Protocol title: A pilot study of oxidative pathways in MS fatigue

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8.Statistical consideration and analysis plan

8.1 Statistical plan for specific aim 1- To test the tolerability and safety of NAC therapy (1250 mg three times a day) compared to placebo for 4 weeks to treat progressive MS fatigue.

Study Subjects: Target population- Adult patients with progressive MS and fatigue seen at UCSF.

The safety outcome will be analyzed by including all subjects who have received at least one dose of study medication. Participants will be analyzed according to the actual treatment received. The assessment of safety will be based on the frequency of adverse events.
The primary efficacy analysis will be done as an intent-to-treat analysis. Consequently, all randomized participants will participate in the analysis as allocated to NAC or placebo.

8.2 Statistical plan for specific aim 2- To obtain preliminary data on changes in fatigue levels and oxidative pathway biomarkers on NAC therapy in subjects with progressive MS fatigue.

In an intent-to-treat analysis, using Student t-test, the mean change in MFIS score from the baseline to week 4 will be compared between the NAC and placebo groups. The per-protocol secondary analysis will take into account treatment regimen. We will use similar models to compare the changes in brain GSH concentration and blood GSH/GSSG ratio, FSS, NeuroQOL fatigue item bank, 9-hole peg test, 25-foot timed ambulation, and Symbol Digit Modalities Test (SDMT) at the baseline and week fourth between the NAC and placebo groups.

Because there is a possibility for imbalance in the baseline values of potential confounding factors (such as EDSS and depression), an a priori secondary analysis is planned. To assess the effectiveness of NAC vs. placebo, we will use a linear regression model, comparing the mean change in MFIS score from the baseline to week 4, adjusting the models for the baseline EDSS and HADS scores.

We will use the same models to analyze the change in the severity of fatigue as measured by MFIS and FSS between week 4 (the end of study drug administration period) and week 6 (2 weeks after patients have been off study drug).

Because of the exploratory nature of these analyses, we will not correct our models for multiple comparison testing. Statistical analyses will be performed by a biostatistician from UCSF biostatistics department blinded to treatment assignment.

Sample size calculation: There are no available data to base power calculation for this aim as such a study has never been performed prior. We will use the data generated for this aim to design future studies.

***Primary efficacy endpoint*:** The primary efficacy analysis will be done as an intent-to-treat analysis. Consequently, all randomized participants will participate in the analysis as allocated to NAC or placebo. Using Student t-test, the mean change in MFIS score from the baseline to week 4 will be compared between the NAC and placebo groups. The per-protocol secondary analysis will take in account treatment regimen.

MFIS consists of 21 items with a total score range of 0 to 84 (higher scores indicate worse fatigue). As the primary efficacy endpoint of the trial, we will compare the mean change in total MFIS score from baseline to week 4 visit between intervention and placebo groups.

Secondary efficacy endpoints **(**differences between treatment groups):

- Per protocol analysis for changes in MFIS from screening /baseline to week 4.
- MFIS subscales changes between screen/baseline and week 4.
- Change in FSS score from baseline to week 4.
- Change in NeuroQOL fatigue item bank score from baseline to week 4.
- Change in 9-HPT score from baseline to week 4.
- Change in 25-FTW from baseline to week 4.
- Change in SDMT score from baseline to week 4.
- Changes in MFIS and FSS between week 4 and 6.

8.3 Statistical plan for specific aim 3- To determine if fatigue severity is associated with baseline spectroscopy and oxidative pathway markers in patients with progressive MS.

We will examine the correlation between biomarkers (spectroscopy and blood oxidation metabolites) and fatigue (MFIS) at baseline. We will use a linear regression model with MFIS score (or its change) as the outcome and the spectroscopy and metabolites as the predictors (in

separate models). Using data obtained from the control subjects (patients with progressive MS and fatigue severity as measured by MFIS<38) we will also perform a case-control analysis (e.g. logistic regression with being fatigued or non-fatigued as the outcome and spectroscopy or blood metabolites as predictors.

Study variables

Demographic variables: Age, sex, self-reported race/ethnicity (based on the National Institute of Health classification), education (in years) and occupation will be recorded at the screening visit. Because the severity of fatigue may fluctuate during the day and based on the ambient temperature, the time of the visit and the outside temperature will also be recorded.

Baseline MS-related information: Years since disease onset, type of MS (primary versus secondary progressive (per medical records), Expanded Disability Status Scale (EDSS) (obtained at screening visit by the examining physician), and 25-foot timed walk (25-FTW), 9-hole peg test (9-HPT) and Single Digit Modalities test (SDMT) (obtained by a trained technician at screening, baseline and week 4).

Fatigue: MFIS and FSS (questionnaires filled by patients after receiving appropriate instructions from study staff, see appendix) at screening, baseline, week 4 (last study drug dose), and week 6 (2 weeks after stopping study drug).

Depression: Hospital Anxiety and Depression Scale (HADS) depression subscale (questionnaires filled by patients after receiving appropriate instructions from a study staff, see appendix) at screening and baseline.

Quality of life: NINDS NeuroQOL fatigue item bank (questionnaires filled by patients after receiving appropriate instructions from study staff, see appendix) at the screening, baseline and week 4.

Safety laboratory assessment: At screening, CBC and differential, creatinine, AST, ALT, urine analysis and urine pregnancy test will be obtained. At week 4, only creatinine, urine pregnancy test, AST and ALT will be measured.

Research laboratory assessment: Plasma level of reduced glutathione (GSH) and oxidized glutathione (GSSG) baseline and at 4 week visit for patients who enter treatment phase, and baseline research samples will be analyzed for patients who are controls in the study

Conventional MRI sequences: The MR experiments will be performed on the GE 7T research scanner in the Surbeck Laboratory using a 32-channel phased array head coil. The 60-minute MR protocol will include standard imaging sequences with calibration scans (3-plane localizer, coil sensitivity maps), gradient recalled-echo, T1-weighted and T2-weighted images, and multiband DWI. Higher order shimming will be performed prior to the spectral data acquisitions. The volume of interest for 3D short TE MRSI will be prescribed to have full coverage of the thalamus that will enable to measure metabolite levels (such as Cho, Cr, NAA, Glutamate,

Glutamine, mI, and GSH) in deep grey matter and surrounding normal appearing white matter. GSH-edited MRS will be acquired as well to provide unobstructed detection of GSH in the selected of regions of interest.

Data collection:

1) Blood samples from participating patients at the baseline and week 4 visits will be collected and analyzed for various metabolites on the oxidative pathway including GSH, GSSG, and response ratio for GSH, and GSSG.

2) 7T MR spectroscopy at the baseline and 4 week visits will be obtained and analyzed for concentrations of N-acetyl-aspartate (NAA), glutamate (Glu), glutathione, myo-inositol (mI), creatine (Cr), and choline (Cho) over the region of interest (ROI) region and concentrations of NAA, Glu, glutathione, mI, Cr, and Cho from normal-appearing white-matter areas within the ROI region.

Power considerations: There are no available data to base power calculation for this exploratory aim as such a study has never been performed prior. We will use the data generated for this Aim to design future studies.

9.Data collection, management and quality assurance

We will use Redcap (http://project-redcap.org/), a secure web application for building online databases, as the trial data management software. The demographic and baseline information will be entered directly from the medical records by study coordinator into the study CRF. The study manger will review CRFs for completeness and enter into Redcap database. UCSF research
fellow (Dr. Nourbakhsh) will double check the data entered into Redcap for accuracy.