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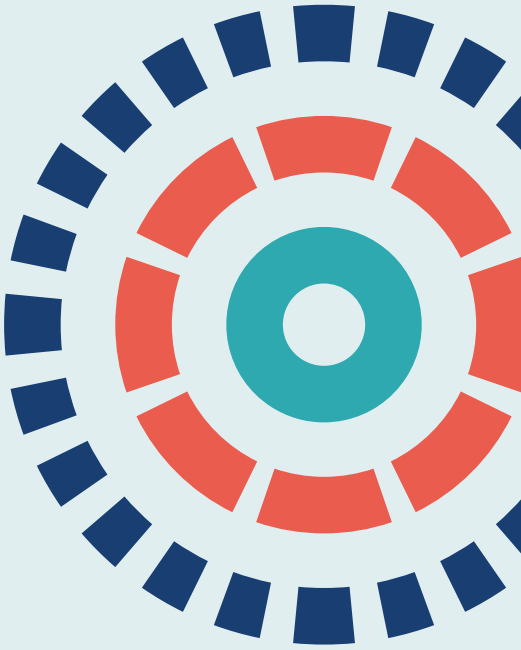

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Minocycline 200 mg or 400 mg versus placebo for mild Alzheimer's disease: the MADE Phase II, three-arm RCT

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Abstract

Minocycline 200 mg or 400 mg versus placebo for mild Alzheimer's disease: the MADE Phase II, three-arm RCT

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Background: Minocycline is an anti-inflammatory drug and protects against the toxic effects of β-amyloid in vitro and in animal models of Alzheimer's disease. To the best of our knowledge, no randomised placebo-controlled clinical trials in patients with Alzheimer's disease looking at the efficacy and tolerability of minocycline have been carried out.

Objectives: The trial investigated whether or not minocycline was superior to placebo in slowing down the rate of decline in cognitive and functional ability over 2 years. The safety and tolerability of minocycline were also assessed.

Design: A Phase II, three-arm, randomised, double-blind, multicentre trial with a semifactorial design. Participants continued on trial treatment for up to 24 months.

Setting: Patients were identified from memory services, both within the 32 participating NHS trusts and within the network of memory services supported by the Dementias and Neurodegenerative Diseases Research Network (also known as DeNDRoN).

Participants: Patients with standardised Mini Mental State Examination scores of > 23 points and with Alzheimer's disease assessed by the National Institute on Aging–Alzheimer's Association's criteria were identified from memory services.

Intervention: Patients with mild Alzheimer's disease were randomly allocated 1 : 1 : 1 to receive one of three treatments: arm 1 – 400 mg per day of minocycline; arm 2 – 200 mg per day of minocycline; or arm 3 – placebo. Patients continued treatment for 24 months. Participants, investigators and outcome assessors were blind to treatment allocation.

Main outcome measures: Primary outcome measures were decline in standardised Mini Mental State Examination and Bristol Activities of Daily Living Scale scores of combined minocycline treatment arms versus placebo, as analysed by intention-to-treat repeated measures regression.

Results: Between 23 May 2014 and 14 April 2016, 554 participants were randomised. Of the 544 eligible participants, the mean age was 74.3 years and the average standardised Mini Mental State Examination score was 26.4 points. A total of 252 serious adverse events were reported, with the most common categories being neuropsychiatric and cardiocirculatory. Significantly fewer participants completed treatment with 400 mg of minocycline [29% (53/184)] than 200 mg [62% (112/181)] or placebo [64% (114/179)] ($p < 0.0001$), mainly because of gastrointestinal symptoms ($p = 0.0008$), dermatological side effects ($p = 0.02$) and dizziness ($p = 0.01$). Assessment rates were also lower in the 400-mg treatment arm: 68% (119 of 174 expected) for standardised Mini Mental State Examination scores at 24 months, compared with 82% (144/176) for the 200-mg treatment arm and 84% (140/167) for the placebo arm. Decline in standardised Mini Mental State Examination scores over the 24-month study period in the combined minocycline arms was similar to that in the placebo arm (4.1- vs. 4.3-point reduction; $p = 0.9$), as was the decline in the 400- and 200-mg treatment arms (3.3 vs. 4.7 points; $p = 0.08$). Likewise, worsening of Bristol Activities of Daily Living Scale scores over 24 months was similar in all trial arms (5.7, 6.6 and 6.2 points in the 400-mg treatment arm, 200-mg treatment arm and placebo arm, respectively; a *p*-value of 0.57 for minocycline vs. placebo and a *p*-value of 0.77 for 400 vs. 200 mg of minocycline). Results were similar in different patient subgroups and in sensitivity analyses adjusting for missing data.

Limitations: Potential limitations of the study include that biomarkers were not used to confirm the diagnosis of Alzheimer's disease, as these and apolipoprotein E (*APOE*) genotyping are not routinely available within the NHS. Compliance was also worse than expected and differential follow-up rates were observed, with fewer assessments obtained for the 400-mg treatment arm than for the 200-mg treatment and placebo arms.

Conclusions: Minocycline does not delay the progress of cognitive or functional impairment in people with mild Alzheimer's disease over a 2-year period. Minocycline at a dose of 400 mg is poorly tolerated in this population.

Future work: The Minocycline in mild Alzheimer's DiseasE (MADE) study provides a framework for a streamlined trial design that can be usefully applied to test other disease-modifying therapies.

Trial registration: Current Controlled Trials ISRCTN16105064 and EudraCT 2013-000397-30.

Funding: This project was funded by the Efficacy and Mechanism Evaluation programme, a Medical Research Council and National Institute for Health Research (NIHR) partnership, and will be published in full in *Efficacy and Mechanism Evaluation*; Vol. 7, No. 2. See the NIHR Journals Library website for further project information.

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List of abbreviations

Aβ	β-amyloid
AD	Alzheimer's disease
ALS	amyotrophic lateral sclerosis
BADLS	Bristol Activities of Daily Living Scale
DeNDRoN	Dementias and Neurodegenerative Diseases Research Network
GP	general practitioner
IMP	investigational medicinal product
MADE	Minocycline in Alzheimer's Disease Efficacy
MMSE	Mini Mental State Examination
MR	modified release
NDPH	Nuffield Department of Population Health
NIA–AA	National Institute on Aging–Alzheimer's Association
NIHR	National Institute for Health Research
NRES	National Research Ethics Service
NSAID	non-steroidal anti-inflammatory drug
PI	principal investigator
QP	qualified person
REC	Research Ethics Committee
SAE	serious adverse event
SD	standard deviation
SLE	systemic lupus erythematosus
sMMSE	standardised Mini Mental State Examination
TSC	Trial Steering Committee

Plain English summary

Alzheimer's disease affects about 700,000 people in the UK and, although there are drug treatments that can modestly improve some of the symptoms, we do not yet have any treatments that slow down the progression of dementia.

Minocycline is an antibiotic that has been shown to protect brain cells in a number of experimental and animal models of Alzheimer's disease. Minocycline is cheap and well tolerated. If it could significantly slow down the course of Alzheimer's disease, it could quickly be made available to large numbers of people with Alzheimer's disease worldwide. Although minocycline is probably one of the best current candidates for Alzheimer's disease modification, the current evidence can only suggest a potential benefit.

A clinical trial was conducted to determine definitively whether or not minocycline is effective in slowing the decline in Alzheimer's disease. Long-term treatment effects of minocycline were investigated, with two doses of minocycline, on decline in cognitive function, including memory, attention and language, and ability to carry out essential functions of daily living, such as getting dressed, grooming and eating.

Unfortunately, the study found that minocycline treatment did not have any measurable effect in slowing down the progression of Alzheimer's disease. Participants who took minocycline showed exactly the same worsening of their cognitive functioning and activities of daily living as those who were allocated to placebo treatment. The trial also established that minocycline at the high dose is poorly tolerated in patients with Alzheimer's disease, whereas the low dose of minocycline is well tolerated, with participants being no more likely to withdraw from trial medication than those taking placebo.

One limitation of the study is that biomarkers were not used to confirm Alzheimer's disease diagnosis, as tests for biomarkers are not routinely available within the NHS. Compliance with medication was also worse than expected, with few patients in the high-dose arm completing 2 years' treatment and only moderate compliance in the low-dose and placebo treatment arms. It was difficult to obtain outcome assessments that resulted in unequal numbers of completed assessments across treatment arms, which could have biased the study's results. Having said that, additional analyses investigating potential bias have, reassuringly, shown the same pattern of results.

Although disappointing, these results are important because they will guide further research into the search for a treatment. There is currently much interest in treating inflammatory changes in the brain in Alzheimer's disease and, as minocycline is a potent anti-inflammatory drug, the study's results will show researchers which pathways they should focus on.

Scientific summary

Background

Alzheimer's disease is a major public health issue, with approximately 700,000 people in the UK suffering from dementia, some 400,000 of whom have Alzheimer's disease. The imperative to discover and develop treatments that can stop or at least delay disease progression is clear. None of the drug treatments licensed for Alzheimer's disease has been shown to affect progression of the illness and, despite a better understanding of the pathogenesis of Alzheimer's disease, clinical trials of potentially disease-modifying treatments so far undertaken have had disappointing results.

There is a substantial body of evidence to indicate that minocycline may be neuroprotective in neurodegenerative diseases such as Alzheimer's disease. Although the primary neuroprotective target of minocycline in the central nervous system is not known, the principal effects of minocycline include inhibition of microglial activation, attenuation of apoptosis and suppression of the production of reactive oxygen species. Minocycline is arguably the most promising off-patent candidate for Alzheimer's disease modification that is not currently in trials. Furthermore, minocycline is cheap and well tolerated.

Objectives

The Minocycline in Alzheimer's Disease Efficacy (MADE) trial was a multicentre, randomised controlled trial in very mild Alzheimer's disease that primarily aimed to determine whether or not minocycline is superior to placebo in affecting the disease course, over a 2-year period, as measured by rate of decline in cognition (assessed via the standardised Mini Mental State Examination score) and function (assessed via the Bristol Activities of Daily Living Scale score). The study also compared the safety and tolerability of minocycline at doses of 200 and 400 mg per day.

Methods

The MADE study was a Phase II, three-arm, randomised, double-blind, multicentre trial with a semifactorial design. Patients with very mild Alzheimer's disease (as assessed by having a standardised Mini Mental State Examination score of > 23 points and assessed by the National Institute on Aging–Alzheimer's Association's criteria) were identified from memory services, both within the 32 participating NHS trusts and within the network of memory services supported by the Dementias and Neurodegenerative Diseases Research Network.

Inclusion criteria were:

- a diagnosis of National Institute of Neurological and Communicative Disorders and Stroke–Alzheimer's Disease Related Disorders Association (NINCDS–ADRDA)-possible or -probable Alzheimer's disease
- a standardised Mini Mental State Examination score of > 23 points
- consent to participate or agreement to participate if capacity to give informed consent was lost
- renal and hepatic function within normal limits
- taking Alzheimer's disease medication (i.e. memantine or cholinesterase inhibitor) on a stable dose for at least 8 weeks.

Exclusion criteria were:

- a known allergy to tetracycline antibiotics
- a serious or unstable medical condition that would represent contraindication to taking trial medication.

Following informed consent and completion of baseline assessment, participants were randomly allocated 1 : 1 : 1 to one of three treatment arms: arm 1 – 400 mg per day of minocycline; arm 2 – 200 mg per day of minocycline; or arm 3 – placebo. Participants continued treatment for 24 months. Participants, investigators and outcome assessors were blind to treatment allocation.

Primary outcome measures were the decline in the standardised Mini Mental State Examination and the Bristol Activities of Daily Living Scale scores of combined minocycline trial arms versus placebo. Outcomes were analysed by intention-to-treat repeated measures regression.

The secondary research objectives were to:

- establish safety and tolerability of minocycline at doses of 200 and 400 mg per day in patients with mild Alzheimer's disease
- establish whether or not 400 mg per day of minocycline offers superior neuroprotection than 200 mg per day of minocycline
- estimate the magnitude of effect sizes on cognitive and functional decline associated with any statistically significant positive treatment effects that will inform the design and powering of a future Phase III trial of definitive clinical effectiveness within the NHS.

Results

Between 23 May 2014 and 14 April 2016, 554 participants from 32 UK memory clinics were randomised. For the 544 eligible participants, the mean age was 74.3 years and the average standardised Mini Mental State Examination score was 26.4 points. Significantly fewer participants completed 400 mg of minocycline treatment (29%, 53/184) than 200 mg of minocycline treatment (62%, 112/181) or placebo (64%, 114/179) ($p < 0.0001$), mainly because of gastrointestinal symptoms ($p = 0.0008$), dermatological side effects ($p = 0.02$) and dizziness ($p = 0.01$). Assessment rates were also lower in the 400 mg of minocycline treatment arm for standardised Mini Mental State Examination scores at 24 months [68% (119/174 expected) for 400 mg of minocycline vs. 82% (144/176) for 200 mg of minocycline vs. 84% (140/167) for placebo]. Decline in the standardised Mini Mental State Examination scores over the 24-month study period in the combined minocycline trial arms were similar to those in the placebo arm (4.1- vs. 4.3-point reduction; $p = 0.9$), as was the decline in the 400 and 200 mg of minocycline treatment arms (3.3 vs. 4.7 points; $p = 0.08$). Likewise, worsening of Bristol Activities of Daily Living Scale scores over 24 months was similar in all treatment arms (5.7 for the 400 mg of minocycline treatment arm, 6.6 for the 200 mg of minocycline treatment arm and 6.2 for the placebo arm; a *p*-value of 0.57 for minocycline vs. placebo, and a *p*-value of 0.77 for 400 vs. 200 mg of minocycline). Results were similar in different patient subgroups and in sensitivity analyses adjusting for missing data.

Conclusions

The MADE trial has shown that, in patients with mild Alzheimer's disease, 24 months of minocycline treatment at the doses tested does not delay the progress of cognitive or functional impairment, as measured by the well-validated and widely used standardised Mini Mental State Examination and Bristol Activities of Daily Living Scale clinical rating scales. The trial has also established that

minocycline at a dose of 400 mg is poorly tolerated in this population, with fewer than one-third of participants completing 24 months' treatment. By contrast, 200 mg per day of minocycline is well tolerated, with participants allocated this treatment being no more likely to withdraw from trial medication than those taking placebo.

Future work

The MADE study provides a framework for a streamlined trial design that can be usefully applied to test other disease-modifying therapies.

Trial registration

This trial is registered as ISRCTN16105064 and EudraCT 2013-000397-30.

Funding

This project was funded by the Efficacy and Mechanism Evaluation programme, a Medical Research Council and National Institute for Health Research (NIHR) partnership, and will be published in full in *Efficacy and Mechanism Evaluation*; Vol. 7, No. 2. See the NIHR Journals Library website for further project information.

Chapter 1 Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder that currently affects 50 million people worldwide,[1] with projected numbers of affected people reaching 135.5 million by 2050 and associated costs, just for the USA, at US$1.2T.[2] At the Dementia Summit in 2013, the G8 health ministers committed to identifying a cure or a disease-modifying therapy for dementia by 2025,[2] but no treatments have so far been shown to delay the progression of cognitive and functional disability that characterises AD. Failure of treatment approaches directed at preventing the associated build-up of β-amyloid (Aβ) or tau protein deposits has stimulated investigation of alternative treatment approaches, including targeting inflammation in the brain.

The risk of developing AD is associated with immune-related and inflammatory genes, including genes coding for myeloid-specific sialic acid-binding receptor (*CD33*), triggering receptor expressed on myeloid cell 2 (*TREM2*), complement receptor 1 (*CR1*) and bridging integrator 1 (*BIN1*).[3] Microglial activation is increased in AD.[4] Aβ is a pro-inflammatory agent in AD[5] and several microglial surface receptors are also Aβ receptors.[6] In the early stages of AD, microglia clear Aβ by phagocytosis and produce Aβ-degrading enzymes.[7] However, as AD pathology progresses, accumulation of Aβ stimulates the microglial production of pro-inflammatory agents, which drives further neurodegeneration.[7]

Two independent systematic reviews of mechanisms, tolerability, brain penetration, epidemiology and early phase trial efficacy data on several classes of repositioned drugs have identified minocycline among the most promising of these agents to progress to clinical trials in patients with AD.[8,9] Minocycline is an anti-inflammatory tetracycline that crosses the blood–brain barrier and inhibits the pro-inflammatory functions of microglia, and there is a substantial body of evidence to indicate that minocycline may be neuroprotective in neurodegenerative diseases. Although the primary neuroprotective target of minocycline in the central nervous system is not known, the principal effects of minocycline include inhibition of microglial activation, attenuation of apoptosis and suppression of the production of reactive oxygen species.[10] In vitro, minocycline protects against Aβ-induced cell death and prevents fibrillisation of Aβ.[11] In transgenic mice, minocycline prevents Aβ deposition and neuronal death,[12] reduces tau phosphorylation and insoluble tau aggregates,[13] downregulates inducible nitric oxide synthetase, cyclo-oxygenase-2 and Aβ precursor protein-cleaving enzyme 1 (BACE1)[14] and protects hippocampal neurogenesis in the presence of Aβ.[15] Minocycline reduces interleukin and tumour necrosis factor alpha (TNFα) levels in mice,[16] and neuronal death and learning deficits in rats, following Aβ administration.[17] In stroke patients, open-label treatment with 200 mg per day of minocycline for 5 days after infarct has been reported to improve functional outcome.[18] In animal models of Parkinson's disease, studies have reported both reduced microglial activation and neuronal death[19,20] and reduced microglial activation and worsened neuronal death.[21,22] Pilot clinical trials in Parkinson's disease at a dose of 200 mg per day of minocycline over 18 months have shown no effect on symptoms and no significant increase in adverse events.[23]

Minocycline treatment in the superoxide dismutase 1 transgenic mouse model for amyotrophic lateral sclerosis (ALS) delayed the onset of neurodegeneration and muscle strength decline.[24] A completed Phase III trial in ALS, however, reported worse outcomes with minocycline in terms of faster decline in forced vital capacity and manual muscle strength.[25]

Suggested explanations for this faster decline is that the dose of up to 400 mg per day of minocycline may have contributed to fatigue in a highly susceptible population, and that increased levels of glutamate receptor 1 phosphorylation may have promoted glutamate toxicity to motor neurons.[26] In AD, both in vitro and in vivo studies have shown reduced microglial activation, attenuated neuronal death, astrogliosis and improved behavioural performance.[11–13,17,27–31] However, to date, there have been no published clinical trials in AD patients and none is currently registered as recruiting.

The minimum daily dose of minocycline that offers neuroprotection in humans has not been established. A dose of 200 mg per day of minocycline is generally very well tolerated in the long-term treatment of acne[32] and has been shown to be neuroprotective in acute stroke,[18] spinal cord injury[33] and multiple sclerosis.[34] However, 200 mg per day of minocycline, although well tolerated, did not improve outcomes in trials in Parkinson's disease[23] or Huntington's disease.[35] Some authors have argued that one reason for the failure of some trials may be that such doses of minocycline are too low to be neuroprotective, pointing out that the typical effective dose in animal studies would be equivalent to 3–7 g per day in humans.[10] It would not be feasible or ethical to subject AD participants to such very high doses of minocycline, but the Minocycline in Alzheimer's Disease Efficacy (MADE) trial includes a comparison of 400 mg per day with 200 mg per day to investigate the tolerability of 400 mg per day and whether or not the higher dose confers increased efficacy.

Alzheimer's disease is a major public health issue and the imperative to discover and develop treatments that can stop or at least delay disease progression is clear. Symptomatic AD treatments in the form of cholinesterase inhibitors and memantine have been the mainstay of current treatment for > 10 years but do not slow progression of the disease. With a more detailed understanding of the basic biology of the AD process, a wide range of cellular and animal model systems have been developed within which several candidate disease-modifying treatments appear promising, though no such agent has performed successfully in Phase III trials.[36,37] Unfortunately, the development of treatments for AD is a complex and difficult process. The slowness of the neurodegenerative process and the substantial difficulties involved in demonstrating that the process has been changed by treatment are major contributors to this problem. Minocycline was arguably the most promising off-patent candidate for AD modification that was not yet in trials, and it was cheap and well tolerated. The time was known to be right for an adequately powered clinical trial, conducted for a sufficiently long period to demonstrate efficacy on simple cognitive and functional outcomes. The results, even if clearly negative, could be expected to move the field on a significant degree.

Based on this wealth of preclinical research suggesting neuroprotection, this trial investigated whether or not minocycline slows decline in cognitive and functional ability in people with mild AD over a 2-year treatment period. The safety and tolerability of minocycline was also compared at doses of 200 mg and 400 mg per day.

Chapter 2 Methods

Objectives

The MADE study was a multicentre randomised controlled trial with the following objectives.

The primary objective was to determine whether or not minocycline is superior to placebo in slowing the disease course of early AD, over a 2-year period, as measured by rate of decline in cognition [as measured by the standardised Mini Mental State Examination (sMMSE)] and function [as measured by the Bristol Activities of Daily Living Scale (BADLS)].

The secondary objectives of the MADE trial were to:

- compare the safety and tolerability of minocycline at doses of 400 and 200 mg per day
- determine whether or not 400 mg per day offers superior neuroprotection to 200 mg per day
- investigate associated risks of side effects and serious adverse events
- estimate the magnitude of any statistically significant positive treatment effects on cognitive and functional decline and, thereby, inform the design and powering of a future Phase III trial of definitive clinical effectiveness within the NHS.

Trial design

The MADE study was a multicentre, randomised, double-blind trial for patients with very mild AD. It had a semifactorial design in which participants were allocated to one of three treatment arms:

- arm 1 – 400 mg per day of minocycline
- arm 2 – 200 mg per day of minocycline
- arm 3 – placebo.

Trial treatment continued for a period of up to 2 years.

A summary of all changes to the protocol, including amendments to the inclusion/exclusion criteria and safety monitoring protocol, are presented in *Appendix 1*.

Ethics approval and research governance

The MADE trial was conducted in accordance with the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use – Good Clinical Practice guidelines and the Declaration of Helsinki.[38] The study protocol, patient and caregiver information sheets and informed consent forms were approved by East of England/Essex Research Ethics Committee (REC; reference number 13/EE/0063) and the Medicines and Healthcare Products Regulatory Agency (reference number 14523/0246/001-0005). Ethics approval for the trial was given by the National Research Ethics Service (NRES) Committee East of England on 1 May 2013 (reference number 13/EE/0063). Local REC approval and the appropriate site-specific assessments were obtained from each of the 32 participating NHS trusts (see *Acknowledgements* for a full list of participating trusts). The trial was registered with the International Standard Randomised Controlled Trial Register (ISRCTN) with the reference number ISRCTN16105064. Trial conduct was overseen by independent Data Monitoring and Trial Steering

Committees. The protocol for the trial can be found on the National Institute for Health Research (NIHR)'s management information system portal.

Outcomes

Standardised Mini Mental State Examination

The first primary outcome measure was the sMMSE,[39,40] a widely used clinician-rated instrument for assessing cognition. Scores range from 0 to 30 points, with higher scores indicating better cognitive function.[41] The original Mini Mental State Examination (MMSE) was designed as a brief test to detect organic brain disease and quantify the degree of cognitive impairment. It is still probably the most widely used cognitive test in the world[42] and has good psychometric properties.[43] The sMMSE was developed to provide raters with explicit guidelines for administration and scoring, with the aim of improving the reliability of the instrument. The sMMSE differs from the MMSE in four main areas: serial sevens are omitted, the order of the time orientation questions is changed, for all questions a response time limit is imposed and for each item unambiguous scoring rules are given. The sMMSE score is considered to be of clinical relevance, with the minimum clinically important difference estimated to be 1.4 points.[44] The sMMSE has been shown to be sensitive to the effects of anti-dementia drug treatment in previous AD clinical trials.[45–47]

Standardised Mini Mental State Examination data were collected at screening and at 6, 12, 18 and 24 months.

Bristol Activities of Daily Living Scale

The second outcome measure is the BADLS,[48] used to assess functional ability (activities of daily living). Scores range from 0 to 60 points, with higher scores indicating greater impairment. The BADLS was specifically designed for use with dementia patients living in the community and participating in clinical trials. The BADLS is sensitive to change, correlates well with economic outcomes and, despite being a carer-rated instrument, appears to have good test–retest reliability. The levels of disability between which the scale aims to discriminate were also carer generated, giving some perspective on the value of change, with the minimum clinically important difference estimated to be 3.5 points.[44] The BADLS has also been shown to be sensitive to change across a wide range of functional disability in previous AD clinical trials.[45,49]

Bristol Activities of Daily Living Scale data were collected at baseline and at 6, 12, 18 and 24 months.

Participants

Patients were identified from NHS Memory Services at 32 university and general mental health trusts in England and Scotland. The inclusion and exclusion criteria used to select patients are detailed in the next two sections.

Inclusion criteria

The inclusion criteria include patients:

- with a diagnosis of possible or probable AD by National Institute on Aging–Alzheimer's Association's (NIA–AA) criteria[50]
- with a sMMSE score of > 23 points, with no upper limit
- giving informed consent to participate
- aged ≥ 50 years
- who have a potential informant who will assist in the administration of the BADLS.

Exclusion criteria

The exclusion criteria include patients:

- with a known allergy to tetracycline antibiotics
- of childbearing potential, that is, female patients who have not been surgically sterile (via hysterectomy, bilateral salpingectomy/oophorectomy) for a minimum of 6 months or undergone bilateral tubal occlusion/ligation at least 6 months prior or been postmenopausal for at least 1 year
- who have an uncontrolled serious concomitant illness
- who have a known chronic kidney disease stages 3b–5
- with moderate liver disease (based on the Child–Pugh Classification for Severity of Liver Disease)
- with abnormal serum chemistry laboratory values at screening, which are deemed to be clinically relevant by the chief investigator
- who withhold consent for the study team to inform his/her general practitioner (GP)
- having systemic lupus erythematosus (SLE)
- participating in another clinical trial of an investigational medicinal product (IMP) in the previous 28 days.[41]

Recruitment procedure

Patients with very mild (as defined by a sMMSE score of > 23 points) AD (as defined by NIA–AA criteria[50]) were identified from memory services, both within the participating NHS trusts (see below for a comprehensive list of all participating NHS sites) where the principal investigators practised and within the network of memory services supported by the Dementias and Neurodegenerative Diseases Research Network (DeNDRoN). Potentially eligible patients were approached by a clinician who knew them and were provided with an opportunity to hear more about research. The Join Dementia Research recruitment tool was also used. Those patients interested met with a member of the research team, who provided further information about the study. Potentially interested individuals were given an opportunity to review the information, ask questions over the telephone and arrange a date for interview. Written informed consent was obtained prior to commencing the screening assessment for the trial and after the individuals had received the information sheet.

Screening

The diagnosis and provisional eligibility for the study was first confirmed using the inclusion/exclusion criteria listed in *Inclusion criteria* and *Exclusion criteria*. The sMMSE was performed and this score was also used as the baseline value. Blood was taken for full blood count and biochemical profile analysis. The screening blood analysis results and concomitant medicines were reviewed and recorded to confirm eligibility before randomisation.

Trial treatment

Trial treatment consisted of oral minocycline modified-release (MR) capsules or identically appearing placebo packed into treatment cartons sufficient for 13 weeks' treatment (with a small overage). The dosing regimens for the three treatment arms were:

- arm 1 – minocycline (400 mg): two MR 100-mg capsules of minocycline in the morning and two MR 100-mg capsules in the evening
- arm 2 – minocycline (200 mg): one MR 100-mg capsule of minocycline plus one minocycline placebo capsule in the morning and one MR 100-mg capsule of minocycline plus one minocycline placebo capsule in the evening
- arm 3 – placebo minocycline: two minocycline placebo capsules in the morning and two placebo minocycline capsules in the evening.

Treatment packs were supplied on a 3-monthly basis for a total treatment duration of 24 months. Participants, their carers, prescribing clinicians, outcome assessors and all MADE trial staff (except statisticians) were masked to trial arm assignment.

Treatment preparation

Modepharma Ltd (Beckenham, UK) was responsible for arranging the IMP's manufacture, as well as project management and assistance relating to the IMP for the trial, including preparation of the IMP Dossier (IMPD). The actual manufacturing of placebo, all IMP packaging and labelling, and final qualified person (QP) release of the IMPs were undertaken by Piramal Healthcare UK Ltd (licence number 29595; Morpeth, UK).

Acnamino™ (Dexel®-Pharma Ltd, Daventry, UK) MR 100-mg capsules were used as the active treatment. These capsules were procured and supplied to Piramal Healthcare for IMP packaging by Modepharma. Acnamino MR 100-mg capsules are hard gelatin capsules each containing one pink film-coated tablet and one peach enteric-coated tablet. Each capsule contained 100 mg of the active substance minocycline as minocycline hydrochloride. Placebo tablet intermediates were made using similar tablet tooling and film-coating colour to the tablets in the active Acnamino MR 100-mg capsule. The blinding of the placebo product was achieved by using the same capsule size and similar gelatin capsule body and cap colours as the Acnamino MR 100-mg capsule. Placebo blister strips and patient treatment packs matched those of the active substance and were labelled in the same way.

Packaging and labelling

Both active and placebo IMPs were packaged under QP control by Piramal Healthcare UK Ltd. Capsules were packed in blister strips of 27 capsules, with a colour-coded Annex 13-compliant label.[51] Seven blister strips (i.e. 189 capsules) were placed in a carton, each with its own individual randomisation number (or treatment pack number) and colour-coded Annex 13-compliant label. Patients were given two cartons, each containing seven blister strips, at randomisation and every 3 months subsequently. Two cartons made up a 3-month (13-week) supply. Patients were directed to take one capsule from one carton and one capsule from the other carton every morning and every evening. As the capsules and blisters looked the same, for patients' ease, the labels used on the first and second cartons (and blisters) had two different colours.

Storage and dispensing

Batches of treatment packs were distributed to participating trial pharmacies by Polar Speed (Leighton Buzzard, UK). Drug supplies were kept in a secure, limited-access storage area, in their original packaging and under the authorised storage conditions for the Acnamino MR 100-mg capsules, and instructions stated 'Store in the original package'. Trial participants were advised to store medication at ambient temperature and out of the reach of children. All unused medication was destroyed by the site pharmacy. Receipt, usage and destruction was monitored and documented throughout the trial on the respective forms. Account was given for discrepancies.

Unblinding

Investigators and patients remained blinded to the treatment allocation throughout the trial. Unblinding was not normally necessary as serious side effects were dealt with on the assumption that the patient was on active minocycline treatment. Study medication was omitted rather than unblinded. If considered urgently necessary for patient management, a 24-hour unblinding service was available (personal communication).

Concomitant medication

Patients could be randomised into the MADE study while taking a cholinesterase inhibitor or memantine. Cholinesterase inhibitors and memantine could also be commenced or discontinued during the course of the study at the discretion of the responsible consultant.

Other concomitant medications that may interact with minocycline [listed in the summary of product characteristics (SmPC) for minocycline and summarised below] were recorded at each visit and the prescriber informed:

- angiotensin-converting enzyme (ACE) inhibitors – absorption of minocycline decreased by quinapril tablets
- antacids, adsorbents and vitamin/mineral supplements – absorption of minocycline is impaired by the concomitant administration of antacids, iron, calcium, aluminium, magnesium and zinc salts (interactions with specified salts, antacids and kaolin) unless taken 3 hours apart; dosages should be maximally separated
- antibacterials – minocycline can decrease the effectiveness of penicillins
- anticoagulants – tetracyclines depress plasma prothrombin activity and reduced dosages of concomitant anticoagulants may be necessary
- diuretics – may aggravate nephrotoxicity by volume depletion
- ergotamine and ergometrine – increased risk of ergotism
- retinoids – administration of isotretinoin should be avoided shortly before, during and shortly after minocycline therapy as the combined administration of the two drugs increases the risk of benign intracranial hypertension
- ulcer-healing drugs – absorption of minocycline is decreased by sucralfate and bismuth salts.

Side effects

If side effects were reported their significance was discussed with the study doctor. Depending on severity, participants were asked to continue with the study drug if possible and a review by the study doctor arranged in 2 weeks. If, at the time of the review, the side effects were severe enough to warrant withdrawal from the study, participants were advised to omit the morning dose and a further review arranged in 2 weeks. If side effects persisted, participants were advised to take a temporary (e.g. 2-week) break from IMP treatment and were reminded to restart once the symptoms resolved. If side effects persisted, then participants were advised to stop taking the study drug.

Treatment compliance

Treatment compliance was monitored by capsule count at the 6-, 12-, 18- and 24-month visits and monitored over the telephone at week 2, and then at months 3, 9, 15 and 21. Participants were asked to bring any unused study medication at each follow-up visit and at the end of the trial. Unused study medication was obtained from the carer at these assessments. The local principal investigator (PI) or research worker kept a log of study medication returns, return date and amount of study medication returned and entered the information on the case report form. Once returned medication had been logged, it was destroyed by the local pharmacy. Carers were also questioned regarding study drug compliance at all interim assessments. The study-specific prescriptions were maintained in the pharmacy file for audit purposes.

Drug accountability

The study drug was dispensed by the local site pharmacy and full accountability of dispensing by the pharmacy and of returns (at each face-to-face visit) was undertaken by the PI or one of the research team. The pharmacy departments at each site maintained a study medication dispensing log, which included date dispensed, batch number, expiry date and number of capsules dispensed. In addition, the unique code numbers assigned to the treatment pack and trial patients were recorded. The study-specific prescriptions were maintained in the pharmacy file for audit purposes. Once the unused or partially used drug supplies were verified they were destroyed by the local pharmacy.

Assessments

Screening and randomisation were performed prior to the assessments described below.

Baseline

The baseline assessment took place within 28 days of screening. The BADLS was then administered to complete the primary outcome assessments. Three months' study drug was supplied via the trial pharmacy.

2-week assessment

A telephone assessment of safety, tolerability, compliance and concomitant medicines was made.

3-, 9-, 15- and 21-month assessments

An assessment of safety, tolerability, compliance and concomitant medicines was made. Blood was taken for analysis at the clinic or patient's home. Three months' study drug was supplied via the trial pharmacy.

6-, 12- and 18-month assessments

Compliance and safety was assessed and concomitant medicines recorded. Primary outcomes were administered and blood was be taken for analysis at the clinic or patient's home. Three months' study drug was supplied via the trial pharmacy.

24-month assessment

Primary outcomes were administered. Compliance and safety were assessed and concomitant medicines recorded. See *Table 1* for further details on the administration of the study assessments.

Safety and tolerability

Our secondary objectives focused on the safety and tolerability of the treatment and, therefore, data on safety parameters [including blood monitoring of haematological, renal and hepatic function as well as documentation of skin reactions, gastrointestinal and neurological symptoms and concurrent infections (i.e. bacterial enteritis, *Clostridium difficile* and orogenital candidiasis)] were also assessed and recorded every 3 months. To monitor renal function, the Modification of Diet in Renal Disease (MDRD) formula was used to calculate the estimated glomerular filtration rate (eGFR) at baseline and changes in creatinine levels were used to monitor renal function post baseline. In particular, the following guidelines (which were generated with approval by the Trial Management Group and in consultation with renal physicians) were used:

> *Any patient with a follow-up creatinine [level] of ≥ 25% and < 50% higher than their baseline value can remain on treatment, but will have a repeat blood sample in 2–3 weeks. If creatinine [levels] remain the same or higher, then a further check will be required. Any patient with a follow-up creatinine [level] of ≥ 50% higher than baseline can remain on treatment but will have a repeat blood sample within 10 days. If creatinine [levels] remain the same or higher, then study treatment will be stopped (unless an obvious alternative cause is identified, e.g. NSAID [non-steroidal anti-inflammatory drug] use, other illness).*

TABLE 1 Study time–event chart

Event	Time point										
	Screening	Baseline	Week 2	Month 3	Month 6	Month 9	Month 12	Month 15	Month 18	Month 21	Month 24
Diagnosis	**										
Inclusion	**										
Exclusion	**										
Concomitant medicines	**		*	**	**	*	**	*	**	*	**
Consent	**										
Random	**										
sMMSE	**				**		**		**		**
BADLS		**			**		**		**		**
Dispense		**		**	**	*	**	*	**	*	
Compliance			*	*	**	*	**	*	**	*	**
Safety check			*	*	**	*	**	*	**	*	**
FBC/ biochemistry	**			**	**	**	**	**	**	**	

FBC, full blood count.
Notes
Assessments marked with two asterisks (**) represent visits, whereas those marked with one asterisk (*) could be conducted over the telephone. All visits have a permissible window of ± 7 days for telephone calls and ± 14 days for face-to-face visits.

Randomisation and blinding

Once informed consent had been given, and baseline assessments completed, participants were randomly allocated, using a centralised randomisation service, to one of three trial arms: 400 mg of minocycline, 200 mg of minocycline or placebo. Treatment packs were allocated centrally by the MADE study office. The minimised randomisation procedure aimed to balance treatment allocation overall, and by four stratification variables: centre, duration of symptoms prior to randomisation (< 6 months, ≥ 6 months), baseline sMMSE score (24–26, 27–30 points) and age (< 65, 65–74, ≥ 75 years). Participants were enrolled by their clinicians or appropriately trained clinical study officers or research nurses. These staff also administered the outcome assessments. Patients, caregivers, clinicians, outcome assessors and investigators were blinded to treatment allocation.

The person randomising participants completed the MADE randomisation form and had to answer a set of prespecified questions over the telephone. Alternatively, completed randomisation forms were faxed – or scanned and e-mailed – to the MADE randomisation service, which provided a call-back service with a treatment allocation. After all the necessary details had been provided, a MADE patient trial number was assigned and two treatment pack numbers were allocated. Allocated treatment packs of 400 mg per day of minocycline, 200 mg per day of minocycline or matching placebo were dispensed to patients via a local pharmacy and/or researcher.

Ethics considerations

Any neuroprotective benefit from minocycline is likely to outweigh the risks. Minocycline is routinely used at doses of 200 mg per day in the long-term treatment of acne and is considered safe in this indication.[31] Rare side effects include acute renal failure, irreversible skin pigmentation and, very rarely, SLE.

The trial applied for multicentre research ethics approval and local research governance approval for the studies. The study personnel, co-investigators and collaborators [the management group and independent Trial Steering Committee (TSC)] ensured that the study was conducted within appropriate NHS and professional ethics guidelines. Information was kept strictly confidential and held in accordance with the Data Protection Act 1998.[52] Data are held on a secure database on a password-protected university computer. Access to data was restricted to the research team.

Participation in the MADE trial carried only a 1 in 3 risk of randomisation to placebo and patients did not have to forgo treatment with a cholinesterase inhibitor or memantine if their responsible clinician considered that they would benefit from such treatment.

Potentially eligible patients were approached by a clinician who knows them, and given the opportunity to hear more about research activities. The study was also advertised directly to patients (via posters, leaflets, etc.). Those individuals who were interested in learning more about research were referred to a member of the research team, who provided an information sheet with full study details, including possible benefits and risks. The potentially eligible patients were offered the opportunity to ask questions and discuss any queries with the carer/relative/doctor and make a date for the eligibility interview. Interested patients were sent the information sheet with the invitation to screening and written informed consent obtained prior to commencing the screening assessment.

All researchers were trained in gaining informed consent through DeNDRoN or the Mental Health Research Network training course. NRES guidance on the content and format of patient information and consent documentation was followed. Consumer representatives were involved to ensure that these documents and all other written trial materials were fit for purpose. Information about the study was mailed to potential participants and their caregivers.

The main potential ethics issue in dementia trials is that the disease may interfere with an individual's ability to give informed consent. Because the trial was studying people with mild dementia, most of the participants had capacity to give informed consent for their involvement. Consequently, fully informed written consent was obtained from patients entering the MADE study. However, as patients remained in the study for up to 2 years it was likely that some may have lost capacity over this period. Patients were therefore also asked what they would want to happen in the event of them losing capacity during the course of the study. These patients were given the option of either withdrawing at this point or a decision being made on their behalf by their personal legal representative in line with the Medicines for Human Use (Clinical Trials) Regulations 2004.[53] This person was usually the patient's main carer, who would have the best knowledge of the individual's attitudes and stated preference to research and, consequently, was best placed to judge whether or not they would have wished to continue if they had capacity. In this situation the patient's agreement to participate was still obtained to their best level of understanding and they did not remain in the study if they refused or showed significant distress.

Patient and public involvement

The study ensured that patients, carers and members of the general public were closely involved in the trial. During the design of the trial all relevant documentation, including participant information sheets and consent forms, were reviewed by our patient and public involvement (PPI) representatives. A representative was invited from the Alzheimer's Society to sit on the TSC and they were involved

in all ongoing trial matters including review of progress, group meetings, review of results and dissemination stages.

Statistical considerations

The study aimed to randomise 560 participants in a semifactorial (i.e. 2 x 1) design, that is, 1 : 1 : 1 between minocycline (400 or 200 mg) and placebo. Based on previous studies, it was estimated that 24-month assessments would be available on at least 80% of surviving participants (i.e. ≈ 390), which would provide 90% power at a *p*-value of < 0.05 to detect a small to moderate [0.35 standard deviation (SD)] effect size for minocycline (any dose) compared with placebo on the primary outcome measures. With outcome assessments on 130 patients allocated 400 mg of minocycline and 130 allocated 200 mg of minocycline, the study would have 80% power at a *p*-value of < 0.05 to detect a 0.35 SD treatment effect of 400 mg compared with 200 mg of minocycline at 24 months.

Only participants who received at least one capsule of the study treatment drug or placebo were to be included in the analyses of primary and secondary outcomes. The primary analyses of the effect of minocycline on rate of decline of sMMSE and BADLS scores, and subgroup analyses, used repeated measures regression methods, adjusted for baseline scores. These analyses use all available assessment data to maximise statistical power to detect any differences between treatments, and to minimise the impact of missing outcome data. For both primary outcomes, the difference in the rate of decline between minocycline (any dose) and placebo, and between patients allocated 400 mg and 200 mg of minocycline, was compared using a time-by-treatment interaction test, with time modelled as a continuous variable. Comparisons of time on trial medication over the 24-month follow-up period split by treatment arms are displayed in Kaplan–Meier curves, with statistical significance determined by log-rank tests. Participants who died were censored at the last assessment time point before death. Reasons for stopping trial medication and adverse events are tabulated by treatment arm. The study used SAS® software version 9.3 (SAS Institute Inc., Cary, NC, USA) for all statistical analyses. The independent data monitoring committee and REC reviewed the unblinded accumulating data and the safety of patients in the study at approximately yearly intervals.

Literature search

Prior to analysing the trial results, a literature search was carried out in 2018 to assess whether or not minocycline affects disease progression in AD, but no relevant studies were found at this stage (see *Appendix 2*, *Table 6*).

Chapter 3 Results

Between 23 May 2014 and 14 April 2016, 554 participants were entered from 32 NHS memory services in England and Scotland. Ten patients did not start trial medication and, as prespecified in the protocol, were excluded from all analyses (*Figure 1*); one participant had been allocated to 400 mg of minocycline, four to 200 mg of minocycline and five to placebo. Baseline characteristics of the 544 eligible participants were well balanced across the three treatment trial arms (*Table 2*).

The mean age of participants was 74.3 years, 57% (303/544) of whom were male and 84% (455/544) of whom were living with a spouse, partner or relative. The average duration of symptoms was 24 months and the average sMMSE score at baseline was 26.4 points.

The sMMSE assessments were obtained for 542 (99.6%) of the 544 participants at baseline, 498 (92%) of the 544 participants at 6 months, 453 (84%) of the 537 participants at 12 months, 420 (80%) of the 528 participants at 18 months, and 403 (78%) of the 517 participants at 24 months (see *Appendix 4*, *Table 8*). There were fewer BADLS than sMMSE assessments, because BADLS assessments are not valid for participants in residential care.

Minocycline at a daily dose of 400 mg was poorly tolerated by participants, with just 29% (53/184) of those allocated 400 mg of minocycline completing 2 years of treatment, significantly fewer participants than in the 200 mg of minocycline (62%; 112/181) or placebo arm (64%; 114/179) ($p < 0.0001$; see *Figures 1* and *7*). By contrast, 200 mg of minocycline was well tolerated by participants, with similar discontinuation rates with 200 mg of minocycline and placebo ($p = 0.56$). When reasons for stopping trial treatment were compared (*Table 3*), more participants allocated to minocycline than to placebo stopped because of gastrointestinal symptoms ($p = 0.0008$), dermatological side effects ($p = 0.02$) and dizziness ($p = 0.01$).

As a consequence of the higher treatment withdrawal rate, fewer assessments were obtained for the 400 mg of minocycline treatment arm than for the 200 mg of minocycline and placebo arms (see *Appendix 4*, *Table 8*). For sMMSE assessments at 24 months, 68% of assessments (119 of the 174 expected) were received for the 400 mg of minocycline arm, 82% (144/176) for the 200 mg of minocycline arm and 84% (140/167) for the placebo arm. Return rates for BADLS assessments were similarly lower for the 400 mg of minocycline arm than for the 200 mg of minocycline and placebo arms (see *Appendix 4*, *Table 8*).

The change from baseline in sMMSE scores over time, with standard error bars, is shown in *Figure 2*. There was an average 4.1-point reduction in the combined minocycline arms compared with a 4.3-point reduction in the placebo arm over the 24-month study period ($p = 0.90$). The reduction in sMMSE score in the 400 mg of minocycline arm over 24 months was less than that observed in the 200 mg of minocycline arm (i.e. 3.3 vs. 4.7 points), but this difference was not significant ($p = 0.08$).

Likewise, the worsening of BADLS scores over 24 months was similar in all treatment arms: 5.7, 6.6 and 6.2 points in the 400 mg of minocycline, 200 mg of minocycline and placebo trial arms, respectively. There were no significant differences in BADLS scores between participants allocated minocycline and those allocated to the placebo arm ($p = 0.57$) or between those participants allocated 400 mg of minocycline and those allocated 200 mg of minocycline ($p = 0.77$; *Figure 3*).

To assess how the higher number of missing outcome assessments in the 400 mg of minocycline treatment arm than in the 200 mg of minocycline or placebo arms (see *Appendix 4*, *Table 8*) might have affected outcome comparisons, various sensitivity analyses were run to investigate potential

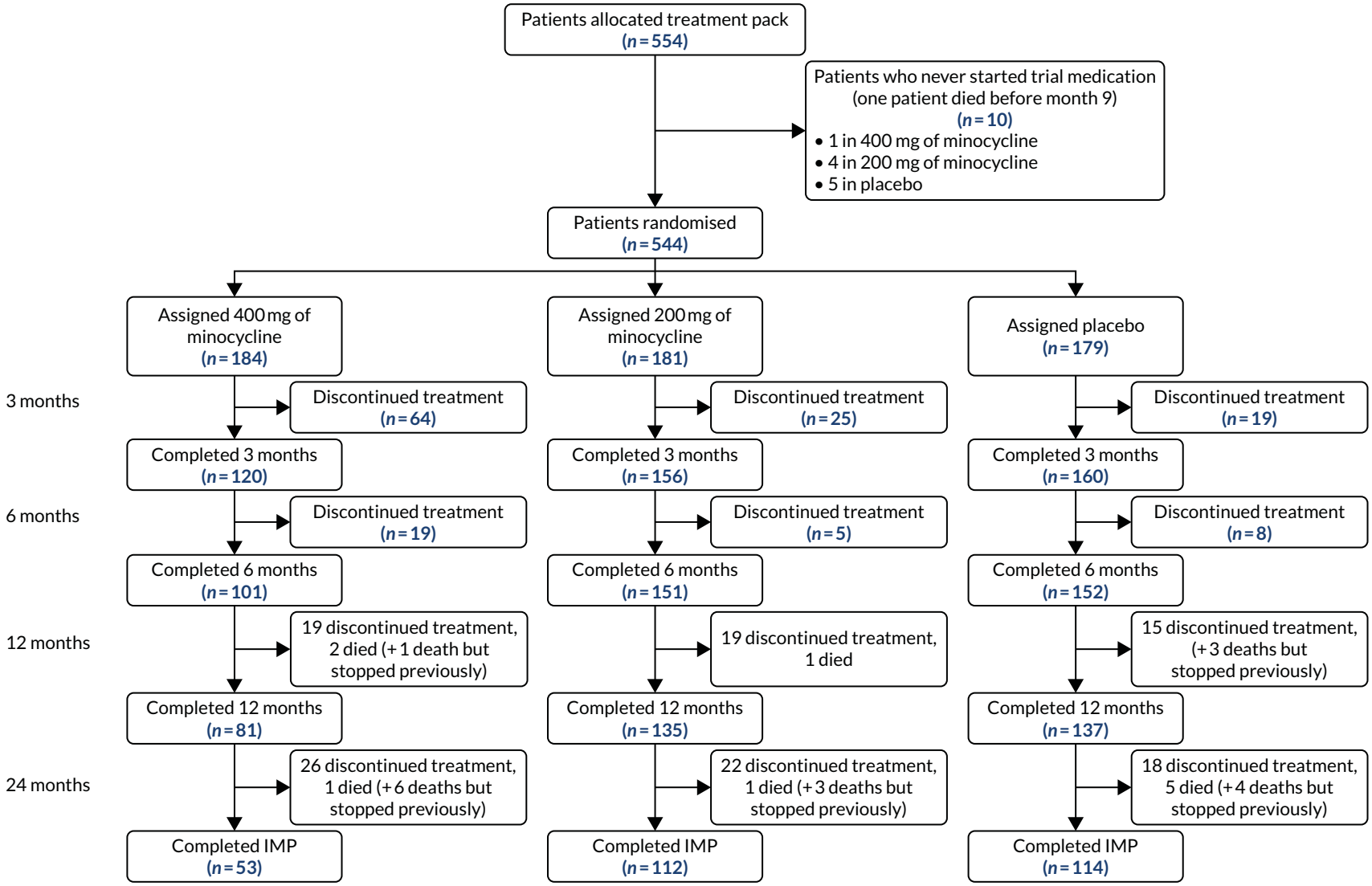

FIGURE 1 Flow chart of participants through the trial.

TABLE 2 Baseline characteristics, by treatment allocation, for the 544 eligible patients

Characteristic	Treatment arm		
	400 mg of minocycline (*N* = 184)	200 mg of minocycline (*N* = 181)	Placebo (*N* = 179)
Age (years)			
< 65, *n* (%)	22 (12)	22 (12)	21 (12)
65–74, *n* (%)	68 (37)	66 (36)	66 (37)
≥ 75, *n* (%)	94 (51)	93 (51)	92 (51)
Mean (SD)	74.3 (8.0)	74.1 (8.4)	74.6 (8.1)
Gender, *n* (%)			
Male	104 (57)	100 (55)	99 (55)
Female	80 (43)	81 (45)	80 (45)
Home circumstance, *n* (%)			
Living with spouse/partner/relative	153 (83)	153 (85)	149 (83)
Alone	31 (17)	28 (15)	29 (16)
Duration of symptoms			
< 6 months, *n* (%)	20 (11)	20 (11)	20 (11)
≥ 6 months, *n* (%)	164 (89)	161 (89)	159 (89)
Mean (SD)	23.5 (18.3)	23.1 (17.8)	24.2 (18.0)
sMMSE score (points)			
24–26, *n* (%)	100 (54)	97 (54)	96 (54)
27–30, *n* (%)	84 (46)	84 (46)	83 (46)
Mean score (SD)	26.4 (1.9)	26.5 (1.9)	26.4 (1.8)

TABLE 3 Reasons for stopping treatment by treatment allocation

Reasons for stopping	Treatment arm (*n*)				
	400 mg of minocycline (*N* = 184)	200 mg of minocycline (*N* = 181)	Placebo (*N* = 179)	Total (*n*)	Minocycline vs. placebo *p*-value
GI symptoms	42	15	10	67	0.00080
Dizziness	14	3	1	18	0.01000
Dermatological symptoms	10	5	1	16	0.02000
Haematological	5	3	1	9	0.16000
Impaired renal function	2	5	4	11	0.81000
Infection	1	2	2	5	0.74000
Shortness of breath	6	0	0	6	0.08000
Worsening dementia	1	3	3	7	0.57000
Depression or anxiety	4	2	2	8	0.63000

continued

TABLE 3 Reasons for stopping treatment by treatment allocation (*continued*)

Reasons for stopping	Treatment arm (*n*)			Total (*n*)	Minocycline vs. placebo *p*-value
	400 mg of minocycline (*N* = 184)	200 mg of minocycline (*N* = 181)	Placebo (*N* = 179)		
Joint or muscle pain	2	0	2	4	0.47000
Concomitant disease/illness	9	6	7	22	0.91000
General deterioration in physical health	2	0	2	4	0.47000
Unknown	1	0	0	1	0.48000
Unspecified side effect	5	2	7	14	0.17000
Patient or carer choice	23	21	18	62	0.49000
Total	127	67	60	254	0.00002

GI, gastrointestinal.

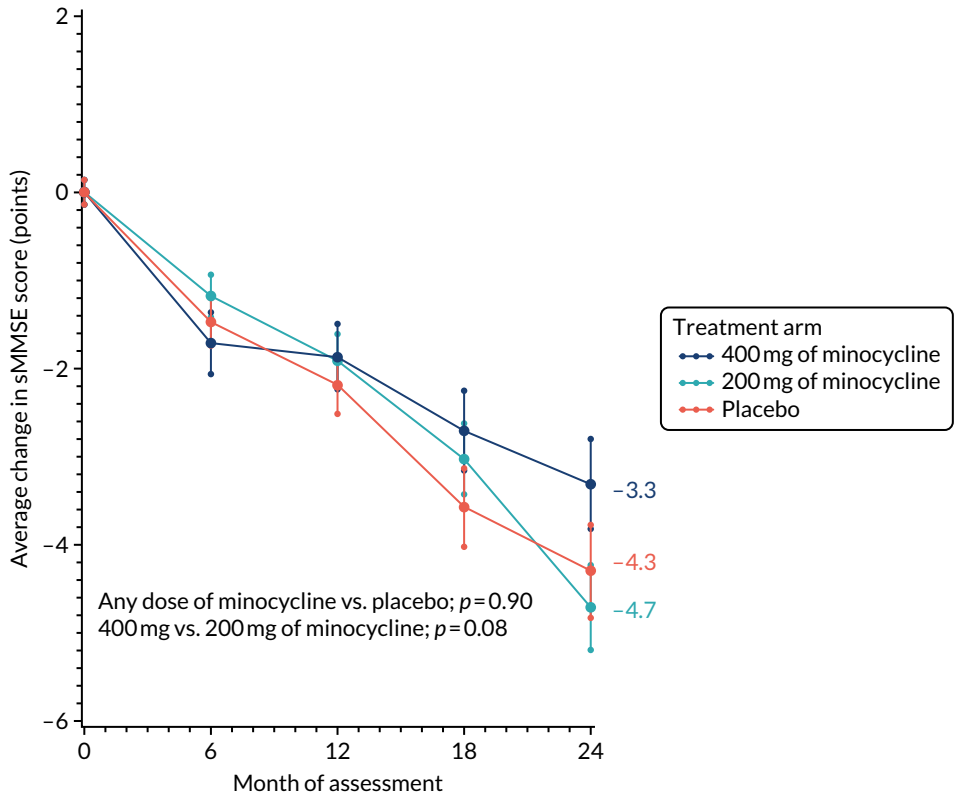

FIGURE 2 Change in sMMSE scores: baseline to 24 months' follow-up. The graph shows change in mean sMMSE score with standard errors; baseline scores are set to zero; *p*-values are from tests of the time-by-treatment interaction from repeated measures analyses.

bias from non-random dropout. In particular, there were 41 participants who had a baseline sMMSE score but no further assessments, so did not contribute any information to the primary analysis (see *Appendix 4*, *Figure 8*). Those participants who discontinue treatment in AD trials are often atypical, usually having worse cognitive and functional ability than those who continue.[20] This is evident from the scores of the 41 participants with a 6-month sMMSE score but no later assessments.

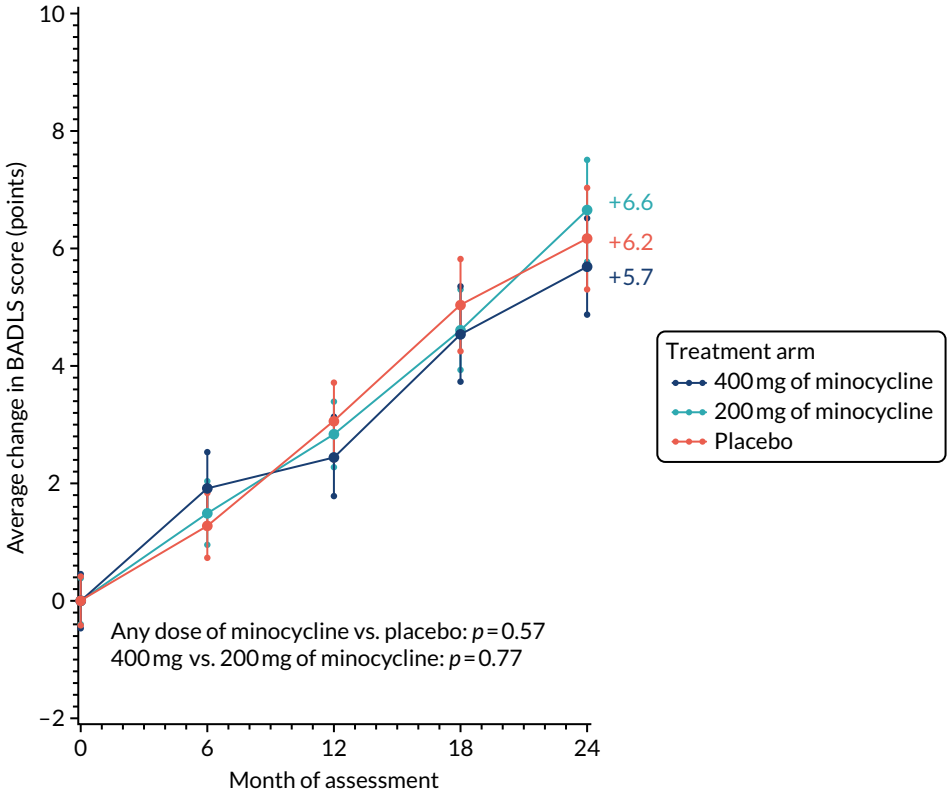

FIGURE 3 Change in BADLS scores: baseline to 24 months' follow-up. The graph shows change in mean BADLS scores with standard errors; baseline scores are set to zero; *p*-values are from tests of the time-by-treatment interaction from repeated measures analyses.

In total, there were 252 reported serious adverse events (SAEs), with the most common categories being neuropsychiatric and cardiocirculatory. The number of SAEs was somewhat higher in the placebo arm than in the 400- and 200-mg minocycline treatment arms (*Table 4*). Given that gastrointestinal symptoms were the main reason for stopping trial treatment, it is reassuring that the numbers of gastrointestinal SAEs in the minocycline arms were low, and no higher than in the placebo arm. Similarly, though more skin-related toxicities, particularly pigmentation, were reported with minocycline than with placebo [36% (130/365) vs. 21% (38/179); $p = 0.0007$], few participants stopped trial treatment because of such toxicities (see *Table 3*) and only six skin toxicities were considered severe (three in participants allocated to any dose of minocycline and three in participants to placebo; see *Appendix 4*, *Table 9*). There were no differences in the numbers of participants stopping treatment because of impaired renal function, which had been a prior concern, nor in numbers of renal SAEs. Twenty-eight patients died during the study: 10 allocated to the 400-mg minocycline treatment arm, six to the 200-mg minocycline treatment arm and 12 to the placebo arm (see *Appendix 4*, *Table 10* and *Figure 11a*). Fifteen of these 28 patients had stopped trial treatment prior to dying. One additional patient died without starting trial treatment. Rates of care home admission were low in this mild AD population, with no difference in numbers between trial arms (see *Appendix 4*, *Figures 11b* and *11c*).

The average decline in sMMSE score from baseline to 6 months in this subset of patients (i.e. those patients without any post-baseline assessments) was 3.9 points, a rate of decline three times higher than the 1.3-point average decline among the 498 patients who had a 6-month sMMSE assessment and went on to complete later assessments. It seems likely, therefore, that those patients without any post-baseline assessments, who do not contribute to the estimate of the rate of decline, also had a worse than average decline in cognitive and functional ability.

TABLE 4 Serious adverse events by treatment allocation

SAE class	Treatment arm, counts of SAEs reported			Total counts of SAEs reported	Minocycline vs. placebo *p*-value
	400 mg of minocycline (*n* = 184)	200 mg of minocycline (*n* = 181)	Placebo (*n* = 179)		
Gastrointestinal	3	8	10	21	0.1400
Respiratory	8	8	10	26	0.5400
Mechanical injury	6	11	13	30	0.2100
Endocrine and metabolic	2	1	9	12	0.0020
Cancer	12	3	11	26	0.0200
Haematological/thrombosis	3	1	2	6	0.9800
Dermatological	0	1	0	1	0.4800
Neuropsychiatric	10	13	16	39	0.2600
Cardiocirculatory	14	9	11	34	0.9400
Renal	3	2	2	7	0.4000
Infection	10	1	19	30	0.0003
Other	7	11	2	20	0.0300
Total	78	69	105	252	–

Differences were compared with the chi-squared test with associated *p*-values (two-sided).

To estimate what impact the missing outcome data from the 41 participants with no post-baseline assessments might have had on the trial results, the study's sensitivity analyses made two different assumptions:

1. It was assumed that for the first 6 months the scores declined at a rate of 3.9 points (as did those scores for participants who had a 6-month sMMSE but no further assessments) and then declined at the average rate of 1.1 points every 6 months for the rest of the trial.
2. It was assumed that for patients who had no postbaseline assessments the scores declined at the average rate of those participants with assessments, that is, 1.3 sMMSE points for the first 6 months and then 1.1 points every 6 months subsequently.

The results from imputation methods 1 and 2 are shown in *Appendix 4*, *Figures 9a* and *9c*. The results are not qualitatively different from those of the primary analyses. The only borderline-significant ($p = 0.06$) differences seen in these sensitivity analyses were between 400 and 200 mg of minocycline. However, with 400 mg of minocycline a little better and 200 mg of minocycline a little worse than placebo, and no difference between any dose of minocycline and placebo, this is probably a chance finding.

Because return rates for BADLS assessments were also lower for the 400 mg of minocycline arm than the 200 mg of minocycline and placebo arms, similar sensitivity analyses were run. There were 39 participants with no BADLS assessment post baseline who did not contribute to the primary analysis. Imputation method 1 assumed that their BADLS score worsened (i.e. increased) by 3.7 points over the first 6 months and then by 1.9 points every 6 months for the rest of the trial. Method 2 assumed that their BADLS score worsened by 1.5 points over the first 6 months and then by 1.9 points subsequently. Because BADLS is valid only for community-resident patients, BADLS scores for those who went into residential care were imputed only up until the last time point before moving into care.

Results for imputation methods 1 and 2 are shown in *Appendix 4*, *Figures 9b* and *9d*. Again, results were not qualitatively different from those of the primary analyses of BADLS assessment scores.

To investigate whether or not the efficacy of minocycline varied by baseline characteristics, subgroup analyses of change in sMMSE over 24 months for minocycline (any dose) versus placebo by duration of symptoms, baseline sMMSE, age and gender were conducted (see *Appendix 4*, *Figure 10*). There were no indications of any benefit from minocycline treatment in those participants with shorter or longer durations of symptoms, lower or higher baseline sMMSE scores, or in men or women. There was a borderline-significant (i.e. $p = 0.04$) trend towards greater efficacy in younger than older patients, but this unanticipated finding could be a chance occurrence given the number of subgroup investigations.

Chapter 4 Discussion

The MADE trial has shown that, in patients with mild AD, 24 months of minocycline treatment at the doses tested does not delay the progress of cognitive or functional impairment, as measured by the well-validated and widely used sMMSE and BADLS clinical rating scales. The trial has also established that minocycline at a dose of 400 mg is poorly tolerated in this population, with fewer than one-third of participants completing 24 months of treatment. By contrast, 200 mg per day of minocycline is well tolerated, with participants allocated this treatment being no more likely to withdraw from trial medication than those taking placebo.

The failure of minocycline to slow the progression of cognitive and functional decline in mild AD is disappointing given the evidence suggesting that neuroinflammation is instrumental in AD progression,[7] minocycline's established anti-inflammatory and neuroprotective effects and the positive data from several experimental animal models of AD.[11–17] NSAIDs have similarly failed to slow disease progression in clinical trials,[54] despite long-term use being associated with a lower risk of developing AD in observational studies[55] and promising data from transgenic animal models.[56] The study's findings also parallel those of clinical trials of minocycline in other neurodegenerative disorders in which, despite preclinical research suggesting neuroprotection, minocycline worsened outcomes in ALS,[25] had no effect in Huntington's disease[57] and multiple system atrophy,[58] had negative symptoms in schizophrenia[59] and had only short-term benefits in multiple sclerosis.[60]

Therefore, there could be three broad potential explanations for the negative results of this trial. First, although there is good evidence for neuroinflammation in AD,[7] this may be more a reaction to pathology than an important driver of progressive neurodegeneration, particularly in patients who are still at the mild stage of dementia. Second, even if neurodegeneration is accelerated by neuroinflammation, minocycline at the doses administered in the MADE study may not have had sufficient activity against these processes to show efficacy. Animal studies, from which much of the evidence for minocycline's activity as an anti-inflammatory and anti-AD agent has come, have generally used much higher doses of minocycline than used in the MADE trial (i.e. typically equivalent to 3–7 g per day in humans)[10] and, so, it could be that trial participants were not exposed to a sufficiently high dose for efficacy. However, if doses of 200–400 mg per day are insufficient for neuroprotection, the difficulties with tolerability experienced by participants allocated 400 mg of minocycline indicate that use of higher doses in this patient population is not feasible.

Minocycline is potentially neuroprotective through several anti-inflammatory processes (suppression of microglial proliferation and activation, reduced release of interleukins 1β and 6 and of tumour necrosis factor alpha, decreased chemokine expression and decreased activity of metalloproteases) as well as anti-apoptotic and anti-oxidant effects.[11–17] A study of 15 patients with traumatic brain injury found reduced microglial activation, as visualised with ^{11}C-PBR28 positron emission tomography (PET),[61] following 12 weeks of treatment with 200 mg of minocycline per day, indicating that the dose ranges used in the MADE trial can have a measurable effect on anti-inflammatory targets. The relationship between minocycline-sensitive microglial activation and neurodegeneration may, however, be complicated. Minocycline treatment in the traumatic brain injury study[61] was also associated with increased plasma levels of neurofilament light, considered a marker of neurodegeneration. The faster progression seen with minocycline in ALS[25] also suggests that some activated microglia might have a reparative function so that their inhibition could accelerate neurodegeneration. This study's results do not suggest that reduced microglial activation with minocycline worsens neurodegeneration in AD.

A third plausible explanation for the negative results of the MADE study could be that minocycline did have some efficacy against progression of AD but treatment effects were too small to be detectable in the trial. It is difficult to discount this possibility. The MADE trial was, however, powered to detect minimal clinically important differences between minocycline and placebo, so smaller differences might not be considered of clinical relevance.

This pragmatic trial had a number of strengths. It was based in a broad network of academic and NHS memory services and the wide eligibility criteria facilitated the recruitment of participants who were representative of patients with very mild AD diagnosed within the NHS. Outcome measures were limited in number, practical and easy to administer reliably by trial staff and chosen because any differences between minocycline and placebo treatment would have unambiguous clinical relevance. The trial recruited to target and was sufficiently large to detect a clinically meaningful treatment effect, and the trial arms were well matched on potentially important variables at baseline. This streamlined trial design could usefully be applied to test other putative disease-modifying therapies.

Potential limitations of the study include that biomarkers were not used to confirm AD diagnosis, because these are not routinely available within the NHS and, therefore, there was no access to these data. Nonetheless, no diagnoses were revised during the study and rates of decline were as expected in a mild AD population. Compliance was also problematic, with few patients in the 400-mg arm completing 2 years of treatment and only moderate compliance in the 200-mg and placebo arms. A recommendation to take trial medication once rather than twice daily in the event of perceived side effects helped improve compliance but was introduced only late in the trial when the problem with 400-mg compliance was identified.

Although the trial protocol specified that outcome assessments should be obtained irrespective of treatment compliance, this could not always be achieved despite the vigorous efforts of the trial team. Consequently, differential follow-up rates could have biased the study's results. However, despite the large number of treatment withdrawals in the 400-mg arm, and consequent loss to follow-up of some participants, results were essentially unchanged in sensitivity analyses investigating potential bias from missing data. Thus, the study's conclusion that 2 years of minocycline treatment for patients with mild AD does not result in any clinically meaningful difference in the rate of decline of cognitive and functional ability is disappointing but robust.

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Appendix 1 Protocol changes

Table 5 is a summary of the changes made to the original MADE trial protocol, which have been approved by the REC and the Medicines and Healthcare products Regulatory Agency (where relevant).

TABLE 5 Research Ethics Committee- and Medicines and Healthcare products Regulatory Agency-approved amendments

Change to protocol	Date
Increased leeway window for final assessments from ± 14 days to ± 30 days	22 March 2018
Guidance on restarting IMP added, with participants able to restart IMP at any time after break	29 July 2016
End-of-study participant letter added	29 July 2016
End-of-study GP letter added	29 July 2016
Increase recruitment from 480 to 560 participants by March 2016	30 October 2015
Adding JDR as additional recruitment tool	21 January 2015
Inclusion criterion 'severe liver disease' modified to 'moderate liver disease'	4 February 2015
Added a trial leaflet	3 September 2014
Added a trial recruitment advertisement poster	3 September 2014
Added a GP letter of invitation	3 September 2014
Add inclusion criterion: participants must have a potential informant to assist with administration of the BADLS assessment	9 January 2014
Exclusion criterion 'Patients with creatinine clearance < 50 ml/minute at screening, according to the Cockcroft and Gault equation must be excluded' was added	7 August 2014
Exclusion criterion 'Known chronic kidney disease stages 3–5′ was amended to 'Known chronic kidney disease stages 3b-5'	7 August 2014
Guidance for renal functioning monitoring updated	9 January 2014
Further guidance for monitoring side effects and loss to follow-up added	9 January 2014
Additional exclusion criterion added: uncontrolled serious concomitant illness	9 January 2014
Removed from exclusion criteria: pregnancy and lactation; diagnosis of MCI; lacks capacity to give informed consent; and severe liver disease	9 January 2014
Broadened recruitment strategy by allowing members of the research team to get patient consent after patients had been initially approached by the study doctor	14 October 2013
Guidance on reporting SAEs/SUSARs added	28 August 2013
Changed frequency of safety checks from every 6 months to every 3 months	28 August 2013
Broadened recruitment strategy by advertising the study and allowing potentially interested individuals to contact the research team directly	28 August 2013
Added age range (≥ 50 years) to inclusion criterion	28 August 2013

JDR, Joint Dementia Research; MCI, mild cognitive impairment; SUSAR, suspected unexpected serious adverse reaction.

Appendix 2 Literature search

Table 6 presents a summary of the number of articles identified by each search engine.

The following URL provides a link to the PROSPERO record for the search:

www.crd.york.ac.uk/prospero/display_record.php?RecordID = 90377 9 (accessed April 2018).

The following search terms were used in March 2018 to perform the search:

minocycline AND (AD OR alzheimer OR MCI OR "mild cognitive impairment" OR dementia).

TABLE 6 Literature search

Search engine	Number of articles identified
PubMed	1549
The Cochrane Library	19
Web of Science	121
Ovid (including EMBASE, PsycARTICLES and PsycINFO)	1471

Appendix 3 Outcome measure response sheets and sample participant responses

TABLE 7 The sMMSE outcome measure response sheet

Question		Time allowed	Score
1	***What year is this?***	10 seconds	
	Which season is this?	10 seconds	
	What month is this?	10 seconds	
	What is today's date?	10 seconds	
	What day of the week is this?	10 seconds	
2	***What country are we in?***	10 seconds	
	What province are we in?	10 seconds	
	What city/town are we in?	10 seconds	
	IN HOME – What is the street address of this house?	10 seconds	
	IN FACILITY – What is the name of this building?		
	IN HOME – What room are we in?	10 seconds	
	IN FACILITY – What floor are we on?		
3	**SAY:** ***I am going to name three objects. When I am finished, I want you to repeat them. Remember what they are because I am going to ask you to name them again in a few minutes.*** Say the following words slowly at 1-second intervals – **ball/car/man**	20 seconds	
4	***Spell the word WORLD. Now spell it backwards.***	30 seconds	
5	***Now what were the three objects I asked you to remember?***	10 seconds	
6	**SHOW** wristwatch. **ASK:** ***What is this called?***	10 seconds	
7	**SHOW** pencil. **ASK**: ***What is this called?***	10 seconds	
8	**SAY:** ***I would like you to repeat this phrase after me: No ifs, ands or buts.***	10 seconds	
9	**SAY:** ***Read the words on the page and then do what it says.*** Then hand the person the sheet with CLOSE YOUR EYES on it. If the subject reads and does not close their eyes, repeat up to three times. Score only if subject closes eyes	10 seconds	
10	**HAND** the person a pencil and paper. **SAY:** ***Write any complete sentence on that piece of paper.*** (Note: The sentence must make sense. Ignore spelling errors)	30 seconds	
11	**PLACE** design, eraser and pencil in front of the person. **SAY:** ***Copy this design please*** Allow multiple tries. Wait until person is finished and hands it back. Score only for a correctly copied diagram with a 4-sided figure between two 5-sided figures	1 minute	
12	**ASK** the person if they are right- or left-handed. Take a piece of paper and hold it up in front of the person. **SAY:** ***Take this paper in your right/left hand*** (whichever is non-dominant), ***fold the paper in half once with both hands and put the paper down on the floor***. Score 1 point for each instruction executed correctly: • Takes paper correctly in hand • Folds it in half • Puts it on the floor	30 seconds	
Total test score		**/30**	

Bristol Activities of Daily Living Scale (BADLS)

This questionnaire is designed to reveal the everyday ability of people who have memory difficulties of one form or another.

For each activity, statements a-e refer to a different level of ability. Thinking of the last 2 weeks, tick the box that represents your relative's/friend's ability.

If patient has never performed this activity even when well score – e (Not Applicable).

Only 1 box should be ticked for each activity.

If in doubt about which box to tick choose the level of ability which represents their average performance over the last 2 weeks.

			Scoring
1.	**Food**		
a.	Selects and prepares food as required	☐	0
b.	Able to prepare food if ingredients are set out	☐	1
c.	Can prepare food if prompted step by step	☐	2
d.	Unable to prepare food even with prompting and supervision	☐	3
e.	Not applicable	☐	0
2.	**Eating**		
a.	Eats appropriately using correct cutlery	☐	0
b.	Eats appropriately if food made manageable and/or uses spoon	☐	1
c.	Uses fingers to eat food	☐	2
d.	Needs to be fed	☐	3
e.	Not applicable	☐	0
3.	**Drink**		
a.	Selects and prepares drinks as required	☐	0
b.	Can prepare drinks if ingredients left available	☐	1
c.	Can prepare drinks if promoted step by step	☐	2
d.	Unable to make a drink even with prompting and supervision	☐	3
e.	Not applicable	☐	0
4.	**Drinking**		
a.	Drinks appropriately	☐	0
b.	Drinks appropriately with aids (beaker/straw etc).	☐	1
c.	Does not drink appropriately even with aids, but attempts to	☐	2
d.	Has to have drink administered (fed)	☐	3
e.	Not applicable	☐	0

FIGURE 4 Bristol Activities of Daily Living Scale: outcome measure responses sheet. (*continued*)

5.	**Dressing**		
a.	Selects appropriate clothing and dresses self	☐	0
b.	Puts clothes on in wrong order or back to front or dirty clothing	☐	1
c.	Unable to dress self but moves limbs to assist	☐	2
d.	Unable to assist and requires total dressing	☐	3
e.	Not applicable	☐	0
6.	**Hygiene**		
a.	Washes regularly and independently	☐	0
b.	Can wash self if given soap, flannel, towel, etc.	☐	1
c.	Can wash self if prompted and supervised	☐	2
d.	Unable to wash self and needs full assistance	☐	3
e.	Not applicable	☐	0
7.	**Teeth**		
a.	Cleans own teeth/dentures regularly and independently	☐	0
b.	Cleans teeth/dentures if given appropriate items	☐	1
c.	Requires some assistance, toothpaste on brush, brush to mouth, etc	☐	2
d.	Full assistance given	☐	3
e.	Not applicable	☐	0
8.	**Bath/Shower**		
a.	Bathes regularly and independently	☐	0
b.	Needs bath to be run/shower turned on, but washes independently	☐	1
c.	Needs supervision and prompting to wash	☐	2
d.	Totally dependent, needs full assistance	☐	3
e.	Not applicable	☐	0
9.	**Toilet/Commode**		
a.	Uses toilet appropriately when required	☐	0
b.	Needs to be taken to the toilet and given assistance	☐	1
c.	Incontinent of urine or faeces	☐	2
d.	Incontinent of urine and faeces	☐	3
e.	Not applicable	☐	0
10.	**Transfers**		
a.	Can get in/out of chair unaided	☐	0
b.	Can get into a chair but needs help to get out	☐	1
c.	Needs help getting in and out of a chair	☐	2
c.	Totally dependent on being put into and lifted from chair	☐	3
e.	Not applicable	☐	0

FIGURE 4 Bristol Activities of Daily Living Scale: outcome measure responses sheet. (*continued*)

11.	**Mobility**		
a.	Walks independently	☐	0
b.	Walks with assistance, i.e. furniture, arm for support	☐	1
c.	Uses aids to mobilize, i.e. frame, sticks etc. .	☐	2
d.	Unable to walk	☐	3
e.	Not applicable	☐	0
12.	**Orientation—time**		
a.	Fully orientated to time/day/date etc.	☐	0
b.	Unaware of time/day etc. but seems unconcerned	☐	1
c.	Repeatedly asks the time/day/date	☐	2
d.	Mixes up night and day	☐	3
e.	Not applicable	☐	0
13.	**Orientation—Space**		
a.	Fully orientated to surroundings	☐	0
b.	Orientated to familiar surroundings only	☐	1
c.	Gets lost in home, needs reminding where bathroom is, etc.	☐	2
d.	Does not recognise home as own and attempts to leave	☐	3
e.	Not applicable	☐	
14.	**Communications**		
a.	Able to hold appropriate conversation	☐	0
b.	Shows understanding and attempts to respond verbally or with gestures	☐	1
c.	Can make self understood but difficulty understanding others	☐	2
d.	Does not respond to or communicate with others	☐	3
e.	Not applicable	☐	0
15.	**Telephone**		
a.	Uses telephone appropriately, including obtaining correct number	☐	0
b.	Uses telephone if number given verbally/visually or predialled	☐	1
c.	Answers telephone but does not make calls	☐	2
d.	Unable/unwilling to use telephone at all	☐	3
e.	Not applicable	☐	0
16.	**Gardening/Housework**		
a.	Able to do housework/gardening to previous standard	☐	0
b.	Able to do housework/gardening but not to previous standard	☐	1
c.	Limited participation even with a lot of supervision	☐	2
d.	Unwilling/unable to participate in previous activities	☐	3
e.	Not applicable	☐	0

FIGURE 4 Bristol Activities of Daily Living Scale: outcome measure responses sheet. (*continued*)

17.	**Shopping**		
a.	Shops to previous standard	☐	0
b.	Only able to shop for 1 or 2 items with or without a list	☐	1
c.	Unable to shop alone, but participates when accompanied	☐	2
d.	Unable to participate in shopping even when accompanied	☐	3
e.	Not applicable	☐	0
18.	**Finances**		
a.	Responsible for own finances at previous level	☐	0
b.	Unable to write cheques but can sign name and recognises money values	☐	1
c.	Can sign name but unable to recognise money values	☐	2
d.	Unable to sign name or recognise money values	☐	3
e.	Not applicable	☐	0
19.	**Games/Hobbies**		
a.	Participates in pastimes/activities to previous standard	☐	0
b.	Participates but needs instruction/supervision	☐	1
c.	Reluctant to join in, very slow, needs coaxing	☐	2
d.	No longer able to willing to join in	☐	3
e.	Not applicable	☐	0
20.	**Transport**		
a.	Able to drive, cycle or use public transport independently	☐	0
b.	Unable to drive but uses public transport or bike etc	☐	1
c.	Unable to use public transport alone	☐	2
d.	Unable/unwilling to use transport even when accompanied	☐	3
e.	Not applicable	☐	0
		BADLS Total	☐

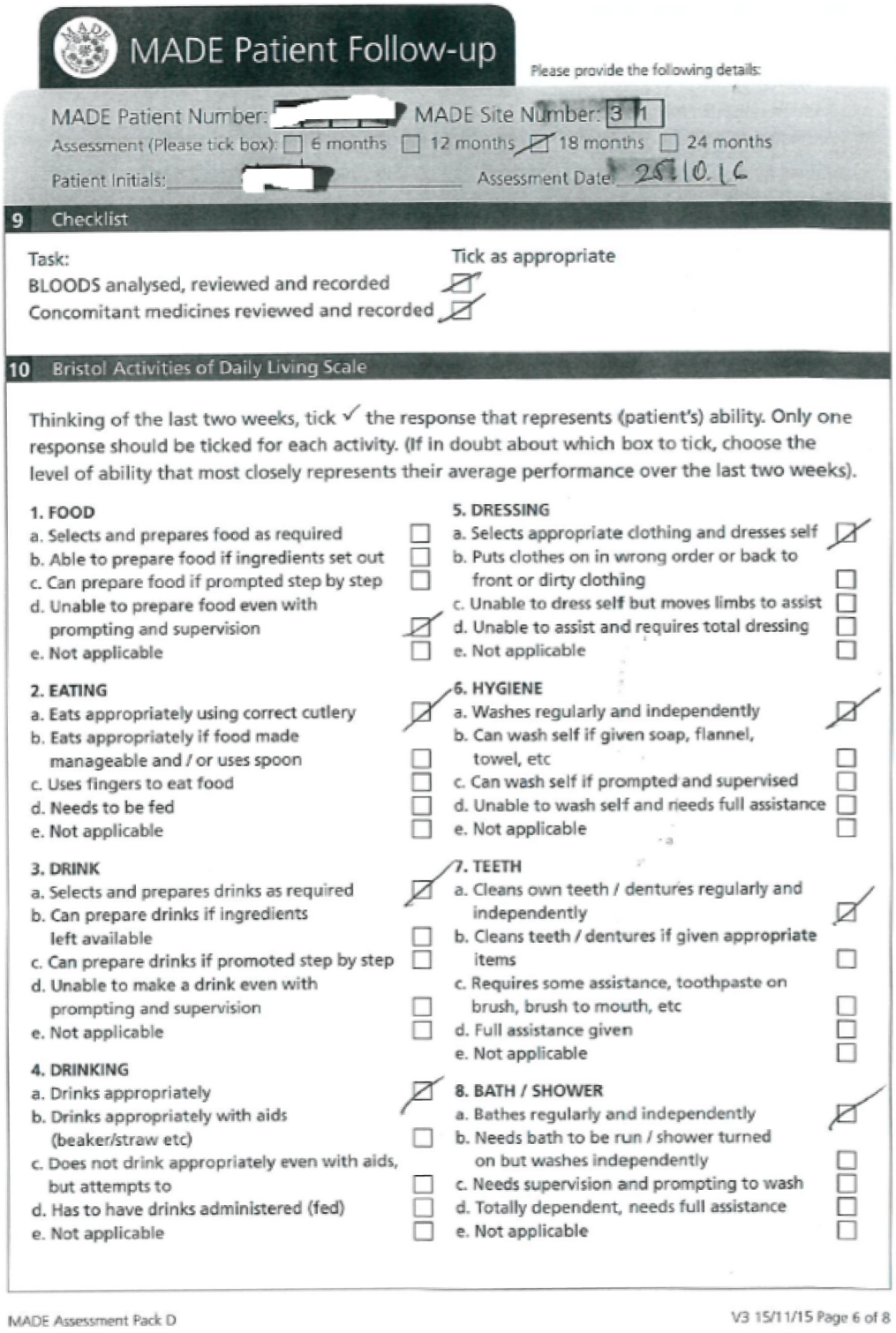
Thank you for taking the time to complete this questionnaire

Patient's name_____

Your name_____

Date_____

FIGURE 4 Bristol Activities of Daily Living Scale: outcome measure responses sheet.



MADE Patient Follow-up

Please provide the following details:

MADE Patient Number: [redacted] MADE Site Number: 3 1

Assessment (Please tick box): ☐ 6 months ☐ 12 months ☑ 18 months ☐ 24 months

Patient Initials: [redacted] Assessment Date: 25.10.16

9 Checklist

Task:	Tick as appropriate
BLOODS analysed, reviewed and recorded	☑
Concomitant medicines reviewed and recorded	☑

10 Bristol Activities of Daily Living Scale

Thinking of the last two weeks, tick ✓ the response that represents (patient's) ability. Only one response should be ticked for each activity. (If in doubt about which box to tick, choose the level of ability that most closely represents their average performance over the last two weeks).

1. FOOD
- a. Selects and prepares food as required ☐
- b. Able to prepare food if ingredients set out ☐
- c. Can prepare food if prompted step by step ☐
- d. Unable to prepare food even with prompting and supervision ☑
- e. Not applicable ☐

2. EATING
- a. Eats appropriately using correct cutlery ☑
- b. Eats appropriately if food made manageable and / or uses spoon ☐
- c. Uses fingers to eat food ☐
- d. Needs to be fed ☐
- e. Not applicable ☐

3. DRINK
- a. Selects and prepares drinks as required ☑
- b. Can prepare drinks if ingredients left available ☐
- c. Can prepare drinks if promoted step by step ☐
- d. Unable to make a drink even with prompting and supervision ☐
- e. Not applicable ☐

4. DRINKING
- a. Drinks appropriately ☑
- b. Drinks appropriately with aids (beaker/straw etc) ☐
- c. Does not drink appropriately even with aids, but attempts to ☐
- d. Has to have drinks administered (fed) ☐
- e. Not applicable ☐

5. DRESSING
- a. Selects appropriate clothing and dresses self ☑
- b. Puts clothes on in wrong order or back to front or dirty clothing ☐
- c. Unable to dress self but moves limbs to assist ☐
- d. Unable to assist and requires total dressing ☐
- e. Not applicable ☐

6. HYGIENE
- a. Washes regularly and independently ☑
- b. Can wash self if given soap, flannel, towel, etc ☐
- c. Can wash self if prompted and supervised ☐
- d. Unable to wash self and needs full assistance ☐
- e. Not applicable ☐

7. TEETH
- a. Cleans own teeth / dentures regularly and independently ☑
- b. Cleans teeth / dentures if given appropriate items ☐
- c. Requires some assistance, toothpaste on brush, brush to mouth, etc ☐
- d. Full assistance given ☐
- e. Not applicable ☐

8. BATH / SHOWER
- a. Bathes regularly and independently ☑
- b. Needs bath to be run / shower turned on but washes independently ☐
- c. Needs supervision and prompting to wash ☐
- d. Totally dependent, needs full assistance ☐
- e. Not applicable ☐

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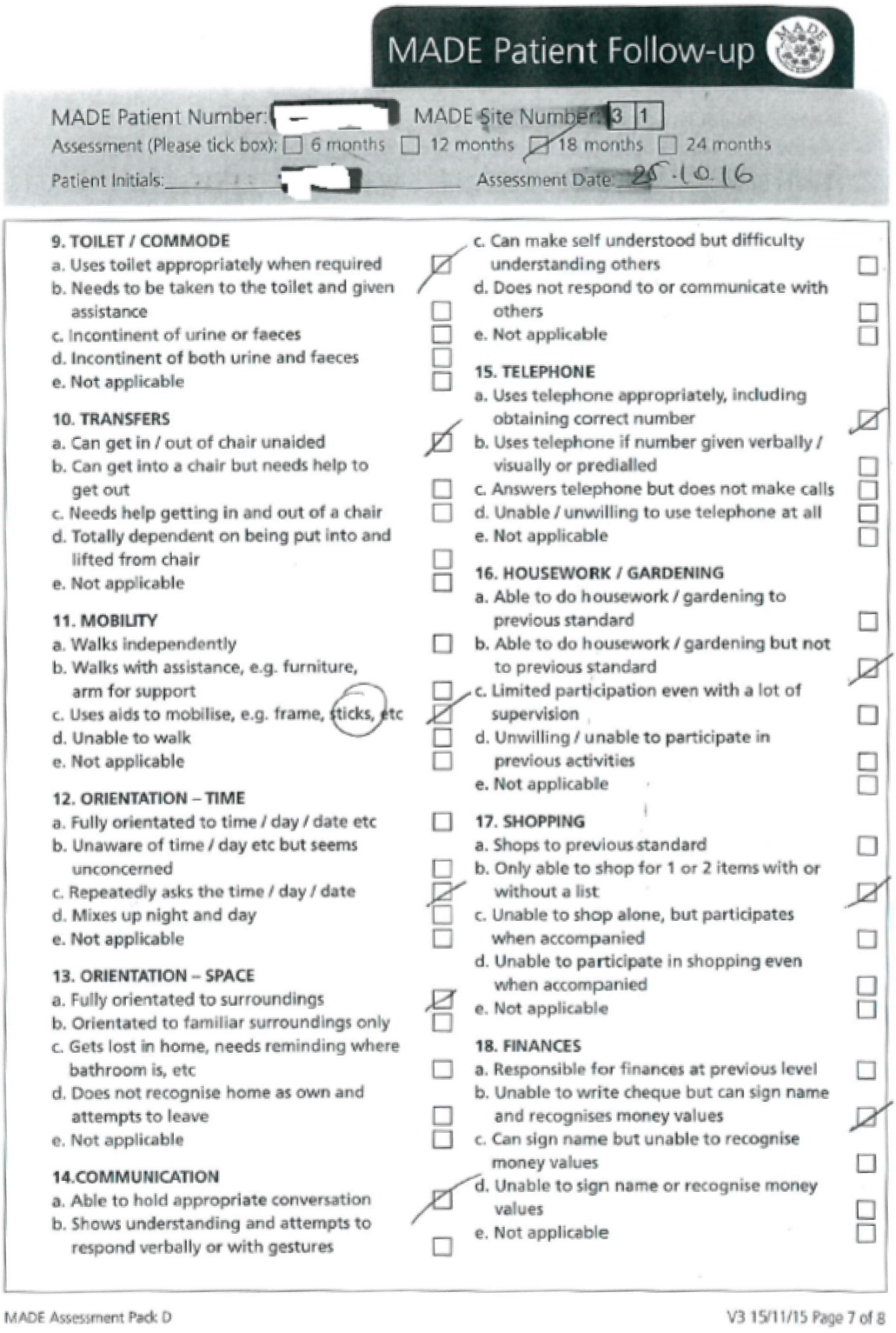
FIGURE 5 Sample participant responses: BADLS responses for 12 months' follow-up. (*continued*)



MADE Patient Follow-up

MADE Patient Number: [redacted] MADE Site Number: 3 1

Assessment (Please tick box): ☐ 6 months ☐ 12 months ☑ 18 months ☐ 24 months

Patient Initials: [redacted] Assessment Date: 25.10.16

9. TOILET / COMMODE
- a. Uses toilet appropriately when required ☑
- b. Needs to be taken to the toilet and given assistance ☐
- c. Incontinent of urine or faeces ☐
- d. Incontinent of both urine and faeces ☐
- e. Not applicable ☐

10. TRANSFERS
- a. Can get in / out of chair unaided ☑
- b. Can get into a chair but needs help to get out ☐
- c. Needs help getting in and out of a chair ☐
- d. Totally dependent on being put into and lifted from chair ☐
- e. Not applicable ☐

11. MOBILITY
- a. Walks independently ☐
- b. Walks with assistance, e.g. furniture, arm for support ☐
- c. Uses aids to mobilise, e.g. frame, sticks, etc ☑
- d. Unable to walk ☐
- e. Not applicable ☐

12. ORIENTATION – TIME
- a. Fully orientated to time / day / date etc ☐
- b. Unaware of time / day etc but seems unconcerned ☐
- c. Repeatedly asks the time / day / date ☑
- d. Mixes up night and day ☐
- e. Not applicable ☐

13. ORIENTATION – SPACE
- a. Fully orientated to surroundings ☑
- b. Orientated to familiar surroundings only ☐
- c. Gets lost in home, needs reminding where bathroom is, etc ☐
- d. Does not recognise home as own and attempts to leave ☐
- e. Not applicable ☐

14.COMMUNICATION
- a. Able to hold appropriate conversation ☑
- b. Shows understanding and attempts to respond verbally or with gestures ☐
- c. Can make self understood but difficulty understanding others ☐
- d. Does not respond to or communicate with others ☐
- e. Not applicable ☐

15. TELEPHONE
- a. Uses telephone appropriately, including obtaining correct number ☑
- b. Uses telephone if number given verbally / visually or predialled ☐
- c. Answers telephone but does not make calls ☐
- d. Unable / unwilling to use telephone at all ☐
- e. Not applicable ☐

16. HOUSEWORK / GARDENING
- a. Able to do housework / gardening to previous standard ☐
- b. Able to do housework / gardening but not to previous standard ☑
- c. Limited participation even with a lot of supervision ☐
- d. Unwilling / unable to participate in previous activities ☐
- e. Not applicable ☐

17. SHOPPING
- a. Shops to previous standard ☐
- b. Only able to shop for 1 or 2 items with or without a list ☑
- c. Unable to shop alone, but participates when accompanied ☐
- d. Unable to participate in shopping even when accompanied ☐
- e. Not applicable ☐

18. FINANCES
- a. Responsible for finances at previous level ☐
- b. Unable to write cheque but can sign name and recognises money values ☑
- c. Can sign name but unable to recognise money values ☐
- d. Unable to sign name or recognise money values ☐
- e. Not applicable ☐

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FIGURE 5 Sample participant responses: BADLS responses for 12 months' follow-up.

FIGURE 6 Sample participant responses: sMMSE responses for 12 months' follow-up.

Appendix 4 Additional figures and tables

TABLE 8 Follow-up rates for sMMSE and BADLS by treatment arm and time point

		Assessment					
		sMMSE			BADLS		
Time point	Treatment arm	Received (*n*)	Expected[a] (*n*)	%	Received (*n*)	Expected[b] (*n*)	%
Screening	400 mg of minocycline	183	184	99.5	183	184	99.5
	200 mg of minocycline	181	181	100	181	181	100
	Placebo	178	179	99.4	177	178	99.4
	Total	542	544	99.6	541	543	99.6
6 months	400 mg of minocycline	159	184	86	159	184	86
	200 mg of minocycline	172	181	95	172	181	95
	Placebo	167	179	93	164	176	93
	Total	498	544	92	495	541	91
12 months	400 mg of minocycline	139	181	77	140	180	78
	200 mg of minocycline	158	180	88	157	178	88
	Placebo	156	176	89	155	171	91
	Total	453	537	84	452	529	85
18 months	400 mg of minocycline	127	179	71	128	178	72
	200 mg of minocycline	146	177	82	146	169	86
	Placebo	147	172	85	148	167	89
	Total	420	528	80	422	514	82
24 months	400 mg of minocycline	119	174	68	118	170	69
	200 mg of minocycline	144	176	82	142	167	85
	Placebo	140	167	84	137	154	89
	Total	403	517	78	397	491	81

a Expected numbers of sMMSE assessments excluding those participants who withdrew prior to starting treatment (i.e. those participants not effectively randomised and those who died prior to the assessment).
b Expected numbers of BADLS assessments also excluding those participants who were admitted to care.

TABLE 9 Skin toxicity incidence and severity by treatment arm

Treatment arm	Toxicity rating	Number of patients
400 mg of minocycline	Mild	33
	Moderate	27
	Severe	1
	Subtotal	61
200 mg of minocycline	Mild	38
	Moderate	29
	Severe	2
	Subtotal	69
Placebo	Mild	22
	Moderate	13
	Severe	3
	Subtotal	38

TABLE 10 Causes of death

Treatment arm	Cause of death	Weeks until death	Stopped treatment ≥ 28 days previously?
Infection			
Placebo	Infection	64	Yes – 17 weeks
Placebo	Pneumonia	36	No
Placebo	Pneumonia and pulmonary oedema	28	Yes – 23 weeks
Placebo	Pneumonia	66	No
Placebo	Chest infection	83	No
200 mg of minocycline	Pneumonia	56	No
400 mg of minocycline	Pneumonia	86	Yes – 2 weeks
Neuropsychiatric			
Placebo	Dementia	95	No
Placebo	AD/Lewy body dementia	92	Yes – 87 weeks
400 mg of minocycline	Progression of AD	58	Yes – 7 weeks
Cardiovascular			
Placebo	Myocardial infarction	102	No
Placebo	Myocardial infarction	72	No
Placebo	Heart attack	64	No
200 mg of minocycline	Cardiac event	50	No
200 mg of minocycline	Heart attack	58	Yes – 51 weeks
400 mg of minocycline	Heart attack	37	No
400 mg of minocycline	Heart failure	100	Yes – 88 weeks
400 mg of minocycline	Heart attack	91	No

TABLE 10 Causes of death (*continued*)

Treatment arm	Cause of death	Weeks until death	Stopped treatment ≥ 28 days previously?
Cerebrovascular			
200 mg of minocycline	Unknown (stroke on 21 March 2017)	103	Yes – 84 weeks
400 mg of minocycline	CVA	42	Yes – 3 weeks
400 mg of minocycline	Stroke	36	No
Renal failure			
Placebo	Chronic renal failure	32	Yes – 12 weeks
400 mg of minocycline	Lung and kidney failure	103	Yes – 1 week
Other cause			
Placebo	Complications after bowel surgery	89	Yes – 44 weeks
200 mg of minocycline	General health decline	56	Yes – 29 weeks
200 mg of minocycline	Large abdominal tumour causing kidney failure	28	Never started
400 mg of minocycline	COPD	57	Yes – 11 weeks
Unknown			
400 mg of minocycline	Unknown	77	Yes – 17 weeks

COPD, chronic obstructive pulmonary disorder; CVA, cerebrovascular accident.

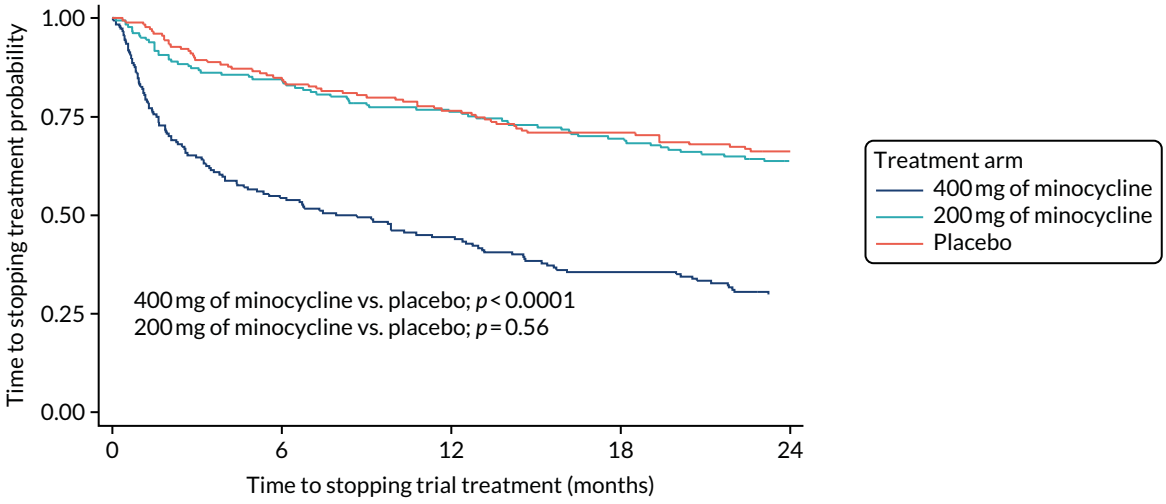

FIGURE 7 Proportion of participants taking trial treatment over time: Kaplan–Meier plot.

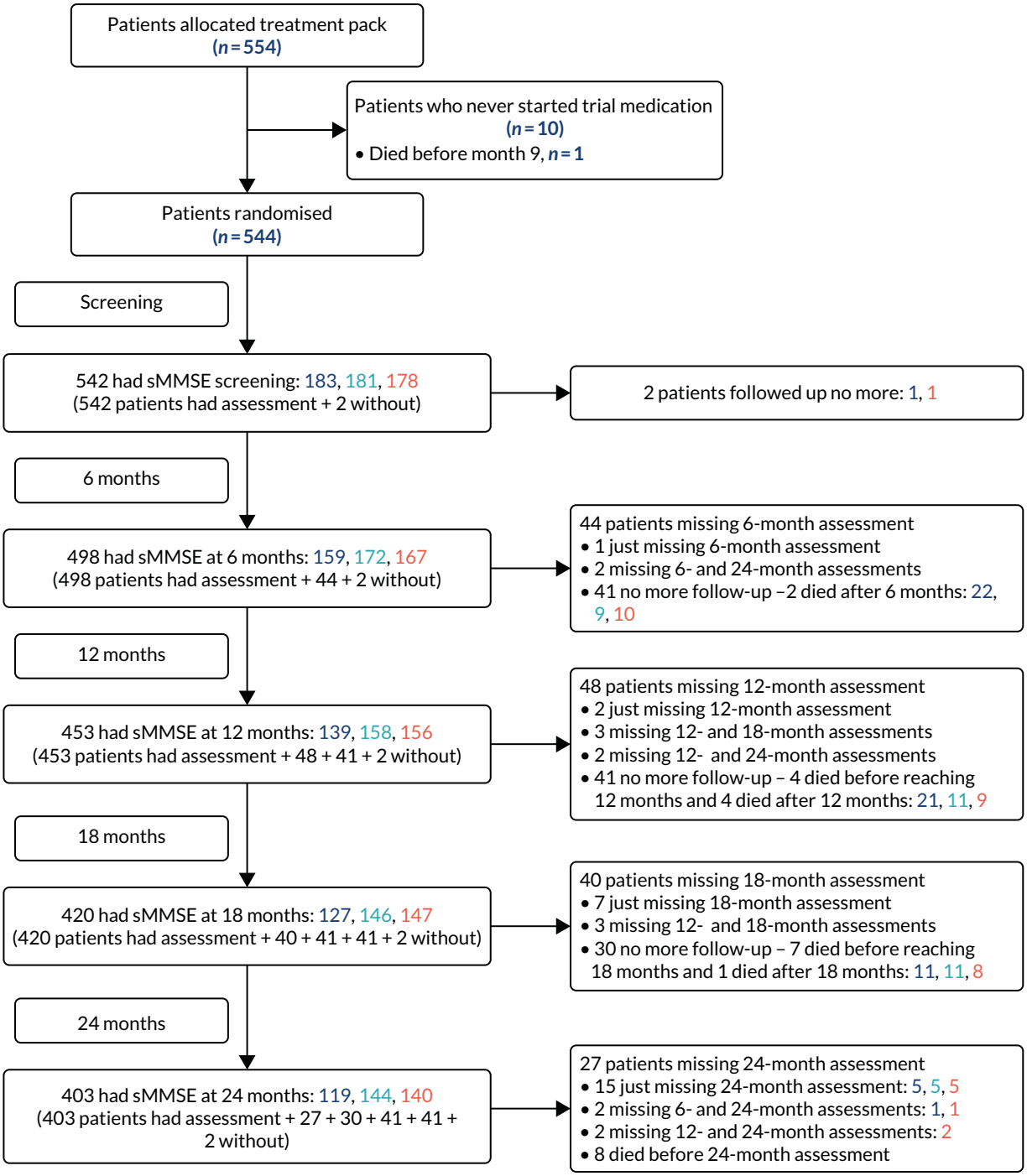

FIGURE 8 Flow chart showing the completeness over time of participant follow-up. Colour coding to show assessments split by treatment arm: navy font is 400 mg of minocycline, light blue font is 200 mg of minocycline and red font is placebo.

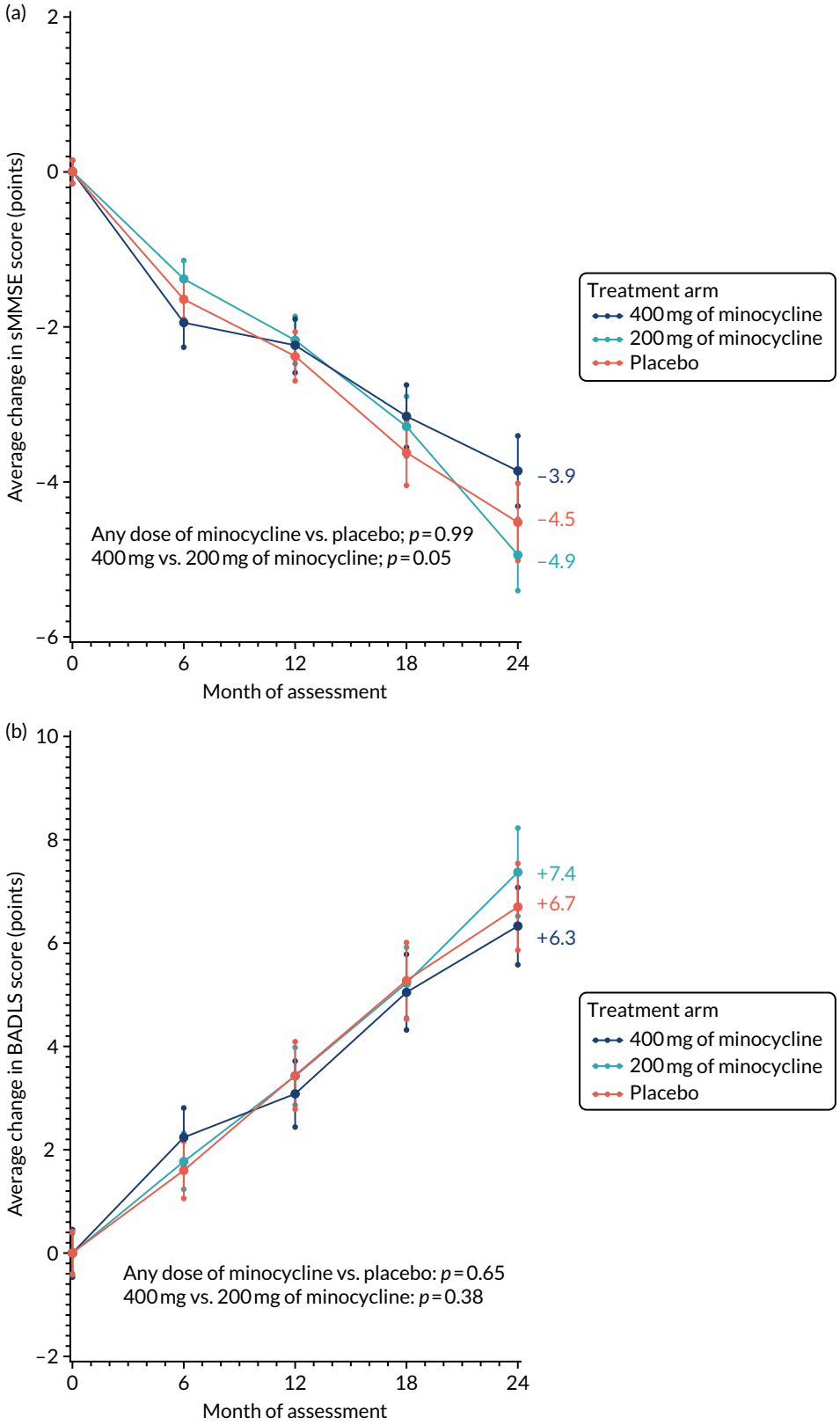

FIGURE 9 Change in sMMSE and BADLS scores from baseline to month 24 using imputation methods 1 (a and b) and 2 (c and d). A to estimate scores for patients with no follow-up past baseline. Graph shows change in mean sMMSE and BADLS scores with standard errors. Baseline scores are set to zero and *p*-values are from tests for time-by-treatment interaction from repeated measures analyses. (a) Average change in sMMSE score from baseline to month 24, using imputation (baseline scores are 26.3 points for 400 mg of minocycline, 26.5 points for 200 mg of minocycline and 26.4 points for placebo). (b) Average change in BADLS score from baseline to month 24, using imputation (baseline scores are 5.6 points for 400 mg of minocycline, 4.9 points for 200 mg of minocycline and 5.5 points for placebo). (c) Average change in sMMSE score from baseline to month 24 using imputation. (d) Average change in BADLS score from baseline to month 24, using imputation. (*continued*)

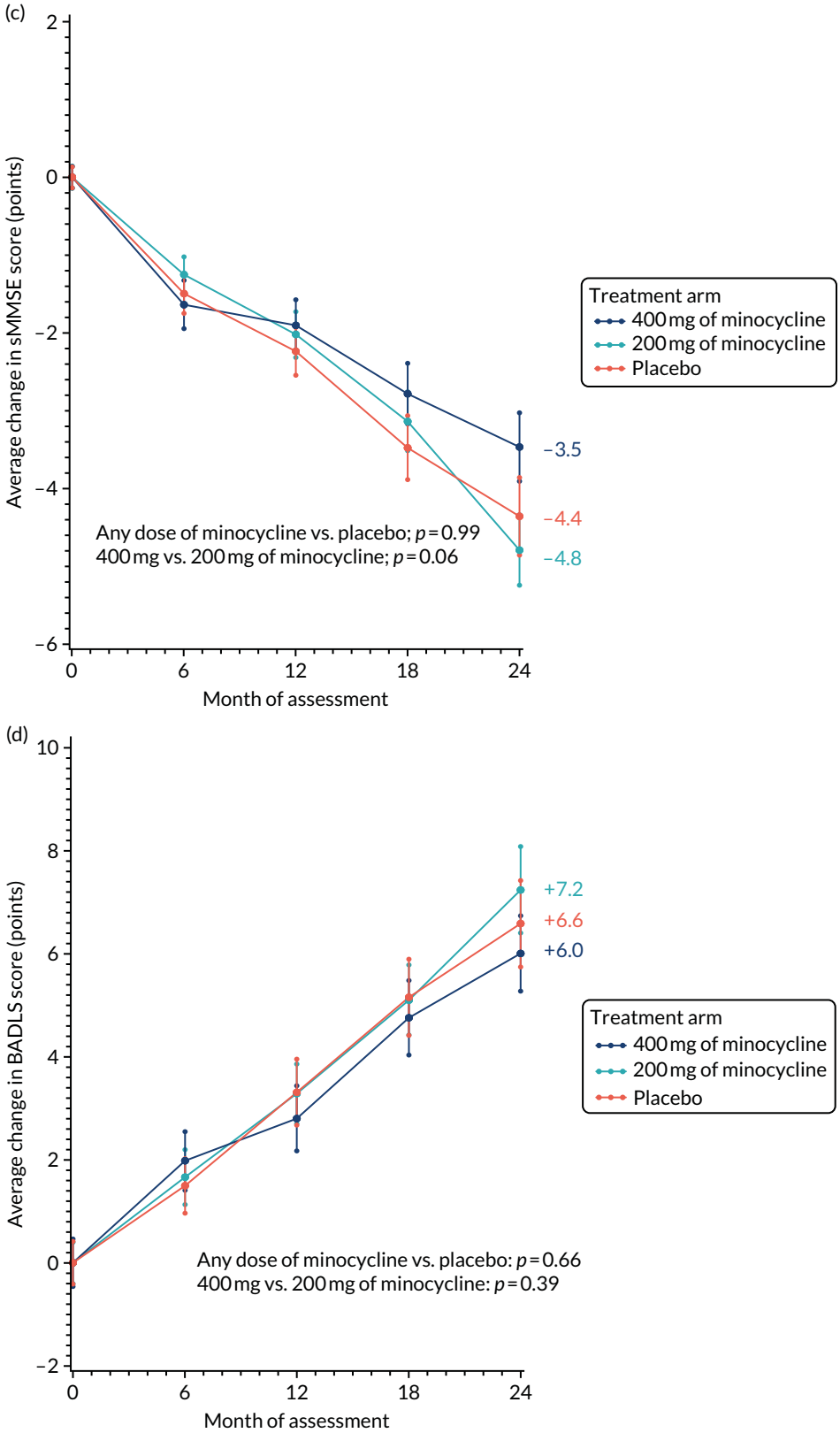

FIGURE 9 Change in sMMSE and BADLS scores from baseline to month 24 using imputation methods 1 (a and b) and 2 (c and d). A to estimate scores for patients with no follow-up past baseline. Graph shows change in mean sMMSE and BADLS scores with standard errors. Baseline scores are set to zero and *p*-values are from tests for time-by-treatment interaction from repeated measures analyses. (a) Average change in sMMSE score from baseline to month 24, using imputation (baseline scores are 26.3 points for 400 mg of minocycline, 26.5 points for 200 mg of minocycline and 26.4 points for placebo). (b) Average change in BADLS score from baseline to month 24, using imputation (baseline scores are 5.6 points for 400 mg of minocycline, 4.9 points for 200 mg of minocycline and 5.5 points for placebo). (c) Average change in sMMSE score from baseline to month 24 using imputation. (d) Average change in BADLS score from baseline to month 24, using imputation.

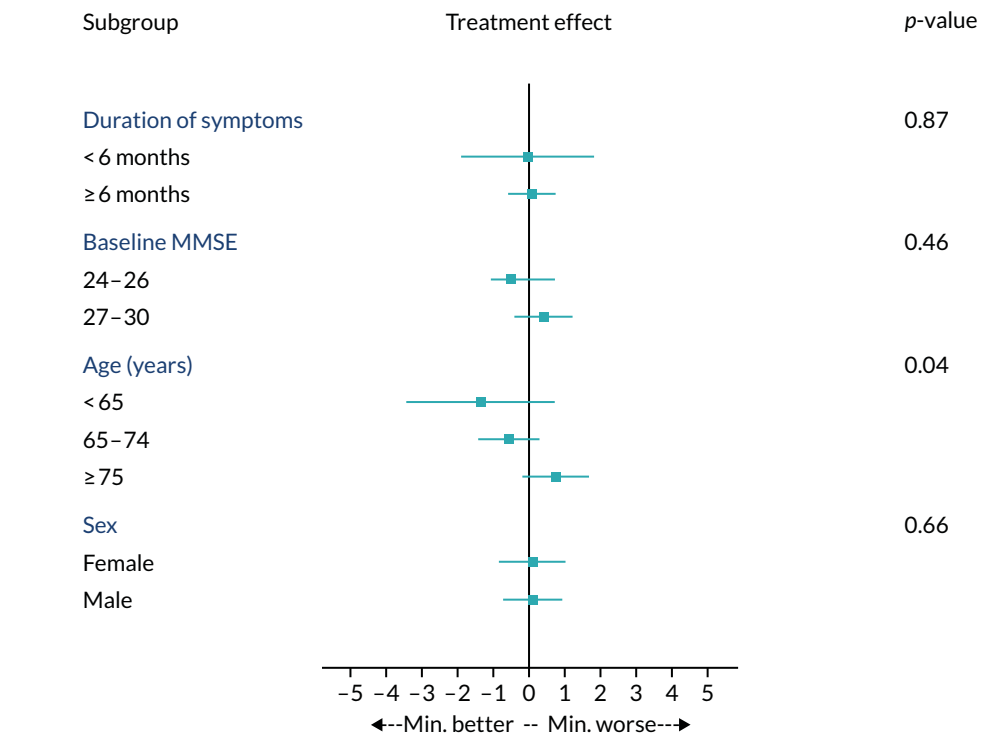

FIGURE 10 Subgroup analyses of change in sMMSE score over 24 months for minocycline (any dose) vs. placebo by baseline characteristics: duration of symptoms, baseline sMMSE score, age and gender. Results are derived from a repeated measures model, with *p*-values from tests for interaction between treatment and the selected subgroup. Min., minimum. The *p*-value is from the test statistic for testing the interaction between the treatment and any subgroup variable.

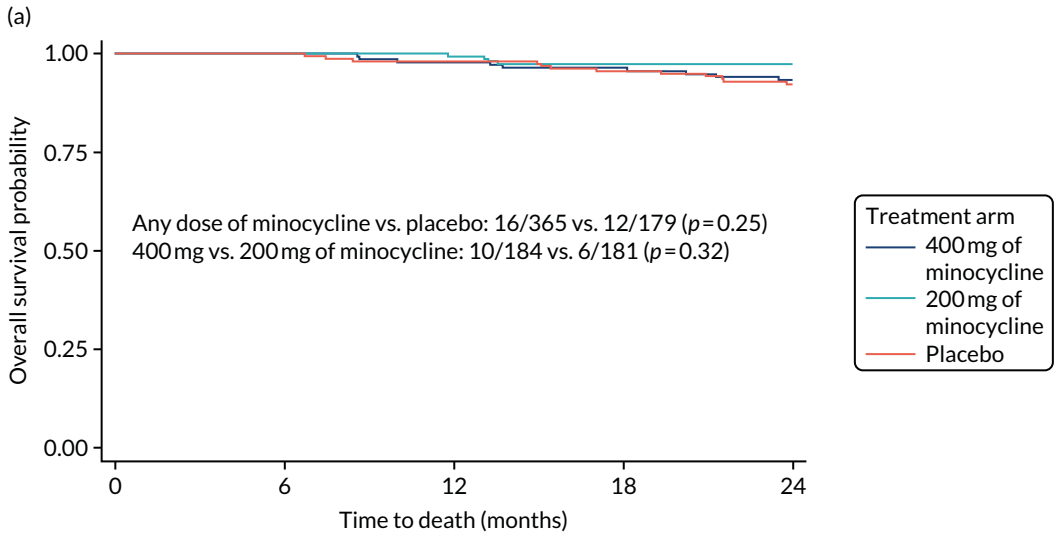

FIGURE 11 Probability of (a) overall survival, (b) institutionalisation and (c) time to death or institutionalisation by treatment arm: Kaplan–Meier survival plots. (*continued*)

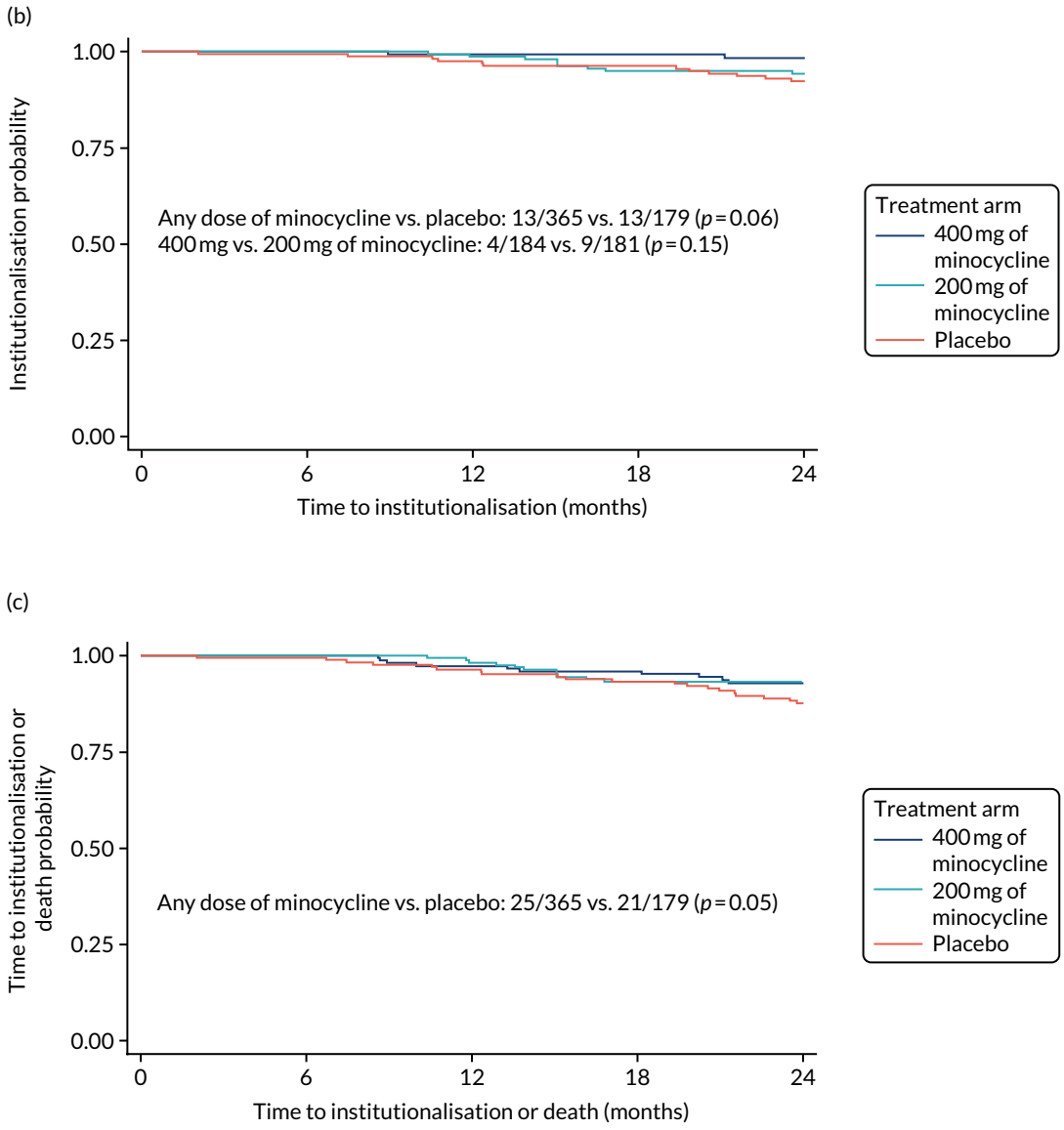

FIGURE 11 Probability of (a) overall survival, (b) institutionalisation and (c) time to death or institutionalisation by treatment arm: Kaplan–Meier survival plots.

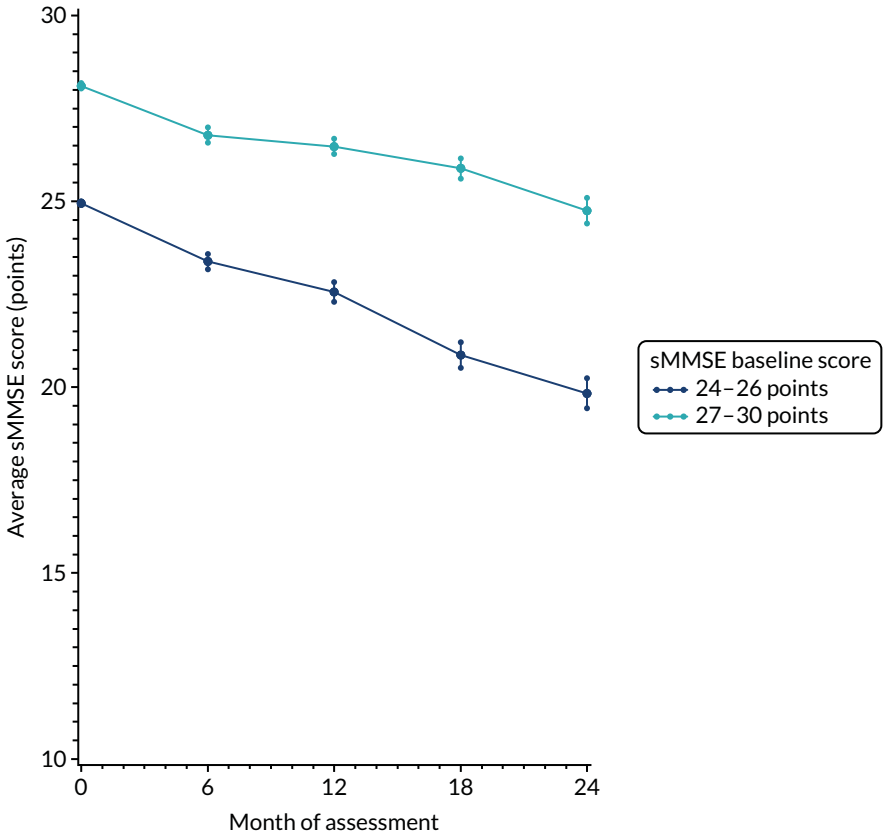

FIGURE 12 Average decline of sMMSE score split by baseline sMMSE score (i.e. a score of 24–26 or 27–30[a] points). a, Eight patients start with a sMMSE score of 30 points and have a 24-month sMMSE score of 30 points.

TABLE 11 Line-by-line listings of categorised[a] SAEs

Reference number of SAE	SAE	On treatment?
Gastrointestinal		
SAE031	Gastroenteritis	Yes
SAE067	Constipation. Was taken to hospital, patient described medication	Yes
SAE064	Gastroenteritis	Yes
SAE077	Sigmoid volvulus with faecal impaction	Stopped > 28 days ago
SAE075	Diverticulitis and impacted bowel	Yes
SAE087	Deranged LFTs/stomach ulcer with gastrointestinal bleed	Yes (stopped same time)
SAE149	Abdominal distension pain	Stopped > 28 days ago
SAE091	Constipation. Admitted to hospital with sickness/stomach pains	Stopped < 28 days ago
SAE095	Gradual internal bleeding of the stomach lining	Yes
SAE092	Diarrhoea, vomiting and weight loss. Ambulance called and patient was admitted to hospital overnight. Hospital-requested RNI scan	Yes
SAE106	Under investigation – severe diarrhoea. Bowels going into spasms	Yes
SAE112	Patient admitted to hospital with severe abdominal pains	Stopped > 28 days ago

continued

TABLE 11 Line-by-line listings of categorised[a] SAEs (*continued*)

Reference number of SAE	SAE	On treatment?
SAE120	Undiagnosed stomach pains. Investigations into possible stomach ulcer or recurrence of bowel cancer	Yes (stopped same time)
SAE138	Diverticulitis	Yes
SAE181	Gastroenteritis	Yes
SAE164	Appendicitis	Yes
SAE187	Death from complications after bowel surgery	Stopped > 28 days ago
SAE209	Secondary, adhesion bowel obstruction – resulted in death	Stopped > 28 days ago
SAE194	Bowel obstruction	Yes
SAE182	Cyst on small intestine updated diagnosis previously bowel obstruction	Yes
SAE228	Obstruction of the common bile duct	Yes
Respiratory		
SAE001	Pneumonia	Yes (stopped same time)
SAE002	Wheezing and shortness of breath	Yes (stopped same time)
SAE012	COPD	Yes
SAE020	Pneumonia	Yes
SAE018	Pneumonia – resulted in death	Yes (stopped same time)
SAE022	Suicide attempt and subsequent aspiration pneumonia and pulmonary oedema – resulted in death	Stopped > 28 days ago
SAE025	Community-acquired pneumonia	Yes (stopped same time)
SAE048	Sepsis secondary to community-acquired pneumonia	Yes (stopped same time)
SAE043	Pneumonia	Yes
SAE047	Suspected pneumonia/further investigation	Yes
SAE079	Pneumonia and pleural effusion – resulted in death	Yes
SAE085	Pneumonia	Yes (stopped same time)
SAE116	Died – COPD	Stopped > 28 days ago
SAE114	Pneumonia – died in hospital	Yes (stopped same time)
SAE117	Pneumonia preceded by declining neutrophil count which then rose before ceasing IMP	Yes
SAE136	Community-acquired pneumonia	Yes
SAE122	Pneumonia	Yes
SAE156	Increased shortness of breath	Stopped < 28 days ago
SAE163	Breathlessness and extreme thirst	Yes
SAE184	Pneumonia	Yes
SAE200	COPD	Yes
SAE226	Admitted to hospital after fall and contracted pneumonia while in hospital	Yes
SAE266	Admitted to hospital after fall and contracted pneumonia while in hospital. Had fractured a rib	Yes

TABLE 11 Line-by-line listings of categorised[a] SAEs (*continued*)

Reference number of SAE	SAE	On treatment?
SAE243	Pneumonia	Stopped > 28 days ago
SAE270	Pulmonary fibrosis	Yes
SAE215	Pneumonia – resulted in death	Stopped > 28 days ago
Mechanical injury		
SAE004	Fall resulting in skull fracture	Yes
SAE015	Fall and closed fracture of rib	Yes (stopped same time)
SAE021	Fall out of bed resulting in head and neck injury	Stopped < 28 days ago
SAE034	Fall sustaining cuts, bruising and reduced mobility	Yes
SAE053	Unwitnessed fall out of bed	Yes
SAE068	Fractured pubic rami from a fall	Yes
SAE046	Fracture of right femur from a fall	Yes (stopped same time)
SAE054	Fractured wrist from a fall	Yes
SAE080	Patient fell and fractured pelvis, related to increased dizziness, less steady on feet since starting MADE trial treatment	Stopped < 28 days ago
SAE063	Possible bruised, cracked or broken ribs following a fall as a result of tripping	Yes
SAE073	Shoulder surgery following fall and dislocation	Yes
SAE065	Fractured right humerus from a fall	Yes
SAE093	Cerebral concussion and cut to head from a fall	Yes
SAE124	Fractured right neck of femur	Yes (stopped same time)
SAE155	Road traffic accident – patient hit by car	Stopped > 28 days ago
SAE119	Fall and admission to hospital overnight	Yes
SAE139	Fractured left femur and underwent left hemiarthroplasty	Yes
SAE175	Fractured ribs from a fall	Yes
SAE117	Broken left hip	Yes
SAE196	Fracture of left neck of femur	Yes
SAE199	Knee replacement operation	Yes
SAE222	Fall	Yes
SAE232	Fracture of metacarpal	Yes
SAE235	Facial injury from a fall	Stopped > 28 days ago
SAE237	Collapse and facial injury and nasal fracture	Yes
SAE254	Fractured hip	Yes
SAE255	Fall causing pubic ramus and wrist fracture	Yes
SAE258	Vertigo/dizziness – leading to head injury from a fall	Stopped > 28 days ago
SAE256	Fracture to middle finger and left hand	Yes
SAE263	Mechanical fall and back pain	Stopped > 28 days ago

continued

TABLE 11 Line-by-line listings of categorised[a] SAEs (*continued*)

Reference number of SAE	SAE	On treatment?
Endocrine and metabolic		
SAE008	Diabetes mellitus management impairment – resolved	Yes
SAE007	New medical diagnosis of type 2 diabetes mellitus	Yes
SAE060	Low sodium levels	Yes
SAE061	Pituitary adenoma	Stopped > 28 days ago
SAE056	Diabetic ketoacidosis	Stopped > 28 days ago
SAE072	Hypoglycaemia	Stopped > 28 days ago
SAE118	Hypoglycaemia	Yes
SAE132	Syndrome of inappropriate anti-diuretic hormone	Yes
SAE135	Low potassium levels as a result of bowel preparation for CT bowel scan	Yes
SAE189	Admitted to hospital after feeling weak and faint. Diagnosed with low sodium levels	Yes
SAE190	Admitted to hospital following low sodium levels and generally feeling weak	Yes
SAE249	Inflammatory arthropathy – probably due to gout	Yes
Cancer		
SAE005	Recurrence of bladder cancer	Yes
SAE032	Tumour on kidney – right kidney/part of liver removed	Yes
SAE024	Colon cancer	Yes
SAE040	Colon cancer (open anterior resection surgery)	Yes
SAE066	Chronic lymphocytic leukaemia	Yes
SAE096	Diagnosis of myeloproliferative neoplasm JAK1	Yes
SAE078	Working diagnosis – colon cancer. Patient feels well so patient/family do not want further tests	Yes
SAE083	Bowel cancer – resulted in death	Yes
SAE011	Cancer of oesophagus	Stopped > 28 days ago
SAE123	Suspected kidney cancer, diagnosis of left renal tumour	Stopped > 28 days ago
SAE229	Chronic lymphoid leukaemia	Stopped > 28 days ago
SAE102	Recent lung cancer diagnosis. Further investigation of cancer shows it to be terminal with secondary cancers in the liver	Yes
SAE109	Bowen's disease	Yes
SAE183	MDS	Yes
SAE147	Bowel cancer	Stopped > 28 days ago
SAE130	Probable lung cancer, will not undergo treatment for cancer	Stopped > 28 days ago
SAE142	Patient diagnosed with prostate cancer	Yes
SAE161	Prostate cancer with pelvic metastasis	Yes
SAE191	Colonic primary tumour, with extensive liver metastasis	Stopped < 28 days ago
SAE193	Complex atypical hyperplasia	Yes (stopped same time)

TABLE 11 Line-by-line listings of categorised[a] SAEs (*continued*)

Reference number of SAE	SAE	On treatment?
SAE206	Lung tumour and secondary cancers	Yes
SAE212	Appearances consistent with lung malignancy. Given comorbid condition for best supportive/palliative care	Yes
SAE217	Vulvar cancer	Yes
SAE238	Tonsillectomy as a result of cancer	Yes
SAE251	Prostate cancer	Stopped > 28 days ago
SAE253	Basal cell carcinoma	Yes
Haematological/thrombosis		
SAE038	Low levels of platelets	Yes (stopped same time)
SAE045	Blood transfusion for suspected bleed, following low levels of haemoglobin	Yes
SAE110	DVT	Yes (stopped same time)
SAE129	Neutropenia	Yes
SAE145	Anaemia	Yes
SAE236	Blood clot	Yes
Dermatological		
SAE245	Minocycline type 2 pigmentation on face	Yes
Neuropsychiatric		
SAE009	Hospitalisation following seizures	Yes
SAE019	Psychosis secondary to dementia. Also mild UTI	Yes (stopped same time)
SAE029	Admitted to psychiatric unit following relapse in psychotic symptoms with agitation	Stopped > 28 days ago
SAE128	Patient confused, lacked co-ordination and had been experiencing more falls for a few weeks	Yes
SAE160	Hospital admission with severe Alzheimer's dementia, with significant behavioural disturbance	Stopped > 28 days ago
SAE050	Admission to hospital as a result of loss of consciousness	Yes
SAE158	Death as a result of dementia	Yes
SAE016	Subdural haematoma	Yes (stopped same time)
SAE152	AD/Lewy body disease – resulted in death	Stopped < 28 days ago
SAE143	Stroke	Yes (stopped same time)
SAE105	Probable stroke. Also receiving treatment for chest infection	Yes (stopped same time)
SAE055	Minor stroke	Yes
SAE146	Seizure (known epilepsy), cracked bone in ankle	Yes
SAE086	CVA – resulted in death	Stopped > 28 days ago
SAE170	AD	Yes
SAE076	AD	Yes
SAE203	Minor stroke non-haemorrhagic	Yes

continued

TABLE 11 Line-by-line listings of categorised[a] SAEs (*continued*)

Reference number of SAE	SAE	On treatment?
SAE069	Stroke	Yes
SAE059	Left intracranial bleed	Yes (stopped same time)
SAE154	AD	Yes
SAE166	Funny turns followed by suspected TIA. Patient hospitalised	Stopped > 28 days ago
SAE150	Stroke	Yes
SAE167	Bleeding on brain	Yes
SAE159	Mini stroke (TIA)	Yes
SAE169	Suspected stroke/seizure	Yes
SAE172	Stroke – resulted in death	Yes
SAE088	Small left frontal lobe cortical haemorrhage	Yes
SAE153	Subdural haemorrhage/blood clot	Yes
SAE121	Admitted following falls, is due to being discharged home with end-of-life/full-time carers. MRI scanning showed chronic subdural haematoma	Yes (stopped same time)
SAE134	Seizure, no diagnosis given	Stopped < 28 days ago
SAE218	Delirium	Yes (stopped same time)
SAE219	Dementia in AD	Yes
SAE198	Confusion, slurred speech, unsteady on feet. Admitted to hospital	Yes
SAE230	Patient in acute psychiatric ward on section 2	Yes
SAE231	Suspected stroke. Patient died	Stopped > 28 days ago
SAE239	Possible TIA	Yes
SAE247	Delirium	Yes
SAE252	Progression of AD – resulted in death	Yes (stopped same time)
SAE265	CVA	Yes (stopped same time)
Cardio-circulatory		
SAE036	Suspected myocardial infarction	Yes
SAE030	Shortness of breath and suspected MI	Yes
SAE010	Cardiac abnormalities: long QT on ECG and impaired left ventricular function	Yes
SAE062	Cardiac event – resulted in death	Yes (stopped same time)
SAE035	Myocardial infarction	Yes
SAE099	Syncope attributed to GTN spray overdose (accidental)	Yes
SAE090	Swollen ankles, shortness of breath, ambulance called. Admitted to hospital for 11 days. Diagnosis fluid on lungs and heart murmur	Yes
SAE104	Cardiogenic syncope	Yes
SAE094	Collapsed, thought to be as a result of low blood pressure	Yes
SAE098	Postural hypotension	Yes

TABLE 11 Line-by-line listings of categorised[a] SAEs (*continued*)

Reference number of SAE	SAE	On treatment?
SAE201	Out-of-hospital ventricular fibrillation arrest as a result of anterior myocardial infarction – resulted in death	Yes (stopped same time)
SAE211	Recurrent gradual-onset syncope, junctional bradycardia on implantable loop recorder	Yes
SAE208	Postural hypotension	Yes
SAE039	Hospitalisation – low blood pressure/pulse rate	Yes
SAE157	Hypotension	Yes
SAE125	Chest pain	Yes
SAE049	Suspected heart attack resulting in death	Yes (stopped same time)
SAE137	Severe mitral valve regurgitation, which resolved on rate-limiting control of AF and LV improvement in function	Yes
SAE144	Currently unknown – heart-related problems	Yes
SAE151	Death from MI	Yes (stopped same time)
SAE168	Death – coronary atherosclerosis hypertension	Yes (stopped same time)
SAE171	Cardiac arrest	Yes
SAE207	Heart problems. Cardiac monitor had revealed that heart had stopped for short time	Yes
SAE202	Heart failure	Stopped < 28 days ago
SAE205	Heart failure	Stopped < 28 days ago
SAE176	Aortic stenosis	Yes
SAE103	Deterioration in cardiac failure plus syncopal episode that led to hospital admission	Yes (stopped same time)
SAE224	Heart failure – resulted in death	Stopped > 28 days ago
SAE241	Heart attack – resulted in death	Stopped > 28 days ago
SAE240	Labile blood pressure/hypertension	Yes
SAE246	Heart failure	Yes
SAE248	Heart attack – resulted in death	Yes (stopped same time)
SAE564	Syncope (probably as a result of bradycardia)	Stopped > 28 days ago
SAE268	Atrial fibrillation	Stopped > 28 days ago
Renal		
SAE057	Kidney stones	Yes
SAE058	Large abdominal tumour causing kidney failure – resulted in death	Never started
SAE084	Chronic renal failure – resulted in death	Stopped > 28 days ago
SAE101	No evidence of bladder cancer. Patient was having tests from a bladder biopsy	Yes
SAE113	Radical left nephrectomy laparoscopy	Stopped > 28 days ago
SAE204	Acute kidney injury	Yes
SAE267	Lung and kidney failure – resulted in death	Stopped > 28 days ago

continued

TABLE 11 Line-by-line listings of categorised[a] SAEs (*continued*)

Reference number of SAE	SAE	On treatment?
Infection		
SAE014	Hospitalisation – chest infection	Yes
SAE017	Hospitalisation – delirium as a result of dehydration and urine infection	Yes
SAE044	Probable UTI, symptoms of confusion, weakness, low mobility	Yes
SAE082	Infection following a foreign body in arm	Yes
SAE051	Chest infection	Yes (stopped same time)
SAE052	Urinary tract infection	Yes (stopped same time)
SAE081	Catheter-associated UTI	Yes (stopped same time)
SAE070	Chest infection	Yes
SAE097	Infection – resulted in death	Stopped > 28 days ago
SAE100	Pruritic rash in the context or urosepsis	Yes
SAE107	Urosepsis	Yes
SAE115	Sepsis, possibly related to gall bladder problems	Stopped > 28 days ago
SAE131	Progressive decline post chest infection	Stopped > 28 days ago
SAE127	Urinary infection	Stopped < 28 days ago
SAE141	Admitted with lower respiratory infection	Yes (stopped same time)
SAE165	Admitted to hospital with very sore throat later diagnosed as thrush	Yes
SAE173	Shortness of breath and chest infection	Yes
SAE174	Shortness of breath and chest infection	Yes
SAE180	Taken to hospital with very low blood pressure and infection	Yes
SAE188	Sepsis	Stopped > 28 days ago
SAE195	On 11 November 2016 wife reports participant has chest infection – resulted in death	Yes (stopped same time)
SAE197	Urinary tract infection	Yes
SAE133	Updated from discharge summary: cellulitis	Stopped > 28 days ago
SAE233	UTI	Stopped > 28 days ago
SAE234	UTI	Stopped > 28 days ago
SAE210	Urinary tract infection, confusion	Yes
SAE216	Oesophageal candidiasis	Yes
SAE213	Admitted with lower respiratory tract infection	Stopped > 28 days ago
SAE22	UTI	Yes
SAE223	UTI	Yes

TABLE 11 Line-by-line listings of categorised[a] SAEs (*continued*)

Reference number of SAE	SAE	On treatment?
Other		
SAE006	Hospitalisation – collapsed in street	Yes
SAE028	Suspected blood clot in legs. Swollen legs/painful. No blood clot found	Yes
SAE033	Suspected thrombosis – investigations complete no diagnosis of thrombosis. Symptoms of swollen legs have been associated with previously known water condition. Symptoms reduced after treatment	Yes
SAE042	Participant drank white spirit in error	Yes
SAE071	Replacement of left knee	Stopped > 28 days ago
SAE111	Sensitivity and tenderness around left nipple	Yes
SAE074	IMP overdose	Yes
SAE089	Patient collapsed following accidental overdose of ranitidine	Yes (stopped same time)
SAE108	General health decline – resulted in death. Patient was in respite care	Stopped > 28 days ago
SAE148	Osteoarthritis	Yes
SAE179	Jaw, back and neck pain – no diagnosis – cardiac problems ruled out	Stopped > 28 days ago
SAE162	Prolonged hospital stay after planned hernia operation	Yes
SAE186	Admitted for elective abdominal hysterectomy and bilateral salpingo-oophorectomy	Yes
SAE178	No acute medical problem identified. Patient felt as if she had severe indigestion. Investigations into whether or not it was a mild heart attack in A&E (stayed overnight). No explanation found	Stopped > 28 days ago
SAE185	TURP	Yes
SAE214	Hip screw removal	Yes
SAE225		Yes
SAE244	Postoperative scrotal oedema	Yes
SAE242	Swollen legs	Yes
SAE250	Unknown – resulted in death	> 28 days ago

A&E, accident and emergency; AF, atrial fibrillation; COPD, chronic obstructive pulmonary disorder; CT, computed tomography; CVA, cerebrovascular accident; DVT, deep-vein thrombosis; ECG, electrocardiogram; GTN, glyceryl trinitrate; JAK1, Janus kinase 1; LFT, liver function test; LV, left ventricular; MDS, myelodysplastic syndrome; MI, myocardial infarction; MRI, magnetic resonance imaging; RNI, radionucleotide; TIA, transient ischaemic attack; TURP, transurethral resection of the prostate; UTI, urinary tract infection.
a Participants can have more than one recorded SAE in each category.

EME
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HTA
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