MS-OPT Trial: Simvastatin in progressive multiple sclerosis

Statistical analysis plan

Protocol number	Version 1.7 dated 18/10/2022 (EudraCT no: 2017-003008-30)
Trial Title	A double-blind, randomised, placebo-controlled single-site study of high dose simvastatin treatment for secondary progressive multiple sclerosis: impact on vascular perfusion and oxidative damage
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The general aim of the study is to determine the effect high-dose Simvastatin treatment has on vascular perfusion and oxidative damage in progressive multiple sclerosis.

Hypothesis: Simvastatin reduces the progression of progressive multiple sclerosis (P-MS) by increasing vascular perfusion and reducing oxidative damage as demonstrated by measuring cerebral blood flow in people with secondary progressive MS SP-MS and primary progressive MS (PP-MS).

1. Aims:

- **Primary**: to test against placebo the efficacy and mechanism of simvastatin in progressive MS.

- **Secondary:** to advance our understanding of the mechanisms of efficacy, and how vascular perfusion and oxidative damage is affected in progressive MS both in the presence and absence of simvastatin.

- To establish whether ASL and AOSLO measurements of blood flow are useful correlates for cerebral blood flow measurement on and off treatment.
- To evaluate whether ASL and high-resolution retinal neuronal structural data including the retinal nerve fibre layer (RNFL) are useful correlates for cerebral blood flow measurement on and off treatment.
- To Investigate the effect statins may have on retinal parameters such as blood flow, oxygen saturation, structure of vascular plexuses, neuronal structure and retinal layer thicknesses.
- To explore whether statin reduce the rate of brain atrophy on MRI (excluding the effect of pseudo-atrophy, which is a temporary response to the drug rather than an actual loss of tissue).
- To explore whether statin modulate the changes in advanced MRI measurements (diffusion imaging, macromolecular tissue volume).
- To examine the clinical effect of simvastatin treatment as reported by the clinician (EDSS, SDMT, MSFC inc. 9HPT and 25FTW) and patient reported outcome measures (MSIS-29v2, and MSWSv2).
- To examine the clinical effect of simvastatin treatment on frontal executive functioning (FAB).
- To collect health economic data (EQ5D5L) to inform future phase III trials.

- **Exploratory objectives:** to advance our understanding of the mechanisms of efficacy, and how vascular perfusion and oxidative damage is affected in progressive MS both in the presence and absence of simvastatin.
  - To investigate phenotypic immune markers in whole blood to determine effect simvastatin has on immune function.
  - To measure biomarkers of blood brain barrier dysfunction, vascular leakage and oxidative damage in participants treated with statins and placebo.
  - To investigate levels of circulating cholesterol.

2. Statistical Analysis Plan:

- **Primary outcome analysis**. The ASL-measured CBF in white matter (), grey matter, deep grey matter, and thalamus will be compared between patients on simvastatin or placebo using *multiple linear regressions* at 16 weeks as the dependent variable, and age, gender or CBF at baseline and treatment group entered as covariates. Whenever the variable 'treatment group' is significant (at 5% significance level), it will be assumed that there is a treatment effect. The same models will be used to investigate the effect of simvastatin on the other imaging outcome measures.

- **Checks for model assumptions**. For all regression-type approaches, we will assess the normality of the residuals (of each one of the fitted models) through q-q plots. Residual homoscedasticity will be assessed through two-way scatter plots of the residuals over fitted values. Once we build the model with all possible predictors as explanatory variables, the assumption of the linear relationship between the dependent variable and each one of the predictors individually, given the presence of the other predictors, will be assessed through investigating different functional forms of the predictor and their impact on the R2 of the model. The independence of observations will be always assumed.

- **Violations of model assumptions**. If the main assumption violated is that of homoscedasticity (i.e. there is residual heteroscedasticity), robust estimations of the standard errors (SE) of the regression parameters will be made, using the sandwich estimator of the SE. Whenever the residual distributions show a clear deviation from normality, bootstrap-based approaches will be used, with at least 1000 replication samples: bias-corrected and accelerated bootstrap-based 95% Confidence Intervals (95% CI) will be computed for each estimated parameter.

- **Secondary outcome analysis**. Metrics for quantifying blood flow from retinal imaging are in development, and have not previously been applied in MS patients. Retinal vessel walls will be assessed in a similar fashion to that outlined by Chui et al (2013). Arterial wall thickness will be assessed as a function of lumen thickness. In the first instance normative data will be obtained from age-matched controls prior to imaging patients with MS. Subsequently appropriate multigroup statistical tests will be applied. Capillary network density will be assessed using similar methods to those described by Tam et al. (2010) and blood velocity will be calculated building up spatio-temporal analysis outlined by Tam et al. (2011).

- **Exploratory analyses**. Exploratory analyses will be performed to assess the associations between ASL changes (dependent variable) and all secondary outcome measures (explanatory variables). An interaction term such as 'secondary outcome measure change' X 'treatment group' will be included in the model as an explanatory

variable. Whenever it becomes significant it will indicate that simvastatin influences the association between primary and secondary outcome measures.

Although the sample size of this study was based on the ASL, we estimated that the number of patients that we would need to detect an increase in neurite density of 0.128 that represents a 20% treatment effect (Magnollay et al., 2013) would require 13 per arm (80% power). Additionally, even if the number of patients per arm will not be sufficient to see a significant treatment effect of this size, this study will provide information on the temporal behaviour of these MRI parameters that can be used to power future neuroprotective trials.

Model assumptions will be also checked as described above and any violations of the model assumptions will be dealt with in a similar way.

- **Sensitivity and other planned analyses**. Baseline clinical, demographical and neuroimaging characteristics of those patients with missing visits will be compared with those of patients without missing values, to investigate the assumption of missing at random (MAR). If there is no evidence against MAR, multiple imputation methods will be performed, and any serious discrepancy between the complete case results and the imputation results will be reported. Worst- and best-case sensitivity analyses will also be performed.

  In general, given that we will use randomisation with minimisation, we do not expect major imbalances at baseline. However, should there be any, we will consider adjusting the models for the imbalanced variable.

- **Interim analysis**. Due to the short-term nature of the study and small sample size, an interim analysis will not be required.

3. CONSORT 2010 Flow Diagram