**Supplemental material 3**

**Lumbar puncture procedure and handling of cerebrospinal fluid**

Lumbar puncture was performed at baseline and week 48 by the trial physicians or other medical doctors using a non-traumatic needle under sterile conditions and with local anaesthesia. 10 – 12 ml of cerebrospinal fluid (CSF) was extracted via a sterile polystyrene tube into a polystyrene vial on ice. Routine analysis for IgG-index and albumin quotient and cell count was performed on fresh CSF at the hospital laboratory. Immediately after the procedure, the CSF was centrifuged (4°C, 400 RPM, 5 mins), and the supernatant was immediately frozen at -80°C for later analysis. The CSF was stored in aliquots of 0.5 ml and thawed on ice before analysis of the remaining biomarkers.

**Analyses of cerebrospinal fluid endpoints**

All analyses of cerebrospinal fluid endpoints were performed by experienced staff at the Research Unit, Danish Multiple Sclerosis Center, Copenhagen University Hospital - Rigshospitalet.

**Neurofilament Light Chain (NFL)**

NFL was measured by enzyme-linked immunosorbent assay (ELISA) using a commercially available kit from Uman Diagnostics, Umeå, Sweden (NF-light® (Neurofilament light) ELISA, 10-7001-CE). Inter-assay CV was 1.6% (n = 3). Intra-assay CV was 1.2%, 1.5% and 1.4% respectively. To account for batch and inter-assay variability, we analyzed all CSF NFL concentrations twice. Once in the screening process to determine whether CSF NFL concentrations fell below the chosen cut-off of 380 ng/L, and once again when comparing baseline and visit 48 concentrations.

**Myelin Basic Protein (MBP)**

MBP was measured with an ELISA DuoSet from R&D Systems (DY4228-05). Manufacturer instructions were followed except for a few modifications. Plates were incubated with capture antibodies (cat no. #844443) using a different coating buffer than the manufacturer specified. The applied coating buffer was a carbonate/bicarbonate buffer with pH 9.2-9.6 (Biolegend, cat no. #421701) to stabilized capture antigens instead of the one specified by the manufacturer (PBS, pH 7.4). CSF was thawed on ice, and plates were incubated overnight (4 °c, 450 RPM). The plates were read on a BioTek ELISA reader. The inter-assay CV (n=4) of low control was 12.8%.

**Chitinase-3-like-1 (CHI3L1), soluble B-cell maturation antigen (sBCMA), soluble CD27 (sCD27), and soluble CD14 (sCD14)**

CSF concentrations of CHI3L1, sBCMA, sCD27, and sCD14 were analysed according to manufacturer instructions (dilution 1:2 for sBCMA and sCD27; and 1:20 for CHI3L1 and sCD14) using an in-house validated Human Magnetic Luminex Assay from R&D Systems (R&D Systems, Minneapolis, US) (Cat. no. LXSAHM-02).1 Plates were read using a Bio-Plex 200 Instrument (Bio-Rad Laboratories, Copenhagen, Denmark), and data were acquired using the Bio-Plex Manager 6.0 software (Bio-Rad Laboratories, Copenhagen, Denmark). Inter-assay CV (n=3) of mid control was 6.7 % for CHI3L1; 6.0 % for sBCMA; 8.5 % for sCD27 and 7.6 % for sCD14.

**References**

1. Mahler MR, Søndergaard HB, Buhelt S, von Essen MR, Romme Christensen J, Enevold C, et al. Multiplex assessment of cerebrospinal fluid biomarkers in multiple sclerosis. Mult Scler Relat Disord. 2020;45.