FINAL REPORT

Study title: **A randomised controlled trial of neuroprotection with lamotrigine in secondary progressive multiple sclerosis**

EudraCT number: **2005-001949-42**
Sponsor's project ID number: **BRD/05/50**
REC reference: **05/Q0505/46**

Section 1: Detailed scientific report

Section A - Original aims and objectives

At present, there is no safe, widely applicable treatment that is capable of reducing the rate at which disability advances in secondary progressive multiple sclerosis (SPMS)[1,2]. There is good evidence that the primary cause of disability is axonal degeneration within the central nervous system[3], so there is considerable interest in developing treatments which can protect axons from degeneration. Several general mechanisms of neurodegeneration have been identified[4-6], including adaptations of the axonal membrane to demyelination which make axons vulnerable to toxic overloading with sodium and calcium ions. This mechanism suggests the possibility that blocking the entry of sodium ions into axons could protect them from degeneration[7-9], and could prevent disability. Our original aim was to test this possibility in a phase II clinical trial, whose main objectives were:

- Primarily, to employ a controlled clinical trial to assess whether partial sodium channel blockade with lamotrigine[10] has a neuroprotective, disease modifying effect on a) the rate of axonal degeneration, measured as the rate of cerebral atrophy, and b) the accumulation of disability in people with SPMS.
- To examine the effects of treatment with lamotrigine on a number of other outcome measures, to assess their potential use in future trials of neuroprotection.
- To assess how well lamotrigine is tolerated when given long term to people with SPMS.

Two further studies were later added on to the main project, to measure promising surrogate markers of neurodegeneration: a) optical coherence tomography (OCT, Prof DH Miller), which enables non-invasive measurement of the thickness of the retinal nerve fibre layer, and b) analysis of tissue fluid biomarkers (Prof G Giovannoni), which offer an alternative way of quantifying axonal damage in the central nervous system. The primary objective of these add-on studies was to determine whether lamotrigine significantly reduced the progression of the relevant surrogate markers.

Study design: Participants were men and women with SPMS aged 18-55 years and an Expanded Disability Status Score (EDSS) of 4 – 6.5, in whom steady progression rather than relapse was the major cause of increased disability in the preceding 2 years. Evidence of progression was either from clinical documentation of steadily increasing disability, or from an increase of at least 1 point in EDSS measurements. Participation was excluded for those eligible for current DMTs, if sodium or calcium channel blocking drugs had been used in the previous 2 weeks, corticosteroids in the previous 2 months, or immunomodulatory drugs in the previous 6 months (1 year for mitoxantrone). Criteria for exclusion also included pregnancy, major systemic disease, or disabling temperature-dependent symptoms related to MS. The study was approved by the Joint UCL/UCLH Committee on the Ethics of Human Research, and all subjects gave written informed consent. The trial was also monitored by an independent Data Monitoring and Ethics Committee (Prof C Hawkins, Dr R Morris and Dr P Rudge).

A double blind, parallel group, randomized controlled trial design was employed, with separate treating and evaluating physicians. Participants were randomized via a website (www.thesealedenvelope.com) by minimization, with age (≤45 yrs, >45yrs), gender, center (National Hospital for Neurology and Neurosurgery or Royal Free Hospital), evaluating physician (JF, TH) and EDSS (≤5.5, ≥6.0) as binary minimization variables. The randomization number and a confidential numbered list were used by a pharmacist to assign participants either to lamotrigine (in a sustained release formulation, Lamictal XR, GlaxoSmithKline), or to placebo (of identical appearance) up to 400 mg daily, depending on the maximum tolerated dose achieved during an initial 8-week dose escalation period, for 24 months. Adherence was assessed after the trial was completed by a) counting tablet returns, and b) measuring serum lamotrigine levels at months 6, 12, 18 and 24. Adherence was defined as a) less than 20% of all tablets returned ('Tablet Compliant', TC), or b) a measurable lamotrigine level in the 24 month sample ('Serum Compliant', SC).

Primary outcome and sample size calculation: The primary outcome was the rate of reduction of partial (central) cerebral volume (CCV) over two years[11, 12]. The sample size calculation was based on a power of 80% to detect a treatment effect of 60% reduction in rate of change of CCV at 5% significance level, using longitudinal data from SPMS patients in a natural history study and from the placebo arm of the European trial of Betaferon in SPMS[13], allowing for combined loss to follow up and non-compliance of 20%. The primary analysis was intention to treat (ITT) but per protocol analyses were also carried out for the Tablet Compliant and Serum Compliant subgroups.

Secondary outcomes: Secondary imaging outcome measurements were whole brain volume (WBV), grey and white matter volumes (GMV, WMV), mean cross-sectional cervical cord area (SCCA), T1 and T2 lesion volumes and magnetization transfer ratio (MTR). Secondary clinical outcome measurements comprised 1) the EDSS, 2) the Multiple Sclerosis Functional Composite (MSFC) and its three separate components, the 25 foot timed walk (TW), 9 hole peg test (9HPT) and Paced Auditory Serial Addition Test (PASAT-3), and 3) the Multiple Sclerosis Impact Scale (MSIS-29).

CCV and the secondary clinical outcomes were measured at months 0, 6, 12, 18 and 24, and the secondary imaging outcomes at months 0, 12 and 24. Imaging was delayed for at least 6 weeks after any corticosteroid treatment[14]. Participants who discontinued the trial medication for any reason were still classified as either active or placebo for the intention to treat analysis and followed up in the usual way.

For the tissue fluid biomarker study, the primary end point analysis was to see whether lamotigine reduced neurofilament levels (axonal injury), NOx (inflammation), GFAP (astrocytic activity), BDNF (neural repair) and NGF (neural repair) at 12 and 24 months relative to baseline, compared to placebo. Secondary outcomes assessed the relationship between these biochemical markers, MRI and clinical scores, and also a cross-sectional analysis of the following biomarkers in the CSF/plasma in lamotrigine treated subjects versus placebo: NOx, osteopontin (inflammation), DJ-1 (oxidative stress), 24-OHC (metabolically active neurones), NfH, NfL, NAA (axonal injury), ferritin (microglial activity), GFAP (astrocytic activity), NCAM, BDNF, NGF (neural repair). Exploratory proteomic analysis of the CSF samples was also to be performed.

During the course of the trial, concern was raised from observations in EAE[15] that withdrawal of sodium channel blockade could lead to clinical deterioration due to a rebound of inflammation in the central nervous system. For this reason, and also to assess the reversibility of any volume changes observed with treatment, we measured CCV, WBV, EDSS and MSFC at month 27 (3 months after treatment was withdrawn) in 69 participants (37 active, 32 placebo) who were then still involved in the trial

MRI protocol: All imaging was performed on a Signa 1.5T machine (General Electric Milwaukee, Wisc, USA) at a single centre (Institute of Neurology, London). The following sequences were acquired: (i) 2D T1 weighted spin echo sequence (TE 15 ms, TR 550ms) in the axial plane yielding 3mm contiguous slices; (ii) a T2 weighted dual fast spin echo (TE 20ms and 80 ms, TR 2500ms) in the axial plane yielding 3mm contiguous slices; (iii) a 3D T1 weighted gradient echo sequence (TR 15 ms, TI 450ms, TE 5ms) yielding 124 contiguous 1.5mm thick coronal slices covering the whole brain; (iv) a 3D IR prepared T1 gradient echo sequence (TR 15 ms, TI 450ms, TE 5ms) of the cervical spine yielding 64 partitions in the sagittal plane of 1mm thick equivalents. The magnetization transfer (MT) sequence was a 2D dual spin echo, generating 28 contiguous 5mm slices interleaved PD and T2 weighted images, both with and without MT weighting pulse, angled in the axial oblique plane to the base of the corpus callosum (TR 1730ms, TE (PD/T2) 30/80ms, matrix 256x128 reconstructed to 256x256, scan time 19mins; MTR excitation pulse of 14.6 μT, 64ms in duration, 2 kHz off resonance with an equivalent flip angle 1430^{o}). This sequence has been used previously with good reproducibility.

Post-acquisition analysis: CCV was measured on the 2D T1-weighted images, based on six contiguous 3mm axial slices with the most caudal at the level of the velum interpositum. A semi-automated thresholding tool, MIDAS (Medical Image Display and Analysis System) was used to segment the brain from surrounding skull and CSF. WBV was measured using fully-automated SIENA (FSL software, Oxford, UK) applied to axially reorientated 3D T1-weighted images. The SIENA algorithm failed in 2 subjects and these were excluded from the analysis. GMV and WMV were measured using 3D T1-weighted sequences reformatted into a pseudoaxial plane and then registered to the 2D proton density images using a normalised mutual information algorithm. Grey and white matter segmentations were performed using an established and automated segmentation tool, SPM5 (Statistical Parametric Mapping, University College London, UK). One subject was excluded from this analysis due to a segmentation failure. T1 and T2 lesion volumes (T1LV, T2LV) were estimated using a semiautomated local thresholding technique on in-house software (DispImage - Plummer, University College London, UK). T2 lesions were contoured on the corresponding proton density image. Manual editing of lesion boundaries was occasionally necessary in poorly defined or confluent lesions. SCCA was measured using an in-house algorithm based on five contiguous 3mm pseudoaxial slices using the centre of the C2/C3 intervertebral disc as a caudal landmark, with slices perpendicular to the spinal cord[16]. For the magnetization transfer ratio (MTR) NAWM and GM masks were first created using SPM5. Due to high lesion loads on the T2-weighted images this segmentation was performed on the 3D T1-weighted images which were initially reformatted into pseudo-axial images and then registered to the T2-weighted images from the MT sequence using a normalised mutual information algorithm. The T2 lesion masks were applied to the reformatted, co-registered T1 weighted images which were then segmented, generating NAWM and GM masks. The MTR was calculated for each voxel on the PD images using the formula: $\{[M_O-M_S]/[M_O]\}$x100 pu where M_O is the intensity of the image with no saturation and M_S is the intensity of the post saturation signal. Prior to this calculation random noise with a value within the range -0.5/+0.5 was added to each pixel to remove the effects of the division of integers resulting in an uneven distribution of quotients. MTR histograms were generated for lesions, NAWM, and GM using the tissue masks described above. In order to minimise the contribution from partial volume voxels in the NAWM and GM segments, we excluded any voxels with an MTR value of <10pu and included a 2 voxel erosion on the WM segments for NAWM measures and a 1 voxel erosion on the GM segments for GM measures. The number of erosions that could be applied to the grey matter segment was limited by cortical thickness.

Statistical analysis

The primary analysis was intention to treat (all participants randomized). Two per protocol comparisons were also carried out: i) Tablet Compliant (TC), within the subgroup of participants who consumed at least 80% of prescribed tablets and were still being prescribed

tablets at 24 months; ii) Serum Compliant (SC), comparing participants in the active group with detectable serum lamotrigine at 24 months with the whole placebo group.

For MRI measures, MSIS, MSFC, TW, 9HPT and PASAT-3, primary comparisons were of rates of change over 0 to 24 months in a linear mixed model[17] in which the outcome measure (including baseline) was the response variable, with pre-specified baseline covariate adjustment comprising all binary minimisation variables apart from treating center. Secondary analyses entered time as a categorical variable in the model, to give cross-sectional comparisons at the 6 to 24-month time points.

The analysis of TW was pre-specified: unsuccessfully completed walk attempts were given a time of 180secs[18], and then the inverse of the within-subject mean time for the two attempts was used as the timed walk measure (this being generally more normally distributed than the usually skewed mean time); finally, when modelling the rate as for other measures above, in order to adjust for use of walking aids additional terms indicating the number of sticks used were added to the model.

The significance of any EDSS treatment effect was assessed by an exact test for trend[19] for 24-month EDSS stratifying by baseline EDSS.

Two approaches were used to explore any non-linearity in CCV atrophy: i) the linear mixed model above was adapted to separately estimate (linear) gradients before and after a posited threshold time (eg 12 months); a significant difference in before vs after threshold gradients is evidence of non-linearity over the whole period; ii) a quadratic term in time was added into the estimating model, with significance of the quadratic term being evidence of a curved, non-linear trajectory.

Analyses were implemented in Stata 10.1 and SAS 9.1.

Section B – Results and Scientific Significance

Of 124 eligible participants, 120 were randomized (Fig 1) to receive lamotrigine (n=61) or placebo (n=59). The participating cohort was representative of SPMS and had an average disease duration of around 20 years, progression from around 8 years before entry, and a median EDSS of 6.0. The active and placebo groups were comparable for all baseline characterisitics (Table 1)

Figure 1 Trial profile
3 participants were withdrawn from the placebo group before starting treatment due to a progression of disability which placed them outside the inclusion criteria, and 7 dropped out of the active arm, due to dose related neurological deterioration in 6 and fatigue in one. 11 participants in the active group withdrew from treatment (due to dose related deterioration of gait and balance in 6, rash in 3, and psychiatric symptoms in one) compared to 5 in the placebo group (due to rash in 2, and nausea, psychiatric symptoms and dose related deterioration of gait and balance in one each).

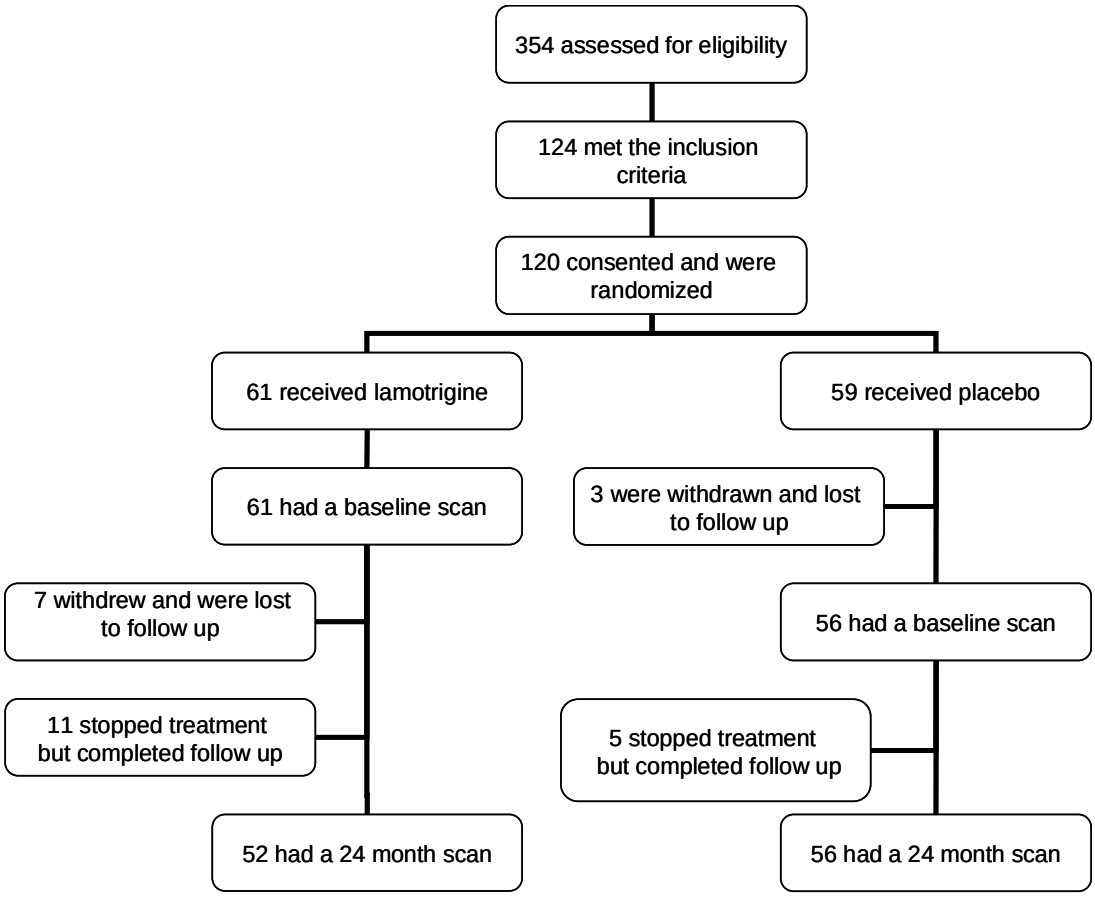


Table 1 Baseline characteristics. *The numbers for the placebo/active groups were as follows: for demographic and clinical variables 57/61; for all MRI measurements GMV and WMV 56/61; and for GMV and WMV 55/61.*

	Placebo	**Active**
Age, years: mean (sd)	50.1 (6.7)	51.9 (7.1)
Gender, F:M	42:17	45:16
EDSS: median (range)	6.0 (4-7.5)	6.0 (4-6.5)
Disease duration, years: mean (sd)	18.9 (8.3)	21.2 (9.2)
Duration of progression, years: mean (sd)	8.0 (5.4)	7.7 (5.4)
MSFC, z-score: mean (sd)	0.023 (0.695)	-0.031 (0.762)
PASAT-3: mean (sd)	44.6 (13.6)	41.7 (14.5)
9-Hole PegTest, secs^{-1}: mean (sd)	0.033 (0.011)	0.033 (0.010)
1/Timed Walk, secs^{-1}: mean (sd)	0.090 (0.063)	0.093 (0.057)
MSIS-29, total n/145: mean (sd)	85.1 (23.1)	84.9 (18.9)
CCV, ml: mean (sd)	255.0 (23.6)	254.8 (24.1)
GMV, ml: mean (sd)	628.7 (63.0)	620.0 (77.5)
WMV, ml: mean (sd)	402.2 (46.5)	395.6 (42.4)
SCCA, mm^2: mean (sd)	63.3 (9.1)	63.9 (8.3)

108 participants underwent imaging for the primary outcome at 24 months (52 active, 56 placebo). 10 participants were lost to follow up. 3 participants dropped out of the placebo group before starting treatment, due to a progression of disability which placed them outside the inclusion criteria, and 7 dropped out of the active arm, due to dose related neurological deterioration in 6 and fatigue in one. A further 16 participants withdrew from treatment, but continued to be followed up (11 active, 5 placebo). The combined rate of dropout and withdrawal from treatment was 22%. The active and placebo groups differed in adherence. At 24 months the proportions in each group who were tablet compliant were: active 31/52 (60%; mean dose 78 mg) and placebo 45/56 (80%; mean dose 240 mg; chi-square p=0.018 for comparison). 25/52 participants (48%) in the active group were serum compliant (mean serum concentration 14.1 mg/L).

1 Analysis of baseline data

Analysis of the baseline data of the 117 participants entering the trial showed that a number of imaging measures (CCV, WBV, GMV, WMV and SCCA) had similar values in the active and placebo groups, and that they all correlated significantly with the MSFC score. The strongest correlation was with the WBV ($r=0.47$, $p<0.001$). WBV and SCCA were the only significant independent predictors of the MSFC in a stepwise regression model containing all the MRI measures, and SCCA was the only MRI measure to show a significant association with the EDSS. GMV had stronger correlations with the clinical variables than WMV, WBV correlated better with clinical impairment than either measure.

In a further analysis of the baseline MTR data of 113 participants entering the study, correlations were found between the MSFC score and the MTR of T2 lesions ($r=0.394$, $p<0.0001$) and of grey matter ($r=0.460$, $p<0.0001$) and NAWM ($r=0.327$, $p<0.0001$). The grey matter histogram mean emerged as the best predictor of MSFC score. None of the measures significantly predicted the EDSS score.

Finally, strong associations were found between grey matter fraction and mean grey matter MTR on the one hand and T1 and T2 lesion volumes and lesion MTR mean on the other ($r=\pm 0.63$-72). In contrast, only weak to moderate correlations were found between NAWM

and lesion measures. In a stepwise regression model, T1 lesion volume was the only independent lesion correlate of grey matter fraction, accounting for 52% of the variance. Lesion MTR mean and T2 lesion volume were independent correlates of mean grey matter MTR, accounting for 57% of the variance.

Scientific Significance: This data extends the existing literature which indicates that measures of atrophy, particularly WBV and SCCA, are clinically relevant and useful disease markers in SPMS, and the MTR data reinforces the concept that grey matter pathology plays an important role in determining neurological impairment. Furthermore, the MTR correlations, as well as those between the T1 lesion volume and grey matter fraction, have implications for the pathophysiology of MS, and suggest that axonal transaction within lesions may be associated with secondary degeneration into the grey matter. A parallel accumulation of demyelinating lesions in the white and grey matter could explain the associations of the T2 lesion volume and lesion MTR with the grey matter MTR. The analyses of these baseline findings have been published[20-22].

2 Analysis of longitudinal changes of imaging and clinical measurements

The volumetric MRI measures for all 56 participants who continued to be followed up in the placebo group were analysed longitudinally to assess their sensitivity, responsiveness, reliability and correlation with disability. The mean annual atrophy rate of whole brain, as measured by SIENA, was 0.59% per year and this was the most responsive atrophy measure. GM atrophy (-1.18%/yr) was greater and more responsive than WM atrophy (0.12%/yr). The SCCA demonstrated the highest atrophy rate at 1.63%/yr (compared to 1.51%/yr for CCV). WBV, GMV and SCCA atrophy all correlated with change in the MSFC z-score (r=0.35, 0.42, 0.34), and GM atrophy was the only correlate of change in the 9 hole peg test and PASAT performance. WBV and SCCA atrophy were significantly greater in subjects with progression of the MSFC z-score, but no MRI measures correlated with EDSS progression. At present we are analysing the correlations identified in this part of the study in more detail using multiple regression analyses, and are also evaluating the relative usefulness of other lesion and MTR measurements. These results were presented at the 2009 ECTRIMS meeting, and are being prepared for publication.
Scientific significance: Until our analysis has been completed, it appears that, whilst WBV change was the most responsive measure in the study, GMV and SCCA atrophy were superior correlates of change in disability. All 3 measures have advantages for use as surrogate markers in secondary progressive MS trials, and improvement in the reliability of the GMV and SCCA measures may enhance these measures further. These results will help to choose the surrogate imaging outcomes, and to help with sample size calculations, when designing future trials of neuroprotective treatments in SPMS.

3 Results of substudies

The biomarker study has serum samples from 118 subjects; 18 of which were excluded from the primary endpoint analysis due to incomplete data points. All five time points (0,6,12,18,24 months) were present in 93 subjects, whilst 62 subjects had blood taken at 27 months off therapy. The baseline sample was unavailable in a single subject. In addition 23 paired CSF and plasma were collected; 9 of which had CSF at approximately 6 and 12 months. 33 subjects participated in the OCT substudy. Measurements were obtained at 6, 12, and 24 months after study entry. Because of the large amount of analysis required for the main, lamotrigine study, the results of these substudies have not yet been analyzed, but this analysis is scheduled to be completed early in 2010.

4 Outcomes of the trial of lamotrigine

The main objective of this project was to test the possibility that partial blockade of sodium channels with lamotrigine might have a neuroprotective effect in SPMS. Our results have suggested a number of treatment effects, and these are described below. At present we have analysed the results of all of the imaging outcomes apart from the T1 lesion volumes and MTR, which we will report shortly.

Intention to treat (ITT) comparisons: Volume measurements: Treatment had no significant effect on the primary outcome, the rate of loss of CCV, nor on the rates of loss of WBV or GMV, over the 24 month treatment period (Fig 2 and Table 2). However, treatment was associated with a borderline significant greater rate of loss of WMV, and a similar trend was seen for SCCA. Both WBV (Table 3) and WMV (active-placebo difference -3.55 ml, p=0.01) were significantly lower in the active group at 12 months, but not at 24 months (WMV difference -0.90 ml, p=0.57). There were also indications that CCV declined more steeply in the active group during the initial 12 months, and of a cross-sectional difference at 18 months (active-placebo difference -2.17 ml, p=0.09). Exploratory analyses of CCV (below) significantly supported these trends, as well as the suggestion that both CCV and WBV rebounded in the active group (but continued to decline in the placebo group) after stopping treatment at 24 months.

Clinical measurements: Treatment had no significant effect on the EDSS at 24 months (Table 2) or on the rates of decline (or 24-month values) of the MSFC, PASAT-3, 9-HPT or MSIS. However, the rate of decline of the TW was significantly slower in the active group, with a corresponding significant adjusted 24-month active – placebo difference (0.013 sec^{-1}, p=0.02). The beneficial effect in the active group remained significant after a sensitivity analysis of the possible influence of missing data points.

Figure 2 Primary outcome

Monthly partial (central) cerebral volume (CCV, mean ± SE) by ITT comparison, including numbers of valid 6-monthly observations (placebo above, active below). There was no significant difference between the two groups in the rate of loss of CCV, while the cross-sectional volume difference was borderline significant only at 18 months (see text and Table 2). CCV was significantly lower in the active group in the serum compliant PP comparison at 18 months (p=0.04) and 24 months (p=0.05), with a similar trend in the tablet compliant PP comparison at 18 months (p=0.15).

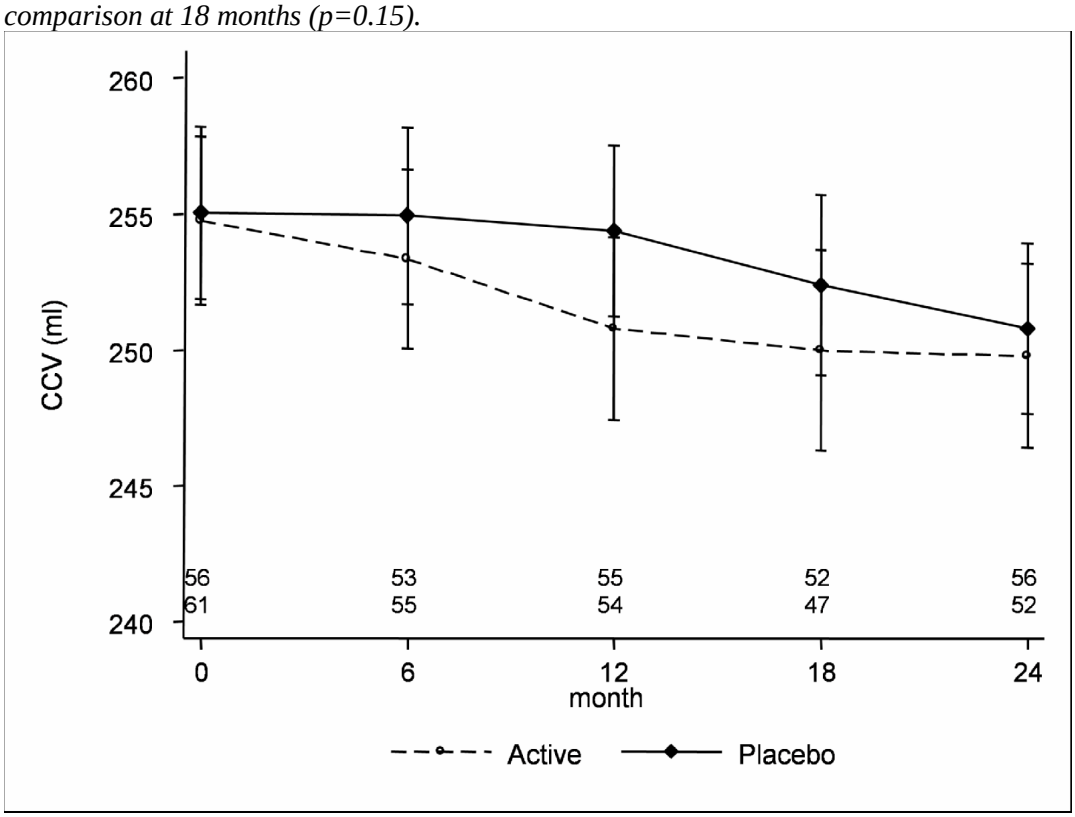


Table 2 ITT comparison for rates of change in outcome measures

The table shows the within-group annual rates of change (also percentage changes from baseline where appropriate), and the planned covariate-adjusted adjusted difference in annual rates. For EDSS the mean (range) 0 to 24 month changes are shown within group, and the between-group p-value for the baseline stratified exact test for trend.

	placebo rate (%) [p-value]	**active rate (%) [p-value]**	**active – placebo difference (CI) [p-value]**
CCV, ml/yr	-2.48 (-0.97) [0.019]	-3.18 (-1.25) [0.006]	-0.71 (-2.56, 1.15) [0.402]
WMV, ml/yr	0.41 (0.10) [0.006]	-0.87 (-0.22) [0.003]	-1.28 (-2.60, 0.05) [0.059]
GMV, ml/yr	-9.24 (-1.47) [0.058]	-9.70 (-1.56) [0.050]	-0.46 (-9.11, 8.18) [0.915]
SCCA, mm^2/yr	-1.26 (-1.99) [<0.0001]	-1.60 (-2.51) [<0.0001]	-0.34 (-0.85, 0.17) [0.184]
EDSS	0.23 (-1.5, 2)	0.21 (-2.5, 2)	[0.725]
MSFC, zscore/yr	-0.18 [0.0002]	-0.17 [0.001]	0.010 (-0.12, 0.14) [0.880]
9 HPT, $secs^{-1}$/yr	-0.00084 (-2.55) [0.029]	-0.00084 (-2.55) [0.022]	-0.000064 (-0.0011, 0.00098) [0.904]
PASAT-3	-1.16 (-2.61) [0.068]	-0.58 (-1.40) [0.369]	0.58 (-1.19, 2.36) [0.518]
1/TW, $secs^{-1}$/yr	-0.0098 (-10.93) [<0.0001]	-0.0035 (-3.74) [0.092]	0.0063 (0.00094, 0.012) [0.022]
MSIS, n/145/yr	0.91 (1.07) [0.451]	-0.27 (-0.31) [0.833]	-1.18 (-4.59, 2.23) [0.495]

Table 3 Measurements of changes of CCV and WBV during different time intervals (months) by ITT comparison.

CCV and WBV in the active group showed a greater decline than placebo in the 0-12 month interval. From 12-24 months the decline of WBV was similar in the two groups, whereas CCV declined less in the active group. From 24-27 months, there were trends for both CCV and WBV to increase in the active group, but to continue to decline in the placebo group.

	CCV (mm^3)				**WBV (%)**			
	placebo	**active**	**difference**	**p value**	**placebo**	**active**	**difference**	**p-value**
0-12	-1461	-3423	-1962	0.12	-0.541	-0.823	-0.282	0.03
12-24	-2841	-1863	978	0.47	-0.647	-0.618	0.029	0.79
0-24	-4252	-5017	-766	0.49	-1.188	-1.343	-0.155	0.29
24-27	-54.2	886.0	940.2	0.49	-0.076	0.014	0.091	0.45

Exploratory analyses: Two longitudinal models to assess non-linearity of CCV gave similar results (Fig 3). First, while there was no evidence of curvature of the trajectory of CCV in the placebo group (p=0.51), there was significant 'concave' curvature in the active group (p=0.024, with p=0.026 for difference from placebo), indicating a more rapid early rate of volume loss compared both to later active and (p=0.025) to early placebo loss. Second, separately estimating linear rates of loss before and after a 12-month 'pivot' indicated in the active group (only) a significantly lower rate in the second than in the first 12-month period (p=0.04). In this analysis, the rate of loss of CCV was non-significantly lower in the active group compared to placebo in the second year of treatment (p=0.21). A similar model further revealed a significant reversal of gradient (p=0.04) of CCV in the active group between 24 and 27 months, representing a switch from volume loss before 24 months to volume gain, as opposed to a continuing, steady linear decline with placebo.

In further analyses, we explored associations between outcomes and serum lamotrigine concentrations in the active group. No correlations were found for volumes, apart from a negative correlation between WMV at 24 months and the mean 0-24 month serum lamotrigine concentration (p=0.03). For clinical outcomes, there was a borderline significant beneficial association between an increased mean 0-24 month lamotrigine concentration and an improved 24 month EDSS, adjusting for baseline EDSS (p=0.06; p=0.01 before adjusting). A beneficial association between a higher 0-24 month mean lamotrigine concentration and a higher timed walk score at 24 months (p=0.03) lost significance after adjusting for walking aids. Interestingly, participants with a lower baseline EDSS tolerated a higher mean serum lamotrigine concentration (p=0.03).

Figure 3 Exploratory analyses of change of partial (central) cerebral volume using longitudinal models

A) Analysis of change of linear gradient and of curvature. There was no significant curvature in the placebo group (solid line, -6.05 $mm^3/month^2$, p=0.16), whereas curvature in the active group (short-dashed line) by ITT comparison was concave by 10.75 $mm^3/month^2$ (p=0.02). The estimated rate of early decline from month 0 was more steep in the active group by -305 $mm^3/month^2$ (p=0.03). Similar, significant results were found in the active group by tablet and serum compliant PP comparisons.

By ITT comparison, the gradient of volume loss is significantly greater in the active group (long-dashed line) before (-283 mm^3/month) compared to after (-66 mm^3/month) a 12 month 'pivot' (p=0.04). Similar, significant gradient changes were found for a 12 month pivot in the serum compliant PP comparison and for a 6 month pivot in the tablet compliant PP comparison. The gradient remains constant throughout in the placebo group (at -140 mm^3/month), with a trend for the rate of loss of volume to be slower in the active group in the second year of treatment (p=0.21).

B) Rebound of volume following withdrawal of treatment by analysis of change of linear gradient. By ITT comparison, there is a change of gradient in the active group (dashed line) of 620 mm^3/month (p=0.04) after compared to before a 24 month pivot, indicating a change from volume loss to volume gain in this group. Similar, significant gains of volume were found in the tablet and serum compliant PP comparisons. Volume continues to decline slowly and linearly in the placebo group (solid line) after treatment is withdrawn.

Figure 3A

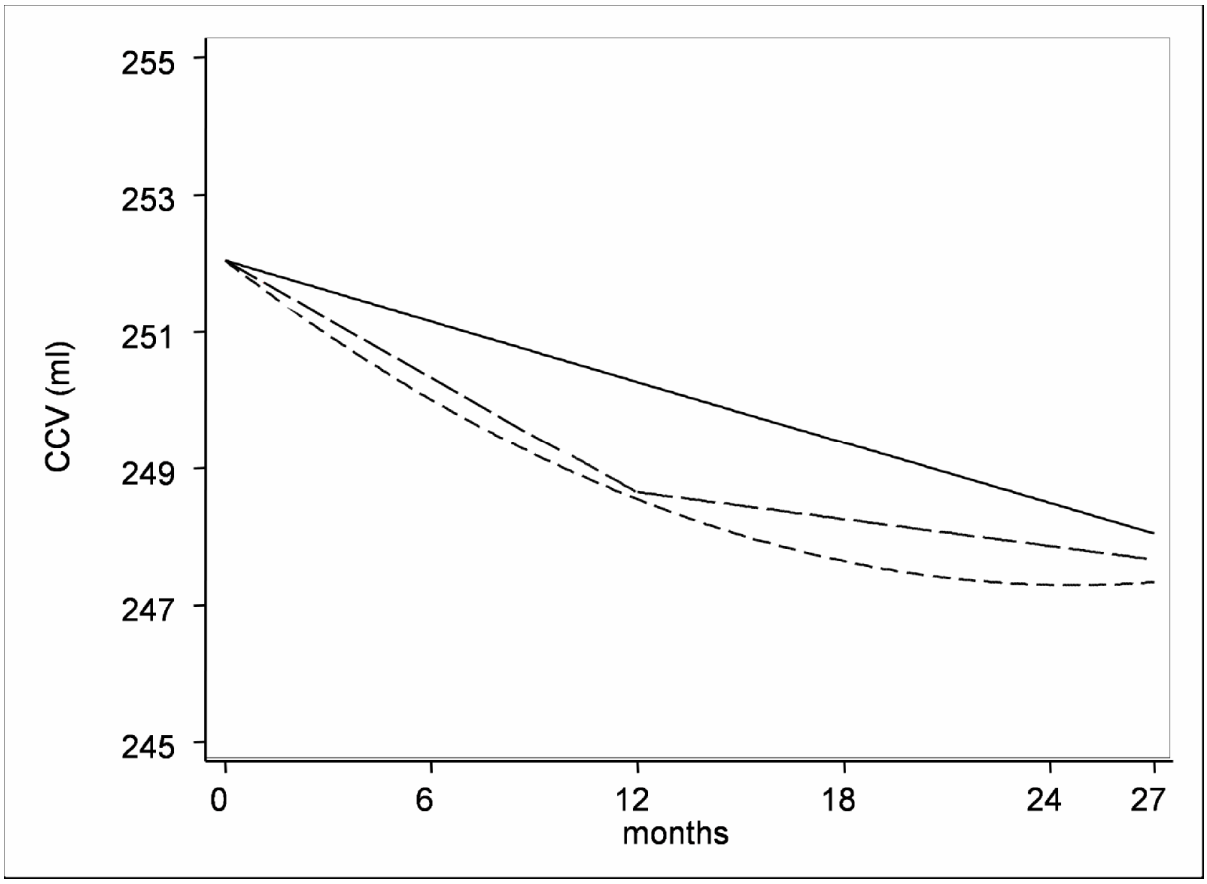
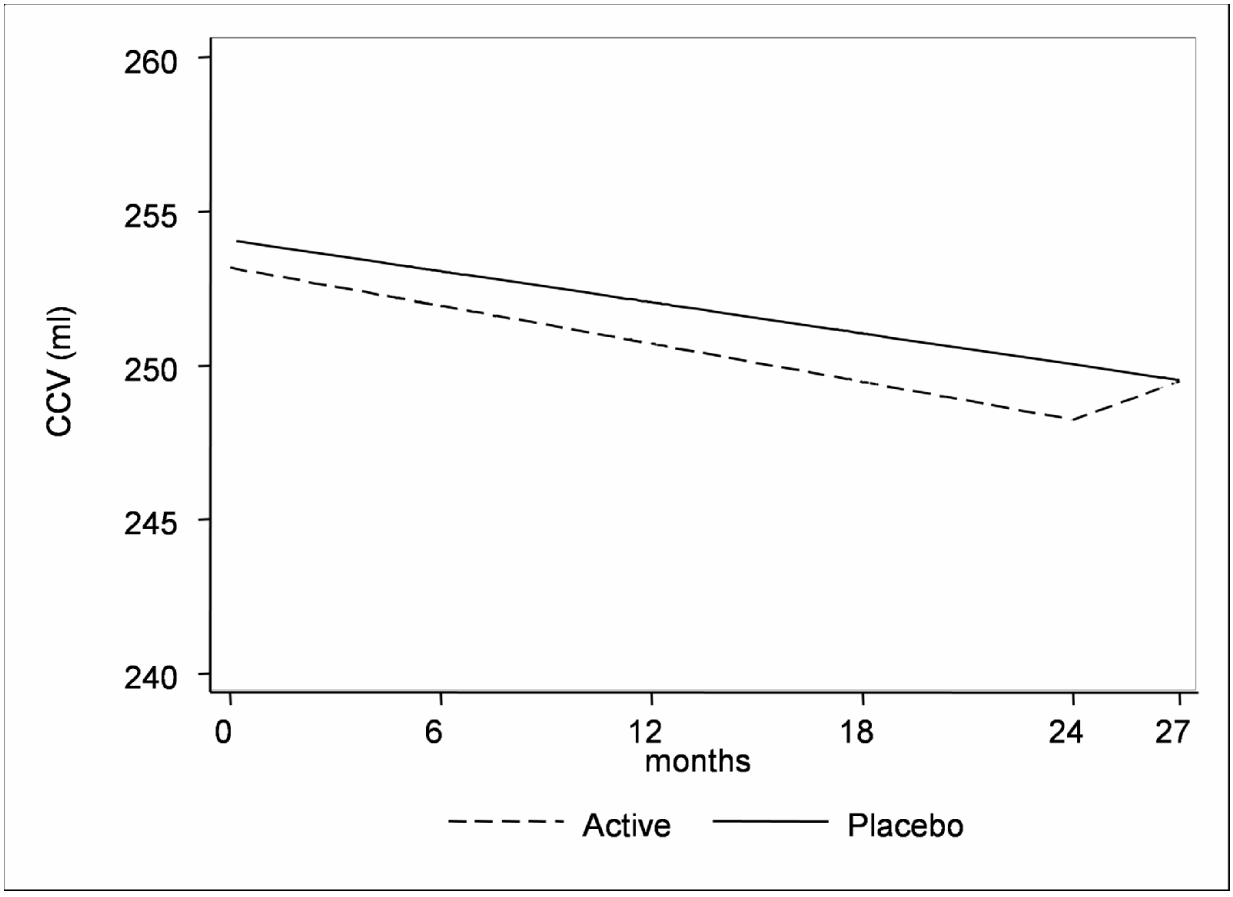


Figure 3B



Per protocol (PP) comparisons:The results of the PP comparisons generally agreed with the corresponding ITT comparisons and the exploratory longitudinal analyses. The only exception was that the rate of decline of WMV was significantly different only for the ITT comparison. SCCA declined at a borderline significant greater rate in the TC and SC active groups compared with placebo (p=0.08 and 0.13 respectively). The reduction of the rate of decline of the timed walk by ITT comparison was also significant by TC comparison (p=0.01) and seen as a trend by SC comparison (p=0.17).

Scientific significance: The main findings from our imaging measurements were that: 1) using cerebral atrophy as a measure of neurodegeneration, we did not observe a neuroprotective effect of treatment with lamotrigine, 2) treatment did not affect GM atrophy, but did lead to greater loss of WMV, 3) treatment led to an accelerated loss of CCV and WBV during the first twelve months, and 4) CCV and WBV began to rebound when treatment was stopped. These findings suggest that lamotrigine has a selective effect on white matter/axons but not on gray matter, and would be consistent with current concepts that the mechanisms of tissue injury may be different in these two compartments[23-25].

Our findings are also consistent with the accelerated, early loss of volume with treatment being related to so-called pseudoatrophy, due to reversible shifts of fluid between tissue compartments, rather than to irreversible tissue injury[26]. The volume loss in the active group began to reverse when treatment was withdrawn, and treatment did not appear to worsen the clinical outcomes. Acute dose-related neurological deterioration was observed, which can be interpreted as an inhibitory effect on axonal conduction from sodium channel blockade, but the rates of deterioration of the EDSS and the MSFC were not altered by treatment. Rather, treatment significantly reduced the extent and rate of deterioration of the timed walk. There was also a suggestion that higher mean serum lamotrigine was associated with improved EDSS.

These findings raise the possibility that sodium channel blockade is indeed neuroprotective in SPMS. In this respect, it is relevant to consider the mechanisms of the pseudoatrophy[26]. The first possibility, that sodium channel blockade could reduce cell volume by reducing the entry of sodium ions and water, seems unlikely because the effect on CCV manifested rather slowly over 6-12 months. Second, pseudoatrophy may have been due to a reduction of inflammatory activity and oedema, as suggested from similar observations of volume changes in clinical trials of immunomodulation[27-30]. In contrast to those studies, however, the relapse rate and T2 lesion volume were not affected significantly by treatment in the present trial (unpublished observations). An effect on immune mechanisms remains possible, particularly within normal-appearing tissue, and would be consistent with growing evidence that the cell membranes of lymphocytes and microglia contain voltage-sensitive sodium channels which can modulate their function[31]. Finally, it is possible that sodium channel blockade reduced the extent of ongoing axonal injury, and that the initial loss of cerebral volume occurred as the phagocytic and inflammatory processes secondary to axonal degeneration subsided to a lower level. The last two interpretations of the volume loss would be consistent with the trend for a substantially slower rate of decline of CCV in the active group in the second year of the trial.
In general, our findings by ITT comparisons were supported by similar, significant changes or trends in the PP comparisons. The interpretation of the PP comparisons was hampered by an approximately 50% rate of non-adherence in the active group, related in part to dose related neurological deterioration. This low rate of adherence may help to explain the differences between the results of some of the PP and ITT comparisons.

Section C – Unplanned outcomes

The main unplanned outcome in this trial was the unexpectedly low rate of aderence with treatment. Although our findings by intention to treat comparisons were supported by similar, significant changes or trends in the per protocol comparisons, the interpretation of these comparisons was hampered by a rate of adherence of approximately 50% in the active group as measured using tablet returns and serum lamotrigine levels. This was most likely due to withdrawal from treatment because of dose related neurological deterioration (see below). Lamotrigine was tolerated poorly in comparison with previous reports of its use in trigeminal neuralgia in MS[32,33]. We found (data not shown) that a lower EDSS at entry correlated with a higher maintenance dose of lamotrigine, suggesting that people with SPMS who have significant pre-existing disability may be particularly sensitive to conduction block. This effect may relate to the reduction of sodium channel expression in chronically demyelinated axons[34] and a consequent reduction of their safety factor for conduction. Future clinical trials using this therapeutic strategy may therefore need to involve less disabled participants. On the other hand, for those participants who did tolerate treatment, the mean serum lamotrigine level of 14.1 mg/L compared favourably with an average neuroprotective serum concentration of 11.6 mg/L in EAE[10]. This concentration was also neuroprotective in a rat model of cerebral ischaemia[35].

Concerning adverse events, there was a significantly higher incidence in the active group by ITT comparison of rash (active 20% vs placebo 5%, p=0.03), gastrointestinal disturbance (active 25% vs placebo 12%, p=0.01), and dose related neurological deterioration, affecting mobility or balance (active 88% vs placebo 53%, p=0.007) . Dose related neurological deterioration reversed promptly as the dose of medication was reduced, in keeping with the possibility that it was related to conduction block in partially demyelinated axons due to sodium channel blockade. The adverse events occurred largely within the first 2 months of treatment.

In all, there were 8 serious adverse events (SAEs) requiring hospital admission, 6 in the active group and 2 in the placebo group. 3 participants in the active group had SAEs after falling – 2 sustained fractured necks of femur requiring surgical repair, and a third developed a small subdural haematoma following minor head trauma, requiring a short hospital admission but no surgery. Although everyone with SPMS is vulnerable to falling, the fact that the use of lamotrigine was associated with deterioration of gait and balance raises the possibility that this particular SAE may be associated with the treatment. One participant in the active group had two cerebral ischaemic events towards the end to the trial which were not thought to be related to the treatment. One participant in the placebo group had an episode of cholecystitis requiring hospital admission and surgery, and another in the active group had an episode of severe constipation, neither considered related to the treatment. One participant in the active group developed anaemia midway through the trial but had in fact stopped the treatment for other reasons many months before. One participant in the placebo group had a urinary tract infection.

Finally, the concerns about the possible exacerbation of inflammatory disease activity after lamotrigine was stopped were not borne out by the findings in the 27 month assessments. There appeared to be no significant increase in the rate of relapse, measurement of disability using the MSFC, or in the CCV or WBV. Indeed, as noted earlier, the MSFC and volume measurements showed an improvement after lamotrigine was stopped.

Section D – Conclusion and implications

In conclusion, the results of the clinical trial suggest that the novel neuroprotective strategy of sodium channel blockade does have an effect on cerebral volume (and particularly white matter volume) reminiscent of so-called pseudoatrophy, and that it may reduce significantly the progression of certain aspects of disability.

While our results may encourage work on ion channel modulation as an effective route to neuroprotection in MS, they also suggest that care will need to be taken to anticipate unforeseen problems with the tolerability of the agents used in this work. Longer trials are also likely to be required which can examine the effects of treatment on surrogate outcomes such as loss of volume after the confounding effects of pseudoatrophy have subsided.

Our results are consistent with the possibility that disability progresses through different pathological mechanisms in the brain grey and white matter and in the spinal cord: not only does the analysis of the baseline and longitudinal data indicate compartment-specific changes of volume and associations with disability, but a treatment effect was found in only one of these compartments, since we present data for all three compartments which argues for a long term trend to preserve white matter volume, with no evident tissue protection in the grey matter or spinal cord. Therefore, further therapeutic strategies may be need to target the dominant mechanisms for neurodegeneration in different compartments of the CNS, once they are better understood.

Section E – Recommendations for future work/research

Our results and experience suggest that work in the following general areas may help to investigate the potential for neuroprotection in multiple sclerosis:

- Further clinical trials of sodium channel blockade may be considered, to test the suggestion from the present work that this strategy can achieve tissue protection. Ideally, these trials should consider using outcome measurements which are sensitive specifically to treatment effects on axonal and glial architecture and on the mechanisms of innate immunity. The trials may also need to involve less disabled cohorts, in whom this treatment may be better tolerated.
- Further research on the mechanisms of tissue injury in MS is also required, to identify additional protective strategies which may be tested alone or in combination in future trials.
- It would be helpful if research on the mechanisms of tissue injury could consider if different mechanisms affect the cerebral grey and white matter and the spinal cord.
- Further work on the design of clinical trials of neuroprotection is also required which takes into account the confounding effects of complicated changes of volume or other surrogate measurements due to the active treatment, as well as the need to extend the period of treatment beyond the phase when these confounding effects occur.
- In addition, trial designs should be developed which detect and adapt to relatively high rates of non-adherence and loss to follow up in disabled cohorts.

References

1 Compston A, Coles A (2002) Lancet 359, 1221-31.
2 Goodin DS et al (2002) Neurology 58, 169-78
3 Frohman EM et al (2007) Arch Neurol 62, 1345-1356.
4 Bechtold DA and Smith KJ (2005) J Neurol Sci 233, 27-35
5 Stys PK (2005) J Neurol Sci 233: 3-13.
6 Garthwaite G et al (2002) Neuroscience 109, 145-55.
7 Lo AC et al (2003) J Neurophysiol 90: 3566-71.
8 Kapoor R et al. (2003) Ann Neurol 53: 174-80.
9 Bechtold DA et al (2004) Ann Neurol 55: 607-16.
10 Bechtold DA et al (2006) J Neurol 253, 1542-51.
11 Losseff N et al (1996) Brain 119, 2009-2019.
12 Molyneux PD et al (2000) Brain 123, 2256-2263.
13 Altmann DR et al (2009) Neurology 72, 595-601.
14 Rao AB et al (2002) Neurology 59, 688-89.
15 Black JA et al (2007) Ann Neurol 62, 21-33.
16 Losseff N et al (1996) Brain 119, 701-708.
17 Verbeke G, Molenberghs G (2000). Linear Mixed Models for Longitudinal Data. New York: Springer-Verlag.
18 Hoogervorst EL et al (2002) Arch.Neurol 59, 113-6.
19 Armitage P et al (2002) Statistical Methods in Medical Research, 4th ed. Oxford: Blackwell Science.
20 Furby J et al (2008) Mult Sclerosis 14, 1068-75.
21 Furby J et al (2009) Mult Sclerosis 15, 687-94.
22 Hayton T et al (2009) J Neurol 256, 427-35.
23 Kutzelnigg A et al (2005) Brain 128, 2705-2712.
24 Fisher E et al (2008) Ann Neurol 64255-265.
25 Fisniku LK et al (2008) Ann Neurol 64, 247-254.
26 Zivadinov R et al (2008) Neurology 71, 136-144
27 Rudick RA et al (1999) Neurology 53, 1698-1704.
28 Frank JA et al (2004) Neurology 62, 719-725.
29 Hardmeier M et al (2005) Neurology 64, 236-240.
30 Miller DH et al (2007) Neurology 68, 1390-1401.
31 Craner MJ et al. (2005) Glia 15, 220-9.
32 Lunardi G et al (1997) Neurology 48, 1714-17.
33 Leandri M et al (2000) J Neurol 247, 556-58.
34 Black JA et al (2007) J Neuropath Exp Neurol 66, 828-37.
35 Crumrine RC et al (1997) Stroke 28, 2230-36.

Section 2: Arrangements for publication and dissemination of research

The baseline clinical and imaging characteristics of the participants have been the subjects of several presentations at meetings, and of three publications in which the relationships between MRI findings and measures of disability have been examined. These presentations and abstracts are detailed below. Further papers on the imaging findings are being prepared for publication, as well as paper reporting the main findings of the trial, and further publications which report additional outcomes are planned. It is expected that the longitudinal natural history data derived from the study will help in the design and sample size calculations of future trials of disease modifying drugs in SPMS.

The results of the project have also been reported in a number of poster and platform presentation at scientific meetings, and the main results of the treatment trial were presented at MS Frontiers (May 2009) the main scientific meeting of the MS Society of Great Britain and Northern Ireland. Presentations were also made at the 2009 ECTRIMS meeting, the main European venue for MS research. Further dissemination of the results of the trial will be undertaken by the MS Society, and through anticipated invited lectures by the co-applicants at future scientific meetings and talks to patient groups.

All participants have been informed personally by phone and letter of the main results of the trial, and of their allocation to the active or placebo group. They have also been given the opportunity to discuss the results of the trial personally with the Principal Investigator.

Bibliographic citations:

Published papers

Kapoor R Neuroprotection in multiple sclerosis: therapeutic strategies and clinical trial design. Curr Opin Neurobiol 2006; 19: 255-59.

Hayton T, Furby J, Kapoor R. The Demyelinated Axon. ACNR 2007; 7: 10-13

Kapoor R. Sodium channel blockers and neuroprotection in multiple sclerosis using lamotrigine. J Neurol Sci 2008; 274: 54-56.

Furby J, Hayton T, Anderson V, Smith KJ, Altmann D, Brenner R, Chataway J, Hughes RAC, Miller DH, Kapoor R. Magnetic resonance imaging measures of brain and spinal cord atrophy correlate with clinical impairment in secondary progressive multiple sclerosis. Multiple Sclerosis 2008; 14: 1068-75

Hayton T, Furby J, Smith KJ, Altmann D, Brenner R, Chataway J, Hughes RAC, Hunter K, Tozer D, Miller DH, Kapoor R. Grey matter magnetisation transfer ratio independently correlates with neurological deficit in secondary progressive multiple sclerosis. J Neurol 2009; 256: 427-35.

Furby J, Hayton T, Smith KJ, Altmann D, Brenner R, Chataway J, Hughes RAC, Miller DH, Kapoor R. Different white matter lesion characteristics correlate with distinct grey matter abnormalities on MRI in secondary progressive multiple sclerosis. Multiple Sclerosis 2009; 15: 687-94.

Presentations and abstracts

The applicants have made numerous presentations on different clinical, imaging and basic aspects of MS, and have described the work funded by this grant in many of these presentations because of its general relevance. In addition, the results of the work funded by the grant have been presented specifically at a number of further meetings, and these are detailed below, together with the bibliographic citation of the meeting abstract, where relevant:

22nd Congress of the European Committee for Treatment and Research in Multiple Sclerosis (September 2006). Poster presentation
Furby J, Hayton T, Smith KJ, Altmann D, Brenner R, Chataway J, Fox N, Hughes RAC, Miller DH, Kapoor R. A randomised controlled trial of neuroprotection with lamotrigine in secondary progressive multiple sclerosis. Multiple Sclerosis 2006; 12: S228.

MS Frontiers (June 2007). Poster presentation
Furby J, Hayton T, Smith KJ, Altmann D, Brenner R, Chataway J, Fox N, Hughes RAC, Miller DH, Kapoor R. The correlation between MR measures of atrophy and disability in secondary progressive multiple sclerosis.

23rd Congress of the European Committee for Treatment and Research in Multiple Sclerosis (October 2007). Poster presentation
Furby J, Hayton T, Smith KJ, Altmann D, Brenner R, Chataway J, Fox N, Hughes RAC, Miller DH, Kapoor R. The correlation between MR measures of atrophy and disability in secondary progressive multiple sclerosis. Multiple Sclerosis 2007; 13: S619.

23rd Congress of the European Committee for Treatment and Research in Multiple Sclerosis (October 2007). Poster presentation
Hayton T, Furby J, Smith KJ, Altmann D, Brenner R, Chataway J, Fox N, Hughes RAC, Miller DH, Kapoor R. The correlation between brain magnetisation transfer ratio and clinical disability measures in secondary progressive multiple sclerosis. Multiple Sclerosis 2007; 13: S624.

TOPS Seminars in Multiple Sclerosis (Palermo, June 2007). Platform presentation.
Kapoor R. From lab to clinic: neuroprotection with lamotrigine.

CORE-MS Meeting: Tissue protective studies in MS (Washington DC, Sept 2007). Platform presentation.
Kapoor R. Lamotrigine trial design. .

European Charcot Foundation Symposium (Fiuggi, December 2007). Platform presentation.
Kapoor R. Sodium channel blockers and neuroprotection using lamotrigine.

American Academy of Neurology (April 2008). Poster presentation
Hayton T, Furby J, Smith KJ, Altmann D, Brenner R, Chataway J, Fox N, Hughes RAC, Miller DH, Kapoor R. Predictors of disability in secondary progressive multiple sclerosis: a multimodal MRI study. Neurology 2008; 70 (Suppl 1): A209.

World Congress on Treatment and Research in Multiple Sclerosis, Montreal (Sept 2008). Poster presentation, and platform presentation as winner of a Best Poster Prize.
Furby J, Hayton T, Smith KJ, Altmann D, Brenner R, Chataway J, Hughes RAC, Miller DH, Kapoor R. Different white matter lesion characteristics correlate with distinct grey matter abnormalities on MRI in secondary progressive multiple sclerosis. Multiple Sclerosis 2008; 14: S104.

MS Frontiers (May 2009). Platform presentation.
Kapoor R, Furby J, Hayton T, Smith KJ, Altmann DR, Brenner R, Chataway J, Hughes RAC, Hunter K, Miller DH Outcomes of a phase II randomised controlled trial of neuroprotection with lamotrigine in secondary progressive multiple sclerosis.

In addition, the following abstracts were presented at the 2009 meeting of the European Committee for Treatment and Research in Multiple Sclerosis:

Hayton T, Furby J, Smith KJ et al Longitudinal changes in magnetization transfer ratio in secondary progressive multiple sclerosis. Poster presentation.

Hayton T, Furby J, Smith KJ et al T1 hypointense lesion volume predicts localized and global brain pathology and correlates with upper limb function in secondary progressive multiple sclerosis. Poster presentation.

Furby J, Hayton TD, Smith KJ et al A longitudinal study of volumetric MRI in secondary progressive multiple sclerosis. Poster presentation.

Kapoor R, Furby J, Hayton T, et al. Outcomes of a phase II randomised controlled trial of neuroprotection with lamotrigine in secondary progressive multiple sclerosis. Platform presentation.