# Articles

# Effect of high-dose simvastatin on brain atrophy and disability in secondary progressive multiple sclerosis (MS-STAT): a randomised, placebo-controlled, phase 2 trial

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# Summary

**Background** Secondary progressive multiple sclerosis, for which no satisfactory treatment presently exists, accounts for most of the disability in patients with multiple sclerosis. Simvastatin, which is widely used for treatment of vascular disease, with its excellent safety profile, has immunomodulatory and neuroprotective properties that could make it an appealing candidate drug for patients with secondary progressive multiple sclerosis.

Methods We undertook a double-blind, controlled trial between Jan 28, 2008, and Nov 4, 2011, at three neuroscience centres in the UK. Patients aged 18–65 years with secondary progressive multiple sclerosis were randomly assigned (1:1), by a centralised web-based service with a block size of eight, to receive either 80 mg of simvastatin or placebo. Patients, treating physicians, and outcome assessors were masked to treatment allocation. The primary outcome was the annualised rate of whole-brain atrophy measured from serial volumetric MRI. Analyses were by intention to treat and per protocol. This trial is registered with ClinicalTrials.gov, number NCT00647348.





Published Online March 19, 2014 http://dx.doi.org/10.1016/ S0140-6736(13)62242-4

See Online/Comment http://dx.doi.org/10.1016/ S0140-6736(13)62641-0

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Findings 140 participants were randomly assigned to receive either simvastatin (n=70) or placebo (n=70). The mean annualised atrophy rate was significantly lower in patients in the simvastatin group (0.288% per year [SD 0.521]) than in those in the placebo group (0.584% per year [0.498]). The adjusted difference in atrophy rate between groups was -0.254% per year (95% CI -0.422 to -0.087; p=0.003); a 43% reduction in annualised rate. Simvastatin was well tolerated, with no differences between the placebo and simvastatin groups in proportions of participants who had serious adverse events (14 [20%] *vs* nine [13%]).

Interpretation High-dose simvastatin reduced the annualised rate of whole-brain atrophy compared with placebo, and was well tolerated and safe. These results support the advancement of this treatment to phase 3 testing.

Funding The Moulton Foundation [charity number 1109891], Berkeley Foundation [268369], the Multiple Sclerosis Trials Collaboration [1113598], the Rosetrees Trust [298582] and a personal contribution from A Pidgley, UK National Institute of Health Research (NIHR) University College London Hospitals/UCL Biomedical Research Centres funding scheme.

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# Introduction

Multiple sclerosis is a major cause of disability, particularly in young adults in temperate climates. Despite much success with drugs that substantially reduce relapse frequency during the initial inflammatory, relapsingremitting phase, more than half of patients eventually develop non-relapsing, secondary progressive multiple sclerosis one to two decades after the onset of relapsingremitting multiple sclerosis. This relentless accumulation of neurological deficit and increasing brain atrophy is thought to be driven by neuroaxonal loss.1 Although several symptomatic treatments are available, progression in secondary progressive multiple sclerosis is presently intractable. Immunomodulatory strategies derived from relapsing-remitting multiple sclerosis have not proven effective when extended into secondary progressive multiple sclerosis (eg, cyclophosphamide,<sup>2</sup> β-interferon,<sup>3,4</sup> myelin-basic protein<sup>5</sup>). Direct neuroprotection strategies (eg, lamotrigine,<sup>6</sup> tetrahydrocannabinol<sup>7</sup>) have also failed.<sup>8</sup> The crucial and as yet unmet challenge is to find effective and well-tolerated treatments for secondary progressive multiple sclerosis.

3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase inhibitors (statins), which inhibit the catalytic conversion of HMG-CoA to mevalonate, are extensively used and well tolerated in the treatment of primary hyperlipidaemia, and for secondary prevention of myocardial and cerebral ischaemia. Clinical benefits noted in these disorders are due to both direct cholesterol lowering, and to cholesterol-independent effects. In murine models, statins inhibit MHC class II-restricted antigen presentation, downregulate T-cell activation and proliferation and induce a shift from a pro-inflammatory Th1 to a Th2 phenotype.<sup>9</sup> Statins also block adhesion molecule expression and inhibit leucocyte migration through the blood-brain barrier, supporting their potential use in early multiple sclerosis.<sup>10-12</sup> However, because statins also have cell protective properties<sup>13</sup> and improve cerebrovascular haemodynamics,<sup>14</sup> they could be used in patients with later stage multiple sclerosis in whom vascular<sup>15</sup> and brain parenchymal cell dysfunction take place.<sup>13</sup>

Initial clinical studies of patients with early stage multiple sclerosis with high-dose (80 mg) simvastatin showed a significant reduction in lesion activity.<sup>16</sup> Subsequent randomised controlled trials of simvastatin<sup>17,18</sup> and atorvastatin<sup>19-21</sup> as add-on therapy to  $\beta$ -interferon in patients with relapsing-remitting multiple sclerosis have yielded conflicting results, although they were hampered by insufficient power. We undertook a randomised controlled trial of high-dose simvastatin to assess the role of statins further in secondary progressive multiple sclerosis.

# Methods

# Study design and participants

We undertook this investigator-led, double blind, placebocontrolled, parallel-group, randomised trial between Jan 28, 2008, and Nov 4, 2011 at three neuroscience centres in London and southeast England.

To be eligible for the study, patients had to be aged 18–65 years, have an Expanded Disability Severity Scale  $(EDSS)^{22}$  score of  $4 \cdot 0$ – $6 \cdot 5$  and fulfil the revised McDonald criteria for multiple sclerosis,<sup>23</sup> and at randomisation have entered the secondary progressive stage.<sup>24</sup> The definition of secondary progressive stage was in keeping with that used in other secondary progressive multiple

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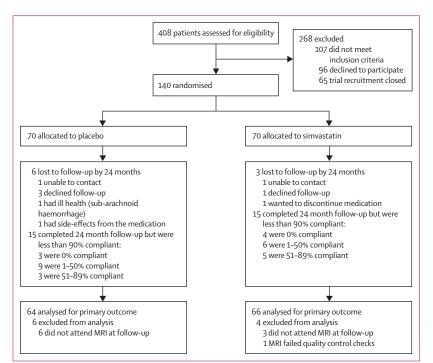


Figure 1: Patient flow diagram

sclerosis trials.<sup>6</sup> Steady progression rather than relapse had to be the major cause of increasing disability in the preceding 2 years, confirmed on the basis of either an increase of at least one point on the EDSS or clinically documented increasing disability. Patients were ineligible if they had primary progressive multiple sclerosis; had a relapse or had been treated with corticosteroids within 3 months of screening; or used immunosuppressants (eg, azathioprine, methotrexate, ciclosporin) or disease modifying treatments (avonex, rebif, betaferon, glatiramer acetate) within the previous 6 months. Detailed inclusion and exclusion criteria are available elsewhere.<sup>25</sup> Patients were seen at months 1, 6, 12, and 24 with telephone follow-up at months 3 and 18.

The study was done in accordance with Good Clinical Practice<sup>26</sup> and the Declaration of Helsinki.<sup>27</sup> The protocol was approved by each study site's institutional review board, and all patients gave informed consent before entering the study.

# Randomisation and masking

Patients were randomised in a 1:1 ratio to receive simvastatin 80 mg, or matching placebo drug (after a first month at 40 mg a day), for 24 months. Randomisation was done by a centralised web-based service, with block size of eight, and minimisation<sup>28</sup> on the following variables: age (<45,  $\geq$ 45 years old); sex; EDSS (4–5·5, 6·0–6·5); centre; and assessing physician. Patients, treating physicians, and outcome assessors (including MRI scan analysts) were masked to treatment allocation. To ensure full masking, both a treating and an independent qualified examining neurologist were involved in the trial.

# Procedures

The predefined primary endpoint was the rate of wholebrain atrophy per year, measured as relative change in brain volume by K-means normalisation brain boundary shift integral (BSI),<sup>29,30</sup> based on each pair of volumetric T1 MRI scans between baseline, 12 months, and 25 months. BSI, like structural image evaluation using normalisation of atrophy (SIENA) is a well-validated and established registration-based, semi-automated, direct measure of volume change that has robust performance characteristics.<sup>31–33</sup> The volumetric scan was acquired twice at baseline, with the best-quality scan chosen for analysis on the basis of expert visual rating of motion and artifact. Scan acquisition sequence parameters were also reviewed to ensure longitudinal consistency. The final scan was 1 month after last medication to minimise any potential effect of changes in artifactual volume, such as pseudoatrophy (a short term reduction in brain volume due to an anti-inflammatory effect);34 thus, both the baseline and end-of study scans were off-medication. The MRI secondary endpoint, assessed at 12 months and 25 months was the number of new and enlarging T2 brain lesions (for acquisition parameters see appendix).

Clinical outcomes assessed at baseline, 12 months, and 24 months were EDSS,<sup>22</sup> multiple sclerosis functional composite scale (MSFC),<sup>35</sup> multiple sclerosis impact scale-29 (MSIS-29)<sup>36</sup> version 2, and relapse frequency. The MSFC *Z* score was normalised with the study baseline scores. Cholesterol levels were taken at baseline and 24 months.

We investigated phenotypic markers with whole blood specimens collected at baseline, 6, 12, and 24 months. Intracellular cytokine expression was examined in CD3+ T cells for IFN- $\gamma$ , IL-4, and IL-17 as markers of Th/Tc1, Th/Tc2, and Th/Tc17 T-cell populations, respectively. Intracellular cytokine (IL-4, IFN- $\gamma$ , IL-10, IL-17) expression on unstimulated T cells and cells stimulated with 50 ng per mL of Phorbol 12-myristate 13-acetate and 1 µg per mL ionomycin was done as previously described (appendix).<sup>37</sup> Coexpression of CD4+ and intracellular expression of FoxP3 and IL-10 were examined as markers of regulatory T cells.

# Statistical analysis

The sample size was chosen to give the trial 90% statistical power (with a conventional two-sided significance level of 5%) to detect a difference of 0.25% a year in whole-brain atrophy between the groups assuming a mean atrophy rate in the placebo group of 0.6% a

year and a standard deviation of 0.4% a year. With these assumptions 54 patients would be needed in each treatment group: in anticipation of dropouts and incomplete follow-up this was increased to 70 patients per group. Analysis of all imaging and clinical outcomes were prespecified in a statistical analysis plan. Primary analyses were by intention to treat. A secondary analysis was undertaken using a per-protocol dataset, which comprised patients who complied with treatment and completed follow-up to 25 months. Participants were considered compliant with treatment if they reported taking, on average, at least 90% of their drug at a dose of 80 mg. Compliance was assessed by examination of the self-reported proportion of capsules taken in the month before the 6, 12, 18, and 24 month assessments. All analyses were done with STATA (version 12.1).

BSI-derived changes in whole-brain volume were converted into a percentage of the baseline whole-brain volume for each available scan interval giving up to three BSI values per participant (change between 0–12 months, 12–25 months, and 0–25 months). A positive value for percentage change shows a decrease in brain volume. We compared mean rates of atrophy in the two treatment groups with the family of linear mixed models developed for the analysis of repeated measures of direct change.<sup>38</sup> These models allow

|                                     | Placebo (n=70) |               |                        | Simvastatin (n=70) |               |                        |  |
|-------------------------------------|----------------|---------------|------------------------|--------------------|---------------|------------------------|--|
|                                     | n (%)          | Mean (SD)     | Median (range)         | n (%)              | Mean (SD)     | Median (range)         |  |
| Women                               | 48 (69%)       |               |                        | 49 (70%)           |               |                        |  |
| Ethnic origin                       |                |               |                        |                    |               |                        |  |
| White                               | 63 (90%)       |               |                        | 69 (99%)           |               |                        |  |
| Black                               | 3 (4%)         |               |                        | 0 (0%)             |               |                        |  |
| Asian                               | 1(1%)          |               |                        | 1 (1%)             |               |                        |  |
| Other                               | 3 (4%)         |               |                        | 0 (0%)             |               |                        |  |
| Relapse in past 12 months           | 12 (17%)       |               |                        | 7 (10%)            |               |                        |  |
| Relapse in past 24 months           | 18 (26%)       |               |                        | 8 (11%)            |               |                        |  |
| Age (years)                         | 70             | 51.1 (6.8)    | 51.5 (36.5 to 64.6)    | 70                 | 51.5 (7.0)    | 51·9 (34·8 to 64·6)    |  |
| Multiple sclerosis duration (years) | 70             | 20.3 (8.8)    | 18·8 (4·2 to 39·6)     | 70                 | 22.1 (8.3)    | 21·8 (4·7 to 39·8)     |  |
| SPMS duration (years)               | 70             | 7.1 (4.8)     | 5·3 (1·6 to 22·6)      | 70                 | 7.3 (5.6)     | 5·4 (2·1 to 25·0)      |  |
| Total cholesterol (mmol/L)          | 67             | 5.62 (0.87)   | 5·6 (3·80 to 7·40)     | 68                 | 5.52 (1.09)   | 5·45 (3·70 to 8·90)    |  |
| EDSS                                | 70             | 5.87 (0.78)   | 6 (4 to 7)*            | 70                 | 5.76 (0.84)   | 6 (4 to 6·5)           |  |
| MSFC Z score                        | 64             | -0.29 (1.48)  | -0.06 (-6.66 to 1.24)  | 64                 | -0.03 (0.92)  | 0·00 (-5·33 to 1·37)   |  |
| Paced auditory serial addition test | 67             | 33.7 (16.1)   | 32 (0 to 59)           | 67                 | 34.8 (13.8)   | 34 (0 to 57)           |  |
| 25ft walk average speed (ft per s)  | 68             | 1.50 (0.87)   | 1·38 (0·14 to 3·47)    | 66                 | 1.67 (0.91)   | 1.58 (0.14 to 3.85)    |  |
| Nine hole peg test (speed per s†)   | 69             | 0.033 (0.010) | 0.035 (0.001 to 0.053) | 67                 | 0.034 (0.009) | 0.034 (0.001 to 0.054) |  |
| MSIS-29                             | 66             | 70·0 (14·5)   | 67·0 (34·0 to 102·3)   | 69                 | 70·2 (14·0)   | 72·0 (38·5 to 107·0)   |  |
| MSIS-29 physical                    | 66             | 51.2 (10.4)   | 49·7 (25·0 to 72·6)    | 69                 | 51.4 (10.6)   | 53·7 (29·5 to 79·0)    |  |
| MSIS-29 psychological               | 66             | 18.8 (5.7)    | 19·0 (9·0 to 36·0)     | 69                 | 18-9 (5-2)    | 18·0 (9·0 to 33·0)     |  |
| Whole-brain volume (mL)             | 70             | 1098 (124)    | 1095 (881 to 1432)     | 70                 | 1104 (118)    | 1106 (891 to 1474)     |  |

MSIS-29 range might take fractional values when a score for a missing item was imputed as the mean score of the completed items in the scale. SPMS=secondary progressive multiple sclerosis. EDSS=Kurtzke expanded disability status scale. MSFC=multiple sclerosis functional composite. MSIS=multiple sclerosis impact scale. \*One patient changed from 6-5 to 7-0 between screening and randomisation. †Speed of pegs placed.

Table 1: Baseline characteristics

simultaneous analysis of the changes over the three time intervals, appropriately allowing for the correlation between these points. Therefore all available atrophy measures were included in this analysis, with participants included in the analysis if they have at least one BSI measure of atrophy.

An interaction between treatment group and time was included in the model as were other interactions between time and the minimisation variables and MRI site. The treatment effect therefore represents the difference between the treatment and placebo group in mean annual percentage change in whole-brain volume adjusted for minimisation variables and MRI site. Analysis of repeated measures with a properly specified linear mixed model including all follow-up data is more robust to dropouts than a complete case analysis. Specifically, the estimated treatment effect is unbiased under the missing-at-random

|                                | Placebo (n=70) | Simvastatin<br>(n=70) | Difference<br>in means* | 95% CI for<br>difference |
|--------------------------------|----------------|-----------------------|-------------------------|--------------------------|
| Change in whole-brain volume   | (% per year)*† |                       |                         |                          |
| Mean (SD) rate                 | 0.584 (0.498)  | 0.288 (0.521)         | -0.254‡                 | -0.422 to -0.087         |
| Number (%) assessed            | 64 (91%)       | 66 (94%)              |                         |                          |
| Number of BBSI measures        | 165            | 175                   |                         |                          |
| EDSS (score 0 to 10)           |                |                       |                         |                          |
| Mean (SD) at 24 months         | 6.35 (0.83)    | 5.93 (1.11)           | -0·254§                 | -0·464 to -0·069         |
| Number (%) assessed            | 61 (87%)       | 67 (96%)              |                         |                          |
| MSIS-29 total (score 29 to 116 | )              |                       |                         |                          |
| Mean (SD) at 24 months         | 76.1 (16.3)    | 70.1 (15.6)           | -4·78¶                  | -9·39 to -0·02           |
| Number (%) assessed            | 57 (81%)       | 66 (94%)              |                         |                          |
| MSIS-29 physical (score 20 to  | 80)            |                       |                         |                          |
| Mean (SD) at 24 months         | 56.3 (11.8)    | 51.7 (11.4)           | -3·73¶                  | -7·18 to -0·28           |
| Number (%) assessed            | 57 (81%)       | 66 (94%)              |                         |                          |
| MSIS-29 psychological (score   | 9 to 36)       |                       |                         |                          |
| Mean (SD) at 24 months         | 19.8 (6.0)     | 18.3 (5.8)            | -1.09                   | -2.83 to 0.84            |
| Number (%) assessed            | 57 (81%)       | 66 (94%)              |                         |                          |
| MSFC Z score                   |                |                       |                         |                          |
| Mean (SD) at 24 months         | -1.21 (2.59)   | -0.78 (2.06)          | 0.289                   | -0.333 to 0.961          |
| Number (%) assessed            | 49 (70%)       | 58 (83%)              |                         |                          |
| MSFC walk (speed ft per s)     |                |                       |                         |                          |
| Mean (SD) at 24 months         | 1.55 (1.19)    | 1.83 (1.61)           | 0.085                   | -0·249 to 0·533          |
| Number (%) assessed            | 54 (77%)       | 62 (89%)              |                         |                          |
| MSFC peg test (speed per s)    |                |                       |                         |                          |
| Mean (SD) at 24 months         | 0.030 (0.014)  | 0.033 (0.010)         | 0.002                   | -0.001 to 0.004          |
| Number (%) assessed            | 54 (77%)       | 61 (87%)              |                         |                          |
| MSFC PASAT (score 0 to 60)     |                |                       |                         |                          |
| Mean (SD) at 24 months         | 35.2 (18.0)    | 38.3 (15.4)           | 4.45                    | -0.11 to 8.84            |
| Number (%) assessed            | 50 (71%)       | 61 (87%)              |                         |                          |

BBSI=brain boundary shift integral. EDSS=Kurtzke expanded disability status scale. MSFC=multiple sclerosis functional composite. MSIS=multiple sclerosis impact scale. PASAT=paced auditory serial addition test. \* Adjusted for minimisation variables and MRI site for change in whole-brain volume; adjusted for minimisation variables and Baseline measurement of outcome for all other outcomes. †Positive values reflect decrease in brain volume. ‡p=0-003. \$p-0-01. #p-0-05. All CIs, other than that for the comparison of atrophy rates, are bias-corrected and accelerated non-parametric bootstrap confidence computed from 2000 bootstrap samples.

Table 2: Changes in whole-brain volume between 0, 12, and 25 months and secondary clinical outcomes at 24 months

assumption rather than the more stringent completelymissing-at-random assumption, which is necessary for a complete case analysis to be unbiased.<sup>39</sup> We judged that because whole-brain atrophy is the primary outcome variable in this trial, the additional statistical complexity introduced by using this approach was justified. However, we also report results from a simple comparison of rates of change over 25 months (adjusting for minimisation variables and site).

For EDSS, MSFC and subscales, and MSIS-29 and subscales, mean score at 24 months was compared between treatment groups using an ANCOVA40 model adjusting for baseline score and minimisation variables. Here a complete case approach was taken for missing data, so participants were included in the analysis only when they had both a baseline and 24 month score for the outcome measure being examined. Because these variables showed divergence from a normal distribution, nonparametric, bias corrected and accelerated, bootstrap CIs were calculated from 2000 replications<sup>41</sup> (bootstrapping is a computer intensive technique that can give valid CIs even when normality assumptions do not hold<sup>42</sup>). A consequence of use of the bootstrap is that exact p values are not calculated: however, whether or not p<0.05 (or p<0.01) was inferred from the 95% (or 99%) CI. We compared incidence of new and enlarging T2 hyper-intense brain lesions and the relapse rate between treatment groups with an over-dispersed Poisson model,43 with adjustment for minimisation variables and adjustment for MRI site where relevant. All available data were used for analysis of relapse rates and new and enlarging lesions, with participants included in the analysis if they had information recorded on at least one follow-up assessment.

Percentage expression of FoxP3 on CD4+ T cells and inducible levels (unstimulated subtracted from the stimulated value) of IFN-y, IL-4, IL-10 and IL-17 on all CD3+T cells were calculated after suitable transformation (natural logarithm or square root). Linear mixed models were used to compare the mean value of each marker between the placebo and simvastatin group at 6, 12, and 24 months, with adjustment for minimisation variables. To make this analysis essentially equivalent to an ANCOVA,44 adjusting for baseline, measures of the marker at baseline were treated as an additional outcome in the model with treatment effects and effects of minimisation variables constrained to be zero at baseline.44 This approach allows use of all available measures of the marker for each participant to be included in the analysis.

Safety was examined by testing the null hypothesis that there was no increase in the proportion of participants with at least one serious adverse event in the active compared with the placebo group, with a one-sided test.

# Role of the funding source

None of the funding sources were involved in the analysis, writing, or decision to submit the manuscript.

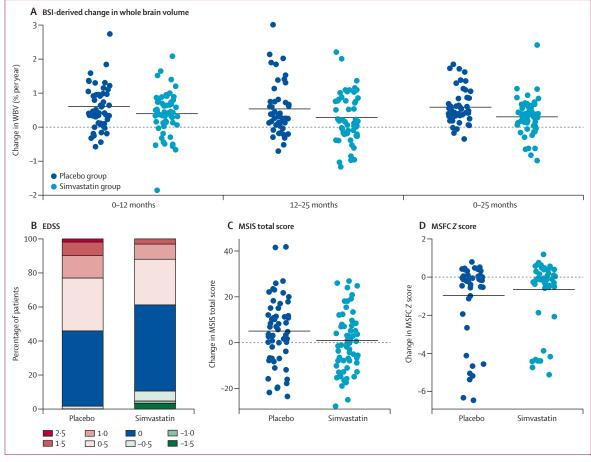
There has been no pharmaceutical company involvement in this study. The corresponding author has had full access to all the data in the study and takes final responsibility to submit for publication.

### Results

Figure 1 shows the trial profile. 140 participants were randomly assigned to receive either simvastatin (n=70) or placebo (n=70). The two groups were very similar on baseline variables used for minimisation and for most other baseline variables, including whole-brain volume (table 1). A slightly higher proportion of the simvastatin group was white and a lower proportion had experienced a relapse in the last 12 or 24 months. Nine participants were lost to follow-up (six in the placebo group and three in the treatment group). Of the patients who completed follow-up, compliance was at least 90% for 49 patients (77%) for placebo and 52 patients (78%) for simvastatin. Cholesterol was significantly reduced from mean  $5 \cdot 5$  (SD  $1 \cdot 1$ ) mmol/L at baseline to  $4 \cdot 1$  (0.9) mmol/L at 24 months in

the simvastatin group (p<0.0001), whereas it did not change in the placebo group from 5.6 (0.9) mmol/L at baseline to 5.6 (1.0) mmol/L at 24 months (p=0.93).

In the prespecified intention-to-treat analysis mean atrophy rate was lower in the simvastatin group at 0.288% (SD 0.521) per year than in the placebo group at 0.584% (0.498) per year. The adjusted difference in atrophy rate between the groups was -0.254% per year (95% CI -0.422 to -0.087; p=0.003), which is a 43% reduction in annualised rate of atrophy (table 2). This reduction in annualised rate of atrophy is readily apparent when examining the individual patient values (figure 2); between 0 and 25 months more than threequarters of patients in the simvastatin group had a lower atrophy rate than the mean rate in the placebo group. There was a similar reduction in the simple mean atrophy rate between baseline and 25 months, which was likewise lower in the simvastatin group at 0.294% (0.508) per year versus the placebo group at 0.565%(0.473) per year (figure 2). Between baseline and



#### Figure 2: Primary MRI outcome and secondary clinical outcomes

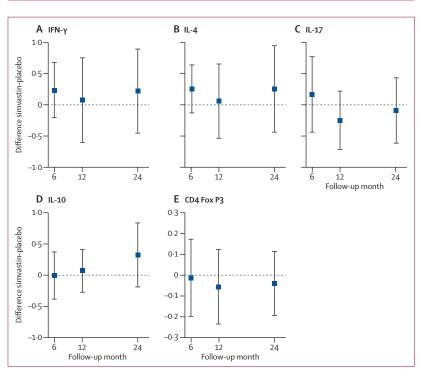
The mean and individual patient values are shown for change in whole-brain volume (A); percentage of patients with a given change in EDSS (B); change 0 to 24 months MSIS-29 (C); and change 0 to 24 months MSFC (D). For panels A, C, and D the mean is indicated by solid bar, placebo group by dark blue points, and simvastatin group by pale blue points. For change in whole-brain volume, EDSS, and MSIS-29, a positive value indicates a worse outcome. A positive value for MSFC Z score indicates an improved outcome. BSI=brain boundary shift integral. WBV=whole-brain volume. EDSS=Kurtzke expanded disability status scale. MSIS=multiple sclerosis functional composite.

12 months, mean rates were similar to those observed between 0 and 25 months, but as expected variability was greater (simvastatin group 0.375% [0.631] per year versus placebo group 0.597% [0.617] per year). The results from the per-protocol analysis of all measured changes were very similar to those found for the intention-to-treat analysis. The mean atrophy rate was lower in the simvastatin group (0.298% [SD 0.562] per year) than in the placebo group (0.589% [0.528] per year), with adjusted difference of -0.279% per year (95% CI -0.488 to -0.071; p=0.009).

|                                 | Placebo    | Simvastatin | Incidence rate<br>ratio* | 95% CI    | p value |
|---------------------------------|------------|-------------|--------------------------|-----------|---------|
| New and enlarging T2 lesions    |            |             |                          |           |         |
| Rate per person-year (N events) | 2.19 (283) | 1.50 (205)  | 0.72                     | 0.45-1.16 | 0.176   |
| Number (%) of patients assessed | 63 (90%)   | 65 (93%)    |                          |           |         |
| Relapses                        |            |             |                          |           |         |
| Rate per person-year (N events) | 0.16 (22)  | 0.20 (28)   | 1.29                     | 0.64-2.60 | 0.473   |
| Number (%) of patients assessed | 70 (100%)  | 70 (100%)   |                          |           |         |
|                                 |            |             |                          |           |         |

\*Adjusted for minimisation variables and MRI site for new and enlarging T2 lesions; adjusted for minimisation variables for relapse rate.

 $\mathit{Table}$  3: Rate of new and enlarging T2 lesions between over 0 to 25 months and relapse rate over 0 to 24 months



# Figure 3: Mean and 95% CI for the difference between simvastatin and placebo groups in immunological markers at 6, 12, and 24 months

The difference between simvastatin and placebo in inducible levels of cytokine expression is shown for square root IFNY (A), natural log IL-4 (B), natural log IL-10 (C), and natural log IL-17 (D). The difference between simvastatin and placebo is shown for natural log of % CD4 Fox P3 expression (E). Analyses of each marker included at least 134 participants, with number at each timepoint between 91 participants (IL-17 at 24 months) and 112 participants (IL-10 at 6 months). Means and 95% CIs are adjusted for minimisation variables and baseline measurement of the marker. The dashed line indicates zero difference between groups.

At 24 months we recorded a statistically significant difference in favour of simvastatin versus placebo for EDSS (difference -0.254; 95% CI -0.464 to -0.069; p<0.01) and total MSIS-29 (-4.78; 95% CI -9.39 to -0.02; p<0.05), in particular the MSIS-29 physical subscale (-3.73; -7.18 to -0.28; p<0.05), with a trend in the MSIS-29 psychological outcome that did not reach formal statistical significance (-1.09; -2.83 to 0.84; p>0.10; table 2). In the MSFC there was no significant difference between the simvastatin and placebo groups, though those on simvastatin had a slightly more favourable MSFC than placebo (0.289; 95% CI -0.333 to 0.961; p>0.10). Changes in score between baseline and 24 months for most secondary outcomes generally reflected worsening over time, particularly in the placebo group (figure 2). There was no significant difference between the simvastatin and placebo group in the rate of new and enlarging lesions (incidence rate ratio 0.72; 95% CI 0.45 to 1.16; p=0.176) or in the rate of relapse (1.29; 0.64 to 2.60; p=0.473; table 3). Results for the perprotocol analyses were similar to those for the intentionto-treat analyses for all secondary outcomes, including

|  | Placebo<br>(n=70) | Simvastatin<br>(n=70) |
|--|-------------------|-----------------------|
| Number with any adverse event            | 54 (77%)          | 49 (70%)              |
| Cause of most common adverse events (>10 | % per group)      |                       |
| Relapse                                  | 17 (24%)          | 17 (24%)              |
| Cramp                                    | 10 (14%)          | 12 (17%)              |
| Headache                                 | 10 (14%)          | 3 (4%)                |
| Pain                                     | 13 (19%)          | 7 (10%)               |
| Worsening mobility                       | 8 (11%)           | 9 (13%)               |
| Increased spasticity                     | 7 (10%)           | 0 (0%)                |
| Urinary tract infection                  | 8 (11%)           | 9 (13%)               |
| Number with drug-related adverse event   | 13 (19%)          | 16 (23%)              |
| Number with serious adverse event        | 14 (20%)          | 9 (13%)               |
| Cause of serious adverse event           |                   |                       |
| Grade 3 relapse*                         | 5 (7%)            | 3 (4%)                |
| Abdominal lesion biopsy                  | 1(1%)             | 0 (0%)                |
| Appendectomy                             | 1(1%)             | 0 (0%)                |
| Depression                               | 0 (0%)            | 1(1%)                 |
| Fall                                     | 1(1%)             | 0 (0%)                |
| Fracture                                 | 2 (3%)            | 1(1%)                 |
| Pneumonia                                | 0 (0%)            | 1(1%)                 |
| Pulmonary embolism                       | 1(1%)             | 0 (0%)                |
| Road traffic accident                    | 1(1%)             | 0 (0%)                |
| Seizures                                 | 0 (0%)            | 2 (3%)                |
| Increased spasticity                     | 1(1%)             | 0 (0%)                |
| Sub-arachnoid haemorrhage                | 1(1%)             | 0 (0%)                |
| Urinary tract infection                  | 3 (4%)            | 2 (3%)                |
| Upper respiratory tract infection        | 0 (0%)            | 2 (3%)                |
| Viral encephalitis                       | 0 (0%)            | 1(1%)                 |

Table 4: Adverse events

clinical outcomes, incidence of relapse, and incidence of new and enlarging lesions.

We noted no significant differences between the simvastatin and placebo groups for inducible levels of IFN- $\gamma$ , IL-4, IL-10, and IL-17 expression on T cells at any timepoint. No significant differences were noted at any timepoint between the placebo and simvastatin-treated groups in expression of FoxP3 on the CD4+ T cells (figure 3).

Of the 70 participants in the placebo group, 54 (77%) had one or more adverse events, with 14 (20%) participants having a serious adverse event. In the simvastatin group, 49 (70%) of 70 participants had an adverse event, and nine (13%) had a serious adverse event. There was no evidence that a greater proportion of participants in the simvastatin group had adverse events during the trial, compared with the placebo group (p=0.873, one-sided for serious adverse events; p=0.831, one-sided for any adverse event; table 4). The treatment was therefore well tolerated with no safety differences between the two groups.

# Discussion

Our findings show that, compared with placebo, simvastatin 80 mg per day reduced the annualised rate of whole-brain atrophy by 43%. To minimise the possibility that unknown changes in imaging volumes could take place (such as pseudo-atrophy), both the initial and final MR imaging were done off-medication. This technique supports the contention that the noted reduction was due to a real effect on ongoing disease-related progression (disease-modifying or neuroprotective), rather than to an indirect and short-term effect of drug presence (eg, on hydration). Furthermore, differences between the two groups were consistently seen over 0-12 months, 12-25 months, and 0-25 months. Moreover, the rate of atrophy in the placebo group was very similar to the 0.64%per year reported in a study of more than 130 patients with untreated secondary progressive multiple sclerosis.45

This effect on brain atrophy rate is positive, given that longitudinal studies have shown a relation between atrophy progression and disability.46 Nonetheless, caution should be taken regarding overinterpretation of brain imaging findings, because these might not necessarily translate into clinical benefit. We also noted a small, but significant, effect in two of the secondary disability outcomes, as assessed from a physician (EDSS) and patient reported (MSIS-29) viewpoint supporting a true effect on disease progression. However, because the study was phase 2, it was not designed to assess the proportions with confirmed EDSS progression. Although the EDSS is a clinically relevant score with well described limitations,47 it remains the favoured outcome of regulators for trials,48 and to discern an effect is encouraging. Simvastatin showed no effect on relapse frequency, but there was a non-significant reduction in T2 lesion accumulation, as recorded in some trials of early multiple sclerosis.<sup>17,18</sup> This study was done in a typical secondary progressive multiple sclerosis cohort<sup>67,44</sup> and supports a biologically plausible relation between MRI-derived whole-brain atrophy rate and disability measures, in secondary progressive multiple sclerosis, as proposed by international expert groups on neuroprotection in multiple sclerosis.<sup>48,49</sup>

Seven randomised controlled trials have been done in early stage multiple sclerosis, using simvastatin and atorvastatin (panel). The relapsing-remitting multiple sclerosis studies, as add-on to  $\beta$ -interferon, showed in totality, neither harm nor benefit on parameters such as relapse rate or MRI measures.<sup>21,50,51</sup> No emergent safety issues were identified. In clinically isolated syndrome the STAyCIS study with atorvastatin, although not meeting the primary endpoint ( $\geq$ 3 new T2 lesions or  $\geq$ 1 relapse), did significantly reduce the proportion with new T2 lesions by 50%.<sup>52</sup> A study of simvastatin in patients with optic neuritis followed-up for 6 months, showed a borderline benefit on contrast sensitivity and significant effects on several other visual secondary outcomes.<sup>53</sup>

The failure to show a robust effect on the inflammatory component of early stage multiple sclerosis could be explained by insufficient power. The largest study SIMCOMBIN (n=307) achieved 65% rather than 80% power for the primary endpoint.18 Other contributory reasons could be that statins might not possess the effective and sustained immunomodulatory properties seen in earlier experimental studies at the dosing schedules used in human trials. Indeed, we did not note any effects of simvastatin on the immune markers tested. Reasons, apart from possible insufficient power, might be drug tolerance (induction of long-term compensatory mechanisms acting before the 6 month assay timepoint), or that the in-vivo statin concentration was lower than that achieved in vitro. The mechanism by which brain penetrant simvastatin causes a significant reduction in the annualised rate of whole-brain atrophy and the improvement in two major disability outcomes in this

#### Panel: Research in context

## Systematic review

We searched for all studies in Medline (from 1946), Embase (from 1980), PubMed, Cochrane Database of Systematic Reviews, CENTRAL, DARE, and the Health Technology Assessment Database up (from 2013) to April, 2013, using the keywords: "multiple sclerosis" AND "statins". We included trials, observational studies, and laboratory studies in humans and animals. The book of abstracts from the meetings of the European Committee for Treatment and Research in Multiple Sclerosis for the previous 8 years was also searched. The resulting papers were examined manually. This search yielded seven randomised controlled trials: one in clinically-isolated syndrome, one in optic neuritis, five in relapsing-remitting multiple sclerosis, and none in secondary progressive multiple sclerosis.

#### Interpretation

This phase 2 study of statins in secondary progressive multiple sclerosis showed that simvastatin at 80 mg a day was safe, well tolerated, and reduced progression of annualised brain atrophy over 2 years. Simvastatin had small but significant effects on two of the secondary clinical outcomes. The mechanism of action needs to be established, but might be due to an effect on vascular function or cell protection.

study therefore remains to be elucidated. Accumulating evidence shows that statins have cell protective properties.<sup>10</sup> For example, inhibition of inducible nitric oxide synthase, thus reducing release of free radicals from activated microglia and astrocytes<sup>13</sup> or exerting a neuroprotective effect by prevention of glutamate-mediated excitotoxic effects.<sup>54</sup> An alternative or additional mechanism could be through an effect on vascular function.<sup>16</sup> Statins acting through endothelial nitric oxide synthase<sup>55</sup> can result in improved cerebral vasomotor reactivity<sup>14,56</sup> protecting against long-term hypoxic damage.<sup>57</sup> Finally, since vascular comorbidity is associated with a substantial risk of disability in multiple sclerosis,<sup>58</sup> the noted benefit might be directly due to the reduction in total cholesterol achieved in this study.

In summary, our results show that oral simvastatin at 80 mg per day might be a treatment option for secondary progressive multiple sclerosis, which is currently untreatable, and warrants further investigation in a larger phase 3 trial.

#### Contributors

JC conceived the study. JC, DC, DM, CB, NF, JG, VC, CF, and RN designed the study. JC and RN did the literature search. JC, AA, DC, DW, and RN collected the data. Data management was done by KH and data analysis by JN and CF. MRI quality control, post-processing, and oversight was done by DM, VA, SC, CN, and NF. Immunological analysis was done by NS, VC, and JG. All authors were involved with the paper writing.

#### Declaration of interests

We declare that we have no competing interests.

#### Acknowledgments

Dafydd Thomas has been fundamental to the origin of this study. We are grateful for the help, support, and encouragement of Alan Thompson and David Miller. We thank all the patients and their families who have given their time for this trial. We thank the senior neuroradiographers at the MRI sites, Philippa Bartlett and Mark Whalley; Joanna Foster for MRI quality control and postprocessing; the assessing physicians Imran Saddiqui, Rosella Padama, Paolo Giannetti and Miriam Mattoscio; the previous trial manager Bella Polito; the trial pharmacists Lucy O'Driscoll and Stephanie Steadman; Tony Brady for blinding and randomisation services; and Grazyna Galatowicz for her expert help with flow cytometry. We thank the members of the Data Monitoring Committee: Richard Morris (Chair), Siddharthan Chandran, and Simon Shields. We thank Imperial College Joint Research Office. The study was sponsored by Imperial College. NF acknowledges support from an Medical Research Council Senior Fellowship, an National Institute for Health Research (NIHR) Senior Investigator award, the NIHR Queen Square Biomedical Research Unit in Dementia and the Wolfson Foundation, IG and VC acknowledge support from the NIHR Biomedical Research Centre at Moorfields Eye Hospital NHS Foundation Trust, and from the UCL Institute of Ophthalmology.

#### References

- Reynolds R, Roncaroli F, Nicholas R, Radotra B, Gveric D, Howell O. The neuropathological basis of clinical progression in multiple sclerosis. *Acta Neuropathol* 2011; 122: 155–70.
- 2 The Canadian Cooperative Multiple Sclerosis Study Group. The Canadian cooperative trial of cyclophosphamide and plasma exchange in progressive multiple sclerosis. *Lancet* 1991; **337**: 441–46.
- 3 Rovaris M, Confavreux C, Furlan R, Kappos L, Comi G, Filippi M. Secondary progressive multiple sclerosis: current knowledge and future challenges. *Lancet Neurol* 2006; 5: 343–54.
- 4 Mantia LL, Vacchi L, Rovaris M, et al. Interferon β for secondary progressive multiple sclerosis: a systematic review. *J Neurol Neurosurg Psychiatry* 2013; 84: 420–26.

- 5 Freedman MS, Bar-Or A, Oger J, et al, and the MAESTRO-01 Investigators. A phase III study evaluating the efficacy and safety of MBP8298 in secondary progressive MS. *Neurology* 2011; 77: 1551–60.
- 6 Kapoor R, Furby J, Hayton T, et al. Lamotrigine for neuroprotection in secondary progressive multiple sclerosis: a randomised, double-blind, placebo-controlled, parallel-group trial. *Lancet Neurol* 2010; 9: 681–88.
- 7 Zajicek J, Ball S, Wright D, et al, and the CUPID investigator group. Effect of dronabinol on progression in progressive multiple sclerosis (CUPID): a randomised, placebo-controlled trial. *Lancet Neurol* 2013; 12: 857–65.
- 8 Humphries C. Progressive multiple sclerosis: The treatment gap. Nature 2012; 484: S10.
- Greenwood J, Steinman L, Zamvil SS. Statin therapy and autoimmune disease: from protein prenylation to immunomodulation. *Nat Rev Immunol* 2006; 6: 358–70.
- 10 Greenwood J, Walters CE, Pryce G, et al. Lovastatin inhibits brain endothelial cell Rho-mediated lymphocyte migration and attenuates experimental autoimmune encephalomyelitis. FASEB J 2003; 17: 905–07.
- 11 Weber MS, Youssef S, Dunn SE, et al. Statins in the treatment of central nervous system autoimmune disease. J Neuroimmunol 2006; 178: 140–48.
- 12 Zamvil SS, Steinman L. Cholesterol-lowering statins possess anti-inflammatory activity that might be useful for treatment of MS. *Neurology* 2002; 59: 970–71.
- 13 van der Most PJ, Dolga AM, Nijholt IM, Luiten PGM, Eisel ULM. Statins: mechanisms of neuroprotection. *Prog Neurobiol* 2009; 88: 64–75.
- 14 Giannopoulos S, Katsanos AH, Tsivgoulis G, Marshall RS. Statins and cerebral hemodynamics. J Cereb Blood Flow Metab 2012; 32: 1973–76.
- 15 Greenwood J, Mason JC. Statins and the vascular endothelial inflammatory response. *Trends Immunol* 2007; **28**: 88–98.
- 16 Vollmer T, Key L, Durkalski V, et al. Oral simvastatin treatment in relapsing-remitting multiple sclerosis. *Lancet* 2004; 363: 1607–08.
- 17 Togha M, Karvigh SA, Nabavi M, et al. Simvastatin treatment in patients with relapsing-remitting multiple sclerosis receiving interferon beta 1a: a double-blind randomized controlled trial. *Mult Scler* 2010; 16: 848–54.
- 8 Sorensen PS, Lycke J, Erälinna JP, et al, and the SIMCOMBIN study investigators. Simvastatin as add-on therapy to interferon β-1a for relapsing-remitting multiple sclerosis (SIMCOMBIN study): a placebo-controlled randomised phase 4 trial. *Lancet Neurol* 2011; 10: 691–701.
- 19 Birnbaum G, Cree B, Altafullah I, Zinser M, Reder AT. Combining beta interferon and atorvastatin may increase disease activity in multiple sclerosis. *Neurology* 2008; 71: 1390–95.
- 20 Lanzillo R, Orefice G, Quarantelli M, et al. Atorvastatin combined to interferon to verify the efficacy (ACTIVE) in relapsing-remitting active multiple sclerosis patients: a longitudinal controlled trial of combination therapy. *Mult Scler* 2010; 16: 450–54.
- 21 Kamm CP, El-Koussy M, Humpert S, et al. Atorvastatin added to interferon β for relapsing multiple sclerosis: a randomized controlled trial. J Neurol 2012; 259: 2401–13.
- 22 Kurtzke JF. Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS). *Neurology* 1983; 33: 1444–52.
- 23 Polman CH, Reingold SC, Edan G, et al. Diagnostic criteria for multiple sclerosis: 2005 revisions to the "McDonald Criteria". *Ann Neurol* 2005; 58: 840–46.
- 24 Lublin FD, Reingold SC, and the National Multiple Sclerosis Society (USA) Advisory Committee on Clinical Trials of New Agents in Multiple Sclerosis. Defining the clinical course of multiple sclerosis: results of an international survey. *Neurology* 1996; 46: 907–11.
- 25 ClinicalTrials.gov. Investigation of simvastatin in secondary progressive multiple sclerosis (MS-STAT). Nov 26, 2012. http:// clinicaltrials.gov/ct2/show/study/NCT00647348 (accessed Feb 17, 2013).
- 26 ICH Expert Working Group. ICH harmonized tripartite guidelines for good clinical practice: E6(R1). June 10, 1996. http://www.ich. org/fileadmin/Public\_Web\_Site/ICH\_Products/Guidelines/ Efficacy/E6\_R1/Step4/E6\_R1\_Guideline.pdf (accessed Feb 17, 2014).

- 27 World Medical Association. Declaration of Helsinki: ethical principles for medical research involving human subjects. (http:// www.wma.net/en/30publications/10policies/b3/17c.pdf).
- 28 Pocock SJ, Simon R. Sequential treatment assignment with balancing for prognostic factors in the controlled clinical trial. *Biometrics* 1975; 31: 103–15.
- 29 Freeborough PA, Fox NC. The boundary shift integral: an accurate and robust measure of cerebral volume changes from registered repeat MRI. *IEEE Trans Med Imaging* 1997; 16: 623–29.
- 30 Leung KK, Clarkson MJ, Bartlett JW, et al, and the Alzheimer's Disease Neuroimaging Initiative. Robust atrophy rate measurement in Alzheimer's disease using multi-site serial MRI: tissue-specific intensity normalization and parameter selection. *Neuroimage* 2010; 50: 516–23.
- 31 Anderson VM, Fernando KT, Davies GR, et al. Cerebral atrophy measurement in clinically isolated syndromes and relapsing remitting multiple sclerosis: a comparison of registration-based methods. J Neuroimaging 2007; 17: 61–68.
- 32 Smith SM, Rao A, De Stefano N, et al. Longitudinal and cross-sectional analysis of atrophy in Alzheimer's disease: cross-validation of BSI, SIENA and SIENAX. *Neuroimage* 2007; 36: 1200–06.
- 33 Durand-Dubief F, Belaroussi B, Armspach JP, et al. Reliability of longitudinal brain volume loss measurements between 2 sites in patients with multiple sclerosis: comparison of 7 quantification techniques. *AJNR Am J Neuroradiol* 2012; **33**: 1918–24.
- 34 Zivadinov R, Reder AT, Filippi M, et al. Mechanisms of action of disease-modifying agents and brain volume changes in multiple sclerosis. *Neurology* 2008; **71**: 136–44.
- 35 Cutter GR, Baier ML, Rudick RA, et al. Development of a multiple sclerosis functional composite as a clinical trial outcome measure. *Brain* 1999; 122: 871–82.
- 36 Hobart J, Lamping D, Fitzpatrick R, Riazi A, Thompson A. The Multiple Sclerosis Impact Scale (MSIS-29): a new patient-based outcome measure. *Brain* 2001; **124**: 962–73.
- 37 Hill T, Galatowicz G, Akerele T, Lau CH, Calder V, Lightman S. Intracellular T lymphocyte cytokine profiles in the aqueous humour of patients with uveitis and correlation with clinical phenotype. *Clin Exp Immunol* 2005; **139**: 132–37.
- 38 Frost C, Kenward MG, Fox NC. The analysis of repeated 'direct' measures of change illustrated with an application in longitudinal imaging. *Stat Med* 2004; 23: 3275–86.
- 39 Little RJA, Rubin DB. Statistical analysis with missing data. Wiley: New York, 2002.
- 40 Frison LJ, Pocock SJ. Repeated measures in clinical trials: analysis using mean summary statistics and its implications for design. *Stat Med* 1992; 11: 1685–704.
- 41 Efron B, Tibshirani RJ. An introduction to the bootstrap. Chapman and Hall: London, 1993.
- 42 Davison AC, Hinkley DV. Bootstrap methods and their application. Cambridge University Press: Cambridge, 1997.

- 43 Breslow NE. Extra-poisson variation in log-linear models. Appl Stat 1984; 33: 38–44.
- 44 Frost C, Kenward MG, Fox NC. Optimizing the design of clinical trials where the outcome is a rate. Can estimating a baseline rate in a run-in period increase efficiency? *Stat Med* 2008; 27: 3717–31.
- 45 De Stefano N, Giorgio A, Battaglini M, et al. Assessing brain atrophy rates in a large population of untreated multiple sclerosis subtypes. *Neurology* 2010; 74: 1868–76.
- 46 Fisher E, Rudick RA, Simon JH, et al. Eight-year follow-up study of brain atrophy in patients with MS. *Neurology* 2002; 59: 1412–20.
- 47 Hobart J, Freeman JA, Thompson AJ. Kurtzke scales revisited: the application of psychometric methods to clinical intuition. *Brain* 2000; **123**: 1027–40.
- 48 Barkhof F, Calabresi PA, Miller DH, Reingold SC. Imaging outcomes for neuroprotection and repair in multiple sclerosis trials. *Nat Rev Neurol* 2009; 5: 256–66.
- 49 Cohen JA, Reingold SC, Polman CH, Wolinsky JS, and the International Advisory Committee on Clinical Trials in Multiple Sclerosis. Disability outcome measures in multiple sclerosis clinical trials: current status and future prospects. *Lancet Neurol* 2012; 11: 467–76.
- 50 Wang J, Xiao Y, Luo M, Luo H. Statins for multiple sclerosis. Cochrane Database Syst Rev 2011; 12: CD008386.
- 51 Bhardwaj S, Coleman CI, Sobieraj DM. Efficacy of statins in combination with interferon therapy in multiple sclerosis: a meta-analysis. Am J Health Syst Pharm 2012; 69: 1494–99.
- 52 Waubant E, Pelletier D, Mass M, et al. Randomized controlled trial of atorvastatin in clinically isolated syndrome: the STAyCIS study. *Neurology* 2012; 78: 1171–78.
- 53 Tsakiri A, Kallenbach K, Fuglø D, Wanscher B, Larsson H, Frederiksen J. Simvastatin improves final visual outcome in acute optic neuritis: a randomized study. *Mult Scler* 2012; 18: 72–81.
- 54 Schmeer C, Kretz A, Isenmann S. Statin-mediated protective effects in the central nervous system: general mechanisms and putative role of stress proteins. *Restor Neurol Neurosci* 2006; 24: 79–95.
- 55 Laufs U, La Fata V, Plutzky J, Liao JK. Upregulation of endothelial nitric oxide synthase by HMG CoA reductase inhibitors. *Circulation* 1998; 97: 1129–35.
- 56 Endres M, Laufs U, Huang Z, et al. Stroke protection by 3-hydroxy-3-methylglutaryl (HMG)-CoA reductase inhibitors mediated by endothelial nitric oxide synthase. *Proc Natl Acad Sci USA* 1998; **95**: 8880–85.
- 57 Lassmann H, van Horssen J, Mahad D. Progressive multiple sclerosis: pathology and pathogenesis. *Nat Rev Neurol* 2012; 8: 647–56.
- 58 Marrie RA, Rudick R, Horwitz R, et al. Vascular comorbidity is associated with more rapid disability progression in multiple sclerosis. *Neurology* 2010; 74: 1041–47.