Minocycline in mild Alzheimer's disease (MADE): a randomised controlled, double-blind trial

Outcomes

sMMSE

The first primary outcome measure is the standardised Mini-Mental State Examination (sMMSE) [40,41], a widely used clinician-rated instrument for assessing cognition. Scores range from 0 to 30 with higher scores indicating better cognitive function.

The original Mini-Mental State (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 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 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[45,46,47].

BADLS

The second outcome measure is the Bristol Activities of Daily Living Scale (BADLS) [48] used to assess functional ability (activities of daily living). Scores range from 0 to 60 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, 51].

Data was collected on the sMMSE at Screening, 6, 12, 18 and 24 months, and for the BADLS at Baseline, 6, 12, 18 and 24 months.

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 (eg 2-week) break from IMP treatment and were reminded to re-start once the symptoms resolved. If side-effects persisted, participants were advised to stop taking the study drug.

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 (bacterial enteritis, clostridium difficile and orogenital candidiasis) were also assessed and recorded every 3 months. To monitor renal function, MDRD formula was used to calculate the eGFR at baseline and changes in creatinine levels were used to monitor renal function post baseline. In particular, the following guidelines were used:

> *Any patient with a follow up creatinine 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 remains the same or higher then a further check will be required. Any patient with a follow-up creatinine of ≥50% higher than baseline can remain on treatment but will have a repeat blood sample within 10 days. If creatinine remains the same or higher then study treatment will be stopped (unless an obvious alternative cause is identified eg, NSAID use, other illness).*

Statistical considerations

We aimed to randomise 560 participants in a semi-factorial (2x1) design 1:1:1 between minocycline (400mg or 200mg), and placebo. Based on previous studies, we estimated that 24-month assessments would be available on at least 80% of surviving participants (i.e. approximately 390) which would provide 90% power at $p<0.05$ to detect a small to moderate (0.35 standard deviation) effect size for minocycline (any dose) compared to placebo on the primary outcome measures. With outcome assessments on 130 patients allocated minocycline 400mg and 130 allocated minocycline

200mg, we would have 80% power at p<0.05 to detect a 0.35 SD treatment effect of 400mg compared to 200mg at 24 months.

Only participants who received at least one capsule of study treatment 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, 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 400mg and 200mg 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. We used SAS software (version 9.3) for all statistical analyses. The independent data monitoring and ethics committee reviewed the unblinded accumulating data and the safety of patients in the study at approximately yearly intervals.

RESULTS

Between May 23, 2014 and April 14, 2016, 554 participants were entered from 32 National Health Service 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 400mg minocycline, four to 200mg, and five to placebo. Baseline characteristics of the 544 eligible participants were well balanced across the three treatment groups (table 2).

Table 2. Baseline characteristics by treatment allocation for the 544 eligible patients

		400mg N=184	200mg N=181	Placebo N=179
Age	<65	22 (12%)	22 (12%)	21 (12%)
	65-74	68 (37%)	66 (36%)	66 (37%)
	75+	94 (51%)	93 (51%)	92 (51%)
Age	Mean (SD)	74.3 (8.0)	74.1 (8.4)	74.6 (8.1)
Gender	Male	104 (57%)	100 (55%)	99 (55%)
	Female	80 (43%)	81 (45%)	80 (45%)
Home circumstance	Living with spouse/partner/relative	153 (83%)	153 (85%)	149 (83%)

		400mg N=184	200mg N=181	Placebo N=179
	Alone	31 (17%)	28 (15%)	29 (16%)
Duration of symptoms	<6 months	20 (11%)	20 (11%)	20 (11%)
	>=6 months	164 (89%)	161 (89%)	159 (89%)
Duration of symptoms	Mean (SD)	23.5 (18.3)	23.1 (17.8)	24.2 (18.0)
sMMSE score	24-26	100 (54%)	97 (54%)	96 (54%)
	27-30	84 (46%)	84 (46%)	83 (46%)
sMMSE score	Mean (SD)	26.4 (1.9)	26.5 (1.9)	26.4 (1.8)

Data are n (%) unless otherwise stated. sMMSE=standardised Mini Mental State Examination

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

sMMSE assessments were obtained for 542 (99.6%) of the 544 participants at baseline, 498 (92%) of 544 at 6 months, 453 (84%) of 537 at 12 months, 420 (80%) of 528 at 18 months, and 403 (78%) of 517 at 24 months (appendix 2 table 1). There were somewhat fewer BADLS than sMMSE assessments as BADLS assessments are not valid for participants in residential care.

Figure 1: Flow chart

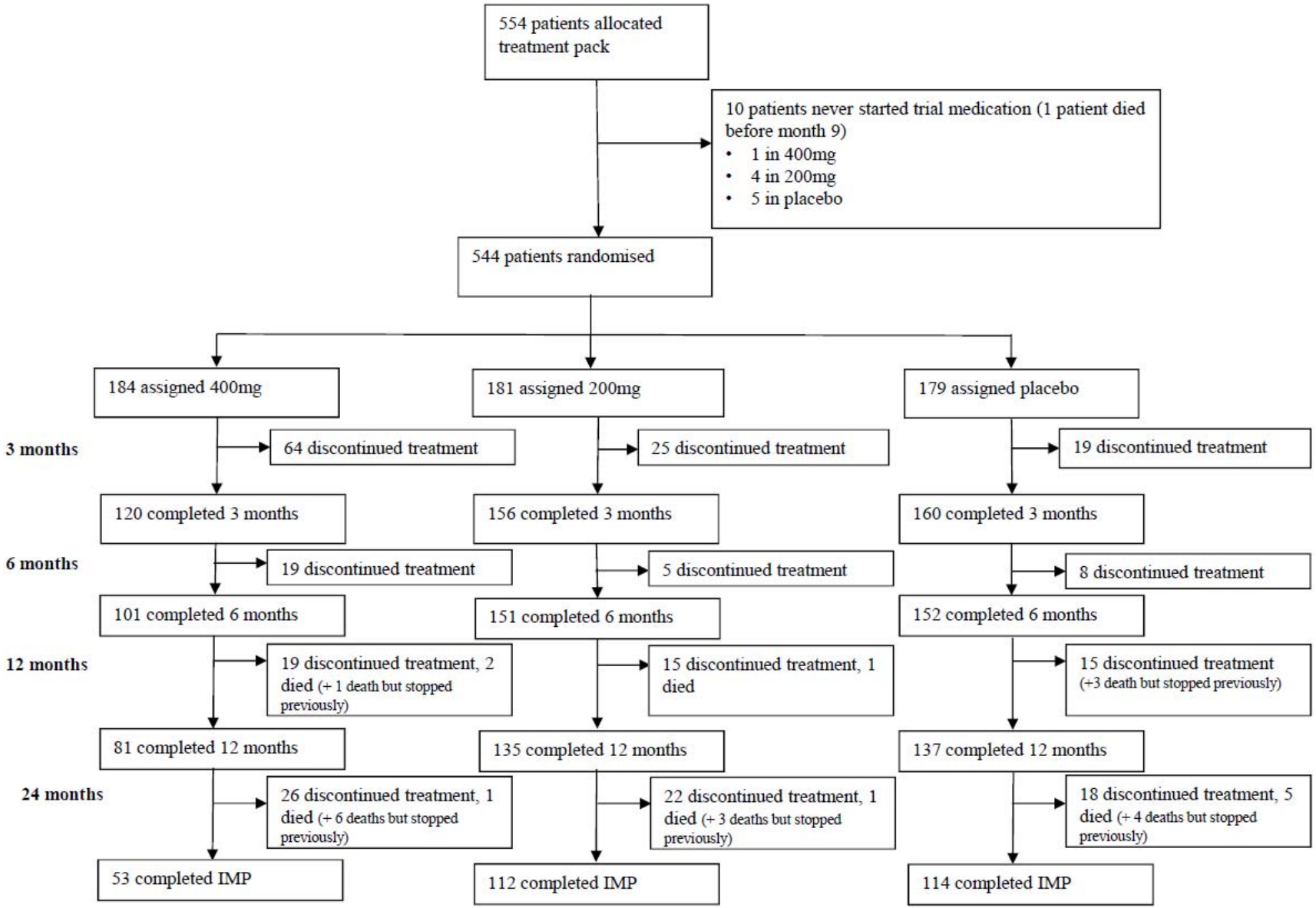
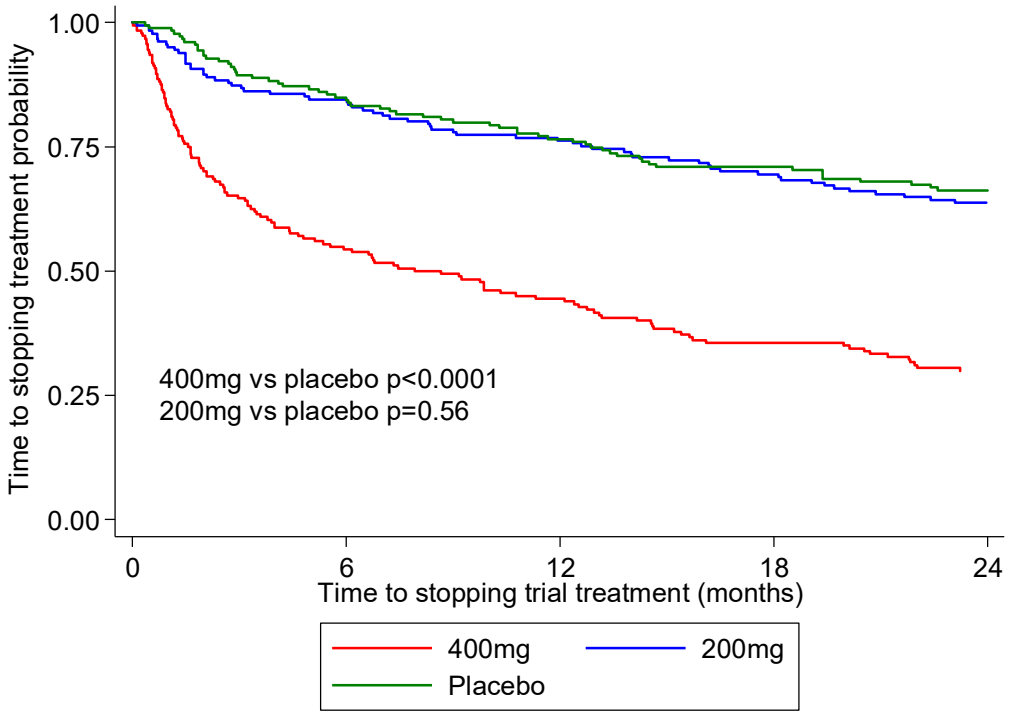


Figure 2. Proportion taking trial treatment over time: Kaplan-Meier plot



Minocycline at a daily dose of 400mg was poorly tolerated with just 29% (53/184) of those allocated 400mg completing 2 years of treatment, significantly fewer than in the 200mg [62% (112/181)], or placebo arms [64% (114/179), p<0.0001, figures 1 & 2]. By contrast, 200mg was well tolerated with similar discontinuation rates with 200mg and placebo (p=0.56). When reasons for stopping trial treatment were compared (table 3A), 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 400mg treatment arm than for the 200mg and placebo arms (appendix 2 table 1). For sMMSE at 24-months we received 68% (119 of the 174 expected) for the 400mg, 82% (144/176) for the 200mg, and 84% (140/167) for the placebo group. Return rates for BADLS assessments were similarly lower for the 400mg arm, than 200mg and placebo arms (appendix 2 table 1).

Change from baseline in sMMSE scores over time, with standard error bars, is shown in figure 3A. There was an average 4.1 point reduction in the combined minocycline groups compared to a 4.3 point reduction in the placebo group over the 24-month study period (p=0.90). The reduction in sMMSE score in the 400mg group over 24 months was somewhat less than that in the 200mg group (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 groups: 5.7, 6.6 and 6.2 in the 400mg, 200mg and placebo groups, respectively, with no significant differences between participants allocated minocycline compared to the placebo group (p=0.57), or between those allocated 400mg and those allocated 200mg minocycline (p=0.77, figure 3C).

Table 3. (A) Reasons for stopping treatment, and (B) Serious Adverse Events by treatment allocation

(A) Reasons for stopping	400mg (n=184)	200mg (n=181)	Placebo (n=179)	Total	Minocycline vs placebo p-value
GI symptoms	42	15	10	67	0.0008
Dizziness	14	3	1	18	0.01
Dermatological symptoms	10	5	1	16	0.02
Haematological	5	3	1	9	0.16
Impaired renal function	2	5	4	11	0.81
Infection	1	2	2	5	0.74
Shortness of breath	6	0	0	6	0.08
Worsening dementia	1	3	3	7	0.57
Depression or anxiety	4	2	2	8	0.63
Joint or muscle pain	2	0	2	4	0.47
Concomitant disease/illness	9	6	7	22	0.91
General deterioration in physical health	2	0	2	4	0.47
Unknown	1	0	0	1	0.48
Unspecified side effect	5	2	7	14	0.17
Patient or carer choice	23	21	18	62	0.49
Total	**127**	**67**	**60**	**254**	**0.00002**

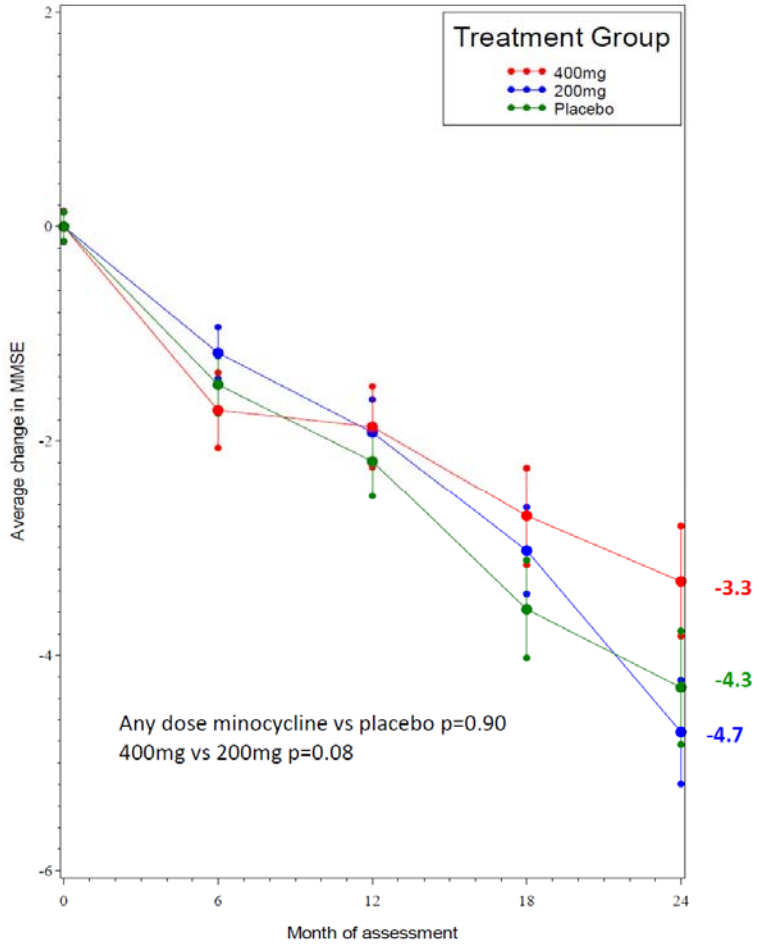
(B) Serious adverse events	Counts of SAEs reported				
SAE class	400mg (n=184)	200mg (n=181)	Placebo (n=179)	Total	Minocycline vs placebo p-value
Gastrointestinal	3	8	10	**21**	
Respiratory	8	8	10	**26**	
Mechanical injury	6	11	13	**30**	
Endocrine and metabolic	2	1	9	**12**	
Cancer	12	3	11	**26**	
Haematological/thrombosis	3	1	2	**6**	
Dermatological	0	1	0	**1**	
Neuropsychiatric	10	13	16	**39**	
Cardio-circulatory	14	9	11	**34**	
Renal	3	2	2	**7**	
Infection	10	1	19	**30**	
Other	7	11	2	**20**	
Total	**78**	**69**	**105**	**252**	

Differences were compared by χ^2 test with associated p values (two sided)

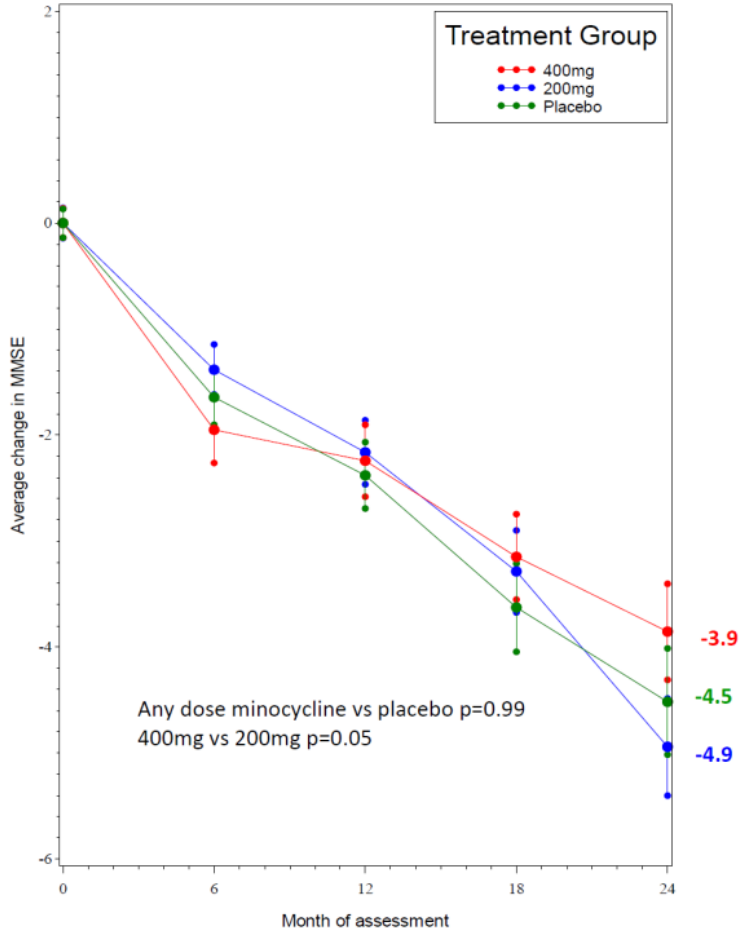
To assess how the higher number of missing outcome assessments in the 400mg treatment arm than in the 200mg or placebo arms (appendix 2 table 1) might have affected outcome comparisons, we ran various sensitivity analyses to investigate potential bias from non-random drop-out. In particular, there were 41 participants who had a baseline sMMSE but no further assessments, so did not contribute any information to the primary analysis (appendix 2 figure 1). Those 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, but no later assessments.

Figure 3. Change in (A, B) sMMSE and (C, D) BADLs from baseline to month 24. (A, C) From data collected, (B, D) using imputation method 1 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; p -values are from tests for time by treatment interaction from repeated measures analyses.

(3A) Change in sMMSE from baseline to month 24, using data collected

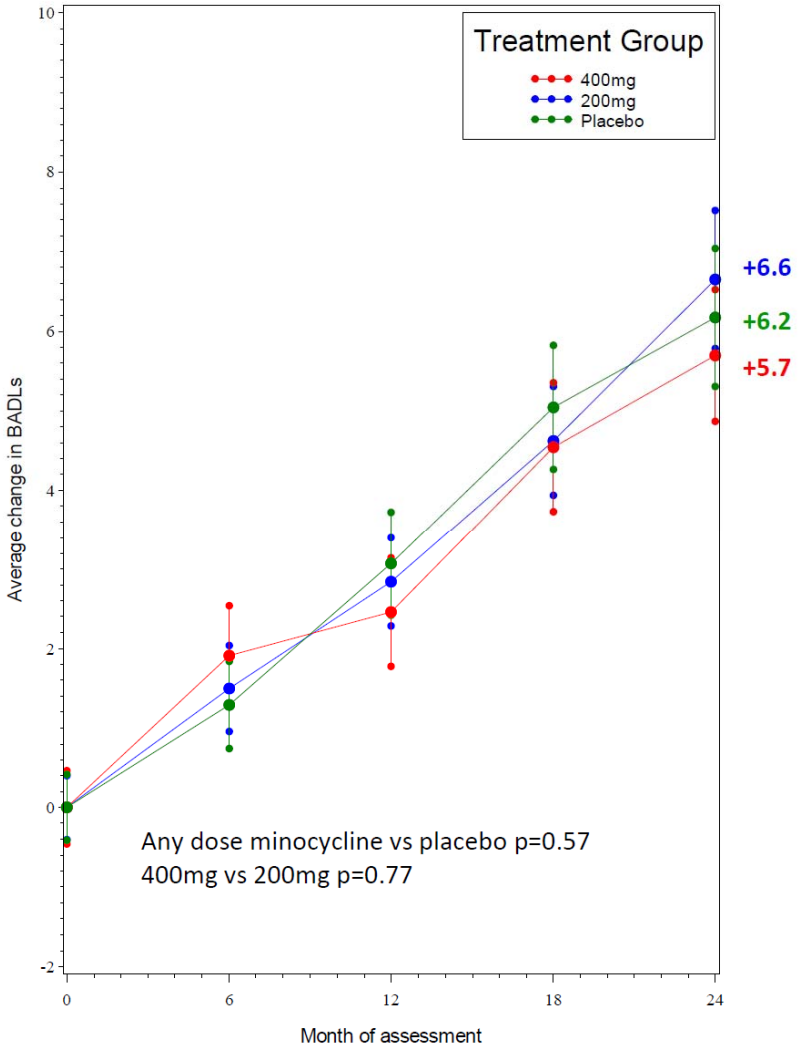


(3B) Change in sMMSE from baseline to month 24, using imputation

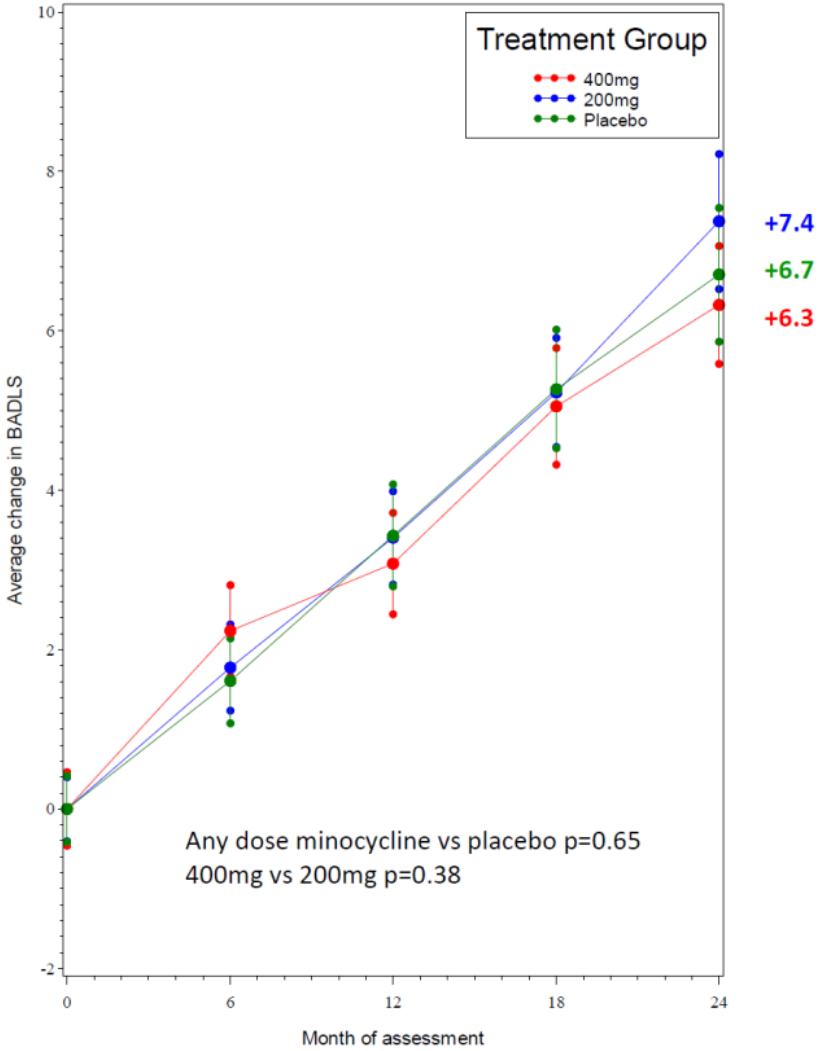


*Baseline scores: 400mg 26.3, 200mg 26.5, placebo 26.4

(3C) Change in BADLs from baseline to month 24, using data collected



(3D) Change in BADLs from baseline to month 24, using imputation



*Baseline scores: 400mg 5.6, 200mg 4.9, placebo 5.5

The average decline in sMMSE from baseline to 6 months in this subset was 3.9 points, a three times higher rate of decline than the 1.3 points 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 worse than average decline in cognitive and functional ability.

To estimate what impact the missing outcome data from the 41 participants with no post-baseline assessments might have had on the trial results, our sensitivity analyses made two different assumptions: (1) we assumed that, for the first 6 months they declined at a rate of 3.9 points (as did those 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. Method (2) assumed that patients with no post-baseline assessments declined at the average rate of those with assessments, i.e. 1.3 sMMSE points for the first 6 months, and 1.1 points every 6 months subsequently. The results from imputation method (1) are shown in figure 3B, and from imputation method (2) in appendix 2 figure 2A. 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 400mg and 200mg minocycline. However, with 400mg a little better and 200mg a little worse than placebo, and no difference between any dose of minocycline and placebo, this is likely a chance finding.

As return rates for BADLs were also lower for the 400mg arm, than 200mg and placebo, we ran similar sensitivity analyses. 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 over the first 6 months, and then by 1.9 points subsequently. As BADLS is only valid for community-resident patients, scores for those who went into residential care were only imputed up until the last time point before moving into care. Results for imputation method (1) are shown in figure 3D, and for imputation method (2) in appendix 2 figure 2B. Again, results were not qualitatively different to those from the primary analyses of BADLS.

To investigate whether the efficacy of minocycline varied by baseline characteristics, we did subgroup analyses of change in sMMSE over 24 months for minocycline (any dose) versus placebo by duration of symptoms, baseline sMMSE, age and gender (appendix 2 figure 3). There was no indications of any benefit from minocycline in those with shorter or longer duration of symptoms, lower or higher baseline sMMSE, or in men or women. There was a borderline significant (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.

In total, there were 252 reported serious adverse events (SAEs), with the most common categories being neuropsychiatric and cardio-circulatory. The number of SAEs was somewhat higher in the placebo arm than the 400mg and 200mg minocycline arms (table 3B). Given that gastrointestinal symptoms were the main reason for stopping trial treatment, it is reassuring that the numbers of gastrointestinal serious adverse events in the minocycline arms were low, and no higher than in the placebo group. Similarly, though more skin related toxicities, particularly pigmentation, were reported with minocycline than placebo [36% (130/365) *vs* 21% (38/179), p=0.0007], few stopped trial treatment because of such toxicities (table 3A), and only six skin toxicities were considered severe (3 allocated any dose minocycline and 3 placebo: appendix 2 table 2). There were no differences in numbers 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 400mg minocycline, 6 200mg, and 12 placebo (appendix 2 table 3 & figure 4A). 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 groups (appendix 2 figure 4b & 4C).

DISCUSSION

The MADE trial has shown that, in patients with mild AD, 24 months 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 400mg is poorly tolerated in this population with fewer than a third of participants completing 24 months of treatment. By contrast, 200mg per day 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] Non-steroidal anti-inflammatory drugs (NSAIDs) have similarly failed to slow disease progression in clinical trials,[52] despite long-term use being associated with a lower risk of developing AD in observational studies,[53] and promising data from transgenic animal models.[54] Our findings also parallel those of clinical trials of minocycline in other neurodegenerative disorders where, despite preclinical research suggesting neuroprotection, minocycline worsened outcomes in amyotrophic lateral sclerosis,[25] had no effect in Huntington's disease,[55] multiple system atrophy,[56] negative symptoms of schizophrenia[57] and only short-term benefits in multiple sclerosis.[58]

We consider that there could be three broad potential explanations for the negative results of our trial. First, although there is good evidence for neuroinflammation in AD,[7] this may be more as a reaction to pathology rather 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 MADE 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 MADE (typically equivalent to 3-7g per day in the human),[59] and so it could be that trial participants were not exposed to a sufficiently high dose for efficacy. However, if doses of 200-400mg per day are insufficient for neuroprotection, the difficulties with tolerability experienced by participants allocated 400mg 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-α, 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 PET,[60] following twelve weeks of treatment with 200mg minocycline per day, indicating that the dose ranges used in MADE 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[60] was also associated with increased plasma levels of neurofilament light, considered a marker of neurodegeneration. The faster progression seen with minocycline in amyotrophic lateral sclerosis[25] also suggests that some activated microglia might have a reparative function so that their inhibition could accelerate neurodegeneration. Our results do not suggest that reduced microglial activation with minocycline worsens neurodegeneration in Alzheimer's disease.

A third plausible explanation for the negative results of MADE 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. MADE was, however, powered to detect minimal clinically important differences between minocycline and placebo, so smaller differences might not be considered of clinical relevance.

Our pragmatic trial had a number of strengths. It was based within 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 Alzheimer's disease 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, 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, since these are not routinely available within the NHS. 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 400mg arm completing two years of treatment, and only moderate compliance in the 200mg 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 only introduced late in the trial when the problem with 400mg 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 our results. However, despite the large number of treatment withdrawals in the 400mg arm and consequent loss to follow-up of some participants, results were essentially unchanged in sensitivity analyses investigating potential bias from missing data. Thus, our conclusion that two years of minocycline treatment for patients with mild AD does not result in any clinically meaningful difference in the rate of decline of

APPENDIX 1

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? ***IN FACILITY – What is the name of this building?***	10 seconds	
	IN HOME – What room are we in? ***IN FACILITY – What floor are we on?***	10 seconds	
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 correctly copied diagram with a 4-sided figure between two 5-sided figures.	1 minute	
12	**ASK** the person if he is 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**	

BADLs outcome measure responses sheet

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.

1.	**Food**		**Scoring**
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
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

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	☐	

014. 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

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 ☐

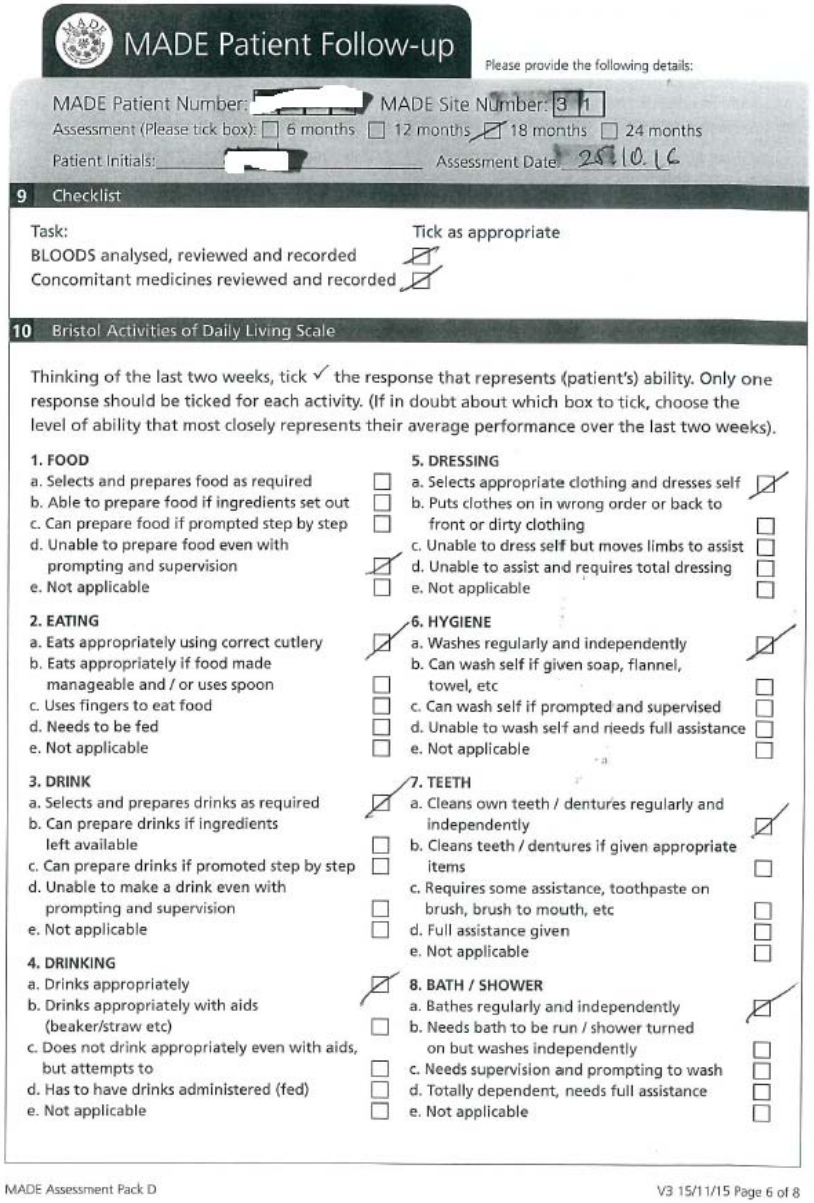
Thank you for taking the time to complete this questionnaire

Patient's name_____

Your name_____

Date_____

Sample participant responses: BADLS responses for a 12 months follow-up



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 ☐

MADE Assessment Pack D V3 15/11/15 Page 6 of 8

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 ☐

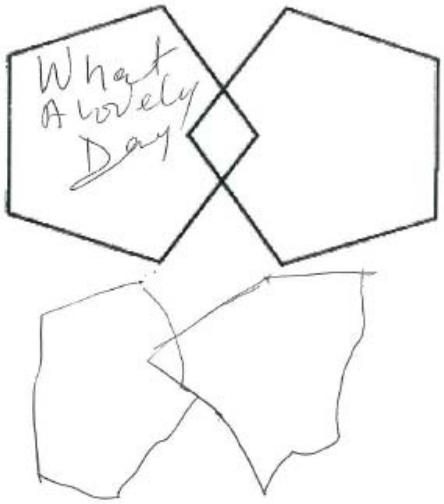
17. SHOPPING
- a. Shops to previous standard ☐
- b. Only able to shop for 1 or 2 items with or without a list ☒
- c. Unable to shop alone, but participates when accompanied ☐
- d. Unable to participate in shopping even when accompanied ☐
- e. Not applicable ☐

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 ☐

Sample participant responses: sMMSE responses for a 12 months follow-up

Write a sentence:

What A lovely Day!



APPENDIX 2

Table 1 - Follow-up rate for SMMSE and BADLS by treatment group and follow-up period

		sMMSE			BADLS		
		Received	Expected*	%	Received	Expected**	%
Screening	**400mg**	183	184	**99.5**	183	184	**99.5**
	200mg	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 Month	**400mg**	159	184	**86**	159	184	**86**
	200mg	172	181	**95**	172	181	**95**
	Placebo	167	179	**93**	164	176	**93**
	Total	**498**	**544**	**92**	**495**	**541**	**91**
12 Month	**400mg**	139	181	**77**	140	180	**78**
	200mg	158	180	**88**	157	178	**88**
	Placebo	156	176	**89**	155	171	**91**
	Total	453	537	**84**	452	529	**85**
18 Month	**400mg**	127	179	**71**	128	178	**72**
	200mg	146	177	**82**	146	169	**86**
	Placebo	147	172	**85**	148	167	**89**
	Total	**420**	**528**	**80**	**422**	**514**	**82**
24 Month	**400mg**	119	174	**68**	118	170	**69**
	200mg	144	176	**82**	142	167	**85**
	Placebo	140	167	**84**	137	154	**89**
	Total	**403**	**517**	**78**	**397**	**491**	**81**

* Expected numbers of sMMSE assessments exclude those who withdrew prior to starting treatment – i.e., those not effectively randomised – and those who died prior to the assessment

** Expected numbers of BADLS* assessments also exclude those who were admitted to care

Table 2 – Skin toxicity incidence and severity by treatment arm

Treatment arm	Toxicity rating	No. patients
400mg	**Mild**	33
	Moderate	27
	Severe	1
	Sub-total	61
200mg	**Mild**	38
	Moderate	29
	Severe	2
	Sub-total	69
Placebo	**Mild**	22
	Moderate	13
	Severe	3
	Sub-total	38

Table 3 – Causes of death

Treatment	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
200mg	Pneumonia	56	No
400mg	Pneumonia	86	Yes, 2 weeks
Neuropsychiatric			
Placebo	Dementia	95	No
Placebo	Alzheimer's/Lewy Body Dementia	92	Yes, 87 weeks
400mg	Progression of Alzheimer's	58	Yes, 7 weeks
Cardiovascular			
Placebo	Myocardial infarction	102	No
Placebo	Myocardial infarction	72	No
Placebo	Heart attack	64	No
200mg	Cardiac event	50	No
200mg	Heart attack	58	Yes, 51 weeks
400mg	Heart attack	37	No
400mg	Heart failure	100	Yes, 88 weeks
400mg	Heart attack	91	No
Cerebrovascular			
200mg	Unknown (stroke on 21/03/17)	103	Yes, 84 weeks
400mg	CVA	42	Yes, 3 weeks
400mg	Stroke	36	No
Renal failure			
Placebo	Chronic renal failure	32	Yes, 12 weeks
400mg	Lung and kidney failure	103	Yes, 1 week
Other cause			
Placebo	Complications after bowel surgery	89	Yes, 44 weeks
200mg	General health decline	56	Yes, 29 weeks
200mg	Large abdominal tumour causing kidney failure	28	Never started
400mg	COPD	57	Yes, 11 weeks
Unknown			
400mg	Unknown	77	Yes, 17 weeks

Figure 1. Flow chart: follow up completeness over time

Colour coding to show assessments split by treatment: red is 400mg, blue is 200mg and green is placebo.

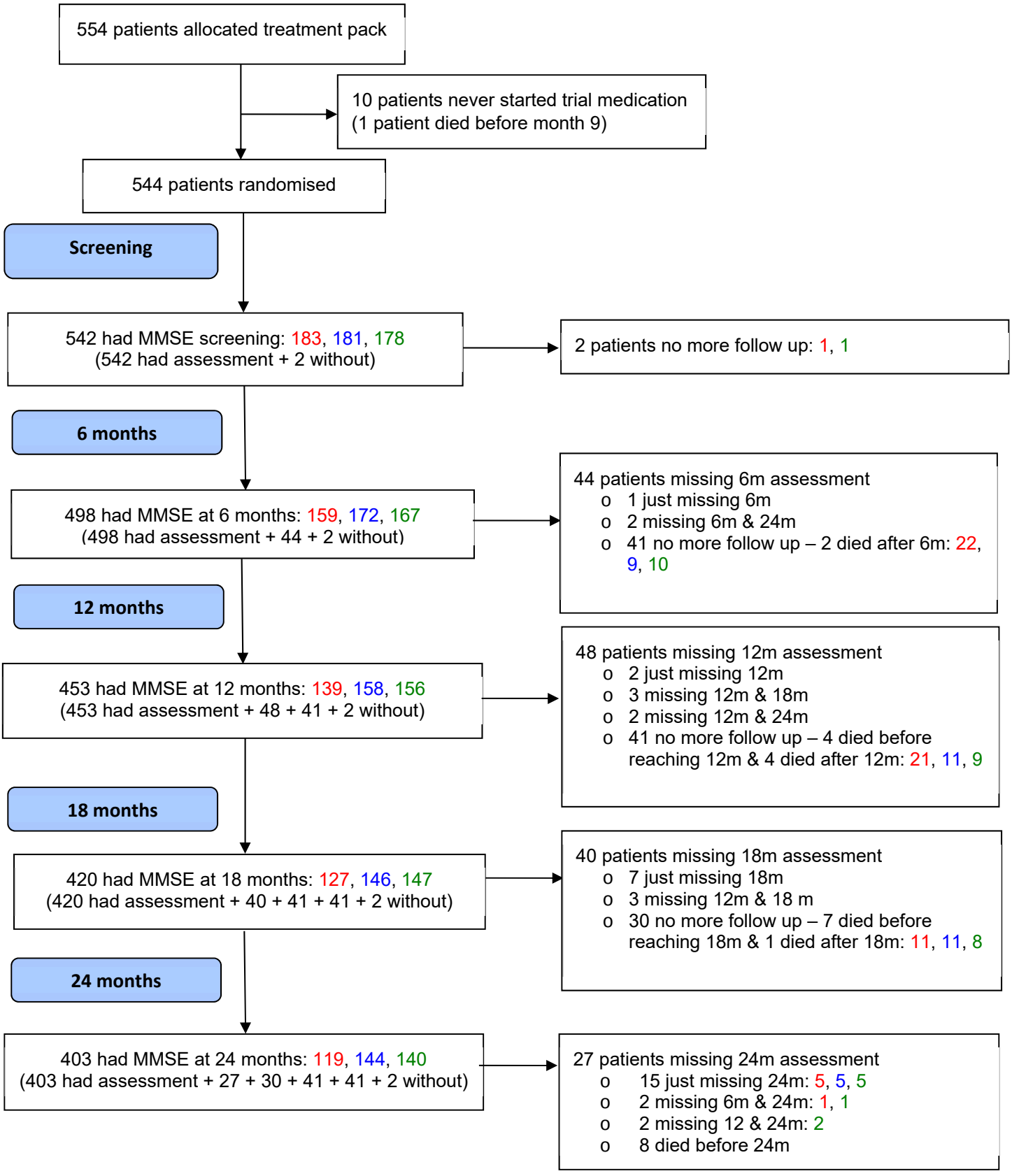
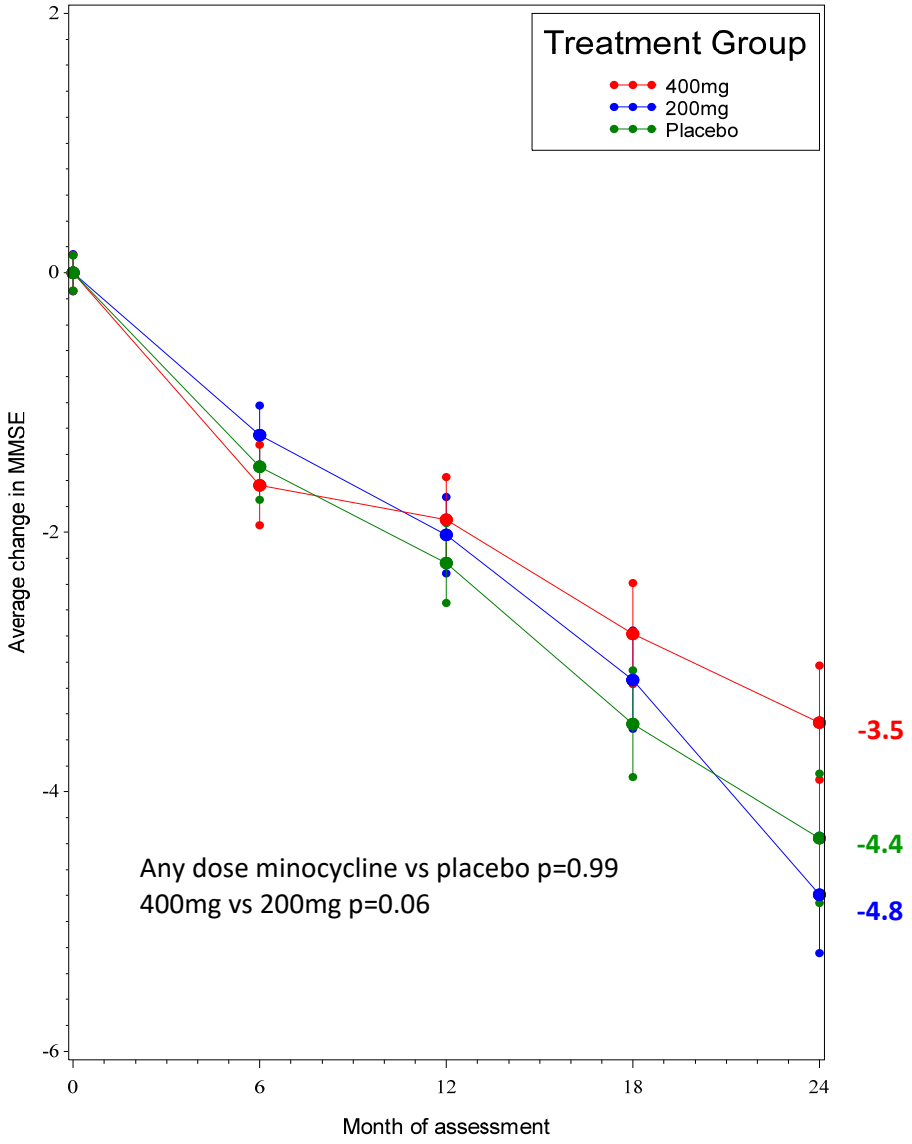


Figure 2. Change in (A) MMSE and (B) BADLS from baseline to month 24 using imputation method (2) to estimate scores for patients with no follow-up past baseline. Graph shows change in mean MMSE scores with standard errors. Baseline scores* are set to zero. p-values are from tests for time-by-treatment interaction from repeated measures analysis.



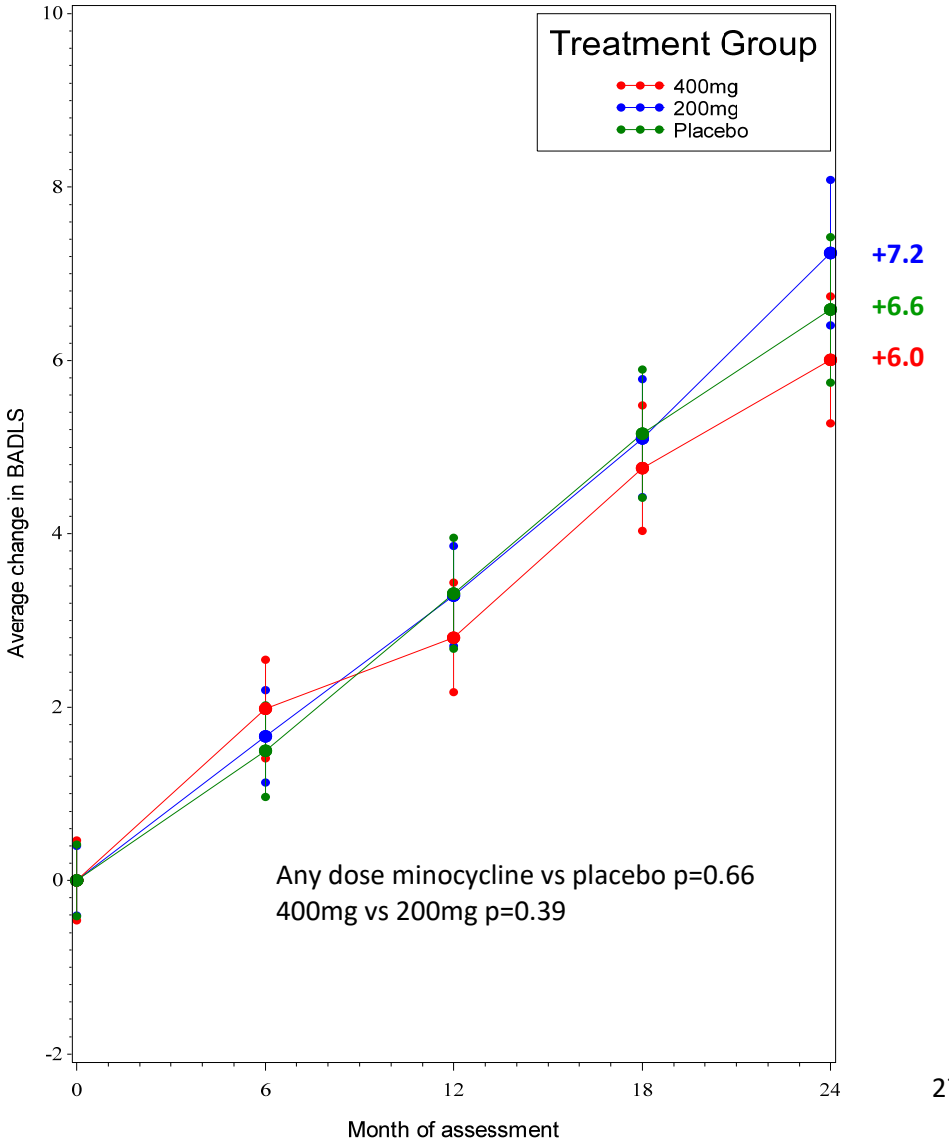


Figure 3 – Subgroup analyses of change in sMMSE over 24 months for minocycline (any dose) versus placebo by baseline characteristics: duration of symptoms, baseline sMMSE, age and gender. Results are derived from a repeated measures model, with p-values from tests for interaction between treatment and the selected subgroup.

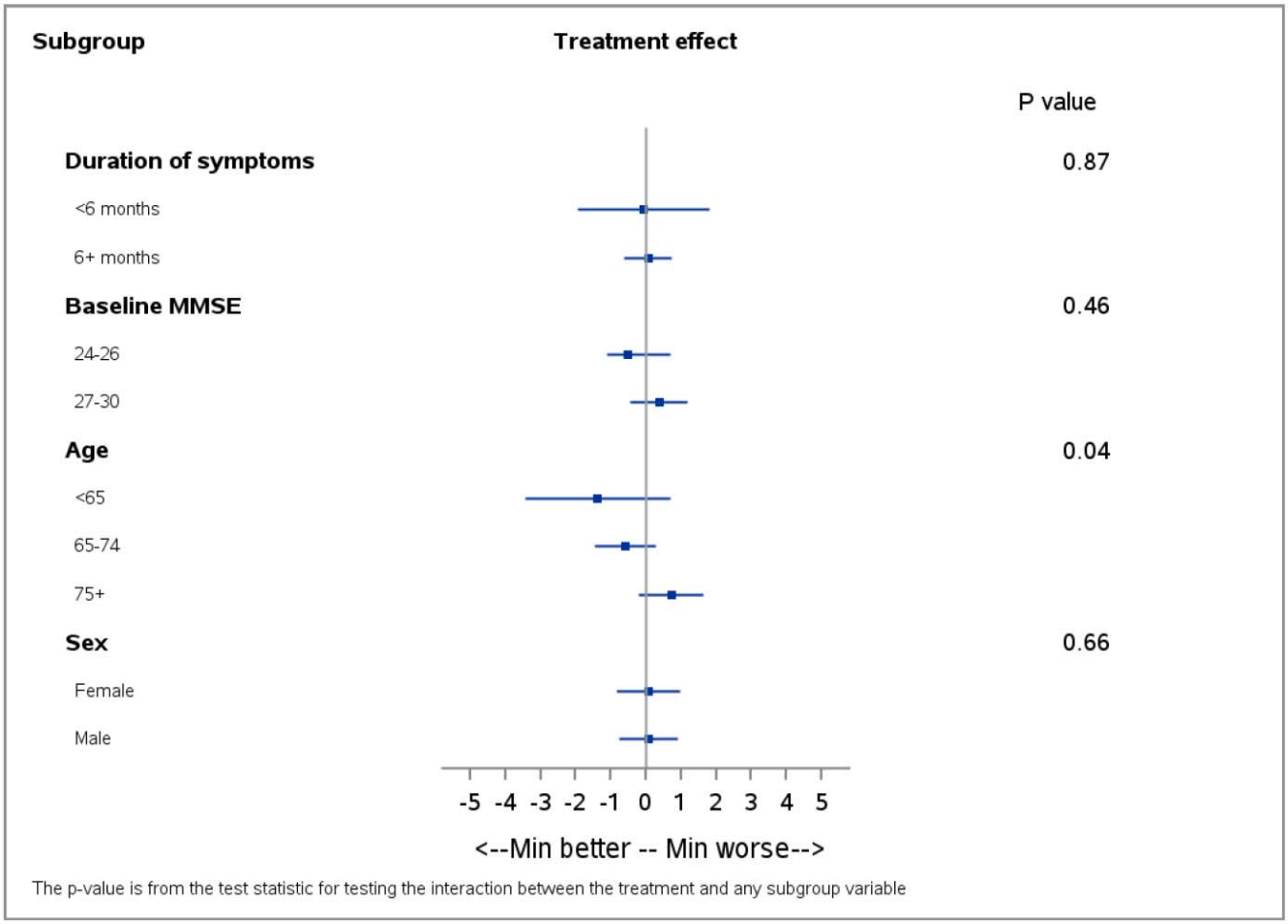
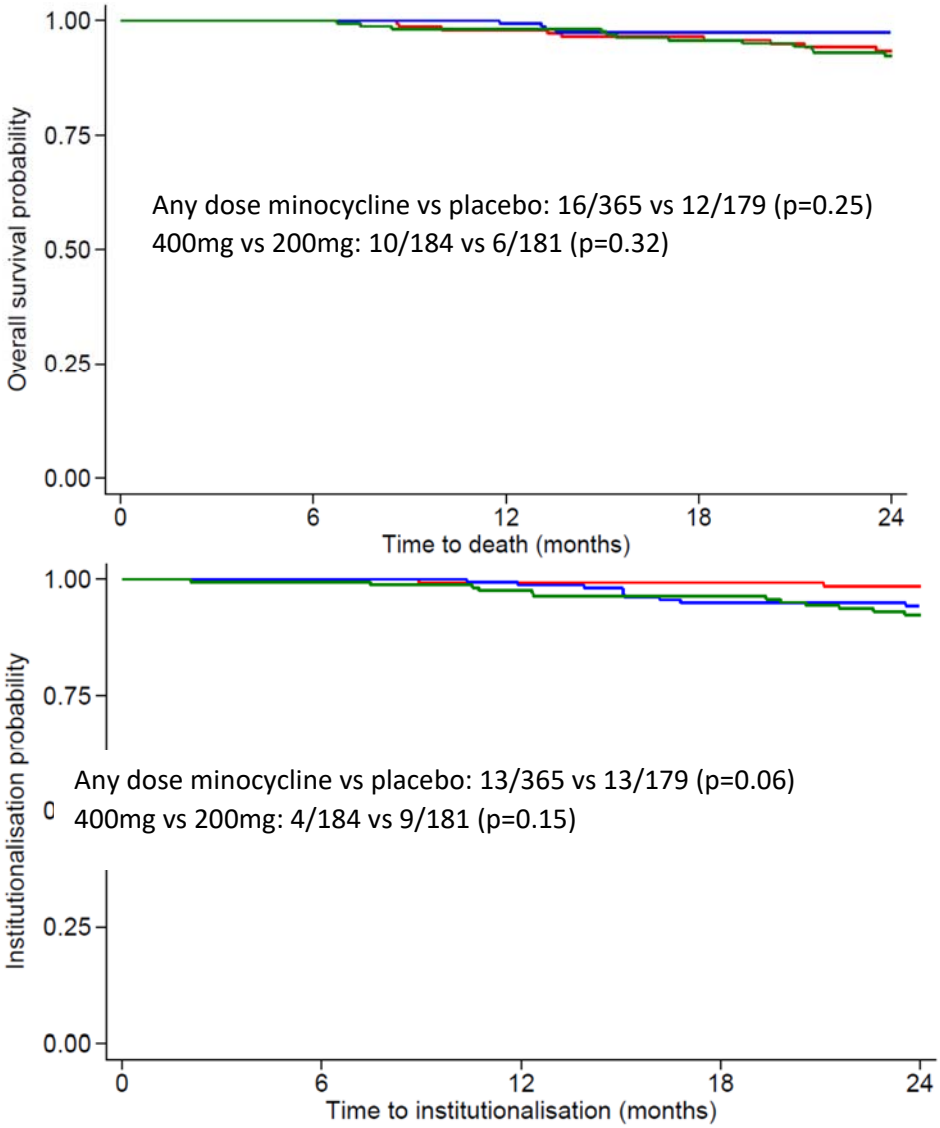
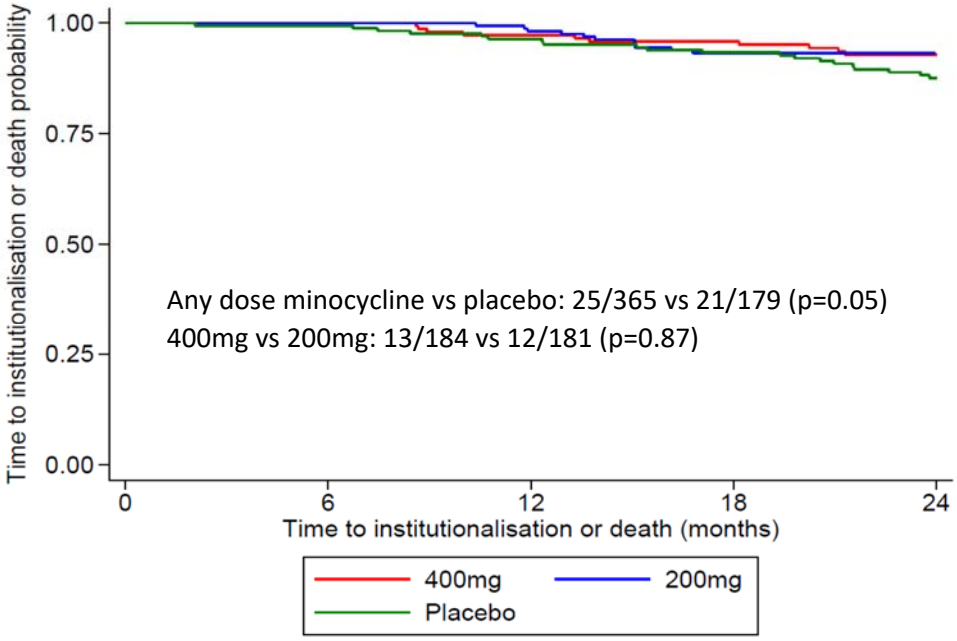


Figure 4: Probability of A) Survival, (B) remaining community resident and (C) being alive and community-resident by treatment allocation: Kaplan-Meier survival plots




Time to institutionalisation or death probability
1.00
0.75
0.50
0.25
0.00
0
6
12
18
24
Time to institutionalisation or death (months)
Any dose minocycline vs placebo: 25/365 vs 21/179 (p=0.05)
400mg vs 200mg: 13/184 vs 12/181 (p=0.87)
400mg
200mg
Placebo