, the trials did not recruit participants with clinically significant symptoms of apathy. Indeed, some apathetic patients were excluded since they tend to be less likely to participate in studies and less easily "engaged" in performing neuropsychological assessments.

The evaluation of dopaminergic agents for the treatment of apathy is a feasible alternative approach. The rationale for the use of dopaminergic agents is based on the strong tie between activity of the dopaminergic mesolimbic brain reward system and the expression of motivated behaviors in brain damaged populations. Evidence for the use of dopaminergic agents also comes from case reports and small open-label studies in nondemented populations (Roccaforte 1990; Galynker 1997; Van Reekum 1995; Corcoran 2004). Furthermore, preliminary data from a double blind, placebo-controlled, single-center crossover pilot study suggest that the dopamine agonist methylphenidate is superior to placebo for the treatment of apathy in AD (Lanctot 2008).

1.6. Methylphenidate as a possible treatment for apathy in AD

Given the prevalence of apathy in this growing patient population, better pharmacologic options are needed. Unfortunately, there have been few randomized, placebo-controlled trials of dopamine agonists that address their efficacy for the treatment of apathy in AD. In 1990, Roccaforte (1990) conducted a comprehensive review of the literature on the use of psychostimulants in the elderly. Taken together, the studies, which began in 1956, included more than 800 patients with poor cognition, depression, and "amotivational syndromes." Doses of methylphenidate ranged from 10-60 mg/day, and one study used 5-20 mg/day of amphetamine. In the dextroamphetamine study, a quarter of the patients dropped out due to side effects (aggression, confusion, delusions), but none of the methylphenidate patients were reported to have dropped out. Two patients were noted to have increased blood pressure and heart rate on 45 mg of methylphenidate, and several others noted "irritability." Overall, methylphenidate appeared to be well tolerated. It was not associated with any significant side effects.

Long-acting methylphenidate is one treatment option. There have been two double-blind controlled trials evaluating the efficacy and safety of non-pediatric use of long-acting methylphenidate: A study by Jain et al. (2007) evaluated 50 patients between the ages of 18 and 60, while a separate study by Reimherr et al. (2007) evaluated 47 patients aged 18 and 65. Both studies describe similar efficacy between long- and short-acting methylphenidate for ADHD. Safety concerns included weight loss and alteration in blood pressure, which is of relevance in this population. Furthermore, there is no reported information on the use of long-acting methylphenidate in the 65 and over population, or in patients suffering from dementia. This is of substantial concern since in these patients, dosage, as well as metabolization issues, will need to

be further studied in normal elderly patients before the drug can be evaluated in a frail, demented population.

From all possible dopamine-enhancing compounds available, short-acting methylphenidate has been one of the most studied compounds in the elderly and presents the best safety profile. However, the efficacy and safety of methylphenidate for the treatment of apathy in AD has not been definitively established.

1.7. Study rationale for Apathy in Dementia Methylphenidate Trial 2 (ADMET 2)

Two small pilot randomized clinical trials showed a positive effect of methylphenidate for the treatment of apathy in AD. The first randomized, placebo-controlled trial of methylphenidate for the treatment of apathy in AD, conducted by Drs. Lanctôt, Herrmann, and Black, reported encouraging results (Lanctot 2007; Lanctot 2008; Herrmann 2008). The results of this study suggest that methylphenidate is modestly effective in a subset of AD patients. They further suggest that improvement in apathy with methylphenidate can be predicted on the basis of attentional response to dextroamphetamine (d-amph) challenge. Response time variability, a measure of the "erraticness" of responses and a component of the Connors' Continuous Performance Task inattention summary score, was also correlated with response to methylphenidate. While d-amph had no effect on the number or types of errors made, it negatively impacted the consistency in response times. The relationship between improvements in apathy with methylphenidate and greater inattention post-d-amph challenge may therefore reflect the behaviorally activating, rather than the subjective or cognitive effects, of psychostimulants in this population.

This study demonstrated a significant benefit of methylphenidate over placebo for the treatment of apathy in AD in patients previously stabilized on a cholinesterase inhibitor. A positive relationship between the acute effects of d-amph was further related to methylphenidate treatment response, implicating a role for dopaminergic dysfunction in the development and treatment of apathy in AD. The study showed a clear efficacy signal at the tested dose as well a benign adverse event profile. The study reported that the two patients who experienced serious adverse events during the study also experienced significant behavioral disturbances, consisting of delusions, agitation, irritability, and appetite change prior to commencing methylphenidate treatment. Thus, patients presenting those symptoms at baseline will be excluded from ADMET 2.

The original Alzheimer's Disease Methylphenidate Trial (ADMET) found that methylphenidate treatment of apathy in AD was associated with significant improvement in two of three efficacy outcomes and a trend toward improved global cognition with minimal adverse events, suggesting that methylphenidate might be a safe and efficacious treatment for apathy in AD. This trial studied 60 participants over 6-week period.

The goal of ADMET 2 is to expand upon this information provided by these two pilot randomized trials by evaluating methylphenidate for the treatment of apathy in 200 AD participants over a 6-month period of time as well as collecting information on the safety of methylphenidate in this population. In addition, the goal of the substudy "Biomarkers of Apathy and Treatment Response" is to understand blood-based biomarker correlates of change in apathy over time, and to identify possible predictors of treatment response.

2. Objectives

Primary objective

- To examine in a masked, randomized trial the efficacy of methylphenidate for the treatment of clinically significant apathy in participants with Alzheimer's dementia. Efficacy will be assessed as the change in Neuropsychiatric Inventory Apathy subscale (NPI apathy) from baseline to 6 months and score on the Alzheimer's Disease Cooperative Study - Clinical Global Impression of Change (CGIC) scale at 6 months.

Additional objectives

- To examine the safety of methylphenidate for the treatment of clinically significant apathy in participants with Alzheimer's disease. Safety will be measured using vital signs, electrolyte panels, adverse event reports, and electrocardiograms. Safety will also be measured by examining neuropsychiatric symptoms other than apathy using the Neuropsychiatric Inventory (NPI).
- To examine changes in other neuropsychological assessments, such as the Dementia Apathy Interview and Rating (DAIR) scale from baseline to 6 months.
- To examine cost-effectiveness by assessing quality of life and economic assessment from baseline to 6 months.
- To examine cognitive changes from baseline to 6 months using a cognitive battery that includes the Mini Mental State Exam (MMSE) and other scales.
- To understand blood-based biomarker correlates of change in apathy over time, and to identify possible predictors of treatment response (with the substudy "Biomarkers of Apathy and Treatment Response," see Chapter 14)
- To assess efficacy as the change in NPI apathy and CGIC apathy from baseline to 2 months.

3. Design

3.1. Overview

This multi-center, parallel, randomized, double-blind, placebo-controlled Phase III, definitive trial will enroll 200 participants with clinically significant apathy from ten clinical centers. Primary outcome measures include the Neuropsychiatric Inventory Apathy subscale (NPI apathy) and the Clinical Global Impression of Change (CGIC). Additional outcome measures include the Dementia Apathy Interview and Rating (DAIR), AD Cooperative Study-Activities of Daily Living Scale (ADCS-ADL), Dependence Scale, and Information on caregiver distress (NPI caregiver distress score). Cost-effectiveness will be measured using health related quality of life (HRQOL; EuroQol EQ-5D- 5L), and resource utilization (Resource Utilization in Dementia Lite, RUD-lite). A cognitive battery of tests will be assessed at baseline and at select in-person follow-up visits (every two months until 6 months) after randomization. Safety will be assessed by measuring vital signs, electrolytes, electrocardiograms (ECGs), and Neuropsychiatric Inventory (NPI). Adverse events reports will be completed as required at each follow-up visit.

These assessments will be administered at baseline and at each in-person follow-up visit (monthly until 6 months) after randomization.

In addition, those participants who consent to participate in the blood-based biomarkers substudy to the main ADMET 2 study will provide a sample of blood biospecimen at baseline and 6 months after randomization according to this schedule:

- Baseline visit – miRNA, oxidative stress, inflammation (cytokines), neuronal loss, and lipidomics[1]
- 6-month follow-up visit - oxidative stress, inflammation (cytokines), and lipidomics

In addition, compliance checks will be conducted at in-person follow-up visits as well as by phone around days 15, 45, and 75 after randomization.

3.2. Sample size and power

Power calculations were conducted for the two primary outcomes: 1) mean difference in change from baseline to 6 months in the NPI apathy subscale scores; 2) odds of having a given rating or better on the ADCS-CGIC ratings at month 6. The power and sample size for the NPI apathy outcome were determined with standard two sample methods for comparing means using SAS 9.2 (Copyright © 2002-2008 by SAS Institute Inc., Cary, NC, USA). The power and sample size for the ADCS-CGIC outcome were determined using the method of Whitehead (1993) for proportional odds logistic regression implemented by the 'popower' in the Hmisc package in R (2011). Both calculations assumed a type I error rate of 0.025 to preserve an overall type I error rate of 0.05 over both primary comparisons.

The sample size calculations assume the difference in NPI apathy change scores (1.8 points) and standard deviation (3.2) of the change scores that was observed in the original ADMET study. The difference in NPI apathy is similar to the difference between mean change in moderate improvement and minimal improvement/no change in ADMET. The mean change in NPI apathy for those with CGIC rating of moderate improvement in ADMET was -5 and for those with minimal improvement or no change was -3.

[1] MiRNA may be collected at any study visit. For those who enroll in the substudy after their baseline visit, these biospecimen may be collected up through the month 3 visit.

There were no deaths observed in the original ADMET; however, in the DIADS-2 study (Martin 2006), which included a similar AD population and was conducted by many of the same investigators proposed here, we observed 4 deaths with 131 participants (3%) that were followed for 6 months. We lost only 5% of the participants in the original ADMET before the final visit (none due to death) but losses may be higher in ADMET 2 due to the longer follow-up time. A sample size of 200 participants will ensure greater than 90% power to detect a difference of 1.8 points in change on the NPI apathy scale even if 15% of the participants are lost before the month 6 visit. We calculated the power for the ADCS-CGIC outcome assuming the overall proportions of ratings in each category from ADMET: 3.5% with marked improvement, 8.8% with moderate improvement, 29.8% with minimal improvement, 54.4% with no change, 3.5% with minimal worsening, 0% with moderate or marked worsening. Assuming an odds ratio for better ratings in methylphenidate of 2.75 (about 25% smaller than the odds ratio observed in ADMET) and 200 participants, the study will have 90% power to detect this odds ratio with 10% losses to follow-up and greater than 85% power with 15% losses.

For the blood-based biomarker substudy, the sample will be one of convenience, based on the availability of study participants in the trial. Possible effect sizes were estimated based upon the projected possible number of participants (see Section 14.10).

3.3. Organization

- Operating units of ADMET 2 are the clinical and resource centers
- Primary decision-making bodies are the Executive and Steering Committees
- Data and safety monitoring will be conducted by an independent Data and Safety Monitoring Committee (DSMC)
- Additional committees are formed for special projects, as needed. These may include Training and certification committee, Quality assurance committee, Publications, presentations, and ancillary studies committee, Policy and protocol committee, and the Biomarkers executive committee.

3.4. Policies

Study policies include:

Governance
Data monitoring
Publication
Adverse events
Ancillary studies
Data sharing
Conflict of interest
Site replacement

4. Eligibility criteria

Men and women, including those in minority groups, will be included with no specific age restrictions. No specific laboratory screenings will be required for inclusion. “Usual care” treatment with methylphenidate does not require laboratory monitoring. Laboratory tests for the purpose of qualifying a participant having Alzheimer’s disease (AD) (e.g., brain imaging, blood and urine test, etc.) will be obtained under usual care clinical practices prior to entry which is consistent with current clinical standards and guidelines.

Inclusion criteria

- Possible or probable Alzheimer's disease (National Institute of Neurological and Communicative Disorders and Stroke - Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) criteria), with Mini-Mental State Exam (MMSE) score of 10-28 inclusive
- Clinically significant apathy for at least four weeks for which either
    - the frequency of apathy as assessed by the Neuropsychiatric Inventory (NPI) is 'Very frequently', or
    - the frequency of apathy as assessed by the NPI is 'Frequently' or 'Often' AND the severity of apathy as assessed by the NPI is 'Moderate' or 'Marked'
- A medication for apathy is appropriate, in the opinion of the study physician
- Provision of informed consent for participation in the study by potential participant or surrogate (with participant assent if the potential participant is unable to provide informed consent) and caregiver
- Availability of caregiver, who spends greater than ten hours a week with the potential participant and supervises his/her care, to accompany the participant to study visits and to participate in the study
- Sufficient fluency, of both the potential participant and caregiver, in written and spoken English to participate in study visits, physical exams, and outcome assessments
- If female, woman must be post-menopausal for at least 2 years or have had a hysterectomy

Exclusion criteria

- Currently meets criteria for Major Depressive Episode, by Diagnostic Statistical Manual of Mental Disorder - IV (TR) criteria
- Clinically significant agitation /aggression for which either
    - the frequency of agitation /aggression as assessed by the NPI is ‘Very frequently', or
    - the frequency of agitation /aggression as assessed by the NPI is ‘Frequently' AND the severity of the agitation as assessed by the NPI is ‘Moderate', or ‘Marked'
- Clinically significant delusions for which either
    - the frequency of delusions as assessed by the NPI is ‘Very frequently', or
    - the frequency of delusions as assessed by the NPI is ‘Frequently' AND the severity of the delusions as assessed by the NPI is ‘Moderate', or ‘Marked'

- Clinically significant hallucinations for which either
  - the frequency of hallucinations as assessed by the NPI is ‘Very frequently', or
  - the frequency of hallucinations as assessed by the NPI is ‘Frequently' AND the severity of the hallucinations as assessed by the NPI is ‘Moderate', or ‘Marked'
- Change to AD medications within the 30 days preceding randomization, including starting, stopping, or dosage modifications
- Change in anti-depressant (except for trazodone used for sleeping difficulties as described below) use within the 30 days preceding randomization or a period of time equal to 5 half-lives of drug, whichever period of time is longer
- Use of trazodone > 50mg or lorazepam > 0.5mg or for indications other than sleeping difficulties within the 30 days preceding randomization or a period of time equal to 5 half-lives of drug, whichever period of time is longer. Other benzodiazepines are prohibited in the past 30 days or within 5 half-lives, whichever period of time is longer
- Failure of treatment with methylphenidate in the past for apathy after convincing evidence of an adequate trial as judged by study physician
- Currently taking any amphetamine product, an antipsychotic, bupropion, or any medication that would prohibit the safe concurrent use of methylphenidate, including but not limited to monoamine oxidase inhibitors and tricyclic antidepressants within the 30 days preceding randomization or a period of time equal to 5 half-lives of drug, whichever period of time is longer
- Need for acute psychiatric hospitalization or is suicidal in the opinion of the study physician
- Significant communicative impairments that would affect participation in clinical trial
- CNS abnormalities (e.g., cerebral aneurysm), seizures (convulsions, epilepsy), Tourette's syndrome or presence of motor tics, or abnormal EEGs
- Lack of appetite that results in significant unintentional weight loss as determined by the study physician in the last three months
- Uncontrolled hyperthyroidism
- Any cardiovascular or cerebrovascular abnormality deemed to be clinically significant by the study physician, tachycardia (heart rate ≥ 100 beats per minute), or uncontrolled hypertension (defined as medication non-compliance or past 3 months with a diastolic reading ≥ 105 mm Hg), at the time of screening
- Closed angle glaucoma or pheochromocytoma
- Women with childbearing potential
- Current participation in a clinical trial or study that may add significant burden or affect study outcomes
- Any condition that, in the opinion of the study physician, makes it medically inappropriate or risky for the potential participant to enroll in the trial, including, but not limited to, contraindication to treatment with methylphenidate

5. Randomization and masking

The Coordinating Center (CC) will generate random treatment assignment schedules using a documented program in SAS. The randomization schedule will be designed to yield an expected assignment ratio of 1:1 for methylphenidate and placebo. Assignments will be stratified by clinical center and schedules will employ permuted block designs, with block sizes to be determined and documented at the CC. Adjustment for residual or other imbalances in the baseline composition of the treatment groups, if needed, will be done using multiple regression techniques at the time of data analysis rather than through further stratification in the design. Treatment assignments will be masked to the participants and the personnel of the clinical centers, but not to a restricted set of personnel at the CC. The CC will also generate a list of randomly ordered medication identifiers which will be linked to the assignment schedule. Documentation of all these processes will be retained at the CC and shall be accessible only to authorized personnel.

Treatments will be assigned using an online program accessible to the clinical centers through the data system. After the entry of specified pre-randomization data, and confirmation of eligibility, each enrolled participant's ID will be irrevocably linked to the next unassigned treatment for that clinical center. The clinical center will be directed to issue a medication kit containing the proper assigned treatment from among those kits available at the center. Treatment assignments will be e-mailed in real-time to the CC. The data system will also check for and prevent duplicate assignments (same participant randomized more than once). The treatment assignment tables in the data system will be encrypted to prevent inadvertent disclosures.

The procedures related to randomization of participants at the clinical centers will be as follows:

- Eligibility and baseline data will be collected on paper forms and entered into the database at the clinical centers
- The data system will confirm eligibility and then issue the next assignment as described above; each assignment will also be e-mailed to the CC
- The data system will automatically store the date and time of assignment, the identity of the clinical center staff person making the assignment, the participant's ID, and the medication identifier to be issued
- Randomization materials, including a visit schedule and allowable time windows for visits, will be generated for the clinical center

Emergency unmasking before the end of the treatment period is expected to be rare. Clinical centers may request an emergency unmasking from the ADMET 2 data system. Clinical centers must contact the CC as soon as possible to report the emergency unmasking.

Optional unmasking may occur for each participant after 6 months follow-up and all data collection is completed. This information will be provided to the participant and caregiver. Treatment assignment will not be revealed to the ADMET 2 staff at the clinical centers.

6. Treatment plan

6.1. Treatment groups

Participants will be assigned to one of the following groups:

- Methylphenidate, target dose of 20 mg per day, administered as two 5 mg over-encapsulated tablets twice a day, and psychosocial intervention
- Matching over encapsulated placebo and psychosocial intervention

6.2. Treatment schedule and titration of study drug dose

The target study dose will be 20 mg per day provided as two 10 mg doses administrated orally. Participants will start on 10 mg daily (i.e., 5 mg twice a day) for three days. Then the dose will be increased to 20 mg per day (i.e., 10 mg twice a day) for six months following a phone call to the participant and/or caregiver that is to occur at day 3. If necessary, study physicians will have the option of decreasing the daily dose preferably a minimum of 10 mg of study drug or placebo per day depending on tolerability. That is, some participants will have unacceptable side effects on 20 mg and will have to be reduced to a lower dose so as continue study treatment. Reductions of dose will only be allowed for clinically significant side effects.

Unmasking for participants may occur after the 6 month visit for the purposes of clinical care. Unmasking before the end of the 6-month period is to occur only in emergency situations (see randomization and masking section 5 for more details).

6.3. Choice of methylphenidate formulation

Because the use of 5 mg allows for finer titration to the 20 mg daily intake and allows for reduction in dose due to possible side effects, the use of 5 mg tablets of methylphenidate is required. However, there is no 5 mg formulation of methylphenidate that is approved and available in both the United States and Canada. The drug will be purchased and distributed centrally in the nine US sites but will be purchased and distributed separately for the Canadian site. Therefore, two generic formulations will be used (one that is approved in the United States and one that is approved in Canada). This is a practical and pragmatic decision that will provide results that are more generalizable than using a formulation that is not approved in one of the countries.

6.4. Psychosocial treatment

All caregivers and the participants (if convenient) will be provided with a standardized psychosocial intervention modeled after the counseling strategies employed in Depression in Alzheimer's Disease Study-2 (Martin 2006). The psychosocial intervention may consist of three components: a counseling session, the provision of education materials, and availability for crises.

The counseling session, in which a trained study clinician will counsel the study caregiver and participant will take place at each study visit at and after the randomization visit. It will last approximately 20-30 minutes. Each counseling session will consist of the following elements:

- Review and adjustment of the participant and caregiver supportive care plans
- Emotional support and opportunity to ventilate feelings
- Counseling regarding specific caregiving skills
- Assistance with problem solving of specific issues that the caregiver brings to the sessions
- Answers for questions regarding the educational materials

The educational materials will consist of:

- A copy of the book The 36-Hour Day
- Dementia Care Guidelines for Families

The caregiver also may be provided with 24-hour phone access to the study nurse or physician for assistance with crises that may arise after hours.

6.5. Adherence to study treatment

To ensure adherence, study medication will be administered to participants under the supervision of the caregivers involved in the study. Study physicians will assess the capacity of each participant to monitor and administer treatments and will involve caregivers as needed to ensure safe use of drug and proper adherence to the treatment protocol. Adherence to assigned treatment will be monitored via participant and/or caregiver interview at each visit and via pill counts. Participants and/or caregivers will be asked to return all study bottles with any unused capsules at each visit. Bottles are to be returned even if all study drug was used.

6.6. Management and reporting of adverse events

Management: All adverse events occurring after randomization and during the 6-month treatment period, regardless of adherence to study treatment, will be recorded at all contacts. A list of common side effects of methylphenidate will be used to monitor for adverse events. At scheduled visits, participants and their caregivers will be interviewed about whether the participant experienced any symptoms or side effects on the list since the last visit. Adverse events, other than those listed as common side effects, will still be recorded. If adverse events are noted, they will be rated as mild, moderate, or severe based on their clinical severity and frequency. Finally, participants and caregivers will be asked about visits to doctors, healthcare providers, and emergency departments for other than routine care.

Center investigators will be responsible for monitoring the safety of participants. They will be responsible for appropriate medical care of participants during the study in connection with study procedures. Safety assessments will include physical exams, vital signs, monitoring of adverse events, and monitoring and maintenance of concurrent medication records. In addition, the medical monitor designated by the Chair's office will provide consultation to all centers regarding medical monitoring. He/she will carry a beeper 24-hours a day to receive protocol questions and serious or problematic adverse events that center investigators believe should be referred to him/her.

Reporting: Supervising Institutional Review Boards (IRBs) will be notified by local investigators of adverse events occurring at their institution, according to their reporting requirements. Investigators also will notify the medical monitor and CC within one day of learning of a death or of an event that is life-threatening, that results in hospitalization or prolongation of hospitalization, or that involves a persistent or significant disability or incapacity. Data collected regarding these serious adverse events will include the treatment provided, outcome, and presumed relationship to study drug and will be updated as new information becomes available; a narrative description also will be provided. CC personnel will review

the data and query the clinical centers for clarification, additional information, or supporting documentation as necessary. Reports and narratives will be forwarded to all investigators for submission to IRBs as necessary. In addition, this information will be provided to the Data and Safety Monitoring Committee (DSMC) as part of their safety review.

Study-wide summary statistics (not broken down by treatment group) of adverse events will be available upon request to all centers on an annual basis for submission to their local IRB.

6.7. Concomitant medications

The use of a wide variety of medications will be allowed, because this study will attempt to represent usual clinical practice. Participants will remain on medications necessary to treat medical co-morbidities. Use of Alzheimer's disease medications will be allowed: (i) if there is no change to AD medications within the month preceding randomization, including, starting, stopping, or dosage modifications, (ii) if there are no current plans to change dosage or discontinue medication, and (iii) if the clinician believes that these medications are not causing or exacerbating the participant's apathy. Changes in Alzheimer's disease medications will be allowed only if the clinician supervising the participant's care determines the change to be clinically required.

To make the efficacy comparison as straightforward as possible, any amphetamine product, antipsychotic, bupropion, or any medication that would prohibit the safe concurrent use of methylphenidate, including but not limited to monoamine oxidase inhibitors and tricyclic antidepressants, will not be allowed while participants are receiving masked study treatment. For the treatment of sleeping difficulties, trazodone up to 50 mg before sleep or lorazepam up to 0.5 mg before sleep may be used nightly, or as needed. Other benzodiazepines should not be used.

If the participant experiences significant agitation (or delusions), the study drug dose can be reduced to one capsule twice a day (10 mg per day in the methylphenidate group). If symptoms persist after reducing the dose then study drug can be temporarily discontinued. If study drug is temporarily discontinued, it can be restarted if:

a. The symptoms have improved, and
b. The participant is able to stop taking any medications used to treat the symptoms (i.e., when study drug is restarted, the concomitant medications must meet baseline eligibility criteria).
   If the participant has been prescribed a selective serotonin reuptake inhibitor (SSRI) or other medication to treat the symptoms, the study drug may only be restarted once the participant has stopped taking the SSRI/other medication for at least 30 days or 5 half-lives, whichever is longer.

If the participant requires initiation or change in an existing antidepressant (i.e. SSRIs) for treatment of depression any time during the study, study drug should be temporarily stopped until the participant is on a stable dose of the SSRI for at least 30 days or 5 half-lives, whichever is longer.

Clinic staff should continue to conduct all participant visits and contacts per protocol regardless of whether the participant is taking study drug.

6.8. Assessment of suicidality and need for acute hospitalization

The assessment of suicidality or need for acute hospitalization will be based on psychiatric assessment at study screening. Specifically, suicidality will be assessed by participant and caregiver interview and by mental status examination of the participant. The examiner will assess for severe hopelessness, passive death wish, suicidal statements, suicidal plan, or behavioral indicators of risk for self-harm. Need for

acute hospitalization will be similarly assessed by a study physician. Acute hospitalization is typically indicated if there is imminent risk of harm.

7. Baseline visit

7.1. Overview

Participants will be typical outpatients with Alzheimer's disease, recruited from clinical settings at the study centers. Residents of nursing homes and assisted living facilities are also eligible. The use of diverse centers will promote representation from ethnic minority groups. The allowable range of cognitive impairment is as broad as possible, sufficient in the high end to establish dementia diagnosis, with sufficient residual on the low end to allow for the quantification of apathy symptoms.

7.2. Overview of consent issues

The participant and caregiver (if required by local IRB) are both to provide consent for this study. Issues of consent are important in this population of participants, as their capacity to give informed consent may be compromised. Consent is to be obtained from participants and their authorized legal representatives using procedures established by the individual centers and their overseeing Institutional Review Boards in accordance with local law. In all cases, prospective participants with dementia will first be assessed for their ability to provide informed consent. Capacity to give consent will be assessed in clinical interviews of participants by clinicians experienced in clinical dementia research. If a potential participant is found not capable of fully providing consent for participation, then his or her surrogate will provide consent and the participant will be asked to provide assent according to the Alzheimer's Association[3] or local IRB guidelines. Potential participants who are found to be able to provide informed consent will be asked to do so and their surrogates will co-sign the consent form as a witness. More details regarding the consent process can be found in the section on the protection of human subjects.

7.3. Review of medical history

A certified study physician must see the participant before randomization. The study physicians are responsible for fully assessing whether the participant has any of the conditions listed as contraindications for the use of methylphenidate, such as closed angle glaucoma, hyperthyroidism, or serious cardiovascular or heart rhythm problems (see exclusion criteria in section 4 for the complete list), through a thorough participant and family medical history or any other means that would be part of standard clinical care before prescribing methylphenidate to a participant. The presence of any contraindicated condition or medication, which are itemized in the baseline eligibility as well as medical history forms, constitutes an exclusion criterion for ADMET 2.

7.4. Baseline visit procedures

The baseline visit may take place across more than one day, but must be completed within the three weeks preceding randomization. The clinical staff should perform and review results of the electrocardiogram (ECG) within the three weeks preceding randomization.

At the baseline visit, study staff will:

- Obtain the name and address of the participant's personal physician, if any
- Record participant and caregiver demographics
- Obtain a medical history and record current medications
- Administer research diagnostic criteria for apathy

- Collect assessments:
  - Clinical Global Impression Worksheet (CGI)
  - Neuropsychiatric Inventory (NPI)
  - Dementia Apathy Interview and Rating (DAIR)
  - Dependence Scale
  - AD Cooperative Study-Activities of Daily Living Scale (ADCS-ADL)
  - Health related quality of life (HRQOL; EuroQol EQ-5D- 5L)
  - Resource Utilization in Dementia Lite (RUD-lite)
- Perform cognitive tests:
  - Mini Mental State Exam (MMSE)
  - Hopkins Verbal Learning Test - Revised (HVLT-R)
  - Digit Span
  - Trail Making Test (A and B)
  - Action Verbal Fluency Test from the PDCRS
  - Category Fluency Task-Animal Naming (from the VCIHS)
  - Short Boston Naming Test
- Perform safety measures:
  - Vital signs
  - Electrolyte panel
  - ECG
  - Gather baseline symptom information
- Obtain blood samples for biomarker analysis of miRNA, oxidative stress, inflammation cytokines), neuronal loss, and lipidomics (for those who consent to the blood-based biomarker substudy, see Chapter 14)
- Obtain the randomized treatment assignment
- Provide the psychosocial intervention
- Dispense study medication and review instructions for medication use
- Review visit schedule, compliance monitoring, and adverse event reporting

8. Follow-up visits and telephone contacts

8.1. Overview of follow-up visits and telephone contacts

Follow-up will include both scheduled and unscheduled visits and contacts. Scheduled follow-up includes:

- Scheduled in-person visits (monthly after randomization)
- Telephone contacts for data collection (days 15, 45, and 75 after randomization)
- Telephone contact for dose adjustments (day 3 and as needed)

Target dates for follow-up visits are calculated from the date of randomization.

8.2. Scheduled in-person visits

At all scheduled in-person visits, study staff will:

- Review study procedures to verify ongoing consent
- Review interval medical history
- Review and record current medications
- Collect assessments:
  - Clinical Global Impression Worksheet (CGI)
  - Neuropsychiatric Inventory (NPI)
  - Dementia Apathy Interview and Rating (DAIR)
- Perform safety measures:
  - Vital signs
  - Electrolyte panel
  - ECG
  - Gather baseline symptom information
- Provide the psychosocial intervention
- Receive and record the amount of unused study drug
- Dispense a new supply of study drug (except month 6)
- Review visit schedule and compliance monitoring
- Refer the participant to a study physician or a primary care physician or specialist if the participant exhibits a notable change in condition or is medically unstable

At months 2, 4, and 6, study staff will also

- Collect assessments:
  - Dependence Scale
  - AD Cooperative Study-Activities of Daily Living Scale (ADCS-ADL)
- Perform cognitive tests:
  - Mini Mental State Exam (MMSE)
  - Hopkins Verbal Learning Test - Revised (HVLT-R)
  - Digit Span
  - Trail Making Test (A and B)
  - Action Verbal Fluency Test from the PDCRS
  - Category Fluency Task-Animal Naming
  - Short Boston Naming Test

At months 3 and 6, study staff will also

- Collect assessments:
  - Health related quality of life (EuroQol EQ-5D- 5L)
  - Resource Utilization in Dementia Lite (RUD-lite)

If not obtained at baseline, at month 1, 2, or 3, study staff may also obtain blood samples for biomarker analysis of miRNA, oxidative stress, inflammation cytokines), neuronal loss, and lipidomics for those who consent to the blood-based biomarker substudy, see Chapter 14).

At month 6, study staff will also obtain blood samples for biomarker analysis of oxidative stress, inflammation cytokines), and lipidomics (for those who consented to this substudy, see Chapter 14).

8.3. Telephone contacts for data collection

To ensure regular contact with study staff during the study, there will be telephone contacts with participants and their caregivers at day 15, 45, and 75 after randomization. The purpose of these contacts is to:

- Collect interim medical history
- Enhance compliance and retention
- Review visit schedule
- Provide medical monitoring
- Collect adverse events

Medical information obtained from participants or caregivers is to be recorded for that contact.

8.4. Telephone contacts for dose adjustments

To aid participants and caregivers, a telephone contact should occur on day 3 or immediately before the day 4 dose adjustment, and at anytime when a dose adjustment is required for clinical purposes. The purpose is to remind the participant and caregiver to make the dose adjustment and to provide medical monitoring. No information will be captured for this contact unless there is a serious adverse event or treatment termination.

8.5. Unscheduled follow-up visits and contacts

In addition to the visits outlined in the above schedule, participants may also be asked to appear for other assessments as needed. Unscheduled clinic visits may occur to evaluate a new or altered medical condition, to assess side effects, to assist with compliance in complex cases, or to provide counseling and behavioral interventions as needed.

Participants may contact the clinical center personnel between scheduled contacts regarding medical or cognitive problems that they are experiencing. Information will be recorded on the nature of the complaints and on any recommendation or referral made by clinical center personnel.

8.6. Data collection by visit

	BL	T1	F1	T2	F2	T3	F3	F4	F5	F6
Months from BL	**0**	**0.5**	**1**	**1.5**	**2**	**2.5**	**3**	**4**	**5**	**6**
Procedures										
Consent	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔
History, or interim history	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔
Demographics	✔	·	·	·	·	·	·	·	·	·
Review of inclusion/exclusion	✔	·	·	·	·	·	·	·	·	·
Psychosocial intervention	✔	·	✔	·	✔	·	✔	✔	✔	✔
Dispensing of study drug	✔	·	✔	·	✔	·	✔	✔	✔	·
Review visit schedule	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔
Review of compliance	·	✔	✔	✔	✔	✔	✔	✔	✔	✔
Assessments										
NPI	✔	·	✔	·	✔	·	✔	✔	✔	✔
CGIC	✔	·	✔	·	✔	·	✔	✔	✔	✔
DAIR	✔	·	✔	·	✔	·	✔	✔	✔	✔
ADL	✔	·	·	·	✔	·	·	✔	·	✔
Dependence Scale	✔	·	·	·	✔	·	·	✔	·	✔
Cognitive battery	✔	·	·	·	✔	·	·	✔	·	✔
EQ-5D-5L	✔	·	·	·	·	·	✔	·	·	✔
RUD-lite	✔	·	·	·	·	·	✔	·	·	✔
Diagnostic Criteria for Apathy	✔	·	·	·	·	·	·	·	·	·
Safety measurements										
Vital signs	✔	·	✔	·	✔	·	✔	✔	✔	✔
Electrolyte panel	✔	·	✔	·	✔	·	✔	✔	✔	✔
Adverse events	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔
Review ECG	✔	·	✔	·	✔	·	✔	✔	✔	✔
Biomarkers blood collection*	✔	·	·	·	·	·	·	·	·	✔

*For those participants consenting to the biomarkers substudy only: MicroRNA, oxidative stress, inflammation (cytokines), neuronal loss, and lipidomics are collected at BL (or F1, F2, or F3 if baseline visit already occurred); oxidative stress, inflammation, and lipidomics are collected also at F6. However, the mRNA portion may be collected at any in-person follow-up visit.

BL = Baseline visit
F=Scheduled follow-up visits
T=Scheduled telephone contact
NPI = Neuropsychiatric Inventory
CGIC = Clinical Global Impression of Change
DAIR= Dementia Apathy Interview and Rating
ADL=Activities of Daily Living
EQ-5D-5L= Euro Quality of Life
RUD-lite= Resource Utilization in Dementia-Lite
ECG = Electrocardiogram

9. Outcomes assessments

The following instruments and measures will be used in ADMET 2.

9.1. Neuropsychology, neuropsychiatry, and other assessments

- *AD Cooperative Study - Clinical Global Impression of Change (CGIC)* (Schneider 1997): The CGIC is a systematic method, developed for the Alzheimer's disease (AD) setting to assess clinically significant change in a clinical trial as viewed by an independent, skilled, and experienced clinician. This will be applied to collect information on the level of apathy. Unlike a targeted symptom scale, this method takes into account the overall level of apathy with emphasis on clinically significant change since the pre-treatment baseline. A trained clinician, not involved in collecting other assessments or procedures, will complete the CGIC using a 7-point Likert scale to rate each participant along a continuum from "very much worse" (7) to "very much improved" (1) with a rating of 4 being "no change." Ratings will be based on an interview with the caregiver and an examination of the participant, without consulting other objective measures, such as cognitive test results. The CGIC requires the assessor to consider a number of aspects of apathy, such as level of initiative, level of interest, and emotional engagement.

- *Neuropsychiatric Inventory (NPI)* (Cummings 1997): The NPI is the most widely-used measure of NPS in dementia clinical trials. This inventory has a strong ability to distinguish medication groups from placebo groups. The NPI assesses type and severity of behavioral disturbances in dementia. The inventory evaluates 12 domains of neuropsychiatric symptoms (NPS): apathy, agitation, delusions, hallucinations, depression, euphoria, aberrant motor behavior, irritability, disinhibition, anxiety, sleeping, and eating. Frequency (1 = occasionally, less than once/week; 2 = often, about once per week; 3 = frequently, more than once a week; 4 = very frequently, once or more/day or continuously) and severity (1 = mild, 2 = moderate, 3 = severe) scales in each domain are scored based on responses from an informed caregiver involved in the participant's life. The NPI also includes a 5-point rating used to quantify caregiver distress with the participant's NPS symptoms. To obtain an NPI score for each domain, the severity score and the frequency score are multiplied, and the caregiver distress ratings of each domain are added up. In ADMET 2, the apathy domain score will be used as an entry criterion to define a level of apathy of moderate or greater severity.

- *Dementia Apathy Interview and Rating (DAIR)* (Strauss 2002): The DAIR is a 16-item, informant rated scale for the assessment of apathy in persons with mild to moderate AD. The DAIR has ability to discriminate apathy from lack of interest due to inability or longstanding, premorbid personality traits. The DAIR evaluates the change in motivation, engagement, and emotional response since disease onset. The minimum score is 0 and the maximum score is 48.

- *Dependence Scale* (Stern 1994): This is a structured scale of the degree of dependence or assistance needed by a participant that was developed for longitudinal studies of AD and has been used as a predictor of nursing home placement. The interview is conducted a certified clinician with the caregiver. The minimum score is 0 and the maximum score is 15. Higher scores indicate increased levels of dependence.

- *AD Cooperative Study-Activities of Daily Living Scale (ADCS-ADL)* (Galasko 1997): This measure is an ADL inventory developed by the ADCS to assess functional performance in patients with AD. In a structured interview format, informants are queried as to whether subjects attempted each of 24 items in the inventory during the prior 4 weeks and their level of performance. The scale discriminates well the stages of severity of AD, from very mild to severely impaired.

- *EuroQol EQ-5D- 5L* (Johnson 1998): This health related quality of life assessment will be collected at baseline, 3 months and endpoint. The EQ-5D-5L is a generic measure of health related quality of life that can be calibrated on a 0 to 1 scale and thus measures utility, allowing for the calculation of Quality Adjusted Life Years (QALYs). The standard EQ-5D measures 5 dimensions: mobility, self-care, usual activities, pain/discomfort, and anxiety/depression graded from level 1 (i.e., no problems) to level 5 (i.e., extreme problems). Use of the 5-levels (EQ-5D-5L) rather than the standard 3 level EQ-5D (EQ-5D-3L) has led to less ceiling effect, greater discriminative ability, and potentially more power to detect differences between groups compared with EQ-5D-3L.

- *Resource Utilization in Dementia-Lite* (Wimo 2013): This is a validated instrument. The costing questionnaire will be generated by the research team, mapping from the resource utilization questionnaire to the costs of those services at fair market value today. The resource utilization questionnaire includes accommodation, informal care, community care, and hospitalizations, enabling estimation of both direct and indirect medical costs.

9.2. Cognitive assessments

The cognitive battery was selected to target tests that measure specific cognitive domains likely to be impacted by methylphenidate treatment. We have also selected measures of memory and naming/lexical retrieval, domains usually impaired in AD. In addition, it is our goal to create a limited efficient battery for practical considerations and to minimize the stress on participants (the battery was designed not to take more than 30 minutes to administer). The battery includes:

- *Mini-Mental State Exam (MMSE)* (Folstein 1975): This is a well-known cognitive screening test for the detection of dementia and estimation of its severity. The particular domains most often affected in AD (orientation, memory) are included in this instrument. Other items include those assessing working memory, naming, following verbal and written commands, spontaneously writing a sentence, and copying two overlapping pentagons. The MMSE has been used as a measure of toxicity in a number of studies evaluating treatments for NPS (Storga 1996). A second factor in choosing the MMSE as a safety measure rather than an efficacy measure was our intention to use separate tests to evaluate safety and efficacy in order to avoid bias and contamination by raters. Therefore, the MMSE will be used as a safety measure in this study.

- *Hopkins Verbal Learning Test – Revised (HVLT-R)* (Brandt 2001): The HVLT-R is used to measure verbal learning, recognition, and delayed recall. In this test, a list of twelve words—four

from each of three semantic categories—is read to participants. Three learning trials are followed, after 20 to 25 minutes, by a delayed recall trial and then a yes/no recognition trial. The minimum score is 0 and the maximum score is 12. Higher scores indicate better performance.

- *Digit Span: The Wechsler Adult Intelligence Scale – Revised Digit Span subtest* (Wheeler 1981) is used to assess auditory attention and working memory. Both forward and backward span is assessed. Both tests consist of six number sequences that the psychometrist reads aloud one at a time. After each sequence is read, the participant must repeat the digits back in the same (forward) or reverse (backward) order.

- *Trail Making Test (A and B)* (Stuss 2001): This timed test is used to measure attention, executive function, and visuo-motor tracking. To complete Trails Part A, the participant must draw lines to connect consecutively numbered circles. To complete Trails Part B, participants must connect consecutively numbered and lettered circles by alternating between the two sequences. The trail making test is discontinued after 5 minutes for both parts A and B. Shorter duration to complete the test and fewer number of errors indicate higher cognitive functioning in the domains tested.

- *Action Verbal Fluency Test from the PDCRS* (Piatt 1999): This test measures executive function, working memory, and information processing speed. The Action Verbal Fluency Test from the Parkinson's Disease–Cognitive Rating Scale (PDCRS) uses action verbal fluencies to differentiate between cognitive groups. The participant is asked to name verbs in 60 seconds without repetition or use of alternate form of verbs or with different endings.

- *Category Fluency Task-Animal Naming (from the Vascular Cognitive Impairment Harmonization Standards)* (Isaacs 1972): This is a test of executive function, working memory, set shifting, and executive control. It is one of the sensitive measures to distinguish normal aging from dementia. It is easy to use, has multiple normative forms, and can be used in different cross cultural settings. The participant is requested to generate words in 60 seconds.

- *Short Boston Naming Test* (Kaplan 1983; Franzen 1995): A modification of the Boston Naming Test measures expressive language. Participants are asked to name 15 line drawings of varying difficulty. The minimum score is 0 and the maximum score is 15. Higher scores indicate better control of expressive language.

9.3. Safety measurements

- *Vital signs*, including blood pressure, pulse, respiratory rate, and weight, will be evaluated at every visit and will be measured using standard clinical procedures. Clinically significant changes will be reported as adverse events.

- *Electrolyte panel* will be assessed at baseline and monthly post-randomization. The panel will include sodium, potassium, chloride, bicarbonate, glucose, urea nitrogen, and creatinine levels.

- *Adverse events* including serious adverse events will be defined using standard approaches. The occurrence of adverse events will be noted by the clinical center teams and recorded in the study documents for analytic purposes.

- *Electrocardiogram (ECG)*: One of the potential adverse events of methylphenidate is an increase in heart rate. This increase could trigger previously undetected conduction problems. Therefore,

we will evaluate ECGs at baseline to allow the investigator to evaluate the clinical significance of potential findings and decide on the inclusion or exclusion of the participant in the study. ECG assessments will also be conducted monthly to evaluate the potential effects of methylphenidate on heart conduction. If clinical manifestations of a cardiac condition are detected through the study, appropriate action will be taken at the investigator's discretion.

10. Analysis

10.1. Analysis overview

All data analyses will be conducted under the oversight of the biostatistician at the Coordinating Center (CC) at Johns Hopkins. All primary analyses will be based on the "intention to treat" principle. Every effort will be made to collect data at the protocol-defined measurement time points, even for participants who have discontinued the study for administrative reasons or due to adverse events. We will include all participants randomized in all analyses whenever possible. When data are not available, we will consider multiple imputation methods; however, in all such cases, these results will be contrasted to those employing participants with complete data. We will also compare participants who are lost to follow-up with those who complete the study on baseline characteristics and treatment assignment to assess characteristics of those who do not complete the study. Unless otherwise specified, tests are two-sided, with significance defined at the $p = 0.05$ level.

Initial analyses will be descriptive in nature, using means, standard deviations, and proportions to describe baseline characteristics of the sample for all participants combined, and by intervention group. Distributions of continuous variables will be examined for symmetry, and transformations will be considered for seriously skewed variables. For continuous variables, t-tests will be used to compare the intervention groups on demographic and other baseline characteristics; the purpose is to assess comparability among the randomly assigned groups. For dichotomous or other categorical variables, chi-square tests will be used to compare intervention groups, and enrolled/excluded groups. Non-parametric methods of comparison will be used for non-normally distributed variables (e.g., rank tests) or data will be transformed to achieve distributions closer to normal. Pearson correlation coefficients (or Spearman rank correlation) will be calculated to assess the strength of the associations among the various outcome measures, and to examine associations of other covariates with outcome measures; such potential confounding variables will be considered for inclusion in secondary analyses involving regression models. Further analyses will utilize general linear modeling approaches to adjust for covariates (especially any on which groups differ at baseline) for continuous variables, and logistic regression analysis for dichotomous variables controlling for within participant dependence. Comparisons also will be made, among treatment groups, of frequency and severity of adverse events, using ANOVA, logistic regression and Chi-square methods. Assuming that these events will be relatively rare, complex analyses will not be appropriate.

For the blood-based biomarker substudy, expoloratory analysis will investigate if there is an association between any biomarker and either level of apathy or level of response to methylphenidate.

10.2. Analysis for specific aims

Specific Aim 1 hypothesis: Methylphenidate will be superior to placebo for the treatment of clinically significant apathy.

Primary Outcomes: Outcome measure 1: Difference in change from baseline to 6 months in the NPI apathy subscale scores as administered by certified personnel to the study caregiver at baseline and each monthly in-person visit.

Analysis: A crude comparison of the difference in mean change scores from baseline to month 6 will be conducted using a t-test. We will also conduct longitudinal analyses of NPI apathy scores to compare treatment groups over time using a saturated means model (including indicators for each visit and each

visit-by-treatment interaction) adjusting for randomization stratification (clinic) by creating a linear mixed effects model with random intercept for each participant to account for multiple measurements over time. All available visit data for each participant will be incorporated into the model according to assigned treatment group. The primary comparison is difference in mean change from baseline to month 6. A transformation of the outcome will be used, if needed, to meet model assumptions. We will conduct planned subgroup analyses to see if treatment effects differ for 1.) The United States verses Canadian clinical centers; 2.) those meeting proposed diagnostic criteria for apathy at baseline verses those who do not meet criteria; 3.) apathy severity at baseline. Planned sensitivity analyses include comparing rate of change in NPI apathy scores over 6 months using target and actual visit times, using a subset of adherent participants in the original model, and comparing proportion of participants with greater than 30% reduction in NPI apathy scores at 6 months. As required by NIH policy, we will conduct valid subgroup analyses of the of the primary outcomes by gender and race/ethnicity subgroups to determine if there is possible subgroup differences (i.e., treatment interactions) in treatment effects.

Outcome measure 2: Ratio of the odds of being at or better than a given category on the ADCS-CGIC at month 6 as evaluated by a study clinician who records his/her clinical impression of change from baseline on a seven-item scale at each in-person follow-up visit.

Analysis: Proportional odds logistic regression will be used to compare the ADCS-CGIC ratings of change (ranging from “marked worsening” to “marked improvement” on a seven point scale) at month 6 between the treatment groups.

The categorical outcome on the ADCS-CGIC of each participant at month 6, which compares each participant's overall apathy outcome at endpoint to that at the baseline visit on a seven point scale (1=marked improvement, 2=moderate improvement, 3=minimal improvement, 4=no change, 5=minimal worsening, 6=moderate worsening and 7= marked worsening) will be compared by assigned randomization group. It is hypothesized that the proportion of participants with better scores on CGIC scale is higher for those assigned to methylphenidate compared with placebo. To capitalize on the ordered categories, the hypothesis will be evaluated with the proportional odds method. This method assumes that the odds ratios Ry are constant across the y categories, i.e. Ry =R. Although this assumption may not necessarily be exactly correct, this method has the correct type I error under the null hypothesis H0 of no treatment effect because, under H0, the proportional odds assumption is satisfied exactly with an odds ratio of 1 between treatment groups across all outcome categories. Moreover, this method is known to be considerably more powerful than unstructured methods for detecting ordered alternative hypotheses to H0, such as our research hypothesis, even when these alternatives do not exactly satisfy the proportional odds assumption. If the data do not meet the proportional odds assumption, a Wilcoxon rank-sum will be used to compare the CGIC ratings. Planned sensitivity analyses include imputation of missing 6 months CGIC outcomes and comparing the proportion of participants with marked or moderate improvement. We will perform the same subgroup analyses described in the previous section.

Secondary Outcomes:

- Outcome measure 1: Difference in change from baseline to 2 months in the NPI apathy subscale scores as administered by certified personnel to the study caregiver at baseline and each monthly in-person visit.

- Outcome measure 2: Ratio of the odds of being at or better than a given category on the ADCS-CGIC at month 2 as evaluated by a study clinician who records his/her clinical impression of change from baseline on a seven-item scale at each in-person follow-up visit.

These secondary outcomes will be analyzed as described above for the primary outcome.

Specific Aim 2 hypothesis: Methylphenidate will be well tolerated.

Safety Outcomes: Adverse events will be collected at each in-person visit using systematic, close-ended questions for known or expected side effects of methylphenidate and by open-ended questions for unexpected side effects, abnormal results of electrolyte or electrocardiogram results. Due to the possible association of anorexia with methylphenidate treatment, a weight loss of 7% or more from baseline at any time during the six months will be considered an adverse event. [The NPI will be measured at each in-person visit and will be used to measure the severity of agitation and sleep difficulties; if there is evidence that there is a difference in effect of treatment on the agitation or sleep difficulties scores by dementia severity (i.e., treatment by baseline MMSE score interaction), we will report these adverse events by dementia severity.] Serious adverse events, including deaths, will be reported to the CC, study investigators and IRBs, regardless of presumed association with study treatment. The proportion of participants experiencing adverse events and serious adverse events will be compared between treatment groups using logistic regression or Fisher's exact test, controlling for baseline imbalances if necessary. As required by NIH policy, we will conduct valid subgroup analyses of the of the adverse event outcomes by gender and race/ethnicity subgroups to determine if there are possible subgroup differences (i.e., treatment interactions) in treatment effects.

Specific Aim 3 hypothesis: Methylphenidate will be superior to placebo with respect to several cognitive, functional and pharmacoeconomic outcomes.

Secondary Outcomes: DAIR will be measured monthly at in-person visits. ADCS-ADL, Dependence Scale, caregiver distress, and caregiver time and the cognitive battery will be measured at baseline and months 2, 4 and 6. Change in the DAIR, ADCS-ADL, Dependence Scale, and cognitive sphere scores from baseline to month 6 will be calculated using a mixed effects model as described above.

Pharmacoeconomic Analysis: EQ-5D-5L and RUD-lite will be assessed at baseline and months 3 and 6. Costs (direct, indirect and caregiver) will be summed in each treatment arm and compared using nonparametric tests as cost data are typically skewed. We will conduct a cost-utility analysis by comparing summed costs in each treatment arm with a summation of utilities derived from the EQ-5D-5L assessment to generate a cost per QALY analysis. This will provide an important assessment of the cost-effectiveness of the intervention and we anticipate that treating with methylphenidate versus placebo will be economically attractive (i.e., potentially cost-saving if improved apathy is associated with lower overall costs and superior levels of functioning and quality-of-life).

For the blood-based biomarkers substudy, data will be analyzed using both nonparametric and parametric statistics with correction for multiple comparisons. As a conservative approach, no assumptions will be made about the distribution of the data and a Wilcoxon rank-sum test will be used to compare MiRNA, oxidative stress, cytokines, neuronal loss, and lipidomics between responders and non-responders. P-values less than 0.05 after adjustment for multiple comparisons will be considered significant. Analyses will also be performed on measures of MiRNA, oxidative stress, cytokines, neuronal loss, and lipidomics among all participants for an association with apathy. As a conservative approach, no assumptions will be made about the distribution of the data, a Spearman rank correlation will be used and the resulting significance adjusted for multiple comparisons. Subsequently, parametric testing will be performed where appropriate.

10.3. Additional considerations

Outcome Attrition: For study participants with any missing outcomes, multiple imputation (MI) will be considered, and analyses will follow the plans described above and the combination rules for MI. Multiple imputation has been recognized as superior to alternatives for addressing attrition in randomized trials because it combines (I) bias reduction, through its prediction of missing values; (ii) relatively low model influence, through the only imputed values; and (iii) accurate expression of uncertainty , through the multiplicity of imputations. To investigate sensitivity to missing values, study participants with and without missing values will be compared by background covariates, and any observed differences will be adjusted for in the analyses.

Treatment non-adherence: Every effort will be made to minimize non-adherence in the trial. With non-adherence, intention-to-treat analysis is unbiased for the effect of randomization, but is not unbiased for the effect of taking the treatment. In addition to the primary analyses, the effect of treatment may be evaluated with an "as treated" analysis, which compares study participants by the received treatment. This is not appropriate if participants who take treatment have different unmeasured prognostic factors independent of treatment, after adjusting prognostic factors.

11. Data monitoring

The Data and Safety Monitoring Committee (DSMC) will act in an advisory capacity to the Steering Committee and the National Institute on Aging (NIA) to monitor participant safety and evaluate the efficacy of methylphenidate. The DSMC will have at least 3 and no more than 5 members who have a background in geriatrics and psychopharmacological research related to Alzheimer's disease, including at least one clinician and at least one biostatistician. The Executive Committee, with approval of the NIA, will select the members.

Monitoring reports of the accumulating data presented to the DSMC by the Coordinating Center (CC) will include treatment group comparisons of baseline characteristics, measures of apathy and cognition, and adverse events as well as clinic performance. DSMC members will not be masked to treatment assignment. The committee may recommend changes to the protocol for performance or safety concerns or early termination of the trial if they observe convincing evidence of a treatment difference in outcomes or safety issues. The DSMC will report to the Steering Committee chair, who will communicate with the rest of the team for reporting to local Institutional Review Boards. The DSMC will function as an independent entity and will be supported financially by the budget of the CC. The CC will develop a draft DSMC charter and the DSMC will refine its policies and procedures in accordance with federal regulations.

The Coordinating Center will also track the collection, shipment, and receipt of blood-based biomarker samples collected in the study. See Chapter 14 for more information.

12. Quality assurance and performance monitoring

12.1. Overview

Quality assurance strategies for ADMET 2 include design strategies and monitoring activities. Design strategies include use of randomization to assign participants to treatment groups, masking data collectors to treatment assignment to the extent possible, requirement of certification of staff and centers, and formal training of staff in study procedures. Activities to monitor quality include performance monitoring, visits to clinical centers, and error detection procedures.

12.2. Certification of centers

Study investigators will be required to complete a clinical center certification form that provides detailed information with regard to the space, facilities, and personnel at the center. One purpose of the form is to serve as a checklist for staff of the resources that need to be in place when participant activities begin. Additional items requested will be a copy of the Institutional Review Board (IRB) notice of approval and copies of the stamped consent statements to be used at the center. The information provided will be reviewed by CC staff prior to certification of a clinical center for data collection.

12.3. Training of staff

Study personnel will train the physicians, coordinators, nurses, assessment raters, and data system operators in the standardized and uniform use of all assessment instruments and/or procedures prior to the randomization of participants in the trial. Training methods will include didactic instruction and clinical demonstrations. Clinicians who administer the psychosocial intervention will be trained in its application and will be provided with training materials. This training will occur at the start of the study and will be reviewed periodically. As appropriate, standardized methods for performing study procedures will be outlined in the handbook.

12.4. Certification of staff

The purpose of the staff certification program is threefold. It identifies to the CC, the study group, and to the staff who will collect and/or record certain items of data for ADMET 2 and who will make decisions relating to eligibility. Second, it makes the data collector aware that he/she is a part of ADMET 2 and has a responsible and identifiable role in the trial. Third, it helps to document a minimum level of competency to perform the functions of the staff person's role.

Personnel to be certified for ADMET 2 include study physicians/clinicians, study coordinators, cognitive assessment raters, psychosocial intervention administrators, and data system operators. Staff will be reminded of their duties and responsibilities to the participants, each other, and the public in adhering to high ethical standards in their interactions with participants and each other, in protecting the privacy of trial participants and the confidentiality of their records, in collecting accurate and reliable data, and in adhering to principles for the analysis and reporting of the data. They will be reminded of their duty to make known to proper authorities any suspicious or wrongful actions in relation to these processes. Each member of the research group will be asked to sign a statement indicating a knowledge and understanding of the above and to voluntarily disclose any potential conflicts of interest. The next best set of assurances lies in the use of design strategies that protect the results from treatment-related biases. Strategies proposed for this trial include random assignment to active treatment or placebo and masked data

collection and outcome assessment. Beyond these strategies, assurances depend on the documents, methods, and procedures used for data collection and monitoring.

12.5. Performance monitoring

Performance monitoring will begin with the initiation of participant screening and will continue throughout the duration of the trial. Centers will be monitored on a regular basis regarding the following:

- Rate of enrollment
- Protocol deviations
- Missed visits
- Losses to follow-up
- Completeness of data
- Percentage of data items requiring edit queries
- Percentage of discrepancies found in audited data items
- Timing of visits

Summaries of the above measures will be provided to the centers and to the DSMC on a regular basis. Review of performance data will be an agenda item for the annual investigators meetings.

12.6. Site visiting

Site visits will be made to each of the clinical centers early in the course of recruitment and at other points in time as needed or desired for quality assurance purposes. The site visitors will review consent forms for enrolled participants and caregivers, study documents, IRB approvals, staffing, adverse event reports, protocol issues, forms management, data management, and study drug accounting records.

12.7. Error detection

The study will employ double data entry and range checks to reduce the occurrence of errors. In addition, some logic checks will be done at the CC, and these checks may be updated throughout the trial to address new data problems as they are discovered. Edit queries will be made to the clinical centers on a regular basis regarding inconsistencies. Periodically, additional batch edits related to consistency of data across forms and over time will be generated.

Periodic audits of subsets of the database will be conducted, both through visits to the centers and through a remote auditing procedure. At on-site visits, participant data will be chosen for verification from source documentation. For the remote auditing procedure, the CC will periodically review participant form sets.

13. Protection of human subjects

13.1. Human subjects

Participants will be adults, who meet clinical and research criteria for dementia of the Alzheimer's type, and their caregivers. There will be no restrictions to participation based on gender, race, ethnicity, or age. We expect to enroll participants who are representative of the ethnic and racial diversity of the geographic and populations of the participating institutions. Eligibility criteria are intended to be as inclusive as possible in order to obtain a clinical sample typical of the participants likely to receive study treatments in the usual clinical situation.

13.2. Monitoring of Institutional Review Board (IRB) approvals

One of the requirements for certification of a center to begin participant activities will be submission to the CC of the center’s notice of IRB approval and a copy of each stamped consent form used at the center. These materials will be reviewed by CC staff for inclusion of appropriate elements.

Centers that have obtained IRB approval for a previous version of the protocol will inform their IRB of changes to the protocol. Protocol amendments and changes to the consent form will be distributed from the CC via numbered memos. These amendments and changes will be submitted by the centers to their IRB in writing.

Reporting local and study-wide adverse events will be done according to each local IRB's policy. A summary of study-wide adverse event data (not by treatment assignment) will be available upon request on an annual basis for submission to local IRBs.

13.3. Consent procedures

Potential participants (or their legal proxies) and caregivers who may potentially fulfill study criteria will be approached by clinic or research staff to assess initial interest. If the potential participant and caregiver are interested, study personnel are to explain the study to them and obtain voluntary written informed consent, which is required for participation in this study. This consent will be obtained from potential participants with Alzheimer's disease (AD) (or authorized surrogates) and from the potential participant's caregiver (if required by local IRB) who will be providing data on themselves as a caregiver. There will also be an additional participant consent form for the blood-based biomarkers substudy for newly or currently enrolled participants.

Even after a participant has provided initial consent to participate, we will obtain assent at each subsequent visit or during implementation of study procedures to assure continuing informed consent on the part of the proxy, to maintain assent by the participants, and to assess capacity. Potential participants and their authorized legal representatives will be included and consented at the study centers using local procedures established by the individual centers and their overseeing IRBs in accordance with local law.

In all cases, prospective participants with dementia will first be assessed for their ability to provide informed consent. Capacity to give consent will be assessed in clinical interviews of potential participants by investigators experienced in clinical dementia research who will also be trained in obtaining consent for the study. In the course of these interviews, these investigators will assess the ability of potential participants to:

- Comprehend the study and its consent form, by asking them to repeat the key elements of the research
- Understand the study and its consent form, by answering questions about the key elements of the research
- Appreciate the personal nature and consequences of what will or could happen to them should they agree to participate

Interviews will take place in the presence of a person who may act as a surrogate research decision maker, if needed. The designation of a surrogate will be governed by local state and IRB rules. In general, this will be, in order of priority: a legal guardian, someone who holds a research advance directive for the participant, a healthcare agent by advance directive, or a healthcare surrogate decision maker by local law or custom, such as a spouse, adult child, or sibling.

If in this process, a potential participant is found not capable of fully providing consent for participation, then the surrogate will provide consent in their place and the participant will be asked to provide assent. Assent is defined according to the Alzheimer's Association (2004) or local IRB guidelines. The process of obtaining consent and assent will be documented in every case. If potential participants are able to provide informed consent, they will be asked to do so and their surrogates will co-sign the consent form as a witness. Caregivers will only be individuals who can provide informed consent for themselves and will be asked to provide informed consent for participation as informants and also to provide data on themselves as caregivers in the course of the study. Caregiver informed consent is required unless otherwise stated by the center's local IRB.

13.4. Potential risks and benefits

Potential risks

The major risks of this study involve the potential adverse effects of methylphenidate and the risk of being assigned to placebo, thus prolonging discomfort and suffering. However, it is not currently knowable in advance whether methylphenidate is efficacious for a particular participant. Indeed, this is the main purpose of the trial. There is a risk that an individual may be initially assigned to placebo and may not improve, or his or her apathy may worsen as a result. However, these risks will be mitigated by the use of psychosocial intervention and the close monitoring of participants. The risk of being in the study with respect to medications is comparable to receiving medication in ordinary clinical settings. The optimal duration of treatment with an anti-apathy medication is not known. In this trial it is possible that some participants may be treated with medication for up to six months. It may be later discovered, as a result of the analysis of this trial and others, that this length of time is too long for some participants, and may have resulted in some unanticipated or unnecessary side effects. Nevertheless, the overall evaluation, clinical care, monitoring, and medication administration are likely to be more intensive and careful than usual clinical care, and to this extent participation in this trial may be associated with less risk.

The dopamine agonist chosen for this study has been evaluated and approved by the Food and Drug Administration (FDA) for clinical use for the treatment of Attention Deficit Hyperactivity Disorder (ADHD) in children and young adults, and is generally well tolerated. Although there is no currently available indication for the treatment of apathy in Alzheimer's dementia, there have been a number of case reports of methylphenidate for the treatment of apathy among adults and elders with major depression (Padala 2005; Masand 1998); Parkinson's disease (Chatterjee 2002) and stroke (Storga 1996). One case report in AD (Kittur 1999) and an open label trial of methylphenidate in AD and vascular

dementia (Galynker 1997) also showed that methylphenidate is effective for the treatment of apathy in dementia.

Despite these findings in 2006, the FDA has issued a warning about sudden death, aggression, and psychotic symptoms in participants with cardiac abnormalities who are taking methylphenidate for the treatment of Attention Deficit Disorder (FDA 2006). While the warning was not aimed at elderly participants, the proposed study will address this issue by closely evaluating cardiac function using electrocardiograms. In addition, participants with psychosis and aggression requiring treatment will be excluded from the study. The FDA also issued a warning about priapism in 2013. The clinic will inform male participants of the possible relationship between priapism and methylphenidate use and will advise them to seek medical care immediately if this condition occurs.

In 2015, the FDA also released a warning about rhabdomyolysis, the breakdown of muscle tissue that leads to the release of muscle fiber contents into the blood. These substances can be harmful to the kidney and often cause kidney damage. The participant will be advised to monitor for general weakness, muscle stiffness or aching (myalgia), muscle tenderness, and changes in urine color (i.e., dark, red, or cola-colored) or output (i.e., decreased) and to report these symptoms to the clinic and seek medical attention as soon as possible.

In 2017, the FDA also released a warning about serotonin syndrome, which could result with the combined use of certain anti-depressant medications with methylphenidate. This rare side effect could result in the production of too much serotonin, causing mild symptoms such as confusion, restlessness, headache, nausea, or diarrhea. More severe symptoms could include high fever, or seizures, and lead to loss of consciousness or even death.

For those participating in the blood-based biomarkers substudy, there is a small risk from providing a blood sample. Taking blood may cause discomfort, bleeding or bruising where the needle enters the body. In rare cases, it may cause fainting. There is a small risk of infection; these are outlined on the consent form.

Potential benefits

Participants will receive treatment for their apathy and will be closely monitored throughout their participation in the study. The participant and caregiver will receive a psychosocial intervention. It will consist of three parts: an educational component, a counseling component, and availability of clinical staff to help caregivers, provided by trained licensed clinicians (nurses, social workers, doctors). All participants and their caregivers will receive education regarding AD, its clinical course, symptomatic behaviors, behavioral management of apathy, and expectations for medication treatments. At the end of the study, an appropriate clinical referral will be made for continued management of dementia and apathy. Failure to achieve meaningful clinical improvement will result in changes in treatment designed to maximize the likelihood of such improvement. The benefits to society of this study will include important new data on the treatment of apathy occurring with dementia.

Risk/benefit ratio

Risks to the participants from the study medication will be similar to those encountered by other elderly participants with dementia who receive treatment with these drugs. Risks to the participants from lack of treatment efficacy will be minimized by the several regular assessments and by the investigators' abilities to discontinue or change medicines or to treat participants openly if they are not benefitting from their current protocol-specified treatment. The benefits that participants will receive in the form of free and

systematic treatment, in addition to the societal benefits of important treatment data concerning a disorder with substantial morbidity and mortality, outweigh the risks to participants. The projected outcomes following participation in this trial are similar to what the participants may expect in the absence of participation, except that treatment is likely to be monitored more carefully, and optimized. Therefore, the anticipated net benefits from participation are likely to be at least as great as those to be expected in the absence of participation.

13.5. Safety monitoring

Study personnel will have frequent contact with participants both by phone and in-person. Participants will be monitored regularly for signs or symptoms of adverse effects. In addition, the DSMC will regularly review and evaluate accumulating safety data and may recommend termination of the trial if the risks become unacceptable.

Methylphenidate has a short acting nature and, if unexpected adverse events emerge, the investigator will simply reduce or discontinue the use of the study medication. If the unexpected adverse events disappear, the participant will re-start the study medication under close clinical supervision, including inpatient care if necessary. If the symptoms continue, it will be concluded that it is unlikely that the symptoms are related to the study medication. In that case, the participant will be treated as deemed clinically appropriate by the treating physician. The continuation or discontinuation of the drug will be decided by the center investigator in conjunction with the treating clinician based on the risk benefit consideration for each individual participant. For this reason, there will be no "rescue medication" used in this study.

The risks and benefits of methylphenidate, of specific-study procedures, and of the study as a whole will be explained in detail to participants, caregivers, and responsible parties. After a medical and psychiatric history, participants will undergo physical, neurological, and psychiatric examinations to assure the clinical appropriateness and safety of their participation. Close clinical monitoring will ensure the appropriateness and safety of their continued participation. Close monitoring will include several components: a) careful education of caregivers to monitor participants at home, including provision of written materials on how to contact the team and what side effects to look for (in lay language); b) in-person visits or telephone contacts; c) contact with an experienced clinician, at minimum; d) in-clinic visits with a prescribing physician; and e) availability of the study team by 24-hour beeper for assistance with crises and urgent or emergent matters and for personal support.

Center investigators will be responsible for appropriate medical care of participants during the study, in connection with study procedures, and for monitoring the safety of participants. The medical monitor will provide medical monitoring to all the centers. He/she or his/her physician designee, will carry a beeper 24-hours a day for protocol questions and problematic or serious adverse events that center investigators believe should be referred to him. The medical monitor and the Coordinating Center will be notified using a special serious adverse event (SAE) report form within one working day by the investigative center via email and telephone and if any of the following events occur: (1) death, (2) hospitalization, (3) life-threatening events, (4) unexpected event. Monitoring personnel will provide timely evaluation and turnaround of the initial SAE report to meet specifications and regulatory reporting requirements. Coordinating Center personnel will review SAE data and accompanying documents, and query centers for clarification or additional information. They will also consult with the center investigator to determine the event, any treatment provided, outcome, and causality, and to obtain necessary supporting documentation. A report with an SAE narrative will be generated and, following principal investigator review, sent to appropriate outside bodies, such as the sponsor. SAE data will be entered into a database, along with additional follow-up information and clarification obtained from the centers as they become available.

Specifically, safety assessments include the monitoring of:

- Change in vital signs from baseline: Clinically significant changes will be reported as adverse events.
- Change in weight from baseline: weight loss greater than or equal to 7% of the participants' baseline weight will be reported as an adverse event.
- Clinically significant change in Mini-Mental State Exam (MMSE) score from baseline: The MMSE has been used as a measure of toxicity in a number of studies evaluating treatments for neuropsychiatric symptoms.
- Change in Neuropsychiatric Inventory (NPI) scores from baseline: The NPI will be used to determine if methylphenidate causes an increase in neuropsychiatric symptoms other than apathy (such as hallucinations and delusions).
- Change in electrolytes from baseline: Clinically significant change in sodium, potassium, chloride, bicarbonate, glucose, blood urea nitrogen, or creatinine.
- Adverse events: Adverse events and serious adverse events will be defined using standard approaches as was the case with Clinical Antipsychotic Trials of Intervention Effectiveness and Depression of Alzheimer's Disease Study-2 (Martin 2006).
- Change in electrocardiogram (ECG) from baseline: One of the potential adverse events of methylphenidate is an increase in heart rate. This increase could trigger conduction problems. Therefore, an ECG must be performed no more than three weeks prior to randomization to allow the investigator to evaluate the clinical significance of potential findings. ECG assessments will be conducted each month to evaluate the potential effects of methylphenidate on heart conduction. If clinical manifestations of a cardiac condition are detected during the study, appropriate action will be taken at the investigator's discretion.
- Compliance check: Between visits the first three months, there will be 10-minute telephone contacts to obtain information such as medication adherence and potential adverse events.

13.6. Confidentiality of participant and caregiver data

Clinical centers will keep all participant and caregiver data and blood samples in a secure location. Passwords to the website or to access database functions will not be shared with other staff. Names, addresses, and other such personal data will not be part of the central database. Data and blood samples collected from study evaluations and interviews will be identified only by study ID codes, which will be the participant ID and 4-letter code assigned at eligibility evaluation. Caregivers also will be identified by an ID code. Any disclosure of potentially identifiable health information will be done in accordance with the law.

13.7. Data sharing

The Coordinating Center will facilitate data sharing in accordance to NIH Data Sharing Policy. Study data will be provided to all ADMET-2 investigators after data collection is complete. In addition, a limited use dataset and documentation will be prepared for use by qualified investigators outside of the ADMET-2 research group. Data supplied to outside parties will have personal health identifiers removed. Study participants will be informed about data sharing in the consent forms.

14. Blood-based biomarkers substudy

14.1. Title

Biomarkers of apathy and treatment response

14.2. Objectives

The objectives are to understand biomarker correlates of change in apathy over time, and to identify possible predictors of treatment response.

14.3. Background and specific hypotheses

Apathy, one of the most common neuropsychiatric symptoms (NPS) in Alzheimer's Disease (AD), has a documented negative impact on patients and caregivers including being associated with increased mortality (Spalletta 2015; Nijsten 2017). There are currently no approved treatments for this symptom. As previously mentioned, one promising potential treatment is methylphenidate, which, in a Phase II trial (ADMET), showed significant improvement in symptoms of apathy compared to placebo (Rosenberg 2013). As outlined earlier in the protocol, the primary aim of the ADMET 2 study is to determine whether methylphenidate is effective in improving clinically significant apathy in patients with AD in a larger sample, over a longer treatment period.

In the original ADMET trial, a distinct group of approximately one third of the sample had a dramatic response to treatment, but one third showed either minimal improvement or no change, and the final third had no benefit from treatment. Importantly, no significant clinical differences were found between those who responded and those who did not, suggesting that the variability in treatment response may have a biological basis. Personalized interventions, based on biomarkers or genetic information, are a key aspect of neuropsychiatric drug development. It is therefore critical to determine biomarkers that will help distinguish responders from non-responders. This substudy to ADMET 2 will investigate the following specific blood-based biomarkers.

1. MicroRNA (miRNA)
2. Markers of oxidative stress
3. Markers of inflammation (cytokines)
4. Markers of neuronal loss (S100B, NF-L)
5. Lipidomics

Background and specific hypotheses are as follows:

miRNA. MicroRNAs (miRNAs) are endogenous, small (approximately 22 nucleotide) non-coding RNAs found in blood. However, several species of miRNA have been linked to dopamine transporter (DAT) expression and function and catecholamine metabolism, making them of special interest to the ADMET 2 study. Many studies have shown that miRNA species may affect transporters or nuclear receptors and drug response, as summarized by Koturbash et al (2015). In particular, miRNA expression has been shown to influence DAT expression and subsequent dopaminergic function. Working in a human neuroblastoma cell culture model, Jia et al (2016) showed that miRNA-137 and miRNA-491 caused a marked reduction in the expression of DAT, altering neuronal dopamine transport. There was evidence that these miRNA species affected DAT expression at the post-transcriptional level. The effect of non-coding RNA species on dopaminergic signaling in the CNS has been summarized by Carick et al (2016),

miRNAs-30b-5p, 1301, 1972, 6070 have been associated with miRNA binding sites located in polymorphic region of the DAT associated with attention deficit hyperactivity disorders, and miRNA binding sites located in polymorphic region of DAT associated with bipolar disorders include miRNAs-762, 1266, 3127, 3192, and 4259.

miRNA hypotheses:

- Blood levels of miRNA-137, 491,762, 1266, 3127, 3192, and 4259 will be associated with greater response to methylphenidate over placebo. miRNA-137, 491,762, 1266, 3127, 3192, and 4259 are involved in dopamine or dopamine agonist signaling.
- Blood levels of miRNA 208 will be associated with differential response to methylphenidate over placebo. Catecholamines increase miRNA 208 expression and will likely affect dopamine signaling.

Oxidative stress markers: lipid peroxidase, malondialdehyde, 4-hydroxynonenal, and 8-isoprostane. Oxidative stress is an emerging mechanism related to both mood disorders and AD (Marcus 1998; Montine 1998; Vitek 1994; Ozcan 2004; Andreazza 2008). Importantly, oxidative stress and neuroinflammation, including activation of nicotinamide adenine dinucleotide phosphate (NADPH)-dependent oxidases, play a major role in the progression of dopaminergic cell death (Labandeira-Garcia 2012). The hypothesis that oxidative stress contributes to neurodegeneration in AD has been supported in studies of oxidative stress markers in serum lipid fractions and in cerebrospinal fluid (CSF), and by pathology series (Markesbery 1999; Schrag 2013; Zhao 2013). Evidence supports a role for oxidative stress in AD etiology and pathophysiology (Wang 2005; Sasaki 1998; Marcus 1998; Mecocci 1994; Sayre 1997). Elevated oxidative DNA damage has been observed in mitochondrial and nuclear DNA in the temporal, frontal, and parietal lobes of post-mortem AD brain tissue (Wang 2005; Sayre 1997). Furthermore, increased levels of two biochemical markers for oxidative stress (malondialdehyde (MDA) and thiobarbituric acid reactive substances (TBARS)) concentrations were observed in the temporal lobe of AD brain tissue (Marcus 1998; Palmer 1994) and TBARS were also observed to be elevated in the frontal lobe and cerebellar cortex of AD brain tissue (Marcus 1998). Elevated oxidative stress products have also been observed in neurofibrillary tangles (Sayre 1997; Montine 1998) and senile plaques (Sasaki 1998; Vitek 1994), two pathological hallmarks of AD. In addition, antioxidant enzyme activity, as measured by superoxide dismutase and catalase activity, was observed to be significantly reduced in post-mortem AD brain tissue (Marcus 1998), suggesting reduced antioxidant capacity in these brains. Consistent with the emergence of apathy in late life and the strong evidence that apathy predicts AD, oxidative stress and subsequent cellular damage are thought to contribute to biological aging and late-life disorders (Kregel 2007; Cadenas 2000; Reitz 2011).

In support of a possible relationship with apathy, there is also evidence for a role of oxidative stress in mood disorders from observational studies (Ozcan 2004; Sarandol 2007; Bilici 2001; Atmaca 2004; Yanik 2004; Andreazza 2008). MDA, a product of lipid peroxidation of polyunsaturated fatty acids and a marker of oxidative stress, has been observed to be elevated in affective disorders (Ozcan 2004), major depressive disorder (MDD) (Sarandol 2007; Bilici 2001; Khanzode 2003), and social anxiety (Atmaca 2004). Additional lipid peroxidation products such as 4-hydroxynonenal (4-HNE) and isoprostanes have also been observed to be elevated in MDD (Dimopoulos 2008; Selley 2004; Mazereeuw 2015). In bipolar disorder, peripheral concentrations of TBARS, another marker of lipid peroxidation, were found to be elevated (Andreazza 2008). Furthermore, antioxidant capacity might be diminished in mood disorders (Gawryluk 2011; Maes 2000; Cumurcu 2009; Andreazza 2009). This may directly result in neurodegenerative damage, as neurons are particularly susceptible to oxidative stress due to their high oxygen demand and high concentration of polyunsaturated fatty acids (Christen 2000), or reduced

antioxidant capacity might be a by-product of enhanced oxidative stress (Ozcan 2004). Indeed, ratios of peroxide products to antioxidant potential predicted depression severity (Yanik 2004).

Oxidative stress hypotheses:

- Higher levels of lipid peroxidation markers will be associated with greater treatment response (decrease in apathy) in subjects receiving methylphenidate versus placebo.
- Decreased lipid peroxidation markers with treatment will be associated with decreased apathy.

Inflammation (cytokines). Cytokines, chemical messengers between immune cells, have been observed to have direct actions on brain cells (Rothwell 2000; Kuno 2006), and serve as mediators between the immune and nervous systems (Kronfol 2000). Moreover, a bidirectional relationship exists where cells in the nervous system can have direct effect on immune cells via molecules such as neurotransmitters (Wrona 2006; Maier 1998). Cytokines can have pro-inflammatory or anti-inflammatory effects depending on the cell type and specific receptors (Rubio-Perez 2012; Scheller 2011). There is a substantial amount of evidence linking an imbalance in pro-inflammatory and anti-inflammatory cytokines to various psychological disease states including depression (Kohler 2017), bipolar disorder (Kim 2007), psychological stress, and sickness behavior (Anisman 2003). In major depressive disorder patients, meta-analyses have shown that pro-inflammatory cytokines tumor necrosis factor alpha (TNF-α), interleukin (IL)-1 (IL-1), and IL-6 are commonly elevated (Dowlati 2010; Howren 2009), leading to the hypothesis that elevated pro-inflammatory cytokines, acting as neuromodulators, are a cause of the behavioral and neurochemical features of these disorders (Schiepers 2005). Sickness behavior, characterized by a series of symptoms that include lethargy, amotivation, anhedonia, and sleep and appetite disturbances, has also been associated with elevated cytokines (Kronfol 2000). Importantly, reduced motivation is common in sickness behavior and may be responsible for reorganizing the organism's priorities to facilitate recovery from infection and inflammation (Dantzer 2007; Bluthe 1994).

Inflammatory and immune mechanisms involving cytokine production have been frequently reported to play a role in AD pathogenesis (Rubio-Perez 2012). Studies have shown that concentrations of IL-1 and IL-6 are elevated in both the CSF and serum of AD patients (Blum-Degen 1995; Swardfager 2010). In addition, meta-analytic evidence suggests that TNF-α, IL-12, and IL-18 are also elevated in the peripheral blood of individuals with AD (Swardfager 2010). Furthermore, amyloid-beta (Aβ) protein, a protein intimately linked to AD pathogenesis, has been shown to induce microglial expression of IL-1 *in vitro*, further suggesting a role of cytokines in the progression of AD (Araujo 1992). Moreover, both IL-1 and IL-6 have been implicated in the transition of diffuse plaques to neuritic plaques in the brain of AD patients (Hull 1996; Sheng 1997). Although cytokine dysregulation appears evident during different phases of progression of AD, it is unclear whether this is a consequence of the disease, a contributor to the disease, or both.

Cytokines hypotheses:

- Higher levels of inflammatory markers will be associated with poorer response to methylphenidate.
- Decreased inflammatory markers with treatment will be associated with decreased apathy.

Neuronal loss markers (S 100 calcium-binding protein B (S100B), neurofilament light chain protein (NF-L)). Patients with AD and significant apathy show increased neuropathological changes, including increased neuronal loss (Forstl 1993). Two common biomarkers of neuronal loss are S100B and NF-L. S100B is one of the most commonly used blood biomarkers for brain injury (Shahim 2016). Serum levels of S100B have been shown to have a positive correlation with disease severity in patients with AD

(Chaves 2010). S100B has also been shown to be increased in patients with depression and bipolar disorder (Rajewski-Rager 2016). NF-L has been suggested as a putative marker of progression in neurodegenerative diseases (Bacioglu 2016). NF-L, along with the neurofilament medium and heavy proteins, acts as scaffolding of the neural cytoskeleton, with important roles in axonal and dendritic branching and growth (Petzold 2005). Patients with AD have been shown to have a higher serum level of NF-L compared to control elderly patients without evidence of central nervous system damage (Gaiottino 2013). In particular, patients with AD and Aβ pathologic features showed significantly higher levels of plasma NF-L (Mattsson 2017). Higher CSF levels of NF-L have also been found in patients with bipolar disorder (Jakobsson 2014).

While no studies have looked at the relationship between S100B or NF-L and apathy in patients with AD, these markers may provide greater insight into the neurotoxic mechanisms behind apathy in AD.

Neuronal loss hypothesis:

- Increased serum levels of S100B and NF-L (reflecting more brain injury) will be associated with poorer response of apathy to methylphenidate treatment in AD.

Lipidomics. There is an increasing amount of published evidence that suggests brain lipid composition is altered in multiple dementias, and that these disease-associated modifications reflect underlying disease mechanisms. The brain is especially enriched in sphingolipids and membrane lipid composition regulates a variety of biologic processes including synaptic plasticity (Inokuchi 2009; Wheeler 2009; Yang 2000; Zhang 2002), neuronal survival (Arboleda 2009; Schneider 2006; Posse de Chavez 2006; Levonson 2004), glial activation (Jung 2013; Antonucci 2012; Jana 2007; Bassi 2006), and the activity of amyloid processing enzymes such as α-, and β-secretase that regulate the physiological (α-secretase), and pathological (β-secretase) production of amyloid peptides (Li 2010; Puglielli 2003; Tamboli 2005). Animal models of disease and studies with human brain tissues and matched CSF/plasma have shown that circulating lipids appear to reflect changes in brain lipid composition (Cutler 2004a; Cutler 2004b; Haughey 2004), possibly through the release of lipid-rich exosomes from brain that enter into peripheral circulation (Dickens 2017). Characteristic changes in CSF lipid compositions have been associated with increased risk of incident AD (Mielke 2016; Mielke 2012), clinical progression (Mielke 2011), and have been correlated with Aβ and tau levels (Mielke 2014), and with white matter integrity (Gonzalez 2016; Proitsi 2015).

We have a prime opportunity to explore blood-based lipid biomarkers as a surrogate measure for apathy in AD, and their association with the biological response to methylphenidate. As lipidomic methodology becomes more sophisticated and reproducible, we are starting to see replicable findings. For example, a preliminary report of six novel lipid markers reflecting disease state in AD was replicated along with the identification of new candidate lipids (Proitsi 2017). Lipid abnormalities have been shown to be associated with cognitive impairment in human immunodeficiency virus (HIV), AD, Multiple Sclerosis, Parkinson's, and coronary artery disease (Cutler 2004; Haughey 2004; Mielke 2014; Bandaru 2007; Bandaru 2013; Bandaru 2009; Haughey 2008; Kooij 2012; Lu 2001; Mielke 2015; Mielke 2010a; Mielke 2010b; Mielke 2013; Saleem 2013; Vidaurre 2014; Wheeler 2008). Furthermore, preliminary data suggests that severity of apathy is associated with alteration in plasma levels of specific ceramides. In a sample of 130 patients at risk for vascular cognitive impairment, 3 sphingolipids were associated with score on the apathy evaluation scale (**AES**): C22:1 (r=0.243, p=0.009), MHxC16:1 (r=0.219, p=0.018), and MHxC22:1 (r=0.207, p=0.026). In addition, baseline C22:1 concentration was associated with change in AES over 6 months (r=-0.218, p=0.031).

Lipidomics hypotheses:

- A lipid biosignature will be associated with apathy response to methylphenidate treatment in AD.
- Decreases in this lipid biosignature identified will be associated with decreased apathy.

14.4. Organization

Five members, a subset of the ADMET 2 Executive Committee (EC) and Steering Committee (SC) members, will form the Biomarkers EC to oversee the substudy. One EC member will oversee the measurement of the five specific peripheral biomarker components described in this chapter.

14.5. Eligibility criteria

Any person who is enrolled in the main study, may enroll in the substudy. All newly enrolled participants in the main study will be invited to participate in the substudy. Current participants already enrolled in the main study will be invited to enroll if they have not yet completed their month 3 visit. The substudy will only be conducted at those ADMET 2 clinics that have agreed to participate in the substudy. Participants will be asked to volunteer for this substudy. Whether or not they volunteer will not affect their ability to participate in the randomized trial.

14.6. Study design

On newly enrolled participants to the main study, blood will be collected on consenting substudy participants at baseline for all blood-based biomarkers (although, miRNA may be collected at any in-person study visit after baseline). Blood will also be collected at the last study visit (F6) for measurement of oxidative stress biomarkers, cytokines, and lipidomics as illustrated in the schedule of collection in Table 1a below.

Table 1a: Schedule of substudy assessments for newly enrolled participants

	Baseline	F6
Months from baseline	0	6
miRNA	X*	
Oxidative stress	X	X
Inflammation (cytokines)	X	X
Neuronal loss	X	
Lipidomics	X	X

* The collection of miRNA may take place at any in-person visit after baseline, if need-be.

On currently enrolled participants to the main study, who have already had their baseline visit, blood will be collected on consenting substudy participants at the month 1, month 2, or month 3 visits for all blood-based biomarkers (although, miRNA may be collected at any in-person study visit). Blood will also be collected at the last study visit (F6) for measurement of oxidative stress biomarkers, cytokines, and lipidomics as illustrated in the schedule of collection in Table 1b below.

Table 1b: Schedule of substudy assessments for currently enrolled participants who have had their baseline visit but have not yet completed their month 3 visit

	F1, F2, or F3	F6
Months from baseline	1, 2, or 3	6
miRNA	X*	
Oxidative stress	X	X
Inflammation (cytokines)	X	X
Neuronal loss	X	
Lipidomics	X	X

* The collection of miRNA may take place at any in-person visit after baseline, if need-be.

Each biomarker requires a specific collection and storage method as outlined in the ADMET 2 study handbook. All specimen are frozen on dry ice and shipped immediately or monthly to the designated laboratory for analysis.

14.7. Materials and shipments

The ADMET 2 study handbook contains information on materials needed for blood collection as well as the Standard Operating Procedures (SOPs) to measure biomarkers and to obtain and interpret images. The handbook also contains detailed information on labeling and shipping of biospecimens.

The CC will follow the normal quality control procedures including the tracking and monitoring of the collection of specimens. The CC is responsible for preparing and distributing kits containing materials needed by clinical centers to complete and store blood collection. The CC also tracks the acquisition and shipment of relevant biospecimens from clinical centers to the sites completing biochemical analyses.

14.8. Biochemical analyses

Detailed methods to be used for biochemical analyses will be developed by the relevant laboratory and documented as an SOP. A summary of the approach for each biomarker is given here.

Micro RNA. *Methods for RNA extraction and miRNA quantitative reverse transcription polymerase chain reaction* (qRT-PCR). RNA extraction will be based on published methods by Bekris et al (Bekris 2013). Then DNA will be depleted using Qiagen deoxyribonucleic acid (DNA) elimination columns (Qiagen, Valencia, CA) and TURBO DNA-free™ Kit (Applied Biosystems, Austin, TX).

Oxidative stress. *Methods for measuring oxidative stress.* Early-stage oxidative damage to lipids will be evaluated by measuring the levels of lipid hydroperoxide (**LPH**, Cayman Chemical; Item No. 705002) per manufacturer's instructions.

Late-stage oxidative damage to lipids will be evaluated by measuring MDA, 4-HNE, and 8-isoprostane (**8-ISO**, Cayman Chemical; Item No. 516351), which will be quantified using standard competitive sandwich enzyme-linked immunosorbent assays (**ELISA**s) for each marker.

Inflammation (cytokines). Serum will be assessed for cytokine levels, including TNF-α, IL-1, and IL-6, by multiplex ELISA, using a commercial kit per the manufacturer's instructions (Milliplex, Millipore #HMHMAG-34K - #HCYTOMAG-60K).

Neuronal loss. Serum S100B will be measured using an electrochemiluminescence immunoassay and NF-L will be measured using ELISA.

Lipidomics. Lipid analysis is conducted using Data-Independent Lipid Analysis by MS/MS^{ALL} on a TripleTOF^{TM} (**TOF**) 5600 (AB SCIEX, Redwood City, CA) mass spectrometer. This method will identify ~1000 individual lipid species from 12 different classes of lipid that include: Ceramides, monohexosylceramides, dihexosylceramides, sphingomyelins, phospatidylcholine, phospatidylserine, phospatidylethanolamine, phospatidylglycerol, cholesterol esters, monoakayldiacylglyerides, diacylglycerides, and triacylglycersides.

14.9. Outcomes

The endpoints in this substudy are the levels of each of the tested biomarkers (miRNA, oxidative stress biomarkers, inflammation (cytokines), neuronal loss (S100B, NF-L), and lipidomics). The relevant outcomes will include 1) the differences in mean biomarker level between responders and non-responders to the treatment methylphenidate and 2) the correlation between level of biomarker and level of apathy.

14.10. Justification of sample size

Statistical Considerations. Each of the individual sections below contains a summary of the available evidence and rationale for studying the peripheral based biomarker in the ADMET 2 population. Insofar as hypothesized either to predict treatment response or change with apathy, the size of expected change, or of meaningful change remains unknown. Given that these differences would be required to make an accurate statistical power analysis, these aims should be considered exploratory and would likely need to be validated in further studies beyond ADMET 2.

The discovery of peripheral based blood biomarkers of response of apathy in AD to pharmacotherapy would be a major advance in understanding the neurobiology of this NPS in AD. The current biomarker initiative is justified on this basis despite the lack of current data to help guide traditional power analyses.

Power and Sample Size. We expect to enroll at least 60 subjects to measure these blood-based biomarkers. As we expect that for each measure some participant information may be incomplete due to measurement issues or loss to follow-up, we assume here a conservative 10% loss, so that each measure will have approximately 54 participants for analysis. For each, we are interested in comparing those responding to treatment with methylphenidate or placebo to those not responding to treatment. Among 54 participants with complete information, we expect one third to have improvement in apathy (responders), and approximately one third to have worsening apathy over the course of the study (non-responders). With these 36 participants, we determined the detectable difference in Cohen's d (mean difference/standard deviation) given the number of comparisons using a conservative Bonferroni adjustment (Witte 2000). The resulting detectable effect sizes are in Table 2 below. We also estimate the detectable association between each measure and a continuous valued treatment response among all 54 participants with complete data, quantified by a correlation, likewise given the type one error rate adjusted by the number of comparisons using a Bonferroni adjustment (Table , below).

Table 2: Power, sample size, and detectable effect sizes for a type one error (alpha) of 0.05, assuming a test to compare non-responders to responders, when the sample is approximately one third responders and one third non-responders. Bonferroni adjustment is assumed for multiple comparisons.

Measure	Sample size	Number of outcomes/ comparisons	Expected drop out	Effective sample size	Effect size (Cohen's d : mean difference / standard deviation) to compare two groups	Power	Supporting reference*
MiRNA	60	50 (unique miRNA types)	10%	36	1.50	80%	Ou 2016
Oxidative stress	60	4 (1 early stage, 3 late stage)	10%	36	1.20	80%	Andreazza 2008
Cytokines (inflammation)	60	3	10%	36	1.15	80%	Spalletta 2013
S100B (neuronal loss)	60	1	10%	36	0.95	80%	Schroeter 2013
NF-L (neuronal loss)	60	1	10%	36	0.95	80%	Norgren 2003
Lipidomics	60	12 (classes)	10%	36	1.35	80%	Gracia-Garcia 2011

* References shown support that the measure listed in Column 1 of this table is expected to have an effect size that is greater than or equal to the needed effect size as estimated in Column 6.

Table 3: Power, sample size, and detectable effect sizes for a type one error (alpha) of 0.05, assuming a test for the association with a continuous-valued treatment response. A Bonferroni adjustment is assumed for multiple comparisons.

Measure	Sample size	Number of outcomes/ comparisons	Expected drop out	Effective sample size	Effect size (correlation) to detect association	Power
MiRNA	60	50	10%	54	0.540	80%
Oxidative stress	60	4 (1 early stage, 3 late stage)	10%	54	0.450	80%
Cytokines (inflammation)	60	3	10%	54	0.440	80%
S100B (neuronal loss)	60	1	10%	54	0.385	80%
NF-L (neuronal loss)	60	1	10%	54	0.385	80%
Lipidomics	60	12 (classes)	10%	54	0.495	80%

14.11. Data analysis

Data will be analyzed using both nonparametric and parametric statistics with correction for multiple comparisons. As a conservative approach, no assumptions will be made about the distribution of the data and a Wilcoxon rank-sum test will be used to compare MiRNA, oxidative stress, cytokines, neuronal loss, and lipidomics between responders and non-responders. P-values less than 0.05 after adjustment for multiple comparisons (Benjamini 1995) will be considered significant.

Analyses will also be performed on measures of MiRNA, oxidative stress, cytokines, neuronal loss, and lipidomics among all participants for an association with apathy. As a conservative approach, no assumptions will be made about the distribution of the data, a Spearman rank correlation will be used and

the resulting significance adjusted for multiple comparisons. Subsequently, parametric testing will be performed where appropriate.

14.12. Risk to participants and protections against risk

Because obtaining and measuring biomarkers is an ancillary study to the main ADMET 2 study, a separate informed consent statement will be developed that will allow the study participant and caregiver to participate in the biomarkers substudy. The consent will notify potential participants that there is a small risk from blood draw (feeling slight discomfort or pain, with potential of feeling faint or bruising).

14.13. Data confidentiality

As with the main study, clinics will keep all participant and caregiver data in a secure location. Names, addresses, and other such personal data will not be part of the central database. Samples collected from will be identified only by study ID codes, which will be the participant ID number and 4-letter code assigned at screening. Any disclosure of potentially identifiable health information will be done in accordance with the law.

14.14. Biohazards

Blood will be collected for miRNA, lipidomics, neuronal loss, oxidative stress, and cytokines. All personnel involved in collecting and handling biologic specimens are to follow appropriate precautionary procedures as currently recommended by the CDC. Shipping of specimens are to be done in compliance with federal regulations.

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Appendix: Design summary

Title

- Apathy in Dementia Methylphenidate Trial 2 (ADMET 2)

Objectives

Primary objective

- To examine in a masked, randomized trial the efficacy of methylphenidate for the treatment of clinically significant apathy in participants with Alzheimer's dementia.

Secondary objective

- To examine the safety of methylphenidate for the treatment of clinically significant apathy in participants with Alzheimer's disease,
- To examine changes in other neuropsychological and quality of life assessments,
- To examine economic assessments from baseline to 6 months, and
- To examine cognitive changes from baseline to 6 months.

Blood-based biomarker substudy objective

- To understand biomarker correlates of change in apathy over time, and to identify possible predictors of treatment response

Type of trial

- Phase III
- Randomized, multicenter clinical trial
- Two parallel treatment groups
- Double masked

Design variables

- Neuropsychiatric Inventory Apathy subscale (NPI apathy) and Alzheimer's Disease Cooperative Study - Clinical Global Impression of Change (CGIC) Scale

Outcomes

Primary

- Mean change in NPI apathy from baseline to 6 months
- Odds of having a given CGIC rating or better for apathy at 6 months

Secondary

Apathy

- Mean change in Dementia Apathy Interview and Rating from baseline to 6 months
- Mean change in NPI apathy from baseline to 2 months
- Odds of having a given CGIC rating or better for apathy at 2 months

Safety

- Mean change in Mini-Mental State Exam from baseline to 6 months
- Mean change in vital signs from baseline to 6 months

- Number and proportion with abnormal electrocardiogram during follow-up
- Number and proportion with abnormal electrolytes during follow-up
- Number and proportion with significant unintentional weight loss (> 7%)
- Number and proportion of participants with adverse events at any time
- Other neuropsychological assessments
- Distribution of change in NPI sub-scale scores (excluding apathy) from baseline to 6 months

Economic assessments
- Mean change in EuroQol 5D-5L from baseline to 6 months
- Mean change in Resource Utilization in Dementia-Lite from baseline to 6 months

Cognitive tests
- Action Verbal Fluency Test from the PDCRS
- Category Fluency Task-Animal Naming
- Hopkins Verbal Learning Test – Revised
- Trail Making Test (A and B)
- Digit Span: The Wechsler Adult Intelligence Scale – Revised Digit Span subtest
- Short Boston Naming Test

Study population
- 200 participants who meet the criteria for apathy in Alzheimer's disease
- Participants enrolled in ADMET 2 are invited to join the blood-based biomarker substudy

Sample size and power calculations
- Two-sided alpha = 0.025 for each co-primary outcome
- Power greater than 90% with 10% losses to follow-up
- NPI apathy
  - 1.8 point difference in change in NPI apathy scores from baseline to month 6
  - Estimated standard deviation in NPI apathy of 3.2
- CGIC
  - Proportions assumed in each rating:
    - 3.5% marked improvement
    - 8.8% moderate improvement
    - 29.8% minimal improvement
    - 54.4% no change
    - 3.5% minimal worsening
  - Odds ratio = 2.75

Randomization
- 1:1 assignment ratio
- Stratification by clinic

Data analysis
- Primary analysis by assigned treatment group (intention-to-treat principle)
- Initial descriptive analyses
- Regression methods for effect estimates
- Assessment of baseline variables for interaction or confounding

- For blood-based biomarker substudy, exploratory analysis for association between biomarkers and apathy or methylphenidate response

Treatments

- Methylphenidate, target dose 20 mg per day (range 10-20 mg per day), and psychosocial intervention
- Placebo and psychosocial intervention

Masking

- Treatment assignment masked to participants and all personnel

Duration of follow-up

- 6 months

Data collection schedule

- Scheduled in-person visits (monthly after randomization until month 6)
- Telephone contacts for data collection (day 15, 45, and 75 after randomization)

Inclusion criteria

- Possible or probable Alzheimer's disease (National Institute of Neurological and Communicative Disorders and Stroke - Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) criteria), with Mini-Mental State Exam (MMSE) score of 10-28 inclusive
- Clinically significant apathy for at least four weeks for which either
  - the frequency of apathy as assessed by the Neuropsychiatric Inventory (NPI) is 'Very frequently', or
  - the frequency of apathy as assessed by the NPI is 'Frequently' or 'Often' AND the severity of apathy as assessed by the NPI is 'Moderate' or 'Marked'
- A medication for apathy is appropriate, in the opinion of the study physician
- Provision of informed consent for participation in the study by potential participant or surrogate (with participant assent if the potential participant is unable to provide informed consent) and caregiver
- Availability of caregiver, who spends greater than ten hours a week with the potential participant and supervises his/her care, to accompany the potential participant to study visits and to participate in the study
- Sufficient fluency, of both the potential participant and caregiver, in written and spoken English to participate in study visits, physical exams, and outcome assessments
- If female, woman must be post-menopausal for at least 2 years or have had a hysterectomy

Exclusion criteria

- Currently meets criteria for Major Depressive Episode, by Diagnostic Statistical Manual of Mental Disorder - IV (TR) criteria
- Clinically significant agitation /aggression for which either
  - the frequency of agitation /aggression as assessed by the NPI is ‘Very frequently', or
  - the frequency of agitation /aggression as assessed by the NPI is ‘Frequently' AND the severity of the agitation as assessed by the NPI is ‘Moderate', or ‘Marked'
- Clinically significant delusions for which either
  - the frequency of delusions as assessed by the NPI is ‘Very frequently', or
  - the frequency of delusions as assessed by the NPI is ‘Frequently' AND the severity of the delusions as assessed by the NPI is ‘Moderate', or ‘Marked'

- Clinically significant hallucinations for which either
  - the frequency of hallucinations as assessed by the NPI is ‘Very frequently', or
  - the frequency of hallucinations as assessed by the NPI is ‘Frequently' AND the severity of the hallucinations as assessed by the NPI is ‘Moderate', or ‘Marked'
- Change to AD medications within the month preceding randomization, including starting, stopping, or dosage modifications
- Change in anti-depressant (except for trazodone used for sleeping difficulties as described below) use within the 30 days preceding randomization or a period of time equal to 5 half-lives of drug, whichever period of time is longer
- Use of trazodone > 50mg or lorazepam > 0.5mg or for indications other than sleeping difficulties within the 30 days preceding randomization or a period of time equal to 5 half-lives of drug, whichever period of time is longer. Other benzodiazepines are prohibited in the past 30 days or within 5 half-lives, whichever period of time is longer
- Failure of treatment with methylphenidate in the past for apathy after convincing evidence of an adequate trial as judged by study physician
- Currently taking any amphetamine product, an antipsychotic, bupropion, or any medication that would prohibit the safe concurrent use of methylphenidate, including but not limited to monoamine oxidase inhibitors and tricyclic antidepressants within the 30 days preceding randomization or a period of time equal to 5 half-lives of drug, whichever period of time is longer
- Need for acute psychiatric hospitalization or is suicidal in the opinion of the study physician
- Significant communicative impairments that would affect participation in clinical trial
- CNS abnormalities (e.g., cerebral aneurysm), seizures (convulsions, epilepsy), Tourette's syndrome or presence of motor tics, or abnormal EEGs
- Lack of appetite that results in significant unintentional weight loss as determined by the study physician in the last three months
- Uncontrolled hyperthyroidism
- Any cardiovascular or cerebrovascular abnormality deemed to be clinically significant by the study physician, tachycardia (heart rate ≥ 100 beats per minute), or uncontrolled hypertension (defined as medication non-compliance or past 3 months with a diastolic reading ≥ 105 mm Hg), at the time of screening
- Closed angle glaucoma or pheochromocytoma
- Women with childbearing potential
- Current participation in a clinical trial or study that may add significant burden or affect study outcomes
- Any condition that, in the opinion of the study physician, makes it medically inappropriate or risky for the participant to enroll in the trial, including, but not limited to, contraindication to treatment with methylphenidate.

Participating sites

- Study Chair’s Office: Medical University of South Carolina (Charleston, SC)
- Coordinating Center: Johns Hopkins Bloomberg School of Public Health (Baltimore, MD)
- Clinical Centers
  - Banner Alzheimer’s Institute (Phoenix, AZ) (2015 to 2017)
  - Emory University (Atlanta, GA)
  - Johns Hopkins University (Baltimore, MD)
  - Roper–St. Francis Healthcare (Charleston, SC)
  - Sunnybrook Research Institute (Toronto, ON, Canada)
  - University Hospitals- Case Medical Center (Cleveland, OH)

- University of Arkansas (Little Rock, AR)
- University of Rochester (Rochester, NY)
- Wake Forest University (Winston-Salem, NC) (2015 to 2019)
- Yale University (New Haven, CT)