Clinical trial protocol

Dimethyl fumarate treatment of primary progressive multiple sclerosis

(FUMAPMS)

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Dimethyl fumarate treatment of primary progressive MS (FUMAPMS)
Sponsor-investigator Agreement
EudraCT 2016-000283-41

Sponsor-investigator Agreement

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Abbreviations

AE	Adverse event
ALAT	Alanine Amino Transferase
Anti-HBsAG	Anti Hepatitis B surface antigen
Anti-HCV	Anti Hepatitis C Virus
Anti-HIV	Anti Human Immunodeficiency Virus
AR	Adverse reaction
BCMA (TNFRSF17)	B-cell maturation antigen (tumor necrosis factor receptor superfamily member 17)
BID	"Bis in die" from Latin meaning twice a day
BICAMS	Brief International Cognitive Assessment for MS
CGM	Cortical grey matter
CH13L1	Chitinase 3 like 1
CNS	Central nervous system
CRP	C-reactive protein
CSF	Cerebrospinal fluid
C2	Cervical vertebrae number 2
DMF	Dimethyl Fumarate
DTI	Diffusion tensor imaging
EDSS	Expanded Disability Status Scale
ELISA	Enzyme-linked immunosorbent assay
EMA	European Medical Association
FA	Fractional anisotropy
FAE	Fumaric acid esters
FSMC	Fatigue Scale for Motor and Cognitive Functions
GCP	Good Clinical Practice
Gd	Gadolinium
HBsAG	Hepatitis B surface antigen
hCG	Human Chorionic Gonadotropin
HIV	Human Immunodeficiency Virus
9HPT	Nine-Hole Peg Test

IgG	Immunoglobulin
INR	International Normalized Ratio
JCV	John Cunningham Virus
MBP	Myelin basic protein
MD	Mean Diffusivity
MCV	Mean Cell Volume
MRI	Magnetic resonance imaging
MS	Multiple Sclerosis
MSIS-29	Multiple Sclerosis Impact Scale 29
MTR	Magnetization Transfer Ratio
NAGM	Normal Appearing Grey Matter
NAWM	Normal-appearing white matter
NFL	Neurofilament light chain
NFkB	Nuclear Factor Kappa Beta
NPCs	Neural stem/progenitor cells
Nrf2	Nuclear factor (erythroid-derived 2)-Related Factor 2
PBVC	Percentage Brain volume change
PML	Progressive multifocal leukoencephalopathy
PPMS	Primary progressive multiple sclerosis
RRMS	Relapsing remitting multiple sclerosis
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction
sCD14	Soluble CD14
sCD27	Soluble CD27
SDMT	Symbol Digit Modalities Test
sNFL	Serum neurofilament light chain
SPMS	Secondary progressive multiple sclerosis
SUSAR	Suspected unexpected serious adverse reaction
TMF	Trial Master File
T25FW	Timed 25-Foot Walk
UDI	Urinary Distress Inventory

1 PROTOCOL SUMMARY

Title: **Dimethyl fumarate treatment of primary progressive multiple sclerosis**

Study aim: The aim of the study is to evaluate the efficacy and safety of dimethyl fumarate (DMF) treatment in primary progressive multiple sclerosis (PPMS). Patients fulfilling the inclusion criteria are randomized to treatment with dimethyl fumarate or placebo for 48 weeks followed by an open-label treatment phase where all patients are treated with dimethyl fumarate for an additional 48 weeks.

The primary endpoint of the study is to assess the effect of treatment with dimethyl fumarate on:

- Change in the cerebrospinal fluid (CSF) concentration of neurofilament light chain (NFL) in patients treated with Dimethyl fumarate or placebo for 48 weeks

Secondary endpoints are:

- Clinical endpoints: comparison of change from screening to week 48 in patients treated with dimethyl fumarate and placebo for the following variables:
    - Expanded Disability Status Scale
    - Timed 25-Foot Walk
    - Nine-Hole Peg Test
    - Brief International Cognitive Assessment for MS (BICAMS)
        - Symbol Digit Modalities Test
- CSF endpoints: comparison of change from screening to week 48 in patients treated with dimethyl fumarate and placebo for the following variables:
    - IgG-index
    - CSF-serum albumin quotient
    - Concentrations of: chitinase-3-like-1, sCD14, sCD27, BCMA (TNFRSF17) and myelin basic protein

- Magnetic resonance imaging (MRI) endpoints: comparison of change from screening to week 48 in patients treated with dimethyl fumarate and placebo for the following variables:

- Number of new or enlarged T2 lesions
- Fractional anisotropy (FA) in Normal Appearing White Matter (NAWM)
- Lesion volume
- Magnetization transfer ratio (MTR) in lesions
- Thalamic volume
- Percentage brain volume change

Tertiary endpoints are:

- Clinical endpoints: comparison of change from screening to week 48 in patients treated with dimethyl fumarate and placebo for the following variables:
  - Brief International Cognitive Assessment for MS (BICAMS)
    - Brief Visuospatial Memory Test Revised
    - California Verbal Learning Test 2

- MRI endpoints: comparison of change from screening to week 48 in patients treated with dimethyl fumarate and placebo for the following variables:
  - Number of Gd enhancing lesions
  - Total number of lesions
  - Volume of Cortical Grey Matter (CGM), NAWM and the putamen nuclei
  - MTR of CGM, NAWM, the putamen and thalamic nuclei
  - Diffusion tensor imaging (DTI) measures (FA and mean diffusivity) of CGM, NAWM (except FA in NAWM), lesions, the putamen and thalamic nuclei
  - Cross sectional area at C2 level of the cervical spinal cord

- Self-reported outcome measures: comparison of change from screening to week 48 in patients treated with dimethyl fumarate and placebo for the following variables
  - Urinary Distress Inventory

- Fatigue Scale for Motor and Cognitive Functions
- Multiple Sclerosis Impact Scale 29

- Safety:
  - Records of adverse events (AE), serious adverse events (SAE) and serious unexpected serious adverse reaction (SUSAR)
  - Safety blood samples

Tertiary (exploratory) outcome measures from the open label phase (week 48-96) are analyzed separately for patients initially randomized to treatment with DMF or placebo and patients initially not randomized due to low concentrations of NFL. Patients with lower CSF concentrations than required for randomization at screening will be asked to be followed-up per protocol after 48 weeks, and if they show evidence of clinical disease progression at the week 48 visit, they can be treated with dimethyl fumarate from week 48-96. For these patients the outcome measures are comparisons of changes in clinical and self-reported measures and MRI endpoints from week 0-48 and from week 48-96.

Research plan: The trial is a single center phase 2A proof-of-concept study. Participating patients will be treated with DMF or placebo for 48 weeks followed by an open-label treatment phase where all patients, initially enrolled in the study, are treated with dimethyl fumarate for an additional 48 weeks. Clinical studies are conducted at the screening visit (week –6 to –2), week 48 and week 96. Lumbar puncture is conducted at the screening visit and week 48. Safety blood samples are taken at the screening visit, baseline visit, week 12, 24, 36, 48, 60, 72, 84 and week 96. Pregnancy test will be performed as a measure of serum hCG on all women of childbearing age at every visit. MRI is conducted at the screening visit, week 48 and week 96.

Trial population: Patients eligible for randomization have PPMS with or without superimposed relapses with an EDSS score of 6.5 points or less and have a high CSF concentration of NFL at screening above 380ng/l. Patients completing the screening visit but not randomized due to CSF concentration of NFL <380ng/l will be followed up after 48 weeks.

Sample size: 54patients with PPMS fulfilling the inclusion criteria will be randomized to active or placebo treatment.

Trial medication: Dimethyl fumarate 240 mg BID for 48 weeks or matching placebo (week 0-48) and open-label treatment with dimethyl fumarate 240 mg BID for 48 weeks (week 48-96).

2 FLOW CHART

See appendix A.

3 BACKGROUND

3.1 Primary progressive multiple sclerosis

Multiple sclerosis (MS) is a chronic, inflammatory disease of the central nervous system (CNS), and is presumed to be caused by T cell-mediated autoimmune processes [1]. Denmark has a high prevalence of MS, and with approximately 14,000 patients, it is the second most common cause of disability in young adults. Two onset forms of MS exist: relapsing-remitting MS (RRMS, 85%) and primary progressive MS (PPMS, 15%) [2]. During the course of the disease, the majority of patients with RRMS will eventually convert to a secondary progressive disease course (SPMS). Progressive MS forms account for at least half of all patients with MS.

Clinically RRMS is characterized by relapses with neurological symptoms that last from a few days to several months but subsequently improve or remit completely. The pathological correlate is focal, inflammatory lesions in the optic nerve, brain or spinal cord with infiltrating leukocytes, activated macrophages, loss of myelin (demyelination) and damage to axons and neurons[3,4]. The focal lesions can be visualized on magnetic resonance imaging (MRI) as hyperintense areas on T2-weighted images and show evidence of blood-brain barrier disruption when Gadolinium (Gd)-contrast is administered.

Progressive forms of MS are characterized clinically by gradual symptom development. Superimposed relapses may occur, and progressive MS with superimposed relapses or development of new or Gd-enhancing MRI lesions is classified as active progressive MS [2]. Superimposed relapses do not, however, influence the disease course, and the overall disease course is comparable in PPMS and SPMS [5]. In the past decade there has been immense interest in neurodegenerative processes as the primary pathophysiological substrate for disease progression in MS, and it has been argued that PPMS is a purely neurodegenerative form of MS [6]. Pathology studies do, however, indicate that inflammation is also a key feature of PPMS, but inflammation in progressive MS is more diffuse than in RRMS [4,7]. Furthermore, patients with progressive MS show evidence of ongoing inflammation, which correlates with demyelination and neuroaxonal damage in CSF biomarker studies [8]. Most recently, it has been suggested that oxidative damage induced by inflammation may play a key role in the pathogenesis of MS [9–11].

Treatment options for patients with PPMS are very limited. Prophylactic treatment with interferon-β and glatiramer acetate did not show statistically significant efficacy in randomized, controlled trials [12,13]. Furthermore, a recent phase 3 study comparing treatment with fingolimod and placebo in PPMS found no effect on disease progression [14]. A previous phase 2B study with the B cell-depleting monoclonal antibody rituximab found no statistically significant effect of treatment in PPMS, whereas a recent phase 3 study found a statistically significant effect of the B cell-depleting monoclonal

antibody ocrelizumab in PPMS (Hauser, ECTRIMS 2015). The effect was, however, marginal, and it is uncertain whether it is primarily driven by a subgroup of patients with active PPMS. Ocrelizumab is, however, not marketed, and if eventually approved by the EMA, will not be marketed earlier than 2017.

We previously completed a phase 2A proof-of-concept study (the NAPMS study) investigating the effect of the monoclonal antibody natalizumab in patients with PPMS or SPMS. This study showed that treatment resulted in significant decreases in the primary outcome measure (the CSF concentration of osteopontin) and in many secondary outcome measures [15]. The improvement in the primary outcome measure was statistically significant even for the subgroup of patients with PPMS alone and was associated with lower levels of NFL and increases in magnetization transfer ratios, which are indicative of improvements in CNS tissue integrity, presumably due to increases in myelination or decreases in diffuse CNS inflammation. A recent phase 3 study comparing treatment with natalizumab and placebo in SPMS did not find a statistically significant effect on the primary outcome measure of progression (Biogen press release 2015). The study did, however, find a statistically significant effect on progression of upper limb dexterity and disease activity in terms of relapses and MRI disease activity. These results are consistent with the findings from our phase 2A study, where we found that CSF concentrations of MMP-9 and CXCL13, which are both known to be associated with disease activity, normalized upon treatment with natalizumab. In contrast, osteopontin and NFL concentrations were lowered but not normalized, which we interpret as being consistent with the limited clinical efficacy of treatment in the recent phase 3 study.

In conclusion, the results of the NAPMS study as well as another phase 2A study of monthly methylprednisolone treatment of progressive MS indicates that it is feasible to conduct phase 2A trials with CSF measures as the primary outcome in PPMS [16].

3.2 Dimethyl fumarate

Fumarates have long been known to have disease-attenuating effects in psoriasis. They have been in routine use in dermatology in Germany for several decades, and initial studies indicated that fumarates might also have beneficial effects in MS [17,18]. DMF has the interesting property of combining immunological effects, at least partly mediated by interference with nuclear factor κB and other transcription factors, and also anti-oxidative and neuroprotective effects mediated by activation of the transcription factor Nuclear factor (erythroid-derived 2)-Related Factor 2 (NRF2) [19,20]. More recently, the surface receptor HCA_2 was reported to be essential for the effect of DMF in an animal model of MS [21]. DMF is currently approved for treatment of RRMS by the EMA in a dose of 240 mg BID. The effect of treatment has been documented in two phase 3 randomized, controlled trials in RRMS [22,23]. Treatment with dimethyl fumarate was not associated with severe adverse events during up to two years of treatment in clinical trials in MS. This is in line with the experience with fumarate treatment in dermatology. Gastrointestinal side effects and flushing occurred more frequently in patients treated with dimethyl fumarate than in patients treated with placebo but resolved within the first month of treatment in the majority of patients. The development of the severe opportunistic infection progressive multifocal leukoencephalopathy (PML) has, however, been reported in rare cases, usually with long lasting, marked lymphopenia, during long-term treatment with fumarates

including DMF. This has led to an update of the summary of medicinal product characteristics (SMPC) by the EMA, with detailed recommendations on lymphocyte monitoring.
A small study comparing treatment with two types of fumarate in progressive MS showed that DMF was well tolerated and that there was no safety issues occurring [24]. Only 26 patients participated, and the study was not a randomized clinical trial but it warrants further investigation into the possible treatment effect of DMF in progressive MS patients.

3.3 Outcome measures

The diffuse inflammation observed in PPMS is better reflected by the study of biomarkers in CSF than by the conventional measure Gd-enhancing MRI lesions [8,25]. CSF concentrations of NFL correlate with inflammation in patients with progressive MS [8], improve upon treatment with natalizumab in progressive MS and RRMS and are associated with Magnetization Transfer Ratio (MTR) measures of tissue integrity in progressive MS [15,26]. As NFL has emerged as a treatment-responsive biomarker, and has been associated with the long-term prognosis in MS [27,28], we have chosen to use change in this biomarker as the primary outcome measure in the present study. Based on the results of previous studies we have chosen to use changes in BCMA (TNFRSF17), soluble CD14 (sCD14), soluble CD27 (sCD27), Chitinase-3-like-1 (CHI3L1) and Myelin Basic protein (MBP) as secondary outcome measures of intrathecal inflammation.
MRI is a routine method in MS research. In addition to the assessment of focal T2 lesions and active Gd-enhancing lesions, MRI measures such as MTR, Diffusion Tensor Imaging (DTI) and brain atrophy measurements are increasingly used in MS research [29]. Brain atrophy and MTR are associated with the long-term prognosis in PPMS [30–33], and DTI provides additional information about tissue integrity and was used as outcome measure in a recent phase 2 study in patients with PPMS [34]. Based on these studies and other studies in the literature, we have chosen to use the occurrence of new or enlarged T2 lesions, fractional anisotropy (FA) of normal appearing white matter (NAWM) from DTI, lesion volume, MTR of lesions, thalamic volume and percentage brain volume change (PBVC) as secondary MRI outcome in the present study. Furthermore, we include the value from the Symbol Digit Modalities Test (SDMT) from the Brief International Cognitive Assessment for Multiple Sclerosis (BICAMS) panel as secondary outcome measures alongside values from the Expanded Disability Status Scale, the 9-hole-pege test and the Timed 25-foot Walk. The study will also explore the differences between the treatment and placebo group on additional measures from MRI, clinical (two further measures from the BICAMS panel) and self-reported outcome measures.
To examine the relationship between changes in neuroaxonal damage and changes in clinical and inflammatory disease characteristics, we will study the relationship between changes in the primary endpoint (CSF NFL concentrations) and changes in secondary endpoints (clinical measures, MRI measures and inflammatory biomarkers in the CSF. For further information about statistical analysis see section 14.
Data from the open-label phase (W48 to W96) explore the effects of prolonged treatment with dimethyl fumarate. Data from this phase of the study are analyzed independently and will describe time effects of DMF treatment by including all DMF-treated patients during the 96 weeks study phase. Concentrations of serum-NFL from screening, week 48 and week 96 will be analyzed [35], see section 14.

3.4 Study rationale

At present, there are no treatments available for patients with PPMS. In the past decade, much focus in progressive MS research has been based on a hypothetic role of neurodegenerative processes. However, until now there is no evidence that neuroprotective therapy is efficacious in progressive MS. In contrast, there is ample evidence that patients with progressive MS show evidence of systemic immune activation. In a recent study we reported that patients with PPMS have increased activation of circulating, pro-inflammatory T lymphocytes [36] and another study from our group provided evidence of comparable levels of immune activation in blood cells in RRMS, SPMS and PPMS [37]. The role of these cells in the pathogenesis of progressive MS is supported by our study of the effect of natalizumab, which blocks the migration of pathogenic immune cells to the CNS, and was associated with a decrease in inflammation, demyelination and neuroaxonal damage in PPMS and SPMS [15]. Treatment with natalizumab did not, however, normalize neuroaxonal damage, which is in contrast to the effect seen in RRMS [26].

Since DMF may exert a combination of anti-inflammatory, neuroprotective and anti-oxidative effects, we hypothesize that treatment with dimethyl fumarate may decrease neuroaxonal damage in PPMS. We also hypothesize that this is the result of beneficial effects on inflammatory processes in the brain and spinal cord as well as anti-oxidative effects, and that this is associated with beneficial effects on MRI and clinical measures of disease severity.

4 AIM OF THE STUDY

The primary aim of the study is to investigate whether 48 weeks of treatment with dimethyl fumarate can reduce neuroaxonal damage as assessed by the CSF concentration of NFL in patients with PPMS. Secondary endpoints investigate the effects on other CSF measures of demyelination and inflammation and investigate the effects of treatment on clinical and MRI outcome from screening to week 48. Tertiary outcome are the MRI measurements not used as secondary outcome, two outcome measures from the BICAMS panel and the self-reported questionnaires.

4.1 Primary outcome measure

The primary aim of the study is to assess whether 48 weeks of treatment with dimethyl fumarate can decrease neuroaxonal damage in PPMS as assessed by:

- Difference in change in the CSF concentration of NFL from screening to week 48 in PPMS patients treated with dimethyl fumarate or placebo

4.2 Secondary outcome measures

- Clinical endpoints: comparison of change from screening to week 48 in patients treated with dimethyl fumarate and placebo for the following variables:
    - Expanded Disability Status Scale (EDSS)

- Timed 25-Foot Walk (T25FW)
- Nine-Hole Peg Test (9HPT)
- Brief International Cognitive Assessment for MS (BICAMS)
    - Symbol Digit Modalities Test (SDMT)

- CSF endpoints: comparison of change from screening to week 48 in patients treated with dimethyl fumarate and placebo for the following variables:
    - IgG-index
    - CSF-serum albumin quotient
    - Concentrations of: chitinase-3-like-1, sCD14, sCD27, BCMA (TNFRSF17) and myelin basic protein (MBP)
- Magnetic resonance imaging (MRI) endpoints: comparison of change from screening to week 48 in patients treated with dimethyl fumarate and placebo for the following variables:
    - Number of new or enlarged T2 lesions
    - Fractional anisotropy (FA) in Normal Appearing White Matter (NAWM)
    - Lesion volume
    - Magnetization Transfer Ratio (MTR) in lesions
    - Thalamic volume
    - Percentage brain volume change (PBVC)

4.3 Tertiary (exploratory) outcome measures

- Clinical endpoints: comparison of change from screening to week 48 in patients treated with dimethyl fumarate and placebo for the following variables:
    - Brief International Cognitive Assessment for MS (BICAMS)
        - Brief Visuospatial Memory Test Revised (BVMTR)
        - California Verbal Learning Test 2 (CVLT-II)

- MRI endpoints: comparison of change from screening to week 48 in patients treated with dimethyl fumarate and placebo for the following variables:

- Number of Gd enhancing lesions
- Total number of lesions
- Volume of Cortical Grey Matter (CGM), NAWM and the putamen nuclei
- Change in MTR of CGM, NAWM, the putamen and thalamic nuclei
- Change in diffusion tensor imaging (DTI) measures (FA and mean diffusivity) of CGM, NAWM (except FA in NAWM), lesions, the putamen and thalamic nuclei
- Cross sectional area at C2 level of the cervical spinal cord

- Self-reported outcome measures: comparison of change from screening to week 48 in patients treated with dimethyl fumarate and placebo for the following variables
  - Urinary Distress Inventory (UDI)
  - Fatigue Scale for Motor and Cognitive Functions (FSMC)
  - Multiple Sclerosis Impact Scale 29 (MSIS-29)

4.4 Open-label phase outcome (week 48-96)

- Clinical, self-reported, MRI and serum outcome week 48-96 (including screening regarding serum-NFL):
  - EDSS
  - T25FW
  - 9HPT
  - BICAMS (SDMT, BVLTR, CVLT-II)
  - UDI
  - FSMC
  - MSIS-29
  - Number of Gd enhancing lesions
  - Total number of lesions
  - New or enlarged T2 lesions

- Percentage brain volume change (PBVC)
- Volume: lesions, CGM, NAWM , the putamen and thalamic nuclei
- MTR: in lesions, CGM, NAWM and, the putamen and thalamic nuclei
- DTI (fractional anisotropy and mean diffusivity): lesions, CGM, NAWM and the putamen and thalamic nuclei
- Cross sectional area at C2 level of the cervical spinal cord
- Serum endpoint
    - Concentrations of neurofilament light chain (NFL) in serum from screening, W48, and W96 (SIMOA (Quanterix, commercially available))

4.5 Additional exploratory outcome

At the time of MRI (screening, week 48 and week 96) a circle drawing test will be performed.

5 PATIENT POPULATION

Patients are recruited at the Danish Multiple Sclerosis Center at Rigshospitalet, University of Copenhagen. Patients are eligible for inclusion in the study if they have PPMS according to the 2010 revision of the McDonald criteria for MS and the 2014 revision of the Lublin criteria for the clinical course of MS. Patients are selected for the screening visit based on a list of inclusion and exclusion criteria, and must have evidence of disease activity as assessed by the CSF concentration of NFL at the screening visit.

Patients eligible for the study based on the inclusion and exclusion criteria, but not fulfilling the CSF criterion for disease activity, can be followed up after 48 weeks. If such patients show evidence of clinical disease progression after 48 weeks, they are eligible for inclusion in the open-label active treatment phase of the study.

5.1 Inclusion criteria

- Age 18 to 65 years
- PPMS according to the McDonald (2010) and Lublin (2014) criteria
- Disease duration at least one year
- EDSS $\leq$ 6.5
- Written informed consent
- No other signs of significant disease judged by the investigator
- Eligible for randomization to active treatment or placebo as assessed by CSF NFL levels above 380ng/L

- Not eligible for randomization as assessed by CSF biomarker studies but accepts follow-up and open-label treatment per protocol
- Patients not eligible for randomization due to low NFL concentrations in CSF at screening can be followed up after 48 weeks, and are eligible for open-label treatment if they fulfil one of the following clinical criteria of disease progression:
    - 1 point increase in EDSS score from screening to week 48 if screening EDSS <6
    - 0.5 point increase in EDSS score from screening to week 48 if screening EDSS>5.5
    - 2 point increase in a physical functional system
    - Worsening in SDMT, 9HPT or T25FW >20% from screening to week 48

5.2 Exclusion criteria

- Pregnancy or breast feeding
- Lack of effective contraception for women of child-bearing potential
- Relapse within 6 months of inclusion
- Methylprednisolone treatment within 3 months of inclusion
- Treatment with interferon-beta, glatiramer acetate, immunoglobulin G or other immunomodulatory treatment within 6 months of inclusion
- Treatment with mitoxantrone, cyclophosphamide, azathioprine or other immunosuppressive treatment within 6 months of inclusion
- Findings on the screening MRI judged to preclude participation by the treating physician
- Other diseases associated with immunodeficiency
- Other diseases judged to be relevant by the treating physician
- Anticoagulant therapy other than platelet inhibitors
- Active malignant disease in the previous 5 years
- Renal insufficiency or blood creatinine > 150 µmol/l
- Present or chronic infection with hepatitis B virus, hepatitis C virus, HIV (tested in the screening blood samples) or other infections found to be relevant by the treating physician.
- Psychiatric disorders or other disorders impairing the patient's ability to participate in the trial
- Contraindication to MRI
- Known allergy or hypersensitivity to dimethyl fumarate

5.3 Treatment discontinuation

Patients will be informed about their right to withdraw from the study and that this will have no negative consequences for their future treatment in the department.
Treatment with the trial medication will be discontinued in case of:

- Withdrawal of informed consent
- Pregnancy, pregnancy wish or lack of effective contraception (for women of child-bearing potential)
- Poor compliance, e.g. not showing up for scheduled visits
- Treatment with other disease-modifying therapies or immunosuppressive drugs
- At the request of the investigator or sponsor

If treatment is discontinued the patient will, if possible, be seen at an unscheduled visit for clinical assessment and safety blood samples as described in 7.1.4 (screening visit) within 4 weeks unless informed consent has been withdrawn.

6 TRIAL DESIGN

6.1 Trial type
The trial is a single-center, parallel group, randomized, 48 weeks placebo-controlled trial with a 48 weeks open-label treatment extension phase.

6.2 Study endpoints
The primary endpoint of the study is the change in CSF concentrations of NFL from screening to week 48. Secondary and tertiary endpoints are described in detail in section 4.
Safety endpoints include standard measures such as a routine physical examination, pulse, blood pressure, and monitoring of adverse events (AE), serious adverse events (SAE) and serious unexpected serious adverse reaction (SUSAR)

6.3 Time plan for the trial
The study includes a screening phase (2-6 weeks before randomization) with a screening visit divided over 2-3 days (see section 7.1.4), a randomized treatment phase (48 weeks) with a baseline visit (week 0) and 4 study visits (week 12, 24, 36, 48) and an open-label extension phase with 4 study visits (week 60, 72, 84 and 96). It is expected than 1-2 patients will be screened per week, and that 1 patient per week can be randomized.

<u>Plan for patient inclusion:</u>

Planned inclusion of the first patient:	September 2016
Planned period of recruitment:	September 2017
Planned end of trial:	December 2020

6.4 Relapses

The patient is asked to contact the clinic within 3 days from the onset of a suspected relapse and should be examined by the study physician within 10 days from onset of the suspected relapse. If the physician finds that the relapse results in significant impairment, methylprednisolone pulse therapy can be administered (section 9.5).

7 METHODS AND OUTCOME MEASURES

The treating physician (investigator) is clinically responsible, and will prescribe the study drug, conduct the clinical procedures (lumbar puncture and quantitative neurological examinations). The study coordinator will assist with the quantitative neurological examinations, study drug administration and blood samples.

7.1 Study visit procedures

All patients will complete a screening visit. If they fulfil all inclusion criteria and no exclusion criteria at the screening visit, they will enter the randomized part of the study and complete a baseline visit and study visits at week 12, 24, 36, 48, 60, 72, 84 and 96. For full overview of content of each visit see Appendix B

7.1.1 Treatment in the open-label phase

Patients who do not show evidence of disease activity in the screening lumbar puncture, measured as NFL below 380ng/L, can be followed up with a study visit at week 48 if they accept to do so. They are eligible for open-label treatment from week 48 to 96, if there is evidence of clinical disease progression (section 5.1) and will be followed with study visits at week 60, 72, 84 and 96.
As described in the next section, patients with increased CSF concentration of NFL at the screening visit are eligible for randomization to treatment with DMF or placebo for 48 weeks followed by the 48 week open-label extension phase. Open-label treatment extension phases are widely used in MS research in order to obtain additional data on efficacy and adverse events.

The rationale for allowing open-label treatment of patients not eligible for randomization but still showing significant clinical worsening 48 weeks after the screening visit is that this will allow us to collect preliminary data on efficacy in this patient subgroup. Although efficacy of DMF in PPMS has not been established, preliminary evidence support that DMF treatment is safe and well tolerated also in progressive MS [24]. Furthermore, the systematic follow-up of patients not eligible for randomization at baseline will provide further scientific evidence to support the use of NFL measurements for selecting PPMS patients for clinical trials. The threshold for clinical disease progression used to establish eligibility for open-label treatment is based on thresholds validated in previous clinical trials in progressive MS [38].

Although prone to bias from the unblinded administration of the study drug, valid data can still be obtained from the open-label study design since the MRI studies conducted will not be prone to the

same bias. This notion is supported by the treatment effects observed in MRI studies in two previous unblinded phase 2 studies from our group [15,16].

7.1.2 Cut off level of NFL

All patients eligible for inclusion in the study will have a lumbar puncture done at the screening visit. If the level of NFL is increased above 380ng/l, the patient is eligible for randomization, which will take place at the baseline visit. The measurement of NFL in CSF is done using the NF-Light® ELISA assay from Uman Diagnostics (see Appendix D). This is a commercially available kit developed in Sweden and used in most research with NFL as an outcome measure [8,39,40]. The cut-off level of 380ng/l correspond to the previously established upper limit in young adults established for this assay [41]. The same assay has been used in several previous studies from our group[8,15,16,28]. These studies confirm that increased NFL levels predict a poor prognosis in MS, that PPMS patients on average have increased CSF levels of NFL, that NFL concentrations in general are stable over 1 year, and that the use of NFL levels as an inclusion criterion is supported by exploratory reanalyses of data from our previous phase 2 clinical trials in patients with progressive MS [15,16].

7.1.3 Quality assurance of NFL measurements

The NFL immunoreactivity detected with the NF-Light® assay from Uman Diagnostics is highly stable [42]. The assay is used for routine analysis by *Statens Serum Institut* in Copenhagen and is currently being implemented for routine analysis at the Neuroimmunology laboratory at Rigshospitalet, where the ELISA for this study is conducted.

Quality assurance of each assay run is done by running reference samples containing NFL in a high and low concentration alongside the sample from the patient and having a negative control in each assay run. All samples are run in duplicate in order to allow assessment of the assay coefficient of variation (CV).

Intra-assay CV for the assay is generally below 5% and inter-assay CV is generally below 9%. If a sample has a CV above 15% it will be reanalyzed.

For more information about the NFL kit kindly see appendix D.

7.1.4 Screening visit (week –6 to week –2)

Patients eligible for the study will go through the following procedures. The screening visit may be divided into two-three separate visits. MRI can be performed on the same day as the initial visit but can also be done on a separate day. Lumbar puncture will always be performed on a day separate from the initial visit and MRI.

On day one the participants complete the following elements:

- Written informed consent of participation
- Inclusion and exclusion criteria are reviewed for the patient
- Data on the MS history, previous treatment and other diseases are collected
- Routine physical examination, pulse, blood pressure, weight, height

- Neurological examination with EDSS
- T25FW, 9HPT, and BICAMS (SMDT, BVLTR and CVLT-II)
- MSIS-29
- UDI
- FSMC
- Safety blood tests:
    - Hematology: hemoglobin, MCV, thrombocytes, leukocytes with differential count
    - Biochemistry: creatinine, sodium, potassium, ALAT, LDH, bilirubin, basic phosphatase, INR, CRP
    - Anti-HBsAg, HBsAG, anti-HCV, anti-HIV
    - Pregnancy test (in women of child-bearing potential) via serum hCG

On day one or two the participants complete the following elements:

- MRI with Gd contrast (performed at Hvidovre Hospital within two weeks)Circle drawing test at the time of the MRI

On day two or three the participants complete the following elements:

- Lumbar puncture for NFL analysis and other CSF outcome measures

For full overview of study visit content see appendix B.

7.1.5 Baseline visit

- Study drug administration
- Pregnancy test (in women of child-bearing potential) via serum hCG
- T25FW, 9HPT, SDMT

For full overview of study visit content see appendix B.

7.1.6 Study visit type 1 (week 12, 36, 60, 84)

- Study drug administration and accountability
- Pregnancy test (in women of child-bearing potential) via serum hCG
- Safety blood tests:
    - Hematology: hemoglobin, MCV, thrombocytes, leukocytes with differential count
    - Biochemistry: creatinine, sodium, potassium, ALAT, LDH, bilirubin, basic phosphatase, INR, CRP

For full overview of study visit content see appendix B.

7.1.7 Study visit type 2 (week 24 and 72)

- Neurological examination with EDSS
- 9HPT and T25FW (only week 24)
- Study drug administration and accountability
- Pregnancy test (in women of child-bearing potential) via serum hCG
- Safety blood tests:
    - Hematology: hemoglobin, MCV, thrombocytes, leukocytes with differential count
    - Biochemistry: creatinine, sodium, potassium, ALAT, LDH, bilirubin, basic phosphatase, INR, CRP

For full overview of study visit content see appendix B.

7.1.8 Study visit week 48

The week 48 visit may be divided into two-three separate visits. MRI can be performed on the same day as the clinical visit but can also be done on a separate day. Lumbar puncture will always be performed on a day separate from the clinical visit and MRI.

- Routine physical examination, pulse, blood pressure
- Neurological examination with EDSS
- T25FW, 9HPT, SDMT and BICAMS (SMDT, BVLTR and CVLT-II)
- MSIS-29
- UDI
- FSMC
- Study drug administration and accountability
- Pregnancy test (in women of child-bearing potential) via serum hCG
- Safety blood tests:
    - Hematology: hemoglobin, MCV, thrombocytes, leukocytes with differential count
    - Biochemistry: creatinine, sodium, potassium, ALAT, LDH, bilirubin, basic phosphatase, INR, CRP
- MRI with Gd contrast (performed at Hvidovre Hospital week 47 or 48)
- Circle drawing test at the time of the MRI
- Lumbar puncture for CSF outcome measures

For full overview of study visit content see appendix B.

7.1.9 Study visit week 96

The week 96 visit may be divided into two separate visits. MRI can be performed on the same day as the clinical visit but can also be done on a separate day.

- Routine physical examination, pulse, blood pressure
- Neurological examination with EDSS
- T25FW, 9HPT, SDMT, and BICAMS
- MSIS-29
- UID
- FSMC
- Study drug accountability
- Pregnancy test (in women of child-bearing potential) via serum hCG
- Safety blood tests:
    - Hematology: hemoglobin, MCV, thrombocytes, leukocytes with differential count
    - Biochemistry: creatinine, sodium, potassium, ALAT, LDH, bilirubin, basic phosphatase, INR, CRP
- MRI with Gd contrast (performed at Hvidovre Hospital week 95 or 96)
- Circle drawing test at the time of the MRI

For full overview of study visit content see appendix B.

7.1.10 Unscheduled visits

Patients are informed to contact the treating physician if they develop new symptoms or worsening that may constitute a relapse. The treating physician will decide whether the patient has a relapse or, and whether this should be treated with methylprednisolone, or whether there is another cause of the worsening.

If symptoms of other disease occur the treating physician will register this as an AE or SAE, decide whether it is related to the study drug, and whether the study drug should be withdrawn.

7.2 Outcome measures

7.2.1 CSF concentration of neurofilament light chain

NFL is aa cytoskeletal constituent of intermediate filaments and reflect neuronal and axonal death when appearing in CSF. The concentration of NFL is often elevated in CSF of progressive MS patients. NFL is a treatment responsive biomarker and has been associated with long-term prognosis in MS [27]. In this study NFL is measured by ELISA technique as a biomarker of intrathecal neuroaxonal damage (see Appendix C, D and section 7.1.3).

7.2.2 Neurological examination with Expanded Disability Status Scale (EDSS), Timed 25-foot Walk (T25FW), Nine-Hole Peg Test (9HPT) , BICAMS, FSMC, UID and MSIS-29

EDSS will be rated at the screening visit, at week 24, 48, 72 and 96. EDSS assessment is done by standardized neurological examination (neurostatus 03/09). T25FW, 9HPT, SDMT, , BICAMS, FSMC, UID and MSIS-29 will be conducted at the screening visit, week 48 and week 96, except 9HPT and T25FW that will also be performed at week 24.

7.2.2.1 Assessment of disease progression

The patients will routinely be assessed by full neurological examination and EDSS scoring. Disease progression is evaluated from this and patient history. The patients are instructed to contact the treating doctor if new symptoms develop between two routine examinations.

7.2.3 Timed 25-foot walk test (T25FW)

The T25FW is a quantitative mobility and leg function performance test. The patient is directed to one end of a clearly marked 25-foot course and is instructed to walk 25 feet as quickly as possible. Patients may use assistive devices when performing this task [43].

7.2.4 Nine-Hole Peg test (9HPT)

The 9-HPT is a brief, standardized, quantitative test of upper extremity function. Both the dominant and non-dominant hands are tested. It has great sensitivity to detect minor impairments of hand function [44].

7.2.5 Brief International Cognitive Assessment for MS (BICAMS)

BICAMS is an international validated assessment tool for the cognitive impairment seen in MS patients. It consists of 3 individual test: Symbol Digit Modalities test (SDMT), Brief Visuospatial Memory Learning Test Revised (BVMTR) and California Verbal Learning Test 2 (CVLT-II)[45].

7.2.6 Multiple Sclerosis Impact Scale (MSIS-29)

MSIS-29 is a measure of the physical and physiological impact of multiple sclerosis from the patient's perspective [46]. It consists of 29 items of which 20 evaluate physical impact and 9 evaluate psychological impact.

7.2.7 Urinary Distress Inventory (UDI)

UDI is a questionnaire describing to which degree a person is bothered by different symptoms of bladder incontinent[47].

7.2.8 Fatigue Scale for Motor and Cognitive function (FSMC)

FSMC has undergone validation and provides differential quantification and graduation of cognitive and motor fatigue in MS patients. Fatigue is one of the core symptoms in MS [48].

7.2.9 CSF concentration of Chitinase-3-like-1 (CHI3L1)

CHI3L1 is a member of the glycoside hydrolase 18 Chitinase family and are expressed by inflamed tissues in numerous chronic inflammatory conditions. It has been detected in the human brain in several central nervous system (CNS) diseases, including MS. It has been suggested that astrocytes are the principal brain cell population that expresses CHI3L1, corroborating in vitro studies, which has identified CHI3L1 as one of the most abundant proteins released by cultured astrocytes upon exposure to pro-inflammatory components[49]**.** CHI3L1 will be analyzed by a validated in-house Luminex assay (R&D Systems).

7.2.10 CSF concentrations of soluble CD14 and CD27, .

sCD27 is primarily secreted by activated T-cells and has been proved to be superior to other CSF markers in diagnosing patients with intrathecal inflammation, furthermore it has shown good correlations with NFL, and may be more specific for patients with progressive MS. sCD14 is secreted by activated mononuclear phagocytes and provides information about innate immune activation in the CNS[50,51]. Soluble CD14 and soluble CD27 will be analyzed by a validated in-house Luminex assay (R&D Systems).

7.2.11 CSF concentration of soluble BCMA (TNFRSF17)

BCMA is a cell surface receptor found on mature B-cells and plasma cells. It acts as a receptor for the ligands *B-cell activating factor* (BAFF) and *a proliferating-inducing factor* (APRIL). BCMA receptor antagonists suppress EAE in mice, and BCMA may be a strong indicator of disease activity in progressive MS according to a large recent study of proteins in the CSF of progressive MS patients[52]. Also, B-cells are known to play an important role in inducing and sustaining intrathecal inflammation. Soluble BCMA (TNFRSF17) will be analyzed by a validated in-house Luminex assay (R&D Systems).

7.2.12 Serum concentrations of neurofilament light chain

Is done retrospectively as an explorative analysis after the completion of week 96. Analysis is done on stored serum samples from screening, week 48 and week 96. Patients do not undergo lumbar puncture for CSF NFL analysis at week 96. Concentrations of NFL in serum have been shown to be an excellent marker of neuronal damage in multiple sclerosis. Furthermore, the correlation between NFL in CSF and NFL in serum has been good in previous studies[35] . Serum NFL will be measured using single-molecule array analysis (Quanterix) (appendix C).

7.2.13 Myelin Basic Protein (MBP)

MBP is a major protein in CNS myelin. The CSF concentration of MBP is increased in MS patients [53]. MBP will be measured in CSF using ELISA technique (R&D Systems) and is used as an intrathecal biomarker of demyelination (see appendix C).

7.2.14 MRI with Gadolinium (Gd) enhancing lesions

Gd enhancing lesions is the most frequently used marker of inflammation in MS patients. It reflects focal inflammation. Focal inflammation is seen in progressive MS and natalizumab treatment has been shown to reduce inflammation in SPMS [54].

7.2.15 Lesions on T2 weighted MRI

Active demyelinating lesions, chronic inactive lesions and gliosis contributes to T2 weighted lesions. The volume of T2 lesions is a marker for accumulated disease activity in a given study period. The number or increase in volume of lesions is a measure for disease activity. This study uses the volume and the number of new T2 weighted lesions as a marker of disease activity by comparison of MRI before and after treatment.

7.2.16 Percentage brain volume change (PBVC)

Atrophy can be estimated on MRI by normalized brain volume (NBV). There is a good correlation between clinical disease progression and these measures [55]. The degree of atrophy is a well-established measurement of neuronal and axonal damage with great reproducibility in different studies of MS treatment [56] and can be measured over a short period. The rate of atrophy can be used as a measure of treatment effect.

7.2.17 Change in volume of T2 lesions, cortical grey matter (CGM) Normal-Appearing White Matter (NAWM), putamen and thalamus

MRI measures in T2 weighted images are used to detect possible changes in these measures during the trial.

7.2.18 Change in magnetization transfer ratio (MTR) of T2 lesions, putamen, thalamus, CGM and NAWM

MTR is an indirect measurement of the content of macromolecules in a given tissue. MTR is a way of estimating the degree of demyelination and axonal loss in MS. Pathological studies have shown that a low MTR value represents low content of myelinated axons in both focal lesions and in NAWM. A low MTR value in normal-appearing grey matter (NAGM) is one of the best correlations between MRI and the clinical findings [31,57] in patients suffering from progressive MS. In this study, we measure the MTR value for T2 lesions, putamen, thalamus, CGM and NAWM.

7.2.19 Change in diffusion tensor imaging (DTI) measures (fractional anisotropy and mean diffusivity) of T2 lesions, CGM and NAWM, putamen and thalamus

DTI, like MTR, gives a measurement of the integrity of the tissue by measuring the movement of water molecules. DTI is measured in mean diffusivity (MD) and fractional anisotropy (FA). Changes in DTI have been shown to precede the development of Gd enhancing lesions [58] and has in NAWM and NAGM been show to correlate with disease progression in MS patients [59,60]. In this study we will examine DTI values in lesions, putamen, thalamus, CGM and NAWM.

7.2.20 Cross sectional area at the C2 level of the spinal cord

Measures atrophy at this level of the spinal cord.

7.2.21 Circle drawing test

The circle-drawing test is a patient performed task done at the time of the MRI at Hvidovre Hospital. Participants continuously draw superimposed circles (for several seconds) with their right and left hand in a normal handwriting position with the hand resting on the table while drawing. It is done as an exploratory analysis and will not be reported as an outcome at week 48.

7.3 Safety studies

Standard safety measures include full neurological examination, pregnancy test of all women of childbearing age, routine physical examination, hematology and biochemical parameters in blood, monitoring of adverse events and reactions (AE/AR), serious adverse events and reactions (SAE/SAR) and serious unexpected serious adverse reaction (SUSAR).

7.3.1 Neurological examination

A full neurological examination will be performed at the screening visit, week 24, 48,72 and 96 by adherence to neurostatus 03/09.

7.3.2 Pregnancy test

Serum-hCG will be measured on all female patients of childbearing age at every study visit.

7.3.3 Routine physical examination

Routine physical examination consists of examination of the cardiovascular system, the respiratory system, the gastrointestinal system, the musculoskeletal system, the urogenital system, dermatologically and HEENT: head, eyes, ears, nose and throat. This examination is performed at the screening visit and when required.

7.4 Study personnel, sample handling and study biobank

Blood samples and CSF samples will be taken at the multiple sclerosis clinic at Rigshospitalet. An experienced medical doctor from the multiple sclerosis clinic will perform the lumbar puncture.
Safety blood samples are analyzed at the department of clinical biochemistry at Rigshospitalet and hereafter destroyed in accordance to standardized guidelines for Rigshospitalet. Analyzes of CSF is done at department of clinical biochemistry and the Neuroimmunology laboratory (which is a part of the department of multiple sclerosis at Rigshospitalet) and hereafter destroyed in accordance to standardized guidelines for Rigshospitalet.
For further detail about execution and handling of the lumbar puncture at CSF kindly, see appendix C.
All MRI scans are performed at the MRI department at Hvidovre Hospital.

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The Danish Multiple Sclerosis Center will report the establishment of a biobank for the study itself and a distinct biobank to The Regional Scientific Ethical Committee and the Danish Data Protection Agency with regards to the storage of CSF samples for analyzes described in this study and for future research purposes.

7.5 Risk assessment for study procedures

7.5.1 Lumbar puncture

The procedure of a lumbar puncture is associated with some degree of discomfort, and the patient will be offered local anesthesia to reduce it. Lumbar puncture involves the risk of post lumbar headache, which is seen in approximately 13-36%. This type of headache usually subsides within a couple of days but can in some instances require an epidural blood patch [61]. Patients with PPMS are generally older than patients with relapsing-remitting MS and older people have a reduced risk of developing headache after lumbar puncture. The risk of headache is further reduced using atraumatic needle/technique.
Lumbar puncture is also associated with a risk of serious complications namely post lumbar puncture infection (meningitis) and bleeding in the cavity surrounding the spinal cord, but these complications are extremely rare.

7.5.2 MRI scan

MRI does not entail any risk when the right precautions are taken. It is a non-invasive technique, that produce detailed images of the body´s internal structure. MRI systems use a strong static magnetic field, a pulsed gradient magnetic field and radiofrequency energy to obtain the images in selected plains of the body. The subject is placed in a strong magnetic field created by a superconducting magnet surrounding the core of the scanner. The subject lying in the scanner is exposed to brief

pulses of non-ionized radiofrequency radiation from a transmission coil. Grey and white matter can be distinguished whit brain MRI and accurate calculations of the different brain volumes ban be performed.
Certain metallic implants are not MRI compatible and eliminates the patient from having MRI done. The scanner is noisy, and all subjects will therefore be provided with earplugs or earmuffs. The contrast medium used for an MRI scan is gadolinium (Gd). It can have side effects such as nausea, headache, a sensation of warmth and a metallic taste. Allergic reactions are extremely rare. To ensure that there is no risk of development of nephrogenic systemic fibrosis all patients must have normal renal function, which is entailed in the safety blood analyzes. Some patients can get claustrophobic during the MRI. This is easily counteracted by a light sedative i.e. Alprazolam 0,5mg taken 30-60 minutes before the scan is performed.
The MRI will be described clinically by a specialist at Hvidovre Hospital within 7-14 days. If by any reason this description give rise to elimination of a patient from the study the patient will be contacted by phone and if necessary be invited to a consultation with a doctor for detailed explanation and perhaps further examinations and tests if needed.

7.5.3 Blood samples

Having a blood sample done can induces a slight discomfort when the needle penetrates the skin. Bleeding under the skin can occur. Infection is rare.

8 STUDY MEDICATION

8.1 Dimethyl fumarate and placebo

Fumarates exert pleiotropic immunomodulatory effects. Multiple pathways have been implied in mediating these effects.
Fumaric acid esters (FAE) have been used in treating psoriasis since 1959. Tecfidera is an oral formulation of FAE containing the active metabolite dimethyl fumarate (DMF) used for treating MS. Studies of the properties of DMF in rats have shown that the drug possesses neuroprotective abilities and protects different neuronal cell types including neural stem/progenitor cells (NPCs) and differentiated neurons (motor neurons) from oxidative damage. This occurs through increased expression of the transcription factor nuclear factor-erythroid 2-related factor 2 (Nrf2) pathway and subsequently induction of the expression of anti-oxidative proteins [62]. DMF inhibits pro inflammatory cytokine production through inhibition of nuclear transcription factor kappa B (NFκB) independently of Nrf2 pathways (68)[63]. Inflammation but also oxidative stress/damage play a part in both secondary and primary progressive MS. Biomarkers of oxidative stress is significantly elevated in these patients [8].

8.1.1 Study medication

Treatment medication (DMF) is distributed from the Danish Multiple Sclerosis Center, University Hospital Rigshospitalet, Copenhagen. Patients will receive active medication (dimethyl fumarate 120 mg capsules) or identically looking placebo capsules to be taken at a final dosage of 240 mg twice

daily. Patients will be instructed to take the capsules with food to minimize gastrointestinal side effects.

8.1.2 Initial dosing and dose reduction

In order to reduce the side effects of DMF, which usually resolve within the first four weeks of treatment, the study drug is administered as one capsule once daily the first week. Then the dosage is increased to one capsule twice daily the second week, two capsules in the morning and one in the evening the third week, and two capsules twice daily from week 4 to 48. The patient and the study coordinator will document study drug use.

	Morning	**Evening**
Week 1	1 capsule	
Week 2	1 capsule	1 capsule
Week 3	2 capsules	1 capsule
Week 4	2 capsules	2 capsules

8.1.3 Dosing of the study drug

In order to prevent common side effects the dosing of the treatment drug is gradually increased over the span of 4 weeks until full daily dosage of 240mg twice a day has been reached. Patients will be given written information on how to take the medicine.

The dosage can be reduced from this time schedule for up to four weeks at the discretion of the treating physician. If the full dosage of two capsules of study medication twice daily cannot be resumed after four weeks of dose reduction, the patient must be withdrawn from the study.

8.1.4 Side effects

Very common side effects (>10%) include abdominal pain, diarrhea, nausea, flushing and ketones in the urine.

Common side effects (1-10%) include lymphopenia, lymphocytosis, leucopenia, skin rash, skin itching, redness, vomiting, gastroenteritis, gastritis, increased liver transaminases, hot flashes, hyperesthesia, hypersensitivity, albuminuria and proteinuria.

8.1.5 Progressive multifocal leukoencephalopathy (PML)

PML is a rare but severe opportunistic infection caused by John Cunningham virus (JCV). PML can be deadly or result in severe disability.

Lymphopenia is a known risk factor for development of PML and Dimethyl fumarate can cause a mean lymphocyte count decrease of approximately 30% [64].There has been case reports of PML

induced by Dimethyl fumarate in both MS patients [65] and psoriasis patients [66].
In order to minimize this risk all patients will undergo blood test for hematology parameters every 12 weeks and will routinely be examined by a physician at the screening visit, week 24, 48, 72 and 96. All patients are further encouraged to contact the treating physician or study nurses with any new symptoms of disease. Estimation of the risk of PML during DMF treatment cannot be made as it is extremely rare and only case reports of it exist.

8.1.6 Delivery, packing, labelling and randomization of study drug

Both the active study medication (DMF) and the placebo capsules are provided from the manufacture – Biogen. The capsules are sent from Biogen UK and capsules containing DMF and placebo are sent approximately 2 days apart to avoid mix up upon delivery. The delivery of both DMF capsules and placebo capsules are to the Regional Capital Pharmacy in Herlev. The pharmacy handles all packaging according to standard guidelines and they develop the randomization code and deliver the codes in sealed envelopes to the Danish Multiple Sclerosis Centre under the Department of Neurology.
Distribution of the capsules is done from the Danish Multiple Sclerosis Center, Rigshospitalet, University of Copenhagen.

8.1.7 Storage and breakage of the randomization code

The sealed envelopes containing the randomization codes are held in a locked cabinet in a lockable office at the Department of Neurology. The key to the office is held by trained researched nurses. The two research nurses, the sponsor, the primary investigator and persons authorized by these persons are the only personal who can gain access to the locked cabinet. The randomization code will be broken if it is deemed necessary by the sponsor or primary investigator (or other persons authorized by sponsor or the primary investigator) to ensure the safety of the patient or in case a patient withdraws consent and request to know the treatment allocation.

8.1.8 Compliance

The capsules are distributed to the patients at the Danish Multiple Sclerosis Center under the Department of Neurology. The exact number of capsules handed out to any one patient is recorded. All patients are encouraged to keep a detailed diary of their medicinal intake and any discomfort and/or side effects and to bring any leftover medication in addition to the diary at every visit. Trained research nurses keep count of medicine handed out and medicine left over in order to assess compliance.

9 CONCOMITANT DISEASES AND CONCOMITANT MEDICATION

9.1 Definitions

Concomitant disease is any disease present at the screening visit that continues unaltered.
Concomitant medication is any medication, besides the study drug, taken by the patient during the trial inclusive at the screening visit.

9.2 Baseline concomitant diseases and concomitant use of medication

Details regarding concomitant diseases and use of medication, other than the study drug, will be noted in the case report form (CRF) with description of dosage, start and stop date, and reason for the usage. Any other diagnostic or therapeutic intervention, occurring in the trial period is also reported in the CRF. The patients may not take any kind of immunosuppressive or immune-modulating substances when entering the trial and a patient whom begins such a treatment during the trial will be excluded from any further participation (see section 5.2).

9.3 Concomitant disease and concomitant medication during the trial

Medication that is considered necessary for the patient and do not interact with the treatment with DMF can be prescribed by the investigator after assessment. Any preexisting symptomatic treatment, i.e. antispasmodic, must be optimized before trial initiation with the aim of continuing an unaltered dosage throughout the trial period.

Changes in concomitant medication or treatment procedures must be reported in the patients' medical charts and the CRF at every visit. The sponsor must be contacted if the changes influences on the patients' possibility of continuing the trial.

Any worsening of concomitant disease compared to the level at the screening visit as well as any concomitant disease occurring during the trial will be considered as a possible side effect (see section 10) to the trial drug and will be reported as such even when the concomitant disease is judged to have no linkage to the trial medication.

9.4 Precautions/overdose

The patient will receive the necessary symptomatic treatment after assessment by the investigator in case of overdose or suspected overdose.

9.5 Rescue medicine

The patient is asked to contact the clinic within 3 days from the onset of a suspected relapse and should be examined by the study physician within 10 days from onset of the suspected relapse. If the physician finds that the relapse results in significant impairment, methylprednisolone pulse therapy can be administered.

9.6 Symptoms in progressive MS

Symptoms attributed to the condition of primary progressive MS need not be described in detail at the screening visit. It is likewise not necessary to report the naturally occurring fluctuations in progressive MS during the trial as concomitant disease or side effects (see section 10.1).

10 ADVERSE EVENTS, ADVERSE RECTIONS, SERIOUS ADVERSE EVENTS OR REACTIONS AND SUSPECTED UNEXPECTED SERIOUS ADVERSE REACTION

10.1 Definitions

- Adverse event (AE):
  Any untoward medical occurrence in a study participant that does not necessarily have a causal relationship with the allocated treatment. It can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the experimental treatment or product, whether or not the event is considered causally related to the treatment

- Serious adverse event (SAE)
  If an AE meets any of the following criteria, it must be reported to the sponsor as a serious AE (SAE) within 24 hours of the investigator being made aware of it:
  - Results in death
  - Is life-threatening (note: the term life-threatening refers to an event in which the patient was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it was more severe)
  - Requires inpatient hospitalization or extension of existing/preplanned hospitalization
  - Results in persistent or significant disability/incapacity
  - Is a congenital abnormality/birth defect
  - Is a medically important event

- Adverse reaction (AR):
  Any untoward and unintended response to an investigational medicinal product related to any dose administered.

- Serious adverse reaction (SAR):
  An SAE for which a causal relationship to the investigational medicinal product is at least possible i.e. a causal relationship is conceivable and cannot be dismissed

- Suspected unexpected serious adverse reaction (SUSAR):
  An adverse reaction that is both unexpected (not consistent with the applicable product information) and also meets the definition of a Serious Adverse Reaction SAR).

Any adverse event must be registered from the first day of administered trial drug but do not include diseases or other preexisting conditions revealed during the screening procedure. All events must be evaluated with regard to relatedness, intensity and seriousness. The intensity of the events will be reported in accordance to table 10.1 and relatedness will be evaluated in accordance to table 10.2. Seriousness is defined above in *"10.1 Definitions"* under *"Serious adverse events"*. Symptoms that are due to progressive MS and that do not have substantial fluctuations besides the normal for this category of patients is not considered as an adverse event. Progressive neurological symptoms

are a part of the natural course in patients with PPMS but may also reflect progressive multifocal leukoencephalopathy (PML). PML must always be excluded as the course of the progressing neurological symptoms before the symptom can be attributed to the natural course of progressive MS. Relapses is not considered an adverse event and should not be reported as such even if the patient needs hospitalization, unless the treating physician (investigator) deem the symptoms not to be an occurrence of the natural history of evolving progressive MS. A preplanned hospitalization is not considered an adverse event and will not be reported as such, but extension of pre-planned hospitalization, i.e. due to unforeseen complications, must be considered a serious adverse event and consequently be reported as such.

Table 10.1 Intensity of events

Intensity	**Definition**
Mild	Aware of signs or symptoms but they are easily tolerated. The event is often transient, treatment is not necessary, and the event does not hinder the patient's daily activities
Moderate	Noticeable symptoms, moderately hindering the patients' daily activities, but still acceptable. Can usually be alleviated by simple therapeutic treatments
Severe	Significant inconvenience and a hinder of the patients´ daily activities. Unacceptable and usually requires medication or other type of treatment

10.2 Relatedness: Causal connection between event and trial medication

The causal connection between trial medication and events must be examined via following definitions:

Likely	• Good reasons and sufficient documentation to assume a causal connection
Possible	• A causal connection is reasonable and cannot be ruled out
Unlikely	• The event is most likely related to an etiology other than the trial medication

Unknown	• It is impossible to determine, i.e. due to insufficient evidence, inconsistency in data or poor documentation

10.3 Categories and definitions of the outcome of an event

Recovered	• Fully recovered after the event with medical or surgical assistance or recovered to a level observed at the first trial related activity after signing the written consent form
Stabilized	• This term should only be used in cases of cancer or chronic conditions that cannot be normalized with medical or surgical intervention. Should furthermore only be used when the patient has completed the trial period
Recovered with sequelae	• When the patient suffers from lasting and significant invalidity or incapacity for work due to an event occurring during the trial, i.e. been paralyzed, gone blind or deaf. Any event recovered but with sequelae should be considered a serious adverse event
Clinical laboratory event	• A laboratory event is classified as such, if a lab answer is outside the normal range and investigator judges it to be clinically significant

10.4 Registration of events

The patients are encouraged to report any new or worsened symptoms or medical incidences at every visit or via other forms of contact after the screening visit. This will continue until week 96 or until the patient is no longer in the trial setting for any reason. All events observed by the investigator or reported by the patient must be evaluated by the investigator and be reported in the CRF. All serious adverse events or reactions (SAE/SAR) will immediately be reported to the sponsor (see section 10.5). Investigator will perform all necessary measures to examine and possibly cure the event. To help examine whether an event or reaction is expected or unexpected the Summary of Product Characteristics from the European Medicine Agency (EMA) will be used.

For every event, the investigator will report the following variables:

- Description of the event
- Start and stop date
- Intensity
- Severity

- Relatedness
- Precautions taken with regard to dimethyl fumarate
- Outcome

Any necessary medical treatment of the event must be reported in the section about concomitant medication in the CRF. If more than one event occurs for one patient, they must be reported separately. If the intensity of an event increases, then it must be reported as a separate event.

10.5 Reporting serious adverse events and reactions (SAE/SAR) and suspected unexpected serious adverse reactions (SUSAR)

Events that require immediate reporting to sponsor within 24 hours of investigator being made aware of it are the following:

- Serious adverse events (SAE) or reactions (SAR) including death (see table 10.1)
- Any adverse event that results in permanent disruption from the trial even when the event is not considered to be a SAE or SAR

The form for reporting an SAE/SAR and/or the form for discontinuation of the trial (trial discontinuation form) will be sent or delivered by the investigator to:

Sponsor:
Professor Finn Sellebjerg
The Danish Multiple Sclerosis Center
Department of neurology, section 2082
Rigshospitalet

All adverse events and reactions that take place during the trial must be reported in the CRF. The sponsor reports to the Danish Medicine Agency and the Regional Scientific Ethical Committee if the SAE/SAR is deemed to be a SUSAR. If the SUSAR results in death or is life-threatening the report must be sent to the Danish Medicine Agency and the Regional Scientific Ethical Committee within **7 days**. A SUSAR that does not cause death and is not life threatening must be reported to the Danish Medicine Agency and the Regional Scientific Ethical Committee within **15 days**.
After the end of the trial all AE/AR, SAE/SAR and SUSAR´s will be reported in the final safety summary report and sent to the Danish Medicine Agency and the Regional Scientific Ethical Committee. All SAR will be reported annually in a safety report to the Danish Medicine Agency and the Regional Scientific Ethical Committee. All SAE will be reported in the final report after the trial is completed. The final results of the trial will be reported in the EudraCT (see section 18). All events and side effect will be summarized using MedDRA terminology.

10.6 Pregnancy and contraception

There are no or limited amount of data from the use of DMF in pregnant women. Animal studies have shown reproductive toxicity. DMF is not recommended during pregnancy and in women of childbearing potential not using appropriate contraception. Patients that become pregnant or seize to use appropriate contraception during the trial must stop taking the trial medicine and are eliminated from further participation. If the patient has been pregnant during the trial, she will be asked to be seen at follow up visits and the result of the pregnancy including reports on the health and development of the baby will be followed until 8-12 weeks postpartum. The investigator will fill in a form on the pregnancy (pregnancy form). Participating female patients of child baring age must use contraception in the form of either an intrauterine device or hormonal contraceptives during the trial. The terminal half-life of monomethyl fumarate, which is the active component of dimethyl fumarate, is approximately 1 hour and no circulating monomethyl fumarate is present at 24 hours in the majority of individuals. Accumulation of parent drug or monomethyl fumarate does not occur with multiple doses of dimethyl fumarate at the therapeutic regimen.

10.7 follow up

Any serious events that occur during the trial and continues after must be followed up until the condition ceases or it is judged clinically that the condition is stable. The patient will be seen approximately 4 weeks past week 96, if there are any trial related problems at week 96 and continue to be evaluated for as long as it is clinically judged to be necessary.

11 CASE REPORT FORM (CRF)

The CRF will be consistent with the protocol and be established ahead of the trial. The CRF will include all the relevant data as described in the protocol with exception of MRI and CSF information. Investigator is responsible for the data being recorded in the CRF and that all other reports are accurate and complete. In the CRF, a patient will be identifiable by an identification number (screening number) and initials. Investigator must keep copies of the CRF for 10 years after cessation of the trial.

11.1 Investigator study file and archives

The investigator has a trial master file (TMF) from the beginning of the trial. The files in the TMF entail all relevant documents for carrying out the trial. The investigator must keep the TMF a minimum of 5 years after cessation of the trial. Patient files must be kept a minimum of 5 years or longer according to hospital policies. All information regarding the trial must be kept in a secure and lockable facility. Approval of this trial from the Regional Scientific Ethical Committee, the Danish Data Protection Agency and the Danish Medicine Agency will be located in the TMF.

12 MONITORING AND CONTROL VISITS

The GCP Unit of Copenhagen University Hospital monitors the trial. During the course of the trial, the monitor will visit the MS Clinic at regular intervals and can be contacted by e-mail or phone if

needed. The purpose of the monitoring visits is to ensure that the CRF is filled out correctly, that the study protocol is adhered to, and that drug accountability is ensured. Monitor will not have direct access to the patient's electronical medical files. The signed consent form from the patient entails that information regarding health-related issues, private affairs and other confidential information can be passed on to monitor if needed for monitoring visits.
The Danish Medicine Agency will not have direct access to the electronical patient file but can be handed the same kind of information as stated above upon control visits. The mentioned information can only be handed to the GCP-unit and the Danish Medicine Agency by the responsible treating physician. The patients will be made aware that a signed informed consent form entails these issues.

Address of the GCP unit:
Copenhagen University Hospital
GCP-unit
Building 51, 3. Floor
Bispebjerg Bakke 23
2400 Copenhagen NV
Phone: +45 3331 3890

13 DATABASE HANDLING

Staff from the Danish Multiple Sclerosis Center handles the database.

14 STATISTICAL CONSIDERATIONS

14.1 Sample size estimation

The study aims to have 95% power to detect a 30% difference in the CSF concentration of NFL between patients treated with DMF and patients treated with placebo at a two-sided significance level of p=0.05. This requires 21 patients in each treatment arm if the study is enriched with patients who have ongoing neuroaxonal damage. This is ensured by including only patients with increased CSF concentrations of NFL at screening (>380 ng/L). The power calculation is based on the assumption that the standard deviation of change in NFL concentrations is approximately 30% in both arms, and that there is no significant regression towards the mean. These assumptions are supported by our observations in a serial study of patients with progressive MS; the results of the NAPMS study and the COMTIMS study. In order to account for a dropout rate of up to 20% (as observed in some clinical trials in RRMS), we plan to include 54 patients in the study.

14.2 Statistical methods

Analysis will be performed on the changes in the cerebrospinal fluid, clinical, self-reported and MRI outcome measures described in section 4.4. Descriptive statistics will be presented separately for patients randomized to treatment with dimethyl fumarate or placebo (numbers and percentages;

mean, standard deviation; and median, inter-quartile range for data not following a normal distribution). The analyses are performed on the change in each outcome measure over the span of 48 weeks using a general linear model with treatment allocation as factor and the baseline value of each variable as covariate.
Analyses will be done using intention to treat analysis of raw data and using multiple imputation (MI) of missing data. Results will be given with 95% confidence intervals and unadjusted p-values from the MI analyses. A total of 25 datasets will be produced. In case of variables that cannot be appropriately analyzed by a general linear model, logarithmic transformation of data will be applied. If this is still not successful, data in the two treatment arms will be compared by a nonparametric method (Mann-Whitney U-test).
The results of the statistical analyzes will be reported as advised in ICH E3 guidelines regarding structure and content in clinical study reports. A p-value of 0.05 will be used without correction for multiplicity.

1. Primary endpoint

- **Change from screening in the concentration of neurofilament light chain (NFL) measured in the cerebrospinal fluid (CSF)**
  At screening visit and week 48 visit, we will measure the concentration of neurofilament light chain (NFL) in the CSF by the use of a commercially available ELISA kit (UMAN diagnostics, see appendix D). The primary efficacy measure will be the difference in change of the concentration of NFL in the CSF between the treatment and placebo group from screening to week 48. The aim of the statistical analysis is to test the null hypothesis that the observed difference in the change of NFL between the treatment group and the placebo group is caused by the random allocation of patients. We will use a general linear model with treatment allocation as factor and baseline concentrations of neurofilament light chain as covariate. We will report the difference in change at the week 48 visit with 95% confidence intervals and a p-value. Change from screening will be reported in absolute values (ng/L) in compliance with the CONSORT statements[67].

2. Secondary Endpoints

2.1. Cerebrospinal Fluid (CSF) endpoints

2.1.1. Change from screening in myelin basic protein (MBP) concentration in the CSF

We will analyze the difference in change in MBP concentration from screening visit to week 48 between the treatment and placebo group using a general linear model with treatment allocation as factor and screening MBP concentration as covariate.

2.1.2. Change from screening in sCD27 concentration in the CSF

We will analyze the difference in change in sCD27 concentration from screening visit to week 48 between the treatment and placebo group using a general linear model with treatment allocation as factor and screening sCD27 concentration as covariate

2.1.3. Change from screening in BCMA concentration in the CSF

We will analyze the difference in change in BCMA concentration from screening visit to week 48 between the treatment and placebo group using a general linear model with treatment allocation as factor and screening BCMA concentration as covariate.

2.1.4. Change from screening in sCD14 concentration in the CSF

We will analyze the difference in change in sCD14 concentration from screening visit to week 48 between the treatment and placebo group using a general linear model with treatment allocation as factor and screening sCD14 concentration as covariate

2.1.5. Change from screening in CHI3L1 concentration in the CSF

We will analyze the difference in change in CHI3L1 concentration from screening visit to week 48 visit between the treatment and placebo group using a general linear model with treatment allocation as factor and screening CHI3L1 concentration as covariate

2.1.6. Change from screening in IgG-index

We will analyze the difference in change in IgG-index from screening visit to week 48 between the treatment and placebo group using a general linear model with treatment allocation as factor and screening IgG-index as covariate

2.1.7. Change from screening in CSF-serum albumin quotient

We will analyze the difference in change in CSF-serum albumin quotient from screening visit to week 48 between the treatment and placebo group using a general linear model with treatment allocation as factor and screening CSF-Serum albumin quotient as covariate.

2.2. Magnetic Resonance Imaging (MRI) Endpoints

2.2.1. Number of new or enlarging T2 lesions

We will analyze the number of new or enlarging T2 lesions from screening visit to W48 between the treatment and placebo group using negative binomial regression with the number of lesions at baseline as covariate.

2.2.2. Percent brain volume change (PBVC)

We will analyze the difference in percentage change in brain volume from screening visit to week 48 between the treatment and placebo group using a general linear

model with treatment allocation as factor and screening normalized brain volume as covariate.

2.2.3. Change from screening in fractional anisotropy (FA) in normal appearing white matter (NAWM)

We will analyze the difference in change from screening to W48 of FA in NAWM between the treatment and placebo group using a general linear model with treatment allocation as factor and the screening value of FA in NAWM as covariate.

2.2.4. Change from screening in in magnetization transfer ratio (MTR) of T2 lesions

We will analyze the difference in change from screening to W48 in MTR of T2 lesions between the treatment and placebo group using a general linear model with treatment allocation as factor and screening MTR of T2 lesions as covariate.

2.2.5. Change from screening in thalamic volume

We will analyze the difference in change from screening to W48 of thalamic volume between the treatment and placebo group using a general linear model with treatment allocation as factor and screening thalamic volume as covariate.

2.3. Clinical Endpoints:

2.3.1. Change from screening in Expanded Disability Status Scale (EDSS)

We will initially analyze the difference in EDSS change from screening visit to week 48 between the treatment and placebo group using a general linear model with treatment allocation as factor and screening EDSS as covariate.

2.3.2. Change from screening in Timed 25-Foot Walk (T25FW).

We will analyze the difference in T25FW change from screening visit to week 48 between the treatment and placebo group using a general linear model with treatment allocation as factor and the screening T25FW value as covariate.

2.3.3. Change from screening in Nine-Hole Peg Test (9HPT).

We will analyze the difference in 9HPT change from screening visit to week 48 between the treatment and placebo group using a general linear model with treatment allocation as factor and the screening 9HPT value as covariate.

2.3.4. SDMT (from the Brief International Cognitive Assessment for MS (BICAMS) panel)

We will analyze the difference in the SDMT change from screening visit to week 48 between the treatment and placebo group using a general linear model with treatment allocation as factor and the screening SDMT value as covariate.

3. Exploratory endpoints

The data will be analyzed separately for patients randomized to treatment with dimethyl fumarate or placebo. The analyses are performed on the change in each outcome measures over the span of 48 weeks using a general linear model (negative binomial regression for lesion numbers) adjusting for the baseline value of each factor.

Analyses will be done on raw data and using multiple imputation (MI). A total of 25 datasets will be produced. Results will be given with 95% confidence intervals and unadjusted p-values from the MI analyses.

3.1 Clinical endpoints

- Change from screening for Brief Visuospatial Memory Test Revised (BVMTR)
- Change from screening for California Verbal Learning Test 2 (CVLT-II)
    - Both tests are from the BICAMS panel. Each generates one outcome that will be analyzed separately

3.2 MRI-derived Endpoints

- Change from screening in number of Gd-enhancing lesions.
- Number of T2 new lesions
- Number of enlarging T2 lesions
- Change from screening in volume of cortical grey matter (CGM), normal appearing white matter (NAWM), and putamen volume
- Change in Magnetization Transfer Ratio (MTR) of CGM, NAWM, the putamen and thalamic nuclei
- Change in diffusion tensor imaging (DTI) measures (FA and mean diffusivity) of CGM, NAWM (except FA in NAWM), lesions, the putamen and thalamic nuclei
- Cross sectional area at C2 level of the cervical spinal cord

3.3 Self-reported outcome measures

- Urinary Distress Inventory (UDI)
- Fatigue Scale for Motor and Cognitive Function (FSMC)
- Multiple Sclerosis Impact Scale 29 (MSIS-29)

3.4 Correlation analyses of changes in primary and secondary outcome measures

The relationship between changes in primary and secondary outcome measures will be analyzed by exploratory Spearman rank correlation analyses. We will conduct these analyses separately for the DMF and placebo groups as well as for the combined group. Rank correlation coefficients lower than 0.5 are classified as low, 0.5-0.71 as moderate, and above 0.71 as high in the correlation analyses.

14.3 Sensitivity analyses

To test the robustness of the analyses we will conduct primary and secondary outcome analysis with unadjusted linear models, and we will test for highly statistically significant (P<0.01) heterogeneity between the compared groups; and adjust for such variable(s) in a linear model. We will compare the unadjusted analyses with the adjusted analyses to estimate the influence of covariates and thereby the robustness of the models.

The possible influence of missing data and extreme values (outliers) will be assessed by comparing analysis of the multiple imputation data set and the raw data and by exclusion of outliers from the analysis. A per protocol analysis will be conducted for participants who have taken at least 60% and 80% of the study medication. Furthermore, we will conduct analyses of the raw data in order to address whether these results differ from the results of the analyses based on multiple imputation.

14.4 Statistical description of analysis on data obtained after end of the open-label phase

Analysis will be performed on the changes in the clinical, self-reported and MRI outcome measures described in section 4.4. In addition, we will measure the changes in serum concentrations of neurofilament light chain (NFL).

The data will be analyzed separately for patients randomized to treatment with dimethyl fumarate, placebo and the initially non-randomized patients (included at week 48 due to >20% worsening in one or more clinical outcome measures. See section 5.1). This will yield two groups: one group with patients that has received DMF for 96 weeks and one where DMF treatment has been given for 48 weeks. Analysis will focus on the changes in outcome measures using a general linear model adjusting for the baseline value of each factor. Non-adjusted p-values will be used. In addition, focus will be on tolerability and safety of the study medication.

- Description of the effect of treatment with DMF from week 48-96 in patients randomized to placebo or DMF at baseline
- Description of the disease course from baseline to week 48 in patients fulfilling all inclusion criteria except the CSF NFL concentration criterion at screening
- Description of the effect of treatment with DMF from week 48-96 in those patients who show evidence of disease progression in spite of having a low NFL concentration in CSF at screening
- Description of serum NFL measures from screening, week 48 and week 96
- Time to EDSS worsening confirmed after 24 weeks and persisting at week 96 (performed every 24 weeks for patients enrolled at baseline)

14.4.1 Endpoints measured at week 96 (analyzed as difference in changes)

- **Serum endpoints**
  - Concentration of serum NFL at screening, week 48 and 96

15 ETHICAL CONSIDERATIONS

Dimethyl fumarate (DMF) is approved for treatment of MS. It is generally well tolerated, and side effects are rarely severe. Progressive multifocal leukoencephalopathy (PML) is seen extremely rarely during treatment with DMF but is most often described in the literature after longtime treatment and alongside severe lymphopenia. Safety hematology parameters will routinely be measured every 12 weeks and patients will be e neurologically examined for EDSS scoring every 24 weeks. All patients will be asked about new symptoms at visits and encouraged to contact the treating physician regarding new symptoms or suspected side effects outside trial visits.
In this trial, the placebo group will have the possibility of being treated with DMF to also gain the possible positive effects of treatment from week 48-96. There is no approved treatment for primary progressive MS other than symptomatic treatment. A positive outcome of this study will therefore have great impact on the treatment of these patients and the development of new-targeted treatments. Likewise, will a negative result also influence and help develop new treatment strategies. The trial will supply much needed information about patients with primary progressive MS, which is a group of patients not often considered in research.
All participant of the study will be thoroughly informed about the risk of known side effects, PML and the risk of unforeseen side effects when participating in a medical trial. The participants can at any time pull out of the study without any reprisals. The study is done in accordance with the Helsinki Declaration for medical research involving humans, ICH-GCP and after approval of the protocol from the Danish Medicine Agency and the Regional Scientific Ethical Committee. The possible beneficial effects of DMF treatment in primary progressive MS patients are estimated to outweigh the possible side effects. The rationale behind this study is underlined by the fact that there is no effective treatment for progressive MS and the result will be greatly beneficial no matter the outcome. There are no ethical arguments against the execution of this study.

15.1 Recruitment, informed consent, power of attorney and patient information

Recruitment happens through the responsible treating physicians from every MS clinic in Denmark. If a patient is interested in possibly participating in the trial and gives consent to have his or her contact information handed over to the sponsor or investigator for this trial then contact to the patient may follow. All trial related activity takes place at Rigshospitalet or Hvidovre Hospital (only MRI scans).
Ahead of any trial related activity, the investigator must inform the patient in word and in writing about the trial and make sure the patient understands the information. Patients will be contacted via letter containing the request form for participation in the trial and the participation information form. The pamphlet called “before you decide” from the Regional Scientific Ethical Committee and the document concerning the rights of patients participating in medical trials is also in the letter.
The letter contains information on how to reach the doctor responsible for the trial. If the patient is interested in participation or hearing more about the trial a meeting will be set. At the meeting the patient will be given oral information from a doctor about the trial and have ample opportunity to ask any questions that might have arisen. The information will be given by the doctor responsible for the

trial or an appointed doctor. If the patient feels that the information level is sufficient the written consent form can be signed. The written consent form will also be signed by the doctor who has given the oral information and the patient will be given a copy of the double signed consent form. Patients will be thoroughly informed that they have the right to further consideration of at least 24 hours before signing the consent form.
Investigator must make sure that the patient is fully aware and fully informed about the purpose of the trial, the procedures, the potential risks, foreseen discomforts and possible expected benefits. The information interview will take place in an interview room at the multiple sclerosis clinic and the patient will be encouraged to have another person with them for support.
Investigator will make sure to inform the patient that signing the informed consent entail that the GCP-unit and the Danish Medicine Agency can be handed information from their electronical medical files if necessary upon monitoring visits from the GCP-unit and upon control visits from the Danish Medicine Agency (see section 12 for detailed information).
It must be stated that the trial is done on a voluntarily basis and that the patient at any time can withdraw their written consent without any negative consequences to the further treatment at the multiple sclerosis center. The patient must voluntarily give informed consent and sign a consent form before any trial related procedure. Written consent will be obtained at the screening visit.
Patients are reinforced that they at all time can withdraw their consent without any negative reflection on their further treatment at the multiple sclerosis department.
A separate consent form for the biobank for future research purposes must also be signed by the patient and the doctor who gives the information.
Patients with progressive multiple sclerosis are easily fatigued. By sending al written material in one letter we enable the process to be with one less visit to the clinic. If the patient however, feels that he or she needs time to consider before signing the informed consent form one more visit most be established.

15.2 The Regional Scientific Ethical Committee

The Regional Scientific Ethical Committee must approve the protocol, the consent form and the patient information form before the trial is initiated.
The Regional Scientific Ethical Committee must approve any supplements to the protocol. It is investigators responsibility to obtain the necessary approvals and to store all reports to and correspondences with the committee in the Investigator File.
The agency will receive reports on serious adverse events, reactions (SAE/SAR) and SUSAR´s according to normal procedures during the trial.

15.3 The Danish Data Protection Agency

Investigator is responsible for getting approval from the Danish Data Protection Agency.

15.4 The Danish Medicine Agency

Sponsor must send the protocol to the Danish Medicine Agency. The agency must approve the protocol and any additional material before the trial is commenced. The agency will receive reports

on serious adverse events, reactions (SAE/SAR) and SUSAR´s according to normal procedures during the trial.

15.5 Financing and insurance

Biogen finances the trial with the amount of 6.678.875kr. The funds are used for compensation of medical staff, laboratory technicians and to pay for expenses of laboratory analyzes, other analyzes and MRI scans. Specific amount can be seen below. Biogen delivers the trial medication and the placebo. The finances are transferred from Biogen to a research account held by the department of neurology. Sponsor, investigator or any other staff involved in the trial will receive no compensatory payments and have no financial interest in Biogen. No personal fees are paid by Biogen to the involved staff. Biogen have no influence on the conduct, analyses or methods used in the trial. The patients in the trial are protected by law by: “Bekendtgørelse af lov om klage- og erstatningsadgang inden for sundhedsvæsenet” and: ”Bekendtgørelse af lov om produktansvar”.

Specifications of funds:

3%	laboratory analyses kits
3%	medicine management and possibly data management
32%	MRI scans
62%	Salary for personal in the laboratory, the doctors, study coordinator and project nurses

16 STUDY RESPONSIBILITY

Sponsor and investigator are responsible for:

- Development of the CRF
- Handling of data
- Statistical analyzes
- Development and submission of application to the Regional Scientific Ethical Committee
- Development and submission of application to the Danish Medicine Agency
- Development and submission of application to The Danish Data Protection Agency
- Archiving all essential documents in the TMF

The GCP-unit is responsible for:

- Monitoring of the trial

17 BIOBANK

A research biobank will be applied for in connection with this protocol. The application will be sent to the Regional Scientific Ethical Committee and the Danish Data Protection Agency. The purpose of the biobank is extracting and storing CSF during the project. The CSF will be used for analyzes of trial measures.
In addition to the trial related biobank a biobank for future research purposes will be established. This biobank for future research purposes will consist of the CSF that was not used for trial related measures.

Amount of CSF:

- Cerebrospinal fluid: 12ml. Taken twice during the study

12 ml is the amount of spinal fluid necessary to obtain a correct measurement of spinal protein levels [68].
Approximately 2ml of CSF will be used for trial related analysis and the rest will be transferred to the biobank for future research purposes. The patients will be asked to sign an independent consent form regarding the biobank for future research purposes.
The Regional Scientific Ethical Committee must grant permission before any analyses can be performed on material in the biobank for future research purposes. The material in this biobank will be kept for 10 years.
The CSF in the trial related biobank will be kept until all trial related analysis has been completed. Any leftover CSF can at that time be transferred to the biobank for future research purposes but only if the patient has signed the informed consent regarding this specific biobank. If the CSF cannot be transferred to the future biobank it will be destroyed in accordance to standardized procedures at Rigshospitalet.

18 DATA AND PUBLICATIONS

The trial is a single center trial and the results are analyzed and published by sponsor, investigator and other collaborating authors.
Sponsor and investigator have all the intellectual property rights of the data and will make sure that both negative, positive and inconclusive results are published.
The sponsor, all investigators and collaborating authors will have the possibility of reading and commenting on any script before a publication.
All data not published are considered confidential.
Upon cessation of the trial, the sponsor must report this to the Danish Medicine Agency and the Regional Scientific Ethical Committee within 90 days. For the Danish medicine Agency, the EudraCT file “End of Trial” is used and for the Regional Scientific Ethical Committee the "Skema til afrapportering ved afslutning af forsøg" will be used.
The Danish Medicine Agency and the Regional Scientific Ethical Committee must be notified within

15 days if the trial is stop before scheduled.
The result of the trial must be entered in the EudraCT within 1 year after trial cessation. After this, the data will be released at www.clinicaltrialsregister.eu. This replaces the need for a trial report to the Danish Medicine Agency but a full report can be requested by the agency.
The report must be sent to the Regional Scientific Ethical Committee if it is listed as a requirement in the approval of the trial from this agency.

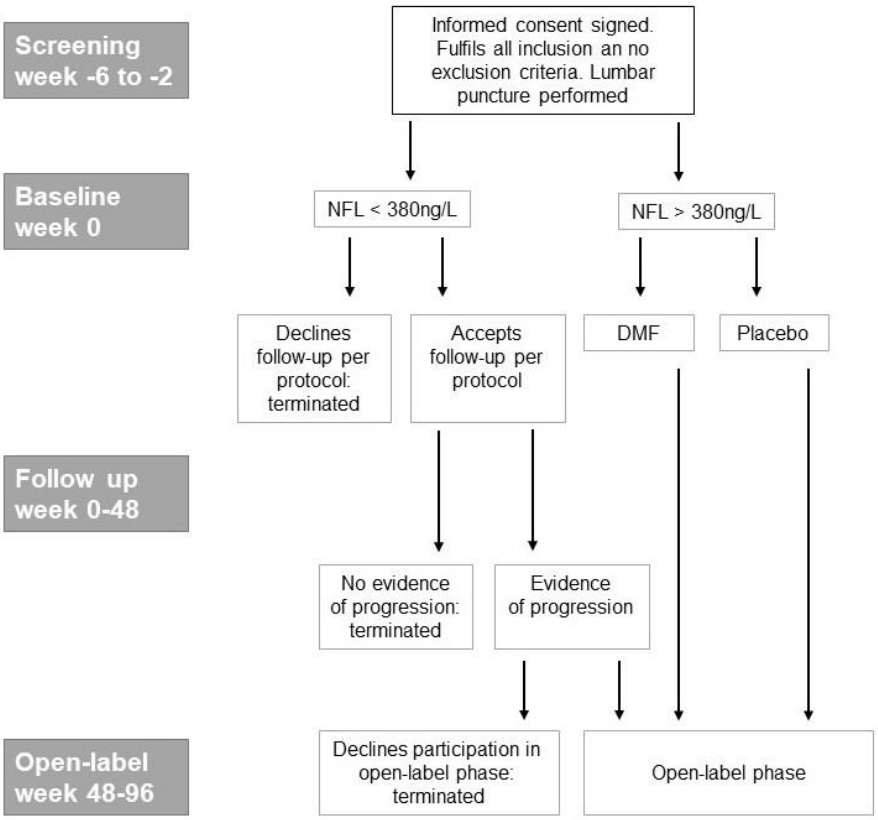
19 STORAGE OF CLINICAL TRIAL DOCUMENTS

Sponsor and investigator are responsible for storage of the list of patient identification and patient files for 10 years after cessation of the trial. Other documents related to the trial must be archived in the Investigator File and be kept for 10 years after cession of the trial. Likewise, all MRI scans are preserved for 10 years.
An access logged electronical file has been established by CIMT (Center for It, Medico og Telefoni). Only people from the neurological department at Rigshospitalet with permission from the investigator can be granted access. The data contained on paper is stored in a lockable office in a lock cabinet.

20 INSURANCE

Participating patients in this trial are insured by: “Bekendtgørelse af lov om klage- og erstatningsadgang inden for sundhedsvæsenet” and: ”Bekendtgørelse af lov om produktansvar”.

21 Appendix A: Study outline



22 Appendix B

	Screening **Week -6 to -2**	**Baseline** **Week 0**	**Week 12**	**Week 24**	**Week 36**	**Week 48**	**e**	**Week 72**	**Week 84**	**Week 96**
MRI	x					x				x
Lumbar puncture	x					x				
T25FW *2	x	x		x		x				x
9HPT*2	x	x		x		x				x
BICAMS*1	X					x				x
SDMT	x 2 random SDMT keys 1-3	x The lacking key from screening and then fixed key no. 4				x Fixed key no. 5				x Fixed key no. 6
UDI	x					x				x
FSMC	x					x				x
MSIS-29	x					x				x
EDSS	x			x		x		x		x
Blood samples	x	x (only s-hCG)	x	x	x	x	x	x	x	x

23 Appendix C

23.1 Handling and analyses of biological material

23.2 Lumbar puncture

23.2.1 Procedure

The lumbar puncture is performed by an experienced medical doctor at the screening visit. It is done using sterile technique with a 0.7mm syringe. The patient will be offered local anesthesia or this will be administered at the treating physician judgment.

23.2.2 Contraindication for lumbar puncture

The lumbar puncture cannot be performed if any of the following contra indications are present:

- Clinical suspicion of increases intracerebral pressure
- Suspected infection in the area of the puncture or clinical significant physical disease
- INR > 1,3
- Platelets < 100 million per ml blood
- The use of antithrombotic treatment other than platelet inhibitors

23.2.3 Handling of the cerebrospinal fluid (CSF)

12 ml of CSF is taken during the lumbar puncture. It is contained in a tube and iced. 1ml is send to analysis for cell count, albumin determination and IgG-index at the department of clinical biochemistry.

The rest of the CSF is handled in the laboratory of Neuroimmunology, where it is centrifuged. The cell free part of the CSF is divided into portions of 0,5ml and stored at minus 80 degrees.

Cells from the CSF are stored for the purpose of possible supplementary analyses as a biobank for future research purposes.

23.3 Cerebrospinal fluid analyses

23.3.1 CSF analyses at the department of clinical biochemistry

Routine analyses of cell count (white blood cells), determination of albumin content and IgG-index

23.3.2 CSF and serum analyses done in the laboratory of Neuroimmunology

Oligoclonal bands, CSF and serum neurofilament light chain (NFL), Chitinase-3-like-1 (CHI3L1), BCMA (TNFRSF17) sCD14, sCD27, and myelin basic protein (MBP)

23.3.3 Neurofilament light chain ELISA analyses

The concentration of neurofilament light chain (NFL) in CSF is analyzed by ELISA technique using a commercially available kit from Uman Diagnostics, Umeå, Sweden (NF-light® (Neurofilament light) ELISA). See Appendix D.

23.3.4 Serum NFL analyses
Concentrations of Neurofilament Light Chain is measured in serum using single-molecule array (SIMOA) analysis (Quanterix, USA, cat. no. 102258).

23.3.5 Chitinase-3-like-1 analyses
Chitinase-3-like-1 is tested in CSF using a validated in-house Luminex assay (R&D Systems).

23.3.6 sCD14 analyses
sCD14 is tested in the CSF using a validated in-house Luminex assay (R&D Systems).

23.3.7 sCD27 analyses
sCD27 is tested in the CSF using a validated in-house Luminex assay (R&D Systems).

23.3.8 Myelin basic protein (MBP) analyses
MBP is tested in CSF using a validated in-house ELISA (R&D Systems).

BCMA (TNFRSF17) analyses
BCMA is analyzed in the CSF using a validated in-house Luminex assay (R&D Systems).

23.4 Blood samples
Blood samples are taken 10 times during the trial. The safety blood samples are analyzed at department of clinical biochemistry at Rigshospitalet and hereafter destroyed in accordance to local standardized guidelines.

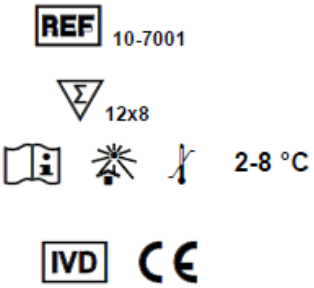
Appendix D

UmanDiagnostics
Rapid detection of brain disease

Instructions for Use

NF-light® (Neurofilament light) ELISA

Enzyme immunoassay for quantitative determination of human Neurofilament light (NF-L) protein in cerebrospinal fluid. The antibodies cross-react with Neurofilament light from rat, bovine and macaque sources and can be used for research on the above species.



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info@umandiagnostics.com
www.umandiagnostics.com

Instructions for use in other languages are available for direct download at company home page.

Dimethyl fumarate treatment of primary progressive MS (FUMAPMS)

Intended use

Enzyme Immunoassay for quantitative determinations of human Neurofilament light (NF-L) protein in cerebrospinal fluid. The antibodies cross-react with Neurofilament light from rat, bovine and macaque sources and can be used for research on the above species.

Introduction

The UmanDiagnostics NF-light® (Neurofilament light) assay is an enzymatic immunoassay designed for quantitative determinations of NF-light in human cerebrospinal fluid. The test uses two highly specific non-competing monoclonal antibodies (Norgren et al., 2002).

Neurofilaments are the main cytoskeletal constituents in neuronal cells. They are important for the maintenance of the axonal caliber and morphological integrity, which affects the velocity and fidelity of neuronal transmissions. Three different neurofilament chains exist, named according to their size. These are Neurofilament light, medium and heavy respectively. The Neurofilament light constitutes the backbone to which the heavier chains co-assemble, forming the neurofilament fibre (Lee et al., 1993). Following injuries of nerve cells due to direct trauma or slow degenerative processes, the content of the cell is released into the surrounding compartment allowing quantitative determinations of the axonal proteins. Increased levels of Neurofilament light have been found in various degenerative diseases such as Amyotrophic lateral sclerosis, Alzheimers disease, Multiple sclerosis and in animal models such as chronic experimental autoimmune encephalomyelitis (Rosengren et al., 1996; Norgren et al., 2003, 2004, 2005).

The assay is a 2-site solid phase sandwich ELISA. One specific monoclonal antibody is coated on a solid surface and binds Neurofilament light. Detection is performed by use of another specific conjugated monoclonal antibody. Quantitative determinations are performed by enzymatic turn-over of a colorless substrate to a colored product, which corresponds to the amount of Neurofilament light in the sample.

Measuring range:	100 pg/mL – 10000 pg/mL
Detection limit:	32 pg/mL
Precision:	Intra-assay CV% < 5
	Inter-assay CV% < 9
Incubation time:	2.5 hours
Sample size:	50 µL/replicate
Storage temperature:	2-8 °C Keep away from heat or direct sunlight
Shelf life:	18 months from date of production

Once opened the NF-light® strip plate should be used within 4 weeks. Make sure that opened strip plate is sealed to avoid humidity.

Warnings and precautions

1. For *in-vitro diagnostic* use only. For professional use only.
2. Before starting the assay, read the instructions for use completely and carefully. Use the valid version of the package insert provided with the kit. Make sure that everything is understood.
3. In case of severe damage of the kit package please contact your supplier in written form no later than one week after receiving the kit. Do not use damaged components. Please keep the damaged components stored for complaint related issues.
4. Do not mix reagents of different lots. Do not use expired reagents.
5. Please use the supplied 15 mL Sarstedt tube (62.554.502) when preparing the conjugate solution.
6. It is advised to run samples, controls and standards in duplicate. If large deviation occurs between replicates please re-assay.
7. Follow good laboratory practice and safety guidelines. Wear lab coats, disposable gloves and protective glasses when necessary.
8. Avoid contact with Stop reagent. It may cause skin irritations and burns. Material Safety Data Sheet for this product is available upon request directly from UmanDiagnostics.
9. Chemicals and prepared or used reagents have to be treated as hazardous waste according to national biohazard and safety guidelines or regulations.
10. Use an orbital ELISA table top shaker at 800 rpm.

Kit contents:

Content	Kit component	Quantity
	Plate cover	2 pcs
	15 mL tube for conjugate dilution	2 pcs
PLATE	Anti NF-light strip plate	12 x 8 wells
STOP	Stop reagent	1 x 6 mL
TMB	TMB substrate	1 x 12 mL
SAMDIL	Sample diluent	1 x 40 mL
CONDIL	Conjugate diluent	1 x 12 mL
CONJ	Conjugate concentrate	1 x 260 µL
50xTRAC	Tracer concentrate (50x)	1 x 260 µL
STAND	Bovine NF-L standard	2 vials
10xWASH	Wash buffer concentrate (10x)	2 x 40 mL

Chromogen and stop reagents
- Enzyme: Horseradish peroxidase (HRP)
- Substrate: Tetramethylbenzidine, TMB
- Stop reagent: H_2SO_4 8% v/v (**Warning corrosive**)

Not included essential equipment:
- Microtiter plate reader 450 nm (reference wavelength 620 - 650 nm)
- Micropipettes 10-1000 µL
- Vortex mixer
- Orbital ELISA table top shaker (800 rpm)
- Deionised water
- Wash bottle, automated or semi-automated microtiter plate wash system
- Absorbent paper, pipette tips and timer
- Polystyrene or polypropylene tubes for standard and sample dilution

⚠**Important information: Use only the supplied 15 mL tube when preparing the conjugate solution.**

Properties of the test

Types of samples

The NF-light® ELISA test is developed for analysis of cerebrospinal fluid samples and cannot in its present form be used for analysis of blood samples.

Temperature dependence

The assay has been developed for room temperature (20 – 25°C).

Measuring range

The standard curve covers the interval 100 -10000 pg/mL NF-L (see curve on page 5). Extrapolation beyond the curve is not allowed with the implication that samples outside the curve have to be further diluted and re-measured.

Reagent preparation

Wash buffer

Dilute the total content of one 10x wash buffer concentrate (10xWASH) bottle with deionised water to a final volume of 400 mL. Diluted unused wash buffer can be stored at room temperature and should be used within two months. The 10x wash concentrate can appear opalescent due to high salt concentration (no effect on assay performance).

Standards

Reconstitution and preparation of standard dilution series should be performed directly before use. The highest standard point (10000 pg/mL) is obtained by reconstituting one vial of lyophilized standard (STAND) with the volume of sample diluent indicated on the bottle label. Vortex briefly and keep in room temperature. Label 6 micro-tubes, one for each standard point (that is 5000 pg/mL, 2500 pg/mL, 1000 pg/mL, 500 pg/mL, 100 pg/mL and 0 pg/mL). Dilute the standard according to the dilution table below:

Standard dilution:

Tube no.	Sample Diluent (SAMDIL)	Standard from tube no.	Concentration pg/mL
Vial	Reconstitute with sample diluent (SAMDIL) according the standard vial label		10000
1	300 µL	300 µL (vial)	5000
2	300 µL	300 µL (1)	2500
3	360 µL	240 µL (2)	1000
4	300 µL	300 µL (3)	500
5	240 µL	60 µL (4)	100
6	300 µL	0 µL	0

Reconstituted standard should be used immediately and can not be re-used.

Tracer

Directly before use, dilute the concentrated tracer (50x TRAC) (biotin labeled anti NF-L mAb) 1:50 with sample diluent (SAMDIL). Mix thoroughly by inverting the tube or by vortexing.

Conjugate

Directly before use, dilute the concentrated conjugate (CONJ) (Streptavdin-HRP conjugate concentrate) in the supplied Sarstedts 15 mL tube according to the vial label with conjugate diluent (CONDIL). Mix thoroughly by inverting the tube or by vortexing.

Assay instructions

All assay reagents should be brought to room temperature prior to use.
After each washing cycle the plate should be tapped dry against absorbent paper.
Agitation of the plate at 800 rpm is of HIGH IMPORTANCE.

1. Dilute the CSF samples with equal amount (1+1) of sample diluent (SAMDIL) to a total minimum volume of 210 µL. The standards reconstituted and diluted according to the standard dilution table are ready to use (i.e. no further dilution should be made).
2. Wash the wells to be used with wash buffer (3x300 µL). The wash buffer added could be either aspirated or removed by knocking the plate against absorbing material immediately before next washing cycle.
3. Add 100 µL of each standard and sample in duplicate. Incubate 1 hour at room temperature (20-25°C) with agitation (800 rpm).
4. Wash the wells with wash buffer (3x300 µL), see point 2.
5. Add 100 µL of freshly diluted tracer antibody to each well. Incubate 45 minutes at room temperature (20-25°C) with agitation (800 rpm).
6. Wash the wells with wash buffer (3x300 µL), see point 2.
7. Add 100 µL of newly diluted conjugate to each well. Incubate 30 minutes at room temperature (20-25°C) with agitation (800 rpm).
8. Wash the wells with wash buffer (3x300 µL), see point 2.
9. Add 100 µL of TMB to each well. Incubate 15 minutes at room temperature (20-25°C) with agitation (800 rpm).
10. Add 50 µL of stop reagent (STOP) to each well and read the absorbance at 450 nm (reference wavelength 620-650 nm).

⚠ **The stop reagent contains diluted sulfuric acid and is corrosive.**

Changes in performance or assay characteristics

Any putative changes in performance and assay characteristics will be described in the instructions for use specific for each kit.

Samples

The CSF should not be contaminated with blood. All patient samples should be considered potentially contagious. After lumbal punction the samples should be kept at -80°C in polypropylene tubes. Repetitive freeze/thawing should be avoided.

Sample stability

The sample stability was evaluated in 5 different clinical samples. The sample reactivity following different treatments was compared to the same sample stored at -80°C.

		Mean % of -80°C control	Mean % range
Freeze-thawing	≤ 4 cycles	98	96-101
Storage	5-8 °C ≤ 1 week	99.7	95-108
	24 h at RT (22°C)	100	91-106
	-20°C 1 month	95.8	89-109

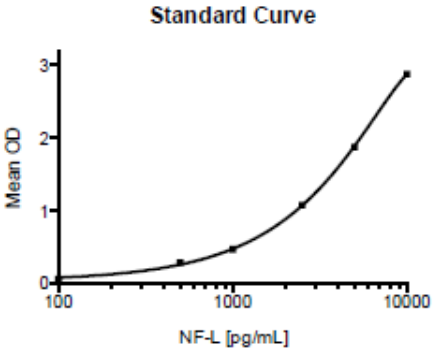
Results

The results can be calculated automatically by using an immunoassay software package. The 4-parameter Marquardt transformation provides the best curve fit (see a typical standard curve below). If no such immunoassay software is available, the concentration of NF-L is calculated from plotting average OD at (λ450 minus λ620) against the known standard concentrations.

All test levels are obtained directly from the immunoassay software package, or from the manually plotted curve. Samples displaying concentrations above the highest standard point needs to be further diluted and

re-assayed. When assaying a sample with expected levels above the standard curve, the sample should first be diluted with an equal amount of sample diluent according to point 1 in assay instructions. This should be followed by a second appropriate dilution to allow the concentration to reach levels covered by the standard curve. The concentration read from the curve needs to be multiplied by the dilution factor applied in the second step. Best results are usually obtained when omitting measurement in the far low and high standard range.

⚠ **The values obtained from the standard curve correspond directly to the NF-L level.**



4-parameter Marquardt transformation

Expected values

CSF from 50 apparently healthy subjects with no known sign of neurological disease was examined. Normal values were measured between 112 and 821 pg/mL. The healthy subjects were divided into tree age groups and the reference levels were defined as mean NF-L level + 2 SD.

Age	Reference level
< 30 years	< 290 pg/mL (n=17)
30 – 39 years	< 380 pg/mL (n=15)
40 - <60 years	< 830 pg/mL (n=18)

The results are only valid if the test has been performed according to the instructions for use and must be correlated to other clinical observations and diagnostic tests. The user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable standards/laws. It is recommended that each laboratory establish its own range of normal values and use samples with known Neurofilament light levels as internal controls.

Performance

Analytical Sensitivity (Limit of Detection)	32 pg/mL		LOB+1.645x SD (100 pg/mL standard)	
Precision	Low (250 pg/mL)		High (5000 pg/mL)	Mean Precision
Intra-assay	4.8 % CV		3 % CV	4 % CV
Inter-assay	9.1 % CV		3 % CV	6 % CV
Recovery (after spiking)	250 pg/mL	500 pg/mL	2500 pg/mL	Mean Recovery
	88.4 %	91 %	98.7 %	93 %
Linearity	Range	Serial dilution	Range	Mean Recovery
	33-14740 pg/mL	1:128	93.4 – 109 %	99.8 %

Warranty

The performance data presented here was obtained using the procedure indicated. Any change or modification in the procedure not recommended by UmanDiagnostics AB may affect the results, in which event UmanDiagnostics AB disclaims all warranties, expressed, implied or statutory, including the implied warranty of merchantability and fitness for use. UmanDiagnostics AB and its authorized distributors, in such event, shall not be liable for any damages, whether direct, indirect or consequential.

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Symbols / Symbole / Symboles / Simboli / Símbolos / Σύμβολα

REF	Cat.-No.: / Kat.-Nr.: / No.- Cat.: / Cat.-No.: / N.ª Cat.: / N.–Cat.: / Αριθμός-Κατ.:
	Use by: / Verwendbar bis: / Utilisé à: / Usado por: / Usar até: / Da utilizzare entro: / Χρησιμοποιείται από:
LOT	Lot-No.: / Chargen-Bez.: / No. Lot: / Lot-No.: / Lote N.º: / Lotto n.: / Αριθμός -Παραγωγή:
	No. of Tests: / Kitgröße: / Nb. de Tests: / No. de Determ.: / N.ª de Testes: / Quantità dei tests: / Αριθμός εξετάσεων:
IVD	In Vitro Diagnostic Medical Device. / In-vitro-Diagnostikum. / Appareil Médical pour Diagnostics In Vitro. / Dispositivo Médico para Diagnóstico In Vitro. / Equipamento Médico de Diagnóstico In Vitro. / Dispositivo Medico Diagnostico In vitro. / Ιατρική συσκευή για In-Vitro Διάγνωση.
	Read instructions before use. / Arbeitsanleitung lesen. / Lire la fiche technique avant emploi. / Lea las instrucciones antes de usar. / Ler as instruções antes de usar. / Leggere le istruzioni prima dell'uso. / Διαβάστε τις οδηγίες πριν την χρήση.
	Keep away from heat or direct sun light. / Vor Hitze und direkter Sonneneinstrahlung schützen. / Garder à l'abri de la chaleur et de toute exposition lumineuse. / Manténgase alejado del calor o la luz solar directa. / Manter longe do calor ou luz solar directa. / Non esporre ai raggi solari. / Να φυλάσσεται μακριά από θερμότητα και άμεση επαφή με το φως του ηλίου.
	Store at: / Lagern bei: / Stocker à: / Almacene a: / Armazenar a: / Conservare a: / Αποθήκευση στους:
	Manufacturer: / Hersteller: / Fabricant: / Productor: / Fabricante: / Fabbricante: / Παραγωγός:
	Caution! / Vorsicht! / Attention! / Precaución! / Cuidado! / Attenzione! / Προσοχή!

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