INMiND-02

CLINICAL RESEARCH PROTOCOL

A double-blind, placebo-controlled study of the effect of a TNFα inhibitor, etanercept (Enbrel), on microglial activation in amyloid PET positive patients with Mild Cognitive Impairment due to AD-Intermediate likelihood

Investigational Product:	Etanercept (Enbrel)
Phase:	II
Sponsor:	University of Southampton Research Governance Office University Road Highfield Southampton SO17 1BJ
Chief Investigator:	Professor Clive Holmes
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1. GLOSSARY AND ABBREVIATIONS

1.1. Glossary

"Mild Cognitive Impairment (MCI) due to Alzheimer's Disease (AD)" and "MCI due to AD- Intermediate likelihood"

The advent of biomarkers and advances in the understanding of AD led the National Institute of Aging and the Alzheimer's Association to form workgroups to review the clinical criteria for the diagnosis of Alzheimer's disease (AD) and amnestic Mild Cognitive Impairment (aMCI), (Jack Cr Jr et al, 2011).

A workgroup was charged with the task of developing criteria for the symptomatic pre-dementia phase of AD, which they have called "Mild Cognitive Impairment- due to AD", (Albert et al, 2011).

The workgroup developed two sets of criteria: 1. Core clinical criteria, and 2. research criteria.

Subjects will be included in this study if they meet the core clinical criteria for MCI due to AD at the screening visit.

Subjects will enter the treatment period of the study if they meet the research criteria for "MCI due to AD- Intermediate likelihood" at the base-line treatment visit.

In order to satisfy the research criteria for "MCI due to AD- Intermediate likelihood" the subjects must have a cortical amyloid load as evidenced by a positive Amyvid PET scan.

Alzheimer's disease (AD)

Subjects participating in this study will be considered to have converted to AD when they no longer satisfy the criteria for "MCI due to AD- Intermediate likelihood", and when they satisfy the National Institute of Aging and the Alzheimer's Association diagnostic guidelines for probable or possible AD (McKhann et al 2011).

Montreal Cognitive Assessment (MOCA)

The MOCA (Nasreddine Z, 2005) is a well validated and widely used measure to assess domains of impairment commonly encountered in MCI. It is a rapid, sensitive,

and easy-to-administer cognitive test which measures 8 cognitive domains within a series of 13 tests: visuospatial/executive function, memory, language, abstraction, delayed recall and orientation. The highest possible score is 30. A score above 26 represents normal cognitive function.

Repeatable Battery for the Assessment of Neuropsychological Status (RBANS)

The RBANS (Randolph C. 1998) is a brief test of immediate and delayed memory, attention, language, and visuo-spatial/ constructional abilities.

The FCSRT-IR (Grober E, Buschke H. 1987) measures episodic memory under conditions that control attention and cognitive processing. The test possesses good psychometric properties and is widely used by clinicians and researchers.

The FCSRT-IR begins with a study phase in which subjects are asked to search a card containing four words for an item that goes with a unique category cue (e.g. item- apple, category- fruit). After all four items are identified immediate recall of just those four items is tested. The search is performed again for items not retrieved. The search procedure is continued until all 16 items are identified and retrieved in immediate recall. This is followed by three trials of recall, each consisting of free recall followed by cued recall for items not retrieved by free recall.

The sum of free and cued recall on each trial is called total recall. Items not retrieved by cued recall are represented during Trial 1 and 2. There is 20 seconds of interference between each trial.

Alzheimer's Disease Cooperative Study Activities of Daily Living Inventory (ADCS-ADL$_{MCI}$)

The ADCS-ADL$_{MCI}$ (Galasko D et al 1997) is a functional assessment adapted for MCI trials.

Cornell Scale for Depression in Dementia

The Cornell Scale for Depression in Dementia is a 19 item scale (Alexopoulos et al 1988) which focuses specifically on aspects of depression in patients with dementia and which has good internal consistency and validity.

Apathy Inventory (IA)

The principle of the Apathy Inventory (IA), (Robert et al 2002), is to obtain information on apathy in patients with brain disorders. There are three versions (carer's version, patient's version, and clinician's version), and each version assesses the same three clinical dimensions of emotional blunting, lack of initiative and lack of interest.

Apathy clinical diagnostic criteria (ACDC)

The Apathy clinical diagnostic criteria (ACDC), (Robert et al 2009), is used for the diagnosis of apathy in patient's with Alzheimer's disease and other neuropsychiatric disorders.

Modified Hachinski Ischaemia Scale (MHIS)

The Modified Hachinski Ischaemia Scale (Hachinski et al 1975) is used for the assessment of risk factors relating to cerebrovascular disease and is widely used in dementia clinical research trials to help clinicians exclude vascular dementia.

1.2. Relevant abbreviations

Abbreviation	**Definition**
Aβ	Amyloid β (protein)
ACDC	Apathy Clinical Diagnostic Criteria
AD	Alzheimer's disease
AE	Adverse Event
ADCS-ADL$_{MCI}$	Alzheimer's Disease Cooperative Study Activities of Daily Living Inventory, Mild Cognitive Impairment
AR	Adverse Reaction
ApoE	Apolipoprotein E
BBB	Blood Brain Barrier
CI	Chief Investigator
CRF	Case Report File
CRP	C Reactive Protein

FCSRT-IR	Free and Cued Selective Reminding Test with Immediate Recall
IA	Apathy Inventory (French origin)
IL-1/ 4/ 6/ 10/ 12/ 13	Interleukin-1, Interleukin-4, Interleukin-6 ; Interleukin-10 ; Interleukin-12 ; Interleukin-13.
IMP	Investigatory Medicinal Product
INMiND	Imaging of Neuroinflammation in Neurodegenerative Disease
LPS	Lipopolysaccharide
MARC	Memory Assessment and Research Centre
MCI	Mild Cognitive Impairment
MHIS	Modified Hachinski Ischaemia Scale
MHRA	Medicines and Healthcare products Regulatory Agency
MOCA	Montreal Cognitive Assessment
NIA/AA	National Institute of Aging/ Alzheimer's Association
NINCDS-ADRDA	National Institute of Neurological and Communication Disorders and Stroke – Alzheimer's Disease and Related Disorders Association
PDAPP	Platelet derived amyloid precursor protein
PET scan	Positron Emission Tomography scan
PI	Principal Investigator
RBANS	Repeatable Battery for the Assessment of Neuropsychological Status
REC	Regional Ethics Committee
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction
SHFT	Southern Health NHS Foundation Trust
Spc	Summary of Product Characteristics
STEADI	Safety and Tolerability of Etanercept in Alzheimer's disease (clinical research trial)
SUSAR	Serious Unexpected Adverse Reaction
TsPO	Translocator Protein

TB	Tuberculosis
TgAD	Transgenic Alzheimer's disease (mouse)
TGF-β	Transforming Growth Factor β
TNFα	Tumor Necrosis Factor-α
UAR	Unexpected Adverse Reaction

2. ADMINISTRATIVE STRUCTURE OF THE STUDY

2.1. Sponsor

The University of Southampton will act as sponsor.

2.2. Financial Support

European Union FP7 Grant as part of Imaging of Neuroinflammation in Neurodegenerative Disease (INMiND). Health 2011.2.21.-2. Understanding the role of neuroinflammation in neurodegenerative diseases. Grant agreement no: 278850.

Two charities "The Alzheimer's Society" UK and the "Alzheimer Drug Discovery Foundation" are co-funding the purchase of the Investigational Medicinal Product (IMP), the manufacture of the placebo, and the randomisation, labelling, packaging and shipping of the Investigational Medicinal Product (IMP) and placebo. Grant Number: 20150501.

2.3. Organisation responsible for the management of the study.

The Host Institution will be the University of Southampton.

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The first clinical research study site is at the Memory Assessment and Research Centre (MARC), Southern Health NHS Foundation Trust/ University of Southampton. Prof Holmes is the Principal Investigator at the first site.

The second clinical research study site is at the Institute of Brain, Behaviour and Mental Health, University of Manchester/Manchester Mental Health and Social Care Trust. Dr Iracema Leroi is the Principal Investigator at the second clinical site.

The neuro-imaging research study site is the Wolfson Molecular Imaging Centre Institute of Brain, Behaviour and Mental Health. Dr Alex Gerhard and Prof Karl Herholz are co-Principal Investigators at the neuro-imaging site.

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3. BACKGROUND AND RATIONALE FOR THE STUDY

3.1. Alzheimer's Disease: A Major Health and Economic Problem

Alzheimer's disease (AD) is the most common cause of dementia in older people and affects around ½ million patients in the UK and around 3 million in the USA. AD is a devastating illness, which causes a progressive decline in cognitive ability, functional capacity and the appearance of a variety of neuropsychiatric complications, resulting in immense distress to patients, their carer's and families.

It is projected that the prevalence of AD will nearly quadruple in the next 50 years, by which time approximately 1 in 45 Americans will be afflicted with the disease (Brookmeyer et al 1998). It is clear that as the population gets older, AD will become an enormous public health problem across the western world. In the UK alone a report by McCrone et al (2008), has calculated the total annual costs of dementia care in 2007 to be £14.8 billion with a projected increase to £34.8 billion by 2026 (40% of the current NHS budget).

For these reasons, AD is at the top of government priorities for research, treatment and service provision, and interventions that could delay or slow down the progression of the disease in its early stages, even modestly, would have a major public health impact.

Current licensed treatments are aimed at the end stage of the disease and act by replacing the neuro-chemical losses of acetylcholine or modifying glutamanergic changes. These changes are the downstream result of a neurodegenerative process that involves amyloid deposition, neurofibrillary tangle formation and neuro-inflammation and at best are thought to delay the progression of the illness by one year (Winblad et al 2004). Drugs aimed at the modification of the development of amyloid deposition in AD subjects have been disappointing and have not shown efficacy on primary outcomes despite around £0.5 billion investment. New approaches to the treatment of AD at the earliest stages are desperately needed.

3.2. Systemic inflammation results in activation of microglial cells in the brain, which in turn causes transient sickness behaviour.

Systemic inflammation is characterized by an acute phase response, which includes increased C Reactive Protein (CRP) production by the liver, and the production of pro-inflammatory cytokines such as interleukin-6 (IL-6) and Tumour Necrosis Factor-α (TNFα) from macrophages that play a role in immune to brain communication.

In subjects *without dementia,* this peripheral signal activates the central innate immune response, including activating cells of the macrophage lineage, the microglia (Perry et al 2007). These signals in turn produce the symptoms of sickness behaviour, including apathy, malaise, anxiety and depression (Dantzer et al 2008). The activation of microglial cells in these subjects is highly regulated due to a range of regulatory mechanisms that dampen down this pro-inflammatory response. Thus, in normal subjects systemic inflammation does not lead to widespread neuronal damage and sickness symptoms are usually transient.

3.3. Imaging studies support the hypothesis that raised systemic pro-inflammatory levels are associated with increases in microglial activation.

Demonstration of the signalling of systemic inflammation to the brain has been shown in Functional MRI studies of healthy human volunteers. Here low dose *Salmonella typhi* endotoxin in a vaccine is associated with significant increases in systemic IL-6 that are associated with slower reaction times and increased neuronal activity in the substantia nigra (Brydon et al 2008) as well as mood reduction associated with enhanced activity in the anterior cingulate cortex (Harrison et al 2009). More recently a PET imaging study in non-human primates, using the TSPO ligand [(11)C]PBR28, a marker of microglial activation, has shown that low dose intravenous Lipopolysaccharide (LPS) gives rise to increased serum IL-1β and IL-6

levels which are positively correlated with an increase in [(11)C]PBR28 binding (Hannestad et al 2012). Another PET imaging study in humans with chronic systemic inflammatory conditions (atherosclerosis; diabetes; smokers) using a different TSPO ligand, [11C](R)-PK-111-95, showed that, in keeping with early rodent models, mildly elevated serum C-reactive protein (CRP) and IL-6 levels in the absence of any evidence of acute infection are also associated with increased microglial activation (Drake et al 2011).

3.4. In circumstances where central microglial cells are already partially activated, or primed, systemic inflammation results in an exaggerated central innate immune response.

In animal models of neuro-degeneration and AD it has been shown that microglia are already partially activated, or primed, by the presence of chronic neurodegenerative changes e.g. by amyloid, resulting in modest increases in central pro-inflammatory cytokine production including interleukin-1 (IL-1) (Griffin et al 1995; Griffin et al 1998); IL-6 (Strauss et al 1992) and TNFα (Tarkowski et al 1999).

It has been shown in a series of animal experiments that in these circumstances where central microglial cells are already partially activated, or primed, that low grade systemic inflammation results in an exaggerated central innate immune response, causing heightened sickness behaviour and increased neuro-degeneration due to the production of reactive oxygen species, that, over time, results in accelerated disease progression (Cunningham et al 2005; Cunningham et al 2008).

3.5. Systemic inflammation leads to an increase in systemic pro-inflammatory cytokine production and leads to an increase in sickness behaviour and an increase in the rate of cognitive decline in patients with AD.

The results of these studies led to the development of the hypothesis that systemic inflammation could lead to an increase in the rate of cognitive decline in patients with AD, where microglial cells are already primed by the presence of amyloid, and other neurodegenerative changes, and that this effect is mediated by an increase in systemic pro-inflammatory cytokine production.

A short term pilot project conducted by Holmes et al supported the hypothesis that cognitive decline is associated with the presence of systemic infections and the presence of systemic IL-1 (Holmes et al 2003).

3.6. The link between systemic inflammation, sickness behaviour, and cognitive decline in patients with AD may be mediated by TNFα.

A further study of 300 subjects with AD showed evidence that systemic inflammation, both acute and chronic, is associated with a greater rate of cognitive decline in subjects with AD, and that systemic inflammation was also associated with an exaggeration of the presence of symptoms characteristic of sickness behaviour. These effects appear to be most clearly associated with the presence of raised serum levels of TNFα (Holmes 2009).

3.7. There is evidence of a genetic predisposition to a higher pro-inflammatory state in patients developing AD.

As previously stated, AD patients with high systemic TNFα levels have more rapid disease progression (Holmes, 2009). TNF-α levels may be elevated in patients as a response to systemic inflammatory insults such as infections; however some patients may also have a genetically inherited predisposition to higher circulating levels of TNFα and other cytokines. Thus, there is an inherited predisposition to higher levels of circulating pro-inflammatory cytokines in the middle-aged off-spring of patients with Alzheimer's disease (Van Exel, 2009).

In addition, recent genome wide association studies of late onset AD have highlighted the importance of genes for components of inflammatory pathways in late-onset Alzheimer's disease (Harold, 2009; Lambert, 2009). These studies suggest that the dysregulation of pro-inflammatory genes towards a more pro-inflammatory state is more common in patients with AD and those predisposed to the condition.

Therefore the inhibition of systemic TNFα could have two beneficial effects, in that it could decrease the cytokine response to stochastic inflammatory insults such as infections or aseptic chronic inflammatory conditions (e.g. atherosclerosis; diabetes) and it could also decrease baseline inherited pro-inflammatory cytokine levels.

3.8. Animal models of anti TNFα agents.

Animal models of AD have largely focussed on genetic modifications of amyloid and the exploration of therapies that may modify amyloid deposition e.g. amyloid vaccines; alpha and gamma secretase inhibitors. As stated previously, interventions aimed at modification of amyloid have not, to date, been successful and these

animal models are of little value in the exploration of anti TNF-α agents because the primary outcome of interest, neurodegeneration, is not a major feature of these models. What work has been done has largely focussed on the effects of reducing central TNF-α levels as a means of reducing neurodegeneration in AD. In an acute model of neuroinflammation, initiated by intra-cerebral injection of Aβ, an intra-cerebral injection of an anti-TNF-α antibody prevented the nitration of proteins in the hippocampus and the impairment of recognition memory induced by Aβ (Alkam et al 2008). In a chronic systemic inflammation model in the 3xTgAD mouse model (Kitazawa et al 2005) chronic inhibition of soluble TNF signalling, using specifically engineered antibodies delivered intra-cerebrally, prevented amyloid-associated neuropathology in 3xTgAD mice and reduced the deposition of intra-neuronal amyloid species (McAlpine et al 2009).

However, there is some evidence to suggest that blockade of peripheral TNFα might, by reducing the signalling and activation of central microglial activation, have beneficial effects without the need for the peripherally active compound crossing the BBB. Thus, inhibition of peripheral TNFα by the peripherally active TNFα inhibitor, Etanercept, blocks sickness behaviour symptoms in a rat model of centrally induced sickness behaviour (Jiang et al 2008). A study of PDAPP mice given peripheral antibody to TNFα also found significant protection against spatial performance and age related behavioral decline (Giuliani et al 2009).

3.9. Early human studies support the hypothesis that TNFα inhibitors are safe and potentially effective treatments for sickness behaviour and cognitive decline in patients with AD.

TNFα inhibitors are widely used in the elderly human population for the treatment of rheumatoid arthritis and ankylosing spondylitis. Thus it is possible to assess the likely safety and potential efficacy of these agents from observations of these populations.

Clinical studies are supportive. A clinical epidemiologically study suggests that patients taking TNFα inhibitor drugs for rheumatoid arthritis are protected against the development of AD, with an odds ratio of around 0.4 (Chou et al 2010). Furthermore, a small open labelled study of patients taking TNFα inhibitor drugs suggests cognitive protection in elderly rheumatoid subjects (Chen et al 2010). The TNFα inhibitor Etanercept also has a substantial effect in alleviating symptoms of depression and fatigue in patients with psoriasis (Tyring et al 2006). These symptoms are key components of sickness behaviour and, notably, the improvements in depression were not well correlated with objective measures of

psoriatic skin clearance or improved joint pain, suggesting a direct central effect of etanercept on sickness behaviour.

Etanercept has also been given as a peri-spinal injection to a small number of patients with AD (Tobinick 2008; 2009), and although there is no evidence that Etanercept crossed the BBB using this method, cognitive scores improved after administration of Etanercept. However, no control group was used in this open-label study and the number of subjects was low (n=15).

A pilot study published as a conference abstract and described as a RPCT of Etanercept in AD patients did not show any major adverse events in the treatment group but there were insufficient numbers in the placebo arm (n=2) to ascertain potential efficacy (Bohac et al 2002).

We have now completed the first randomised placebo controlled double blind trial of AD subjects using the peripheral TNFα inhibitor Etanercept. The study was designed to demonstrate safety and give preliminary data on potential efficacy (STEADI). This study demonstrates that the use of the peripheral TNFα agent is safe in AD subjects and suggests a benefit in a range of behavioural, functional and cognitive clinical outcomes (Butchart et al 2015).

3.10. Systemic inflammation and elevated levels of systemic TNFα may also play a role in the development of AD, in subjects with MCI, suggesting a potential role for TNFα inhibitors as a protective agent in the development of AD.

Genetic evidence as previously stated suggest that elevated systemic pro-inflammatory cytokine levels, including elevated systemic TNFα levels, could play a role in the onset of AD, as well as the progression of AD. In addition, a range of established risk factors for the disease e.g. diabetes; atherosclerosis, obesity; midlife hypertension are associated with increases in systemic inflammation.

Therefore it is possible that in individuals with prodromal AD, or Mild Cognitive Impairment due to AD, whose microglial cells are primed by the presence of amyloid, that systemic inflammation could also act as a driver towards the development of AD by activating microglial cells leading to the production of oxidative species that cause neurodegeneration. Thus, as in AD subjects, TNFα inhibitors may act to dampen down central microglial activation and reduce the likelihood of progression of Mild Cognitive Impairment due to AD to probable or possible AD.

STUDY HYPOTHESIS:

In MCI-due to AD, systemic inflammation and elevated systemic levels of TNFα cause partially activated, or primed, microglial cells, to become fully activated, which can be modulated by the administration of a peripheral TNFα inhibitor, etanercept.

STUDY QUESTION:
Will the administration of a peripheral TNFα inhibitor, etanercept, over a 12 month treatment period, reduce microglial activation in patients with Mild Cognitive Impairment due to AD- Intermediate likelihood, and reduce cognitive and behavioural decline?

4. STUDY OBJECTIVES

4.1. Primary Objective

To ascertain the change in microglial activation on [11C] *(R)*-PK-111-95 PET scans from base-line to the final imaging visit in the treatment group compared to the placebo group.

4.2. Secondary Objectives

1. To ascertain the change in the primary cognitive outcome measure, the Montreal Cognitive Assessment, (MOCA), from baseline to final treatment visit in the treatment group compared to the placebo group.
2. To ascertain the change in cortical amyloid load on AMYVID PET scans from base-line to the final imaging visit in the treatment group compared to the placebo group.

4.3. Exploratory objectives

1. To ascertain the change in the first of the exploratory cognitive outcome measures, the Repeatable Battery for the Assessment of Neuro-psychological Status, (RBANS), from baseline to final treatment visit in the treatment group compared to the placebo group
2. To ascertain the change in the second of the exploratory cognitive outcome measures, the Free and Cued Recall Selective Reminding Test with Immediate Recall (FCSRT-IR), from baseline to final treatment visit in the treatment group compared to the placebo group.

3. To ascertain the change in the Cornell Scale for Depression in Dementia score from base-line to final treatment visit in the treatment group compared to the placebo group.
4. To ascertain the change in the behavioural assessment the Apathy Inventory from baseline to final treatment visit in the treatment group compared to the placebo group.
5. To ascertain the change in the behavioural assessment the Apathy Clinicians Diagnostic Criteria from baseline to final treatment visit in the treatment group compared to the placebo group.
6. To ascertain the change in the Alzheimer's Disease Co-operative Study Activities of Daily Living Inventory Mild Cognitive Impairment (ADCS-ADL MCI) from baseline to final treatment visit in the treatment group compared to the placebo group.
7. To ascertain the change in the levels of plasma markers of neuro-inflammation (pro and anti-inflammatory cytokines: IL-1; IL-6, TNF-α, IFN-γ, IL-4, IL-10, IL-12, IL-13; CRP, and TGF-β) through the study, in the treatment group compared with the placebo group.
8. To study the relationship between the changes in the levels of plasma markers of neuro-inflammation (IL-1; IL-6, TNF-α, IFN-γ, IL-4, IL-10, IL-12, IL-13; CRP, TGF-β) and changes of microglial activation on [11C] *(R)*-PK-111-95 PET scan, the change in cortical amyloid load on AMYVID PET scans and clinical outcomes, in the treatment group compared with the placebo group.
9. To examine the penetration of etanercept across the blood brain barrier in patients with mild cognitive impairment due to Alzheimer's Disease.
10. To examine the effects of etanercept on inflammatory markers in the cerebrospinal fluid (CSF) of patients with mild cognitive impairment due to Alzheimer's Disease, and the relationship with clinical outcomes.

5. STUDY DESIGN

5.1. Summary of the Study Design

A 64 week, multi-centre, randomised, double blind placebo controlled study.

The target population is comprised of subjects who satisfy the core clinical criteria for MCI due to AD and the research criteria for MCI due to AD- Intermediate likelihood, based on a positive Amyvid PET scan.

46 subjects will be included in the treatment period of the study.

Subjects will be randomized on a 1:1 ratio to receive etanercept (Enbrel), or identical matching placebo.

The study duration for each subject is 64 weeks, which consists of an 8 week screening period, a 52 week treatment period and a 4 week follow up period.

There will be two imaging visits. The first imaging visit will be conducted during the screening period and the second imaging visit will be conducted at week 51of the treatment period, one week prior to the last assessment on treatment visit, plus or minus 7 days.

5.2. Study Duration

The estimated study duration is 27 months, with the first patient, first visit, scheduled for September 2015, the last patient, base-line visit, scheduled for September 2016 and the last patient, last visit, scheduled for November 2017.

5.3. Rationale for the sample size

The MOCA will be administered at the screening visit, V0, to aid the diagnosis of MCI due to AD. The guideline scoring range for the diagnosis of MCI due to AD will be 19 to 25 out of 30 points, at the PI's discretion.

It is anticipated that it will be necessary to screen 100 subjects in order to identify 75 subjects who satisfy the core clinical criteria for the diagnosis of MCI due to AD, and who meet all of the pre-imaging inclusion and exclusion criteria, including the requirement that they have no evidence of active or latent Tuberculosis.

It is anticipated that 60%, or 46, of these 75 subjects will be positive for cortical amyloid based on Amyvid PET evidence.

Therefore 46 subjects will be identified as satisfying the core clinical criteria and the research criteria for "MCI due to AD- Intermediate likelihood", and these 46 subjects will enter the treatment period of the study and be randomised to etanercept (Enbrel) or placebo at a 1:1 ratio.

Allowing for 15-20% of the 46 subjects on treatment to withdraw, or be withdrawn, from the study during the 52 week treatment period, 34 - 38 subjects will complete the study.

34 - 38 subjects completing the study gives 80 - 90% power; 5% specificity to determine a 50% change in microglial binding between the control and treatment groups based on [11C] *(R)*-PK-111-95 PET data.

5.4. Statistical analysis

The primary outcome measure is microglial activation as measured by a [11C] *(R)*-PK-111-95 PET scan. The null hypothesis is that patients taking etanercept (Enbrel) will not demonstrate a 50% reduction in microglial activation as measured on a [11C] *(R)*-PK-111-95 PET scan, after one year.

Secondary outcomes include the change in cortical amyloid load and clinical observational outcomes between groups. In the event of a study subject's premature withdrawal from the trial, both observed cases and a last observation carried forward analysis will be done. This "last observation carried forward" analysis will ensure that data concerning subjects that withdrew early because of adverse side effects or because they converted to AD, will not be lost in the final analysis. All data will be examined for possible confounding factors using a mixture of linear and logistic regression analysis.

5.5. Safety Assessments

1. Initial TB and infectious disease screen (including a chest X-ray (CXR) and a QuantiFERON-TB Gold assay for TB exposure, and a hepatitis B surface antigen test).
2. Collection of data on adverse events and concomitant treatment.
3. Clinical examination including blood pressure, heart rate, weight and temperature.
4. Safety bloods will be done at all clinical visits: Full Blood Count, Urea and Electrolytes, Liver Function Tests, C Reactive Protein (CRP).
5. Other bloods to be done at screen, to exclude other causes of cognitive impairment: B12, folate, bone function tests (corrected calcium), glucose (non-fasting), thyroid function tests.
6. Urinalysis.
7. ECG at screen and then repeated when clinically indicated.

5.6. Clinical Assessments

1. Cognitive Function :
   MOCA
   RBANS
   FCSRT-IR

2. Assessment of mood :
   Cornell Scale

3. Functional Assessment :
   ADC-ADL$_{MCI}$

4. Behavioural Assessment :
   AI and ACDC

5. Ischaemia risk Assessment
   MHIS

6. Immunological Response :
   Plasma concentration of inflammatory markers (to include: IL-1, IL-6, TNF-α, IFN-γ, IL-4, IL-10, IL-12, IL-13, CRP, and TGF-β).

7. Baseline blood genotyping for ApoE and other potential genetic markers of MCI progression.

8. (Optional) Cerebrospinal fluid (CSF) examination to establish the degree of penetration of etanercept across the blood barrier and CSF concentration of inflammatory markers.

5.7. Measures to minimise bias

5.7.1. Training

All members of the study team will be trained in:

- ICH Good Clinical Practice (GCP).
- The conduct of the study as described in the protocol.

5.7.2. Measures to ensure the integrity of the neuro-psychological data

Special emphasis will be placed on ensuring the integrity of the neuro-psychological data gathered during the study:

- Rating scales will be administered by trained raters.
- Every effort will be made to ensure that the same rater administers the same scales to the same subject, at every visit.
- Visits will be scheduled to ensure that subjects are tested at a similar time of day at every testing visit.
- Testing will take place in a quiet, low sensory stimulation environment.
- Every effort will be made to ensure that testing will take place in the same room at each visit.
- Note: the MOCA and Cornell Scale are the only scales that will be administered at the screening visit, for the diagnosis of MCI due to AD and the exclusion of clinically significant depression respectively. Consideration was given to administering all of the rating scales at the screening visit to ensure that subjects are familiar with the testing process when asked to do the tests at the base-line visit, but given the 50% anticipated screen failure rate, given that the other scales relate to exploratory outcomes, and bearing in mind that the screening visit is already a long visit, on balance a decision has been made not to do the RBANS, FCSRT-IR, ADCS-ADL$_{MCI,}$ IA and ACDC at the screening visit.

5.7.3. Measures to ensure the blinding of the study drug and placebo

ACE Pharmaceuticals BV (PO Box 1262, NL-3890, BB Zeewolde, Netherlands, Tel: +31 36 5227201, Fax +31 36 5229096) will purchase the Investigatory Medicinal Product, etanercept (Enbrel), and they will manufacture the placebo. They will label and package both the study medication and placebo to ensure blinding.

The company will use a computer to generate a simple random allocation sequence to ensure 23 patients in the treatment group (etanercept (Enbrel)) and 23 patients in the placebo group. The investigators will have no knowledge of the allocation sequence, which will remain concealed throughout the study.

ACE Pharmaceuticals BV will load either etanercept or placebo vials into serially numbered containers according to the allocation sequence. The loaded containers, and the interventions inside them, will be identical in appearance and consistency to ensure concealment of the allocation sequence from the investigators.

ACE Pharmaceuticals BV will ship containers 1 to 31 to the Southampton clinical site, and containers 32 to 46 to the Manchester clinical site.

Participants, who have fulfilled all the inclusion and exclusion criteria and wish to enter the study, will be assigned the container at their site with the next available serial number, in strict chronological order.

It is possible that circumstances will arise in which one site may not be on schedule to reach their recruitment target number, and the other site may make up the shortfall, with the total remaining at 46. The surplus containers from the site recruiting fewer than targeted numbers will be transferred to the site making up the shortfall and thus recruiting greater than targeted numbers and the extra patients at that site will be allocated the next available serial number from the surplus batch of containers, in strict chronological order.

The study will be double-blinded throughout.

5.8. Study schedule

See addendum 1.

5.9. Definition of the end of the trial

In the light of interim data and other evidence from relevant studies, the TSC may indicate that the trial should be terminated early, if in its view, there is proof beyond reasonable doubt that the data indicate that the trial should be terminated. This decision will in part be based on statistical considerations. Appropriate proof beyond reasonable doubt cannot be specified precisely but might include poor recruitment or tolerability of the study procedures resulting in the likelihood that the study will have insufficient power to attain its primary outcome.

Otherwise, the data collection for the trial will end with the last visit of the last participant undergoing the trial.

6. SELECTION AND WITHDRAWAL OF SUBJECTS

6.1. Inclusion Criteria

Subjects will have to meet all of the following criteria at screening to enter the study:

1. All subjects must have the capacity to make an informed decision as to whether they would like to take part in this specific clinical research trial.
2. A subject can be male or female and they must be between 50 to 90 years old, inclusive.
3. A subject must have received a minimum of 7 years of formal education.
4. A subject must be able to hear, read, write and perform study neuro-psychological tests in English.
5. A subject must have adequate visual and auditory acuity to allow neuro-psychological testing, based on the research clinician's judgement.
6. A subject must fulfil the NIA-AA criteria for the diagnosis of Mild Cognitive Impairment due to AD at the screening visit (Albert et al, 2011.) A subject must have a MOCA score of 19 to 25 inclusive at screening, at the discretion of the Principal Investigator.
7. A subject must have a study partner who spends at least eight hours a month with the subject. The study partner may be a close friend or a neighbour and not necessarily a close relative, spouse, son or daughter, and should be present at all visits. Every effort should be made to ensure that the study partner will be the same throughout the study. If it becomes necessary for the study partner to change, the new study partner must satisfy the requirements of this criterion and the change of study partner must be clearly documented.
8. A subject must have been on a stable medication regime for more than 3 months prior to screening.
9. Women of child bearing potential must use adequate contraception to prevent pregnancy during the study and must continue to use contraception for at least four weeks after the last study dose.

6.2. Exclusion criteria

Subjects meeting any of the following criteria during screening or baseline will be excluded from the study:

General criteria

1. Inability or refusal to provide informed consent from subject or study partner.
2. Absence of study partner.

3. Unlikely to cooperate in the study, not able to attend scheduled examinations and visits, or not able to follow study instructions, left to the research clinician's judgement.
4. Participation in another study with administration of any investigational drug in the previous 3 months or already enrolled in another study.

Medical and therapeutic criteria

5. Any contraindications to the use of etanercept (Enbrel) as per the Summary of Product Characteristics (section 4.3): a. Hyper-sensitivity to the active substance or to the excipients in the injection (mannitol (E421), sucrose, trometamol, and water), b. sepsis or risk of sepsis, c. treatment with etanercept (Enbrel) should not be initiated in patients with active infections, including chronic or localised infections.
6. Parkinson's disease, Dementia with Lewy Bodies or clinically significant Parkinsonian symptoms.
7. Vascular disorder (Modified Hachinski Ischaemic Scale score > 4).
8. Recent Transient Ischaemic Attack (TIA) – within the last 3 months.
9. Signs of major cerebrovascular disease on MRI or CT scan prior to entry into study, (i.e. evidence of an established cortical or basal ganglia infarct).
10. Signs of major cerebrovascular disease on the MRI performed at the screening imaging visit prior to the amyloid and microglial PET scans.
11. Any other previous or ongoing chronic or recurrent disease of the central nervous system, including demyelinating disease or psychiatric diseases, that may have an impact on cognitive performance, left to the research clinician's judgement.
12. Any of the following laboratory abnormalities at the screening visit:
    i) Clinically significant Vitamin B_{12} levels less than the lower limit of normal.
    ii) Clinically significant folate levels less than the lower limit of normal.
    iii) Clinically significant thyroid-stimulating hormone (TSH) levels greater than the upper limit of normal and a clinically significant free thyroxine (FT4) level lower than the lower limit of normal.

    (Subjects who are successfully treated for folate and vitamin B12 deficiencies or hypothyroidism may be re-screened after 3 months.)

13. Subjects with a previous or present history of severe medical conditions, or medical conditions which are poorly controlled, such as hypertension or diabetes, left to the research clinician's judgement.
14. History of alcohol or drug dependence or abuse within the last 2 years. Current alcohol >35 units per week for men, or >28 units per week for women, or drug abuse, at the discretion of the research clinician.
15. Surgical intervention planned during the study period.
16. Treatment with immunosuppressive drugs including any systemic corticosteroid drugs. (Topical and nasal corticosteroids and inhaled corticosteroids for asthma are permitted.)
17. Treatment with benzodiazepines within a period of three days prior to [11C] *(R)*-PK-111-95 PET scans imaging.
18. Vaccination or immunization with any live vaccine (e.g.: polio, rubella, yellow fever) within the past 30 days.
19. Pregnancy or breast feeding.
20. Severe hepatic, renal or cardiac disease.
21. Previous use of a TNFα agent.
22. Known skin photosensitivity.
23. Infection in past 4 weeks or active infection.
24. Heart failure: New York Heart Association (NYHA) Grade 3-4.
25. History of blood disorders or current WCC ≤ 3.5 x 10^9/l; platelet count ≤ 100x10^9/l; Hb ≤ 10g/dl.
26. Active or latent tuberculosis.
27. Rheumatoid arthritis; psoriasis; psoriatic arthritis or ankylosing spondylitis.
28. Septic arthritis in past 12 months.
29. Sepsis of prosthesis in past 12 months.
30. Chronic leg ulcers.
31. Indwelling urinary catheter.
32. Pulmonary fibrosis.
33. History of neoplasms / malignancies in past 5 years.
34. Pre-malignant conditions including Barrett's oesophagus; cervical dysplasia; large bowel polyps.

35. Other clinically significant abnormality on physical, neurological, ECG or laboratory examination that could compromise the study evaluations or be detrimental to the patient during the course of the study.

36. Use of experimental medications for AD, or any other investigational medication or device, within 60 days. Patients who have been involved in a monoclonal antibody study are excluded unless it is known that they were receiving placebo in that trial.

Imaging exclusion criteria

37. Subjects with significant cortical or basal ganglia infarct or other significant pathology found on MRI brain scan.
38. Subject with a negative Amyloid PET scan. *NB: Subjects with a positive Amyloid PET scan or a border-line positive PET scan will be included in the study. The final decision on whether the Amyloid PET scan is negative, border-line positive, or positive, will rest with the Principle Investigators at the Wolfson Molecular Imaging Centre.*

6.3. Informed consent

The Principal Investigator, or a person designated by the Principal Investigator, must collect written consent from each subject and their study partner before entry into the study.

The subject and the study partner will be provided with a participant and study partner information sheet and optional lumbar puncture patient information sheet at least 24 hours before the screening visit, so that they will have sufficient time to study the information sheet before attending for the screening visit.

It is the intention that the terms used in the study information sheets to describe the study product and its possible side effects, the method of randomisation, the objectives of the study, the potential advantages and disadvantages of taking part in the study, the request for participants to comply with the visit schedule, the reasons why participants are asked to report adverse events and concomitant medication changes, and information relating to the optional lumbar puncture, will be stated clearly, using terminology that will be familiar to a member of the public.

The information contained in the study information sheets will be re-iterated by the Principal Investigator, or a research clinician designated by the Principal Investigator, at the screening visit and the subject and study partner will be given ample time to ask questions about the study.

If the Principal Investigator, or a person designated by the Principal Investigator, is satisfied that the subject and study partner have the capacity to make an informed decision to take part in the study, and that they wish to do so, they will each be asked to sign and date an informed consent form, and they will sign the informed consent form for their own participation in the study. If the patient agrees to have the optional lumbar puncture, they will be asked to sign the optional lumbar puncture consent form.

The patient and study partner informed consent forms and the optional lumbar puncture consent form (if signed by patient) will be signed and dated by the research clinician responsible for obtaining consent.

The subject and the study partner will be given a copy of the signed consent forms and the study information sheets, and the original signed copies of the informed consent forms will be kept by the Principal Investigator.

Patients who lack capacity to make an informed decision to decide to take part in this study will not be eligible for entry into the study.

Subjects who lose capacity to decide to take part in the study during the study will be withdrawn from the study.

6.4. Participant withdrawal criteria

Criteria for the premature discontinuation of treatment are:

1. Consent withdrawal.
2. The subject loses the capacity to make an informed decision to continue to take part in the study.
3. The subject converts to AD, i.e. subject's presentation satisfies the McKhann 2011 criteria for Alzheimer's disease.
4. Protocol deviation, such as lack of informed consent, poor compliance (left to the research clinician's judgement), or any deviation which could have a significant impact on cognitive functioning, e.g. forbidden concomitant treatment.
5. Any event or circumstance unrelated to treatment, which, in the research clinician's opinion, justifies the discontinuation of treatment.
6. The occurrence of a serious or considered serious, adverse event, including a severe inter-current infection, which in the opinion of the Chief Investigator, site Principal Investigator, the Trial Steering Committee or the Data

Management Committee (in the event that the TSC independent chairman has asked for a DMC to be convened) requires participant withdrawal from the study. When premature discontinuation of treatment is due to an adverse event, at least one early withdrawal follow up visit must be organized to collect the information relating to the outcome of the event.

7. Lost to follow-up: when a research clinician loses contact with a subject, the research clinician must make every effort to contact the subject to establish the reason for the discontinuation of treatment and to suggest the subject comes to an early withdrawal follow up visit. If all reasonable attempts to contact the subject are unsuccessful, the research clinician can then declare the subject “lost to follow-up”. The research clinician should document the details of the attempts to contact the subject.

6.5. Participant withdrawal procedure

The research clinician must record the reasons for the early withdrawal of the subject from the study and the date of the premature discontinuation of treatment in the source notes and the case report form.

If more than one reason is given, the research clinician must indicate the main reason.

In the case of the early withdrawal of a subject from the study, every reasonable effort must be made to conduct an early withdrawal follow up visit, (EWFUV). Whenever possible, the early withdrawal follow up visit should take place within four weeks of the discontinuation of treatment.

The early withdrawal follow up visit will consist of enquiries about concomitant medication use and adverse events, and it will include the safety assessments that would be conducted at the normal study follow up visit (V7), and where appropriate, and if the subject agrees to participate, the tests for the secondary and exploratory outcome measures will also be conducted at this visit.

Adverse events which are not related to the study drug or trial procedures and which are not considered serious by the research clinician, which have not fully resolved by the time of the early withdrawal follow up visit, may be designated as unresolved, or partially resolved, in which instance the patient’s doctor will be informed of the open AE and follow up of the AE will be provided by that doctor.

In the case of a withdrawal from the study due to a serious adverse event, non-serious adverse reaction or serious adverse reaction to the study drug or a study procedure, the research clinician must make every reasonable effort to collect information relating to the outcome of the serious adverse event, non-serious

adverse reaction or serious adverse reaction after the last study visit, until the serious adverse event, non-serious adverse reaction or serious adverse reaction has resolved.

The research clinician should use their clinical judgement to determine how to collect the information relating to the outcome of serious adverse events or adverse reactions. For example, the clinician may ask the subject to attend for a further follow up appointment, or they may collect the information by contacting the subject or study partner by letter or by phone, or by contacting the subject's GP, or by contacting any other doctor involved in the care of the subject.

In the case of the early withdrawal of a subject from the study, the Chief Investigator may decide that a final imaging visit could provide useful data for the study, and if the subject agrees to take part in the visit, a final imaging visit could be conducted.

Whenever possible, the final imaging visit should take place within one week prior to the discontinuation of treatment and prior to the early withdrawal follow up visit.

The research clinician will ensure that the patient is provided with adequate follow up after the end of study treatment.

Withdrawn patients will not be replaced.

7. TREATMENT OF SUBJECTS

7.1. Study products and blinding systems

Following the screening period, subjects will receive etanercept (Enbrel) or placebo.

Subjects will have a 50% chance of receiving etanercept (Enbrel) and a 50% chance of receiving placebo. The randomisation will be done by ACE Pharmaceuticals BV using a randomisation computer programme. This information will be kept by ACE Pharmaceuticals BV until the study is completed, or until individual requests are made for un-blinding on the basis of clinical need.

Participants, study partners and the research teams will all be blinded throughout the study, unless individual clinical circumstances arise which require that a subjects medication must be un-blinded (see section 7. 6. Un-blinding procedure)

At the end of the study, or on early withdrawal from the trial, the choice of on-going treatment will be the responsibility of the referring clinician.

7.2. Product administered: etanercept

Etanercept is a widely used, licensed treatment for chronic inflammatory conditions in the elderly, including rheumatoid arthritis, psoriasis, psoriatic arthropathy and ankylosing spondylitis. Treatment doses for adults with these conditions are usually 50mg per week, although up to 100mg per week is advocated in the early treatment stages for subjects with psoriasis.

Etanercept has been associated with infections including tuberculosis and septicaemia, nausea, vomiting, oesophagitis, cholecystitis, pancreatitis, gastro-intestinal haemorrhage, myocardial ischaemia, cerebral ischaemia, venous thromboembolism, hypotension, demyelinating disorders, seizures, bone fracture, renal impairment, polymyositis, bursitis, lymphadenopathy, abdominal pain, worsening heart failure, hypersensitivity reactions, fever, headaches, depression, lupus erythematosus-like syndrome, pruritus, injection site reactions and blood disorders (including anaemia, leucopenia, thrombocytopenia, pancytopenia and aplastic anaemia). There have been rare reports of lymphoma in patients taking etanercept.

7.3. Preparation and Delivery of Trial Medication

The study drug, etanercept (Enbrel) is produced by Pfizer as a 25mg vial of powder for reconstitution. ACE Pharmaceuticals BV will source and purchase the study drug from Pfizer Netherlands. The dose of the study drug, etanercept (Enbrel), is 50mg once weekly, given as a sub-cutaneous injection; therefore, the weekly dose will need to be drawn up from two 25mg vials of powder.

ACE Pharmaceuticals BV will manufacture the placebo as a powder for reconstitution in an identical vial. ACE Pharmaceuticals BV will label both the placebo vials (two per dose, 53 doses) and the study drug vials (two per dose, 53 doses) to ensure blinding.

Participants in the study will be given either a 50mg (drawn up from 2 x 25mg vials) once weekly subcutaneous injection of etanercept or a subcutaneous injection (drawn up from 2 vials) of the placebo.

Injections will be administered by a trained research nurse or a medical doctor engaged in the study. Injections will be given either at the patient's usual residence or at the relevant clinical site.

7.4. Treatment management

The containers containing the therapeutic units for single patients will be sent to the pharmacist at the Memory Assessment and Research Centre (MARC) in Southampton (containers 1 to 31) and the pharmacist at DeNDRoN, Manchester Mental Health and Social Care Trust, The Rawnsley Building, Hathersage Road, Manchester Royal Infirmary, M13 9WL (containers 32 to 46), and stored in a refrigerator (2 - 8^{O}C) in an area of restricted access. The expiry date will appear on each box. (It is possible that circumstances will arise in which one site may not be on schedule to reach their recruitment target number, and the other site may make up the shortfall, with the total remaining at 46. The surplus containers from the site recruiting fewer than targeted numbers will be transferred to the site making up the shortfall and recruiting greater than targeted numbers.)

Drug management will be the responsibility of the research clinician and/or the pharmacist at the respective clinical site.

The treatments will be dispensed by the pharmacist at the relevant clinical site.

The research pharmacist of the healthcare establishment, or any designated person from the study team, must use the study treatments only for the patients involved in the study. All defects or deterioration of treatments or their packaging are to be reported.

In the event of any anticipated return of treatments to the manufacturer (batch recall), the manufacturer will prepare an information letter intended for the research clinician and/or the pharmacist at both clinical sites. On receipt of the letter the research clinician and/or the pharmacist will identify the patients in possession of the treatment at the moment the incident becomes known, by using, among other tools, the treatment tracking form, and will contact them immediately.

7.5. Treatment dispensing and compliance monitoring

No study drug will be administered during the screening period. The study injection will be administered by a study nurse or doctor once a week during the treatment period from V1, week 0, to the final treatment visit, V6, week 52, making a total of 53 injections.

Patients enrolled in the study will be asked to carry a Patient Alert Card (about the size of a credit card) containing details about the study and a contact number for the study centre. This will provide 24 hour access for advice and un-blinding should it be clinically necessary.

7.6. Un-blinding procedure

See addendum 3.

7.7. Previous and concomitant treatments

During the study subjects should not receive any other treatment prone to interfere with the study evaluations. Particular attention should be paid to psychotropic drugs, treatments likely to interfere with the CNS, and immunosuppressant drugs.

Subjects will be advised that they must not receive any live vaccines during the study, or for at least thirty days after the last dose of study medication. (Live vaccines include measles, mumps, rubella, varicella (chicken pox/shingles) and yellow fever. BCG is a living, attenuated form of TB and thus should also not be taken during the study or for at least thirty days after the last dose of study medication.)

Subjects will be advised that they should continue to receive pneumococcal vaccine as prescribed by their General Practitioner.

Subjects can receive their yearly influenza vaccine as prescribed by their GP.

8. ASSESSMENT OF SAFETY

This study is of a widely used licensed drug with a well established risk profile in the elderly population being evaluated. Whilst Individuals in the study will undergo close monitoring for side effects throughout the study, given the low frequency (around 2%) of significant side effects with etanercept (Garcia et al 2014), the small numbers of subjects exposed to etanercept (Enbrel) (n= 23 randomised) in this study are highly unlikely to yield sufficient data for an independent data monitoring committee to be able to assess any differences between groups. Thus an independent data monitoring committee is not being convened. However, an internal monitor will collate a summary of un-blinded data, including AEs, AR's and recruitment rates, from both clinical sites, every 6 months and report these to an independent Trial Steering Committee. If TSC members have concerns about the un-blinded data, such as a high number of SAEs, then the TSC chair will ask the CI to convene an independent un-blinded Data Monitoring Committee to assess.

8.1 Safety measures

8.1.1. Screening period

The following safety measures will be assessed at screening visit, V0:

- Subject's medical and surgical history.
- Checking that the subject satisfies the inclusion and exclusion criteria.
- Physical examination, including a neurological examination, vital signs (blood pressure, heart rate and temperature), body weight, and height.
- An electrocardiogram (ECG) will be done.
- Laboratory tests: the following parameters will be assessed (not fasting):

    - Full blood count (Haemoglobin, haematocrit, erythrocytes, leucocytes, basophils, eosinophils, neutrophils, monocytes, lymphocytes, mean corpuscular volume (MCV) and platelet count)
    - Urea and electrolytes (Sodium, Potassium, Urea, Creatinine, eGFR)
    - Liver function tests (including Total Protein, Albumin, Bilirubin, AST, GGT, LDH, Alkaline Phosphatase)
    - Glucose (non-fasting)
    - C Reactive Protein (CRP)
    - Bone function tests (Corrected Calcium)
    - Thyroid Function Tests (TSH, T4)
    - B12
    - Folate
    - Hepatitis B surface antigen test
    - Urinalysis (This will be done using urinalysis dipsticks. If the dipstick analysis reveals any abnormal findings, the urine sample will be sent for microscopic analysis.)

    - A tuberculosis (TB) screen will be done in accordance with the British Thoracic Societies recommendations relating to screening subjects for TB prior to starting anti-TNFα therapy which were formulated in 2005 (British Thoracic Society Standards of Care Committee (2005)). The emergence of the QuantiFERON-TB Gold assay for screening for TB means that the requirement for a tuberculin skin test (TST) has been replaced by the QuantiFERON-TB Gold assay in everyday clinical practice. The TB screen will be conducted in two steps. The first step is a QuantiFERON-TB Gold assay and the second step is a chest X-ray. A blood sample for the QuantiFERON-TB Gold assay will be taken at

the screening visit. If the QuantiFERON-TB Gold assay is positive the subject will be excluded from the study, and if the assay is negative, the patient will proceed to the second step of the TB screen and will have a chest X-ray. If the radiologist's report on the chest X-ray excludes active or latent TB, then the subject will be adjudged to have passed the TB screen.

The following safety measures will be done at the screening imaging visit (V0i):

- MRI check-list to exclude metal implants and foreign bodies.
- Exclusion of pregnancy in women of child bearing age.
- Check that inclusion/ exclusion criteria are satisfied.

8.1.2. Treatment period

The subject will receive 53 study injections given by a trained research nurse or doctor during the 52 week treatment period. 6 of the study injections will be given during the study clinic visits V1, V2, V3, V4, V5, and V6. The other 46 study injections will be given in the clinic or in the subject's home, by arrangement with the subject, at "Routine injection visits (RIVs)".

The following safety assessments will be done at all 46 of the routine injection visits (RIVs):

- Checking for adverse events: somatic complaints expressed by the subject spontaneously or upon enquiry by the research clinician, will be assessed and recorded. Particular attention will be given to inspecting the injection site of the previous study injection.
- Checking for any changes in concomitant medication, and ensuring that they are in accordance with the study protocol requirements.

The following safety measures will be assessed at the base-line treatment visit (V1), at the four week treatment visit, (V2), at the thirteen week treatment visit, (V3), at the twenty-six week treatment visit, (V4), at the thirty-nine week treatment visit, (V5), at the fifty-two week treatment visit, (V6), and at the week four follow up visit, (V7), and, if required, at the early withdrawal follow up visit, (EWFUV).

- Checking for adverse events: somatic complaints expressed by the subject spontaneously or upon enquiry by the research clinician, will be assessed and recorded.
- Checking for any changes in concomitant medication, and ensuring that they are in accordance with the study protocol requirements.

- Physical examination, including a brief neurological examination, vital signs (blood pressure, heart rate and temperature), body weight.
- Laboratory tests: the following parameters will be assessed (not fasting):
    - Full Blood Count
    - Urea and electrolytes
    - Liver function tests
    - CRP
    - Urinalysis (This will be done using urinalysis dipsticks. If the dipstick analysis reveals any abnormal findings, the urine sample will be sent for microscopic analysis.)

The following safety measures will be done at the final imaging visit (V6i):

- MRI check-list to exclude metal implants and foreign bodies.
- Exclusion of pregnancy in women of child bearing age.
- Check that inclusion/ exclusion criteria are satisfied.

The optional lumbar puncture will take place after a subject has been on the study drug for six months. This can be done at visit 4, or it can be done on a separate day two weeks either side of visit 4, by arrangement with the patient. The following safety measures will be taken with regard to the optional lumbar puncture:

- Subject and study partners will be informed of potential side effects and what action to take in the event of side effects.
- The LP will be done by a suitably trained doctor.

8.1.3. Follow up visit (V7)/ early withdrawal follow up visit (EWFUV):

- Full Blood Count
- Urea and electrolytes
- Liver function tests
- CRP
- Urinalysis (This will be done using urinalysis dipsticks. If the dipstick analysis reveals any abnormal findings, the urine sample will be sent for microscopic analysis.)

8.2 Safety Measurement Methods

Biological parameters (biochemistry, haematology, and serology) will be assessed by a central laboratory.

The blood samples for biology will be collected by the study staff. Results of the biological analyses of the blood samples taken at the screening visit must be available before inclusion.

In the case of a clinically significant abnormality that the Principal Investigator considers to be incompatible with the study continuation, the subject will be withdrawn from the study.

During the study, all clinically significant abnormal laboratory investigations and vital signs that develop in the included subject will be followed until the subject's value returns to normal or its previous baseline level.

8.3 Adverse Events and Adverse Reactions

8.3.1 Adverse Event (AE)

An **adverse event** is an untoward medical occurrence in a participant in a clinical research trial to whom a medicinal product has been administered, including occurrences which are not necessarily caused by or related to that product.

Note: An adverse event can therefore be any unfavourable and unintended sign (including abnormal lab results), symptoms or disease temporally associated with the use of the medicinal product, whether or not considered to be related to the medicinal product.

8.3.2 Adverse Reaction (AR)

An **adverse reaction** is an untoward and unintended response in a participant in a clinical research trial to an investigational medicinal product, which is related to any dose administered to that participant.

Note: Any adverse event judged by either the reporting investigator or the sponsor as having a reasonable causal relationship to an IMP qualifies as an AR. A reasonable causal relationship exists when there is evidence or argument to suggest a causal relationship.

All adverse reactions are adverse events.

8.3.3 Unexpected Adverse Reaction (UAR)

An **Unexpected adverse reaction** is an adverse reaction, the nature and severity of which is not consistent with the applicable product information as described in the

summary of product characteristics for that product (for an approved investigational medicinal product).

Note: Reports which add significant information on specificity or severity of a known, already documented serious adverse reaction constitute unexpected events. For example, when the outcome of an expected adverse reaction is not consistent with the relevant product information, the event may be considered unexpected.

8.3.4 Serious Adverse Events (SAEs)

An adverse event or adverse reaction is defined as **serious** if it:

(a) Results in death
(b) Is life-threatening [1]
(c) Requires hospitalisation
(d) Prolongs a current hospitalisation
(e) Results in persistent or significant disability or incapacity
(f) Consists of a congenital anomaly or birth defect
(g) Is deliberate self-harm
(h) Other (please specify) [2]

[1] *Life threatening in the definition of an SAE or SAR refers to an event in which the subject was at risk of death at the time of the event; not an event that hypothetically might have caused death if it were more severe.*

[2] *Medical judgements should be exercised in deciding whether other AEs may be considered serious because they jeopardize the patient or may require intervention to prevent one of the other outcomes. Examples include blood dyscrasias or convulsions that do not result in hospitalisation, or development of drug dependency or cancer.*

8.3.5 Serious Adverse Reactions (SARs)

A **suspected serious adverse reaction,** the nature and severity of which is consistent with information about the IMP in question presented in the summary of product characteristics for that product (in the case of a product with a marketing authorisation).

8.3.6. Assessment of Adverse Events and Adverse Reactions

Each AE should be assessed for seriousness, intensity, causality and expectedness. This evaluation may be performed by the PI or another medical doctor, acting on behalf of the Sponsor.

8.3.6.1. Seriousness

This is assessed using the criteria detailed in section 8.3.4.

8.3.6.2. Intensity (severity)

The assessment of intensity will be based on the investigator's clinical judgement using the following definitions:

- **Mild:** An event that is easily tolerated by the participant, causing minimal discomfort and not interfering with everyday activities.
- **Moderate:** An event that is sufficiently discomforting to interfere with normal everyday activities.
- **Severe:** An event that prevents normal everyday activities.

Note: **severity** is often used to describe the intensity of a specific event. This is not the same as ***'Seriousness'***, which is based on participant/event outcome or action criteria.

8.3.6.3. Causality

The relationship between the drug and the occurrence of each adverse event will be assessed and categorised (as detailed below). The investigator will use clinical judgement to determine the relationship. Alternative causes, such as natural history of the underlying diseases, concomitant therapy, other risk factors etc. will also be considered. The investigator will also consult the Summary of Product Characteristics (Spc) or other product information.

- **Not related:** Temporal relationship of the onset of the event, relative to administration of the product, is not reasonable or another cause can by itself explain the occurrence of the event.

- **Unlikely:** Temporal relationship of the onset of the event, relative to administration of the product, is likely to have another cause which can by itself explain the occurrence of the event.

- ***Possibly related:** Temporal relationship of the onset of the event, relative to administration of the product, is reasonable, but the event could have been due to another, equally likely cause.

- ***Probably related:** Temporal relationship of the onset of the event, relative to administration of the product, is reasonable, and the event is more likely explained by the product than any other cause.

- ***Definitely related:** Temporal relationship of the onset of the event, relative to administration of the product, is reasonable, and there is no other cause to explain the event, or a re-challenge (if feasible) is positive.

  Where an event is assessed as **possibly, probably, or definitely related, the event is an **adverse reaction.***

  8.3.6.4. Expectedness

  This only applies to adverse reactions. The expectedness of an adverse reaction shall be determined according to the Summary of Product Characteristics (Spc).

- **Expected:** Reaction previously identified and described in protocol and/or reference documents e.g. Summary of Product Characteristics (Spc).

- **Unexpected:** Reaction not previously described in the protocol or reference documents.

  NB: Adverse reactions must also be considered as unexpected if they add significant information on the specificity or severity of an expected adverse reaction.

8.3.7. Reporting of Adverse Events

All the information received about all the adverse events experienced by a subject during the study and any assessments of this information will be documented in the source notes.

Information on adverse events will be collected by a research clinician from subjects and study partners at all treatment visits after screening (V1, V2, V3, V4, V5, and V6), at the follow up visit, (V7), and in the event of an early withdrawal, at the early withdrawal follow up visit, (EWFUV).

Information about adverse events may also be provided by the subjects usual medical care providers.

The research clinician will collate and record the information received from the subject, the study partner and/or the subjects' usual medical care providers, and the research clinician will then review the adverse events to ascertain whether they meet the criteria for 'serious'.

If the event is assessed as being a Serious Adverse Event, or SAE, then the guidance on reporting SAEs and SARs will be followed (Section 8.3.9. below).

8.3.8. Recording Adverse Events and Adverse Reactions in the Case Report Form (CRF)

For adverse events that are not defined as serious, the research clinician will review the events prior to completing the CRF, in order to assess and record intensity, causality and expectedness of the event.

The date of onset and date of resolution, the intensity, causality, expectedness and seriousness of each event, and the action taken with the study drug and any other actions taken, including the administration of concomitant medication due to the event, will be transcribed into the adverse events record in the CRF.

The adverse events record in the CRF can be amended as new information about the event emerges and is recorded in the source documents.

All adverse events will be monitored until the follow up visit, (V7), or, in the case of an early withdrawal, until the early withdrawal follow up visit, (EWFUV).

If an adverse event is assessed as not serious and not related, or unlikely related, to the IMP or to a study procedure, then any AEs of this description which have not resolved by V7 or the EWFUV can be recorded as "unresolved", or "stable but not resolved", and the subject can be discharged from the trial and the follow up of the AE will be the responsibility of the subject's usual medical care provider.

If an adverse event is assessed as being serious, but not related or unlikely related to the IMP, i.e. if it is a serious adverse event (SAE), then the research clinician must make every reasonable effort to collect information relating to the SAE, until the SAE has resolved.

If an adverse event is assessed as being possibly, probably or definitely related to the study drug, i.e. if it is a non-serious or serious adverse reaction (AR or SAR), then the research clinician must make every reasonable effort to collect information relating to the AR or SAR, until the AR or SAR has resolved.

8.3.9. Reporting Serious Adverse Events and Reactions (SAEs and SARs)

If an adverse event or adverse reaction is assessed as 'serious', a designated study doctor will complete a Serious Adverse Event form and must inform the Sponsor within 24 hours of becoming aware of the event.

The SAE report form should be signed and dated by a doctor within the research team delegated to undertake this task. In the absence of a trial doctor being available to assess an adverse event that qualifies as a SAE, a research worker should complete and sign the form, completing as much information as possible, and send the completed form to the Sponsor.

The research clinician should assess causality to the best of their ability. Any SAE received by the Sponsor will be assumed to be definitely study drug related if no causality assessment is completed and may then enter the SUSAR reporting system.

The sponsor must review every SAE within 24 hours of it being received. The review will consist of a review of the seriousness, expectedness, causality and intensity of the event.

The sponsor will make changes to the assessments as appropriate, sign and date the changes, and counter-sign the form and date their signature.

As soon as possible thereafter, a research worker should enter the SAE data In the CRF so that follow-up information will be tracked until event resolution and any missing data will be noted and resolved.

If an event is assessed as not being 'serious', even if it is an adverse reaction, it does not require expedited reporting to the MHRA.

If an event is 'expected', based on the known effects of the study medications as listed in the Summary of Product Characteristics (Spc), it does not require expedited reporting to the MHRA.

In these cases, the sponsor should log receipt of the SAE form, ensure that it has been signed off as not being a SUSAR, and check it has been entered in the CRF by a research worker.

Follow up information will be collected and monitored via the CRF. The follow-up information should describe whether the event has resolved or continues, if and how it was treated, whether the blind was broken or not, and whether the subject continued in the study or withdrew from study participation.

Serious Adverse Reactions will be extracted from the CRF for the annual Development Update Safety Report (DUSR).

The annual Development Update Safety Report (DUSR) will be compiled by the sponsor and sent to the MHRA by the sponsor.

8.3.10. Reporting of Suspected Unexpected Serious Adverse Reactions (SUSARs)

In the event that the sponsor reviews an event and finds that it is a SUSAR, the event must be reported to the MHRA in an expedited manner.

- **Fatal or life-threatening SUSARs:**

Within **seven days** of becoming aware of the event (and as soon as possible), the sponsor, with support from the Southern Health NHS Foundation Trust (SHFT) Research and Development (R&D) Department if required, will report all SUSARs that are assessed as fatal or life-threatening to:

1. The Medicines and Healthcare products Regulatory Agency (MHRA) on the CIOMS form (available from the R&D office) should be sent by fax to 020 70842060[1].

2. The research ethics committee that granted approval (main REC)

- **All other SUSARs**

Within 15 days of becoming aware of the event, the sponsor, with support from the SHFT R&D Department if required, must report all other SUSARs to:

1. The Medicines and Healthcare products Regulatory Agency (MHRA). The CIOMS form (available from http://www.cioms.ch/cioms.pdf or from the R&D office) should be sent by fax to 0207 084 2060.

2. The research ethics committee that granted approval (main REC).

Both the MHRA and the main REC suggest that the expedited SUSAR report be sent to them in the standard CIOMS format which is appropriate for reporting all three scenarios (7 day, 15 day and follow-up SUSARs).

8.3.11. Reporting other safety issues

In addition, other safety issues also qualify for expedited reporting (15 day time frame) where they might alter the current risk-benefit assessment of the IMP or would be sufficient to consider changes in the IMP administration or overall conduct of the trial, for example:

a. Single case reports of an expected serious adverse reaction with an unexpected outcome (e.g. death).
b. An increase in the rate of occurrence of an expected serious adverse reaction, which is judged to be clinically important.
c. Post-study SUSARs that occur after a subject has completed a trial.
d. A new adverse event related to the conduct of the trial or the development of the investigational medicinal product (IMP) that is likely to affect the safety of subjects.
e. A serious adverse event which could be associated with the trial procedures.
f. A significant hazard to the subject population, such as lack of efficacy of an IMP used for the treatment of a life-threatening disease.
g. A major safety finding (e.g. carcinogenicity) from a newly completed animal study.

These safety issues must be reported to the MHRA and the main REC in the format of a letter titled “Safety Report”, and in addition will be reported to the Research and Development Department of the Southern Health NHS Foundation Trust, the hosting NHS organisation.

The sponsor should retain a copy of the expedited report and associated documentation.

The sponsor via the Trial Steering Committee (TSC), will perform an integrated safety analysis of all adverse event information reported and the sponsor will ensure discussions are held and actions undertaken to secure the safety of all subjects. Discussions may result in the expedited reports being submitted and/or the discontinuation of the trial.

8.4. Follow up of serious adverse events (SAEs), adverse reactions and serious adverse reactions (SARs)

The research clinician must ensure that follow up of the subject is appropriate to the nature of the event.

In the case of all serious adverse events, adverse reactions and serious adverse reactions, whenever it is reasonable to do so, the follow up of the subject should continue until resolution.

If follow-up is not directly performed by the research clinician but by a specialist, the subject's general practitioner or the subject's supervising doctor (if hospitalised), the research clinician must establish and maintain contact with the person/department in charge of the follow-up of the subject, to enable any additional information to be reported.

The research clinician will also ensure that any relevant vital information about the subject's clinical condition, treatment and special characteristics (e.g. allergies) recorded in the research source record is replicated in the main, formal Case Record Form (in accordance with guidance from the Chief Medical Officer, Department of Health Gateway reference 11985, 22nd June 2009).

8.5. Procedure for an event requiring immediate notification

In the case of an event requiring immediate notification that occurs:

- During the study.
- During 28 calendar days after the subject's final study visit.
- Beyond these 28 days, irrespective of the time of onset after the end of the study, if the event is likely to due to the research;

The research clinician must:

- Record in the subject's medical file/ research source notes, the date on which the research clinician learnt of the event.
- Transcribe the information regarding the AE and the assessment of the AE that has been recorded in the source notes, into the adverse event record in the CRF.
- Provide any additional information as required as it becomes available.

8.6. Responsibility of the sponsor

Independently of the regulatory obligations of the research clinician, the sponsor must report the event requiring immediate notification to the appropriate authorities and to all the research clinicians involved, according to the requirements in ICH Good Clinical Practice guidelines and local regulations.

9. ASSESSMENT OF EFFICACY

9.1. Imaging assessments

- [11C] *(R)*-PK-111-95 PET scan
- Amyloid AMYVID PET scan

9.2. Clinical Assessments

- Cognitive Function :
  MOCA
  RBANS
  FCSRT-IR

- Assessment of mood and behaviour:
  Cornell Scale
  Apathy Inventory (IA)
  ACDC

- Functional Assessment :
  ADCS-ADL$_{MCI}$

9.3. Inflammatory marker response

The following plasma inflammatory markers will be examined:

- IL-1; IL-4; TNF-α; IFN-γ; IL-6; IL-10; IL-12; IL-13; CRP; TGF-β.

Blood tests for plasma inflammatory markers will be done at the clinical centres (Southampton and Manchester) at clinical visits V3, V4, and V5 and at the Wolfson Molecular Imaging Centre in Manchester at both imaging visits, V0i and V6i.

9.4. Penetration across blood-brain barrier

At 6 months (Optional):

- Concentration of etanercept in cerebrospinal fluid (CSF)
- CSF concentration of acute phase reactants and inflammatory markers

9.5. Optional consent to store samples for future studies

Participants will be asked to consent for the optional storage of serum and CSF samples, for use in future research studies. These samples will be rendered acellular by centrifugation and will therefore be classed as “non-relevant” under the terms of the Human Tissue Act (2004). The stored samples will keep the subject identification number and will be stored for a maximum of 8 years after the end of the study. Any sample remaining at that time will be destroyed safely and securely in accordance with the Human Tissue Act (2004) and local guidelines.

10. EVALUATIONS BY VISIT

10.1. Screening visit, V0

The following procedures will be performed at the screening visit and during the screening period:

- Informed consent
- Inclusion and exclusion criteria review
- Demographic data
- Medical history
- Concomitant medication
- Modified Hachinski Ischaemia Scale
- MRI check-list to exclude metal implants and foreign bodies

- MoCA
- Cornell

- Confirmation of the diagnosis of Mild Cognitive Impairment- due to AD (NIA/ AA criteria, 2011)

- Physical examination
- 12 lead ECG
- Vital signs (blood pressure, heart rate, temperature)
- Height and weight
- Safety blood tests (FBC, U and E, LFTs, CRP) and screening blood tests, including B12, Folate, Thyroid Function Tests, glucose (non-fasting), bone function tests (corrected calcium), Hepatitis B antigens, QuantiFERON-TB Gold assay.
- Urinalysis

- Subject and study partner education on the potential side effects of etanercept, the need to report adverse events and concomitant medication changes, and education about the visit schedule.

- TB screen: arrange CXR if QuantiFERON-TB Gold assay is negative

10.2. Screening and baseline imaging visit (V0i)

All imaging procedures will be performed at the Wolfson Molecular Imaging Centre (WMIC) at the University of Manchester. The team at the WMIC have extensive experience of all of these imaging modalities and their procedures follow well established approved protocols.

MRI scan

Participants will undergo a T1-weighted and inversion-recovery structural, volumetric MRI scan for grey-white matter segmentation, intra-individual co-registration with the PET scans and volumetric studies on the 1.5T MRI scanner. Additionally a T2 weighted sequence for assessment of potential confounding pathology will be performed. The overall MRI scanning time will be below 30 minutes.

Should the MRI scan show a significant cortical or basal ganglia infarct or other significant pathology other than one would expect in MCI patients the patient will not be eligible for further study participation.

Eligible patients will proceed to the licensed Florbetapir F 18 (Amyvid) PET Scan Amyvid PET scan protocol:

Florbetapir F 18 (Amyvid) PET imaging will be performed on a High Resolution Research Scanner in accordance with a previously approved protocol for this procedure at WMIC (ARSAC number RPC 595/3586/30718).

The patient will be weighed and the subject will be asked to empty their bladder prior to the imaging session. Intravenous access will be obtained using a small venflon.

The patient will be placed on the scanner for acclimatization and subsequently positioned into the camera. A seven minute transmission scan will be started.

370 MBq of florbetapir F 18 {± 10%) will be drawn up and assayed with a dose calibrator. The assay results and assay time will be recorded and 370MBq ± 10% of florbetapir F 18 will be injected as a single bolus within 20 seconds, after the transmission scan, followed by an infusion line flush with 15-20 ml of saline. Emission data over 60 minutes will be acquired in list mode. After reconstruction of the image the amyloid scan will be visually assessed by a trained reader.

If the scan is amyloid positive the subject will be considered to have passed all screening procedures and the patient can proceed with the study. Therefore, the patient will proceed to have a baseline [11C] *(R)*-PK-111-95 PET scan on the following day.

[11C] *(R)*-PK-111-95 PET imaging will be performed on a High Resolution Research Scanner in accordance with a previously approved protocol for this procedure at WMIC (ARSAC 595/3586/24989). Venous access will be obtained using a small venflon preferably on non-dominant arm. A blood sample for storage for later inflammatory protein assays will be obtained from this venflon and the patient will be positioned and secured in the scanner. A transmission scan will be performed for approx. 7 min. 740 MBq of [11C] *(R)*-PK-111-95 (target dose; minimum dose 370 MBq) will be drawn and assayed with a dose calibrator. The assay results and assay times will be recorded. Approx. 7 min after the start of the emission scan, 740 MBq [11C] *(R)*-PK-111-95 will be injected as a single bolus within 30 seconds followed by an infusion line flush with 15-20 ml of saline. Emission data over 60 minutes will be acquired in list mode.

Reconstruction of images will follow previous methods with binding potential values and further quantitative analysis performed using a simplified reference tissue model and supervised cluster analysis as previously described (Sue et al 2013). Binding potential maps will be further interrogated and baseline (and follow-up scans) compared to using a region of interest and Statistical parametric mapping approach.

10.3. Base-line treatment visit, V1

(Should a subject discontinue from the study for any reason after having signed the informed consent form, but before randomization, information regarding the demographics of the patient and the reason for discontinuation will be recorded in the Screening Log.)

- Review of inclusion and exclusion criteria
- Review of concomitant medication
- Review of past medical history
- Review of any adverse events that have occurred since the screening visit

- Confirmation of the diagnosis of Mild Cognitive Impairment due to AD-Intermediate likelihood (NIA/AA criteria, 2011)

- Randomization number assignment

- MOCA
- RBANS
- FCSRT-IR
- ADC-ADL$_{MCI}$
- Cornell
- AI and ACDC

- Physical examination and vital signs
- Weight
- Safety bloods (FBC, U and E, LFTs, CRP)
- Urinalysis

- Administration of the first dose of study medication in clinic
- Vital signs 15 minutes after administration of first dose of study medication

10.4. Routine injection visits, RIVs

- Review of concomitant medication
- Review of any adverse events that have occurred, with particular reference to any injection site reactions.

10.5. Week four treatment visit, V2

- Review of concomitant medication
- Review of adverse events

- Physical examination
- Vital signs
- Weight
- Safety bloods
- Blood for ApoE genotyping and other potential genetic markers of MCI progression
- Optional bloods for future research
- Urinalysis

- Compliance

10.6. Week thirteen treatment visit, V3

- Review of concomitant medication
- Review of adverse events

- MOCA
- RBANS
- FCSRT-IR
- ADC-ADL$_{MCI}$
- Cornell
- AI and ACDC

- Physical examination
- Vital signs
- Weight
- Safety bloods
- Bloods for plasma inflammatory markers
- Urinalysis

- Compliance

10.7. Week twenty-six treatment visit, V4

- Review of concomitant medication
- Review of adverse events

- MOCA
- RBANS
- FCSRT-IR
- ADC-ADL$_{MCI}$
- Cornell
- AI and ACDC

- Physical examination
- Vital signs
- Weight
- Blood for plasma inflammatory markers
- Safety bloods
- Urinalysis

- Compliance

- Optional lumbar puncture for CSF collection. This can either be done at the end of V4, or it can be done on a separate day within two weeks either side of visit 4, by arrangement with the patient.

10.8. Week thirty-nine treatment visit, V5

- Review of concomitant medication
- Review of adverse events

- MOCA
- RBANS
- FCSRT-IR
- ADC-ADL$_{MCI}$
- Cornell
- AI and ACDC

- Physical examination
- Vital signs
- Weight
- Safety bloods
- Blood for plasma inflammatory markers
- Urinalysis

- Compliance

10.9. Final imaging visit (V6i) following same procedures as in 10.2

- MRI scan
- Amyvid PET scan
- [11C] *(R)*-PK-111-95 PET scan
- Plasma inflammatory markers

10.10. Week fifty-two treatment visit, V6

- Review of concomitant medication
- Review of adverse events

- MOCA
- RBANS
- FCSRT-IR
- ADC-ADL$_{MCI}$
- Cornell
- AI and ACDC

- Physical examination
- Vital signs
- Weight
- Safety bloods
- Urinalysis

- Compliance

10.10. Follow-up visit, V7

- Review of concomitant medication
- Review of adverse events

- Physical examination
- Vital signs
- Weight
- Safety bloods
- Urinalysis

10.11 Early Withdrawal Follow up Visit, EWFUV

- Review of concomitant medication
- Review of adverse events

- Administration of neuro-psychological tests where appropriate

- Physical examination
- Vital signs
- Weight
- Safety bloods
- Urinalysis

- Plasma inflammatory markers where appropriate

- Compliance

11. DIRECT ACCESS TO SOURCE DATA DOCUMENTS

The research clinician will allow persons responsible for audit, representatives from the Research Ethics Committee and of the regulatory authorities, to have direct access to source data and documents.

12. QUALITY CONTROL AND ASSURANCE

12.1. Study training before the study

It is the responsibility of the study co-ordinator at each of the two clinical research study sites, and of the Principal Investigator at the imaging site, that all study team members are trained in ICH Good Clinical Practice before undertaking any study procedures.

A site initiation visit (SIV) will be held at each of the clinical research study sites, at which members of the site research study team will receive training in the conduct of the study.

Members of the site research study team who cannot attend the SIV meeting can be trained in the conduct of the study by the site study co-ordinator and/or the site Principal Investigator.

Those members of the research study team who will be administering the neuro-psychological rating scales will receive training in the administration of these scales.

All members of the research study team who will work on the study must sign the site delegate's log prior to administering study procedures.

12.2. Study monitoring during the study

The Principal Investigators will allow persons responsible for audit, representatives from the Ethics committee and of the regulatory authorities to:

- inspect the site, the facilities and the material used in the study
- meet all members of the team involved in the study
- consult all of the documents relevant to the study
- check that the case report forms (CRFs) have been filled out correctly
- directly access source documents for comparison of the source data with the data in the case report forms
- verify that the study is carried out in compliance with the protocol and local regulatory requirements

All information dealt with during these visits, will be treated as strictly confidential.

A TSC will be convened that will receive 6 monthly reports on blinded data, including recruitment rates, AE's and AR's from both clinical sites and will be responsible for the oversight of the progress of the trial, the instigation of further un-blinded investigation of unanticipated numbers of AE's and AR's, and will have the power to prematurely close the trial.

The TSC consists of three independent members, (Professor Peter Passmore, Dr Joe Butchart, and Dr Bernadette McGuiness), the CI Prof Holmes and the PI for the second clinical site, Dr Leroi. Professor Passmore will be the independent chair.

13. REGULATORY AND ETHICS APPROVALS

13.1. Ethics Committee Approval

The study protocol, a participant and study partner information sheet, informed consent forms, and all other relevant/ requested documents will be submitted to an independent Ethics Committee by the CI at the same time as the IRAS application is submitted.

The study will not start before written approval by the corresponding ethics committee has been obtained and the local regulation requirements have been complied with.

13.2. Study conduct

The trial will be conducted in compliance with the protocol, ICH Good Clinical Practice (GCP) guidelines (CPMP/ICH/135/95), the European Union Clinical Trials Directive (2001/20/EC), the associated UK Medicines for Human use (Clinical Trials) Regulations (2004), the Data Protection Act (1998), Ethics Committee and MHRA approvals, The Mental Capacity Act, (2005), and other requirements as appropriate.

13.3. Recruitment and Consent

Recruitment will begin once the approvals from the regulatory authority, the Regional Ethics Committee and the local research authority have been received.

The two clinical site study co-ordinators will be responsible for co-ordinating recruitment at each site and ensuring that recruitment methods comply with the legislation and guidelines described in section 13.2.

Both clinical sites will also be using 'Join dementia research' (JDR) as a recruitment tool. This is an on-line self-registration service that enables volunteers with memory problems or dementia, carers of those with memory problems or dementia and healthy volunteers to register their interest in taking part in research. The purpose of JDR is to allow such volunteers to be identified by researchers as potentially eligible for their studies. Researchers can then contact volunteers, in line with the volunteers preferred method of contact, to further discuss potential inclusion.

JDR is funded by Department of Health working in partnership with the charities Alzheimer's Society and Alzheimer's Research UK and is Health Research Authority (HRA) endorsed. The on-line service and all associated documentation, methods of contacting volunteers and handling of data, were reviewed by a specially convened HRA committee which included experts in research ethics, data protection and information governance. Formal endorsement was issued by the HRA in a letter dated 20 May 2014.

The Principal Investigator, or a person designated by the Principal Investigator, is to collect written consent from each subject and their study partner before entry into the study, as described in section 6.3.

13.4. Modification of the study information sheet and informed consent form

Any change to the study information sheet and informed consent form constitutes an amendment and must be submitted for approval to the Research Ethics Committee and if appropriate the regulatory authorities.

13.5. MHRA Approval and Clinical Trial Authorisation

A EudraCT Number, an International Standard Randomised Controlled Trial Number (ISRCTN), and Clinical Trial Authorisation (CTA) application to the MHRA will be submitted by the Chief Investigator on behalf of the sponsor.

14. DATA HANDLING AND RECORD KEEPING

14.1. Study Data

A Case Report Form (CRF) will be designed to record all the data required by the protocol and which will be collected by the study team members.

A Case Report Form is completed for each patient.

The case report forms will be designed in accordance with the relevant legislation and guidelines detailed in section 13.2.

The case report forms will be completed by the Principal Investigator or a member of the research study team who has been delegated to do this.

At the end of each entry, the research study team member must sign and date the Case Report Form in order to attest to the:

- Authenticity of the data collected in the case report form and,
- The coherence between the data in the case report form and the information in the source documents, with the exception of data that is held in the CRF and is regarded as source data, (see section 14.2.)

All corrections and alterations of data on the case report forms must be signed and dated by the Principal Investigator or a research study team member delegated to enter data in the Case Record Files.

14.2. Source data

Some data, including scales, questionnaires and tests, completed by research study team members, the subject or the study partner, will be recorded directly into the Case Report Form and will be regarded as source data.

14.3. Data management

The study data management will be consistent with legislation and guidelines detailed in section 13.2.

Data from the CRFs will be entered into a statistical data file (SPSS) via a data entry system.

Data entry: Blinded, double data entry with a third person resolving any discrepancy between first and second entry.

The total scores of the tests will be automatically calculated in the data base.

14.4. Archiving

The Principal Investigator will keep all information relevant to the study for at least 15 years after the end of the study.

15. FUNDING

The European Union has agreed to provide funding to achieve the following:

- The development of the study design, the identification and procurement of suitable amyloid and microglial ligands for PET imaging, the recruitment of a suitable imaging site and a second clinical site, and the identification and procurement of a suitable medicinal investigatory product, (IMP).

- Allocation of resources to individual sites to ensure that each site has a sufficient number and type of trained research study staff for the safe and proper conduct of the study.
- Recruitment of subjects.
- Collecting clinical data from subjects.
- Collecting and collating clinical data from subject's records.
- Blood collection for inflammatory marker, biochemical and genetic assays
- Inflammatory marker assays
- Ensuring that sufficient funding is available to pay for the costs of travel and accommodation for those subjects required to travel from Southampton to Manchester for the neuro-imaging visits.

Two charities "The Alzheimer's Society" UK and the "Alzheimer Drug Discovery Foundation" are co-funding the purchase of the Investigational Medicinal Product (IMP), the manufacture of the placebo, and the randomisation, labelling, packaging and shipping of the Investigational Medicinal Product (IMP) and placebo.

16. INSURANCE

The Chief investigator and all staff involved with the project will be covered under the Clinical Negligence Scheme for Trusts operated by the Sponsor, The University of Southampton.

17. PUBLICATION POLICY – OWNERSHIP OF THE RESULTS

This study is an investigator-initiated study which is funded by an independent study grant granted to the Chief Investigator.

The data arising from the study will be made available to the INMiND consortium, but the data will remain the property of the University of Southampton and the publication of the data will be the responsibility of the Chief Investigator, who will be free to place any information from the study into the public domain.

18. PROJECT TIMETABLE

April to May 2015	Discussions with participating sites and confirmation of protocol version 1.0, for submission to Ethics and regulatory authorities.
June to August 2015	Ethics and regulatory authorities' approvals.
September 2015	Study commences. Site initiation visits at the clinical study sites. Recruitment commences. Confirmation from the imaging site that they are ready to proceed with study procedures.
September 2015	First subject, first visit.
November 2017	Last subject, last visit.
December 2017	Cytokine analysis.
December 2017	Data analysis.
January 2018	Paper preparation.

The study will take 27 months from September 2015 to December 2017, with the LPLV in November 2017, dependent upon ethical approval, MHRA approval, SHFT R&D Department approval, University of Manchester/Manchester Mental Health and Social Care Trust R&D Department approval, and ACE Pharmaceuticals in-house procedures.

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20. Addendums

20.1. Addendum 1: Visit schedule

	Screening: clinical V0	Screening imaging visit V0i	Base-line Treatment visit V1	Routine Injection Visits (RIVs)	Treatment visit V2 (+/- 5 days)	Treatment visit V3 (+/- 5 days)	Treatment visit V4 (+/- 5 days)	Treatment visit V5 (+/- 5 days)	Final imaging visit V6i	Final treatment visit V6 (+ 5 days)	Follow-up visit V7 (+/- 5 days)	Early withdrawal follow-up visit EWFUV
	Week -8 to 0	Week -8 to 0	Week 0		Week 4	Week 13	Week 26	Week 39	Week 51	Week 52	Week 56	
Informed consent	x											
Incl/ excl criteria	x	x	x									
Demographic data	x		x									
Medical history	x		x									
Modified Hachinski Ischaemia Scale	x											
Con meds at screen	x											
Confirm diagnosis (a)	x		x									
Con med changes			x	x	x	x	x	x		x	x	x
Adverse Events			x	x	x	x	x	x		x	x	x
MRI check-list	x	x							x			
MOCA	x		x			x	x	x		x		x (c)
RBANS			x			x	x	x		x		x (c)
FCSRT-IR			x			x	x	x		x		x(c)
ADC-ADLMCI			x			x	x	x		x		x (c)
IA			x			x	x	x		x		x (c)
ACDC			x			x	x	x		x		x (c)
Cornell	x		x			x	x	x		x		x (c)
Physical examination	x		x		x	x	x	x		x	x	x
Vital signs	x		x		x	x	x	x		x	x	x
Safety bloods (U and E, FBC, LFT, CRP)	x		x		x	x	x	x		x	x	x
Urinalysis	x		x		x	x	x	x		x	x	x

Weight	x	x	x		x	x	x	x	x	x	x	x
ECG (b)	x											
Height	x											
Other screening bloods (B12, Folate, TFT, Hep B, glucose (non-fasting), bone function tests)	x											
QuantiFERON-TB Gold assay	x											
TB screen: CXR	x											
Inflammatory markers		x				x	x	x	x			x
Genotyping			x									
Optional bloods for future research			x									
CSF sample (optional) (d)							x					
Administration of study medication			x	x								
Vital signs 15 minutes post first dose in clinic			x									
Compliance					x	x	x	x		x		x
MRI		x							x			
Amyvid PET scan		x							x			
[11C](R)- PK-111-95 PET scan		x							x			

(a): Confirmation of the diagnosis of “MCI- due to AD” at screen visit, V0, and then confirmation of the diagnosis “MCI- due to AD: Intermediate likelihood” at base-line treatment visit, V1.

(b): ECG to be done at screen visit, V0, and then it can be repeated at any visit, if clinically indicated.

(c): Neuro-psychological tests will be done at an early withdrawal follow up visit, EWFUV, at the discretion of the PI.

(d): The optional lumbar puncture can be done at the end of visit 4, or on a separate day within two weeks either side of visit 4, by arrangement with the patient.

20.2. Addendum 2: Un-blinding procedure for INMiND-02

Code Break Procedure

Objectives: In an emergency situation where it is in the patient's best interest, it may be necessary to break the code which identifies the treatment a patient taking part in a blinded trial is receiving. This procedure provides information for this situation.

Scope: Location of code breaking information, contact names and numbers, how to request a code break.

Procedure:

1. Location of code breaking information

- A complete set of emergency envelopes, which contain the randomisation numbers and associated treatment group, will be kept in a locked drawer in the Research Manager's office, at the Southampton clinical site. These envelopes will allow un-blinding of the IP for an individual patient, while maintaining the overall study blind.

2. Contact names and numbers

- **Southampton clinical site:** During office hours requests for un-blinding will be received by staff in MARC on 02380475206. Out of hours, requests for un-blinding will be received by the person on-call who would be contacted via the out of hour's telephone on 0777 3355 969.

- **Manchester clinical site:** During office hours requests for un-blinding will be received by staff in the research team at DeNDRoN, Manchester Mental Health and Social Care Trust, The Rawnsley Building, Hathersage Road, Manchester Royal Infirmary, M13 9WL; telephone number 0161 276 3314 / 0161 276 3324.Out of office hours requests for un-blinding will go to the Manchester duty doctor, who would be contacted via the Safire Assessment Unit (0161 9223801). The person receiving the request for un-blinding will phone MARC on the above numbers and pass on the request for un-blinding.

3. Breaking the code

- Locate the emergency envelopes and remove the envelope for the individual patient involved.
- Open the sealed envelope and identify whether the treatment was active or placebo.
- Document on the envelope the date, reason for opening, initial and sign the envelope.
- Document in the patient's file all details relating to the un-blinding including who requested the code break, the reason it was required, the date and time the un-blinding occurred and the result of the code break.
- If the patient is a Manchester patient, the MARC team member responsible for the un-blinding will relay this information directly by telephone to the person making the request for un-blinding from the Manchester team, and the person making the request for un-blinding in the Manchester team must also document in the patient's file all details relating to the un-blinding including who requested the code break, the reason it was required, the date and time the un-blinding occurred and the result of the code break.
- Contact the chief investigator and site principle investigator to notify them that a patient has been un-blinded if this has not already been done.