


RESEARCH ARTICLE

Blood neurofilament light as a potential endpoint in Phase 2 studies in MS

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Introduction

In the development of treatments for multiple sclerosis (MS) magnetic resonance imaging (MRI) measures of inflammatory activity have played a central role as highly predictive endpoints of proof of concept Phase 2 clinical trials. Treatment effects on these MRI measures have been highly predictive of effects on relapses in larger pivotal Phase 3 trials.^{1,2} However, the relationship between conventional MRI measures (new or enlarged T1- and T2-weighted and contrast-enhanced T1-weighted lesions) and neuroaxonal damage, a major determinant of permanent disability in MS,³ is not clearly defined or fully supported

by clinical experience. Repeated cranial MRI examinations do not cover the spinal cord, increase costs in clinical trials and may become burdensome to patients as most Phase 2 clinical trials in MS require monthly MRI scanning. Several candidate laboratory markers studied in MS lack relation to important disease processes, such as neuroaxonal damage, or prognostic value for important outcomes, such as long-term disability.⁴ Neurofilament light chains (NfL) have gained attention among different potential markers because of their specificity for neuroaxonal damage and being measurable both in CSF and peripheral blood.^{5–8} With the recent development of Single Molecule Array (SIMOA) immunoassays, NfL levels in

Abstract

Objectives: To assess whether neurofilament light chain (NfL) could serve as an informative endpoint in Phase 2 studies in patients with relapsing–remitting multiple sclerosis (RRMS) and estimate the sample size requirements with NfL as the primary endpoint. **Methods:** Using data from the Phase 3 FREEDOMS study, we evaluated correlation of NfL at Month 6 with 2-year outcomes: relapses, confirmed disability worsening (CDW), new or enlarging T2 lesions (active lesions), and brain volume loss (BVL). We compared the proportion of treatment effect (PTE) on 2-year relapses and BVL explained by 6-month log-transformed NfL levels with the PTE explained by the number of active lesions over 6 months. We estimated sample size requirements for different treatment effects. **Results:** At Month 6, blood NfL levels (pg/mL, median [range]) were lower in the fingolimod arm (fingolimod ($n = 132$) 18 [8–247]; placebo ($n = 114$) 26 [8–159], $P < 0.001$). NfL at 6 months correlated with number of relapses ($r = 0.25$, $P < 0.001$), 6-month CDW (hazard ratio 1.83, $P = 0.012$), active lesions ($r = 0.46$, $P < 0.001$), and BVL ($r = -0.41$, $P < 0.001$) at Month-24. The PTE (95% CI) on 24-month relapses and BVL explained by 6-month NfL was 25% (8–60%) and 60% (32–132%), and by 6-month active lesions was 28% (11–66%) and 45% (18–115%), respectively. Assuming a 20–40% treatment-related reduction in NfL levels, 143–28 patients per arm will be required. **Conclusions:** Blood NfL may qualify as an informative and easy-to-measure endpoint for future Phase 2 clinical studies that captures both inflammatory- and noninflammatory-driven neuroaxonal injury in RRMS.

blood can be reliably assessed in the full range of possible concentrations and have shown high correlation with CSF NfL levels^{9,10} and with clinical and MRI-related outcomes.^{9–16}

Our study aimed to assess whether NfL could serve as a valid and informative endpoint in Phase 2 clinical trials in relapsing–remitting MS (RRMS), in conjunction with or as an alternative to MRI-based outcomes, and to estimate the sample size requirements for a Phase 2 study with NfL as the primary endpoint.

Methods

Patients and study design

This is a post hoc analysis of data from the placebo-controlled FREEDOMS study. The study design and inclusion/exclusion criteria of the FREEDOMS trial have been previously described.¹⁷ Briefly, FREEDOMS included patients with RRMS (diagnosed according to the 2005 revised McDonald criteria¹⁸) aged 18–55 years who had a score of 0–5.5 on the Expanded Disability Status Scale (EDSS) and ≥ 1 documented relapses in the previous year or ≥ 2 relapses in the previous 2 years. Patients were randomized (1:1:1) to receive fingolimod 0.5 or 1.25 mg/day or placebo for 2 years.¹⁷ Serum NfL levels were measured in a subset of 246 patients enrolled in the FREEDOMS study who had at least two measurements of NfL (at baseline and Month 6; fingolimod 0.5 mg, $n = 132$; placebo, $n = 114$).

Assessments

In the FREEDOMS study, standardized neurological assessments, including determination of EDSS score¹⁹, were conducted at baseline and every 3 months by neurologists blinded to randomization and not further involved in patient care. MRI scans were obtained at baseline and Months 6, 12, and 24, or at the end of the study for patients discontinuing prematurely.¹⁷

Relapses had to be verified by the examining neurologist within 7 days after the onset of symptoms according to defined criteria.¹⁷ MRI lesion activity and brain volume loss (BVL) were assessed by a central reading site (Medical Image Analysis Center, Basel, Switzerland) that remained blinded for clinical data and randomization. MRI protocols and analysis methods have been detailed elsewhere.¹⁷

The percentage brain volume change (PBVC) was assessed using Structural Image Evaluation using Normalization of Atrophy (SIENA).²⁰ Disability worsening was defined as an increase in the EDSS score by ≥ 1 point sustained for ≥ 6 months (or ≥ 1.5 points if baseline EDSS = 0).

Blood samples were collected from consented patients at baseline and Months 6, 12, 18, and 24. NfL measurements were not available for all patients who participated in the FREEDOMS study, as not all patients consented for the biomarker analysis, which required a separate consent. Our analysis included only a subset of patients from the FREEDOMS study with the selection criteria being availability of NfL measurements for both baseline and Month 6 ($N = 246$) to replicate the time-points for a Phase 2 trial. There was no adjustment done for missing data. All available blood samples were analyzed in a blinded manner; clinical data and treatment allocation were not disclosed to the laboratory personnel. The concentration of NfL in plasma samples treated with ethylenediaminetetraacetic acid were measured by an in-house SIMOA immunoassay.¹⁰

Statistical analysis

NfL measurements: For our study, data were analyzed in the intent-to-treat population, defined as all individuals with NfL values available at baseline and Month 6 ($n = 246$). NfL levels were log-transformed to normalize their skewed distribution.

The primary aim of this study was to assess the validity of NfL as a biomarker to be used as a primary endpoint in Phase 2 trials. With this aim, we assessed the following properties of blood NfL as measured at Month 6:

- 1 *Do NfL levels allow detection of a treatment effect at Month 6?* For this assessment, a repeated measures Analysis of Variance was run, with log-transformed NfL levels at baseline and Month 6 as the dependent variables and treatment arm as the independent variable. The significance of the treatment by time interaction coefficient in the model was used to assess whether patients treated with fingolimod had a higher reduction of NfL levels compared to the placebo arm.
- 2 *Do 6-month NfL levels correlate with established longer term (24 months) disease-related endpoints (number of new or enlarged T2 lesions [active lesions], PBVC, number of relapses over 2 years and risk of 6-month confirmed disability worsening, CDW):* These correlations were compared to those between active lesions at 6 months (the standard Phase 2 endpoint in MS clinical trials) and the same disease endpoints at 24 months. Correlations of 6-month measures (NfL and active T2 lesions) with 24-month active lesions, PBVC, and number of relapses were assessed by the nonparametric Spearman coefficient. The association of the risk of 6-month CDW with 6-month measures (NfL and active lesions) was assessed by a Cox model with the independent covariates included in the model

as continuous variables and displayed by Kaplan–Meier survival curves with the independent covariates as binary variables (comparing those with 6-month NfL ≤ 30 pg/mL vs. those with 6-month NfL > 30 pg/mL, and those with 6-month active lesions = 0 vs. those with 6-month active lesions > 0). The NfL cut-off level of 30 pg/mL is close to the geometric mean observed in RRMS patients.^{21–24} The multivariate models for assessing the simultaneous impact of NfL and active T2 lesions at 6 months were a generalized Poisson model for 24-month relapses, an ANOVA model for 24-month PBVC and a Cox model for 6-month CDW.

3 What is the contribution of NfL and active lesions as surrogate markers to the treatment effect on other disease endpoints: For a quantitative assessment of this contribution, an additional measure - the proportion of treatment effect (PTE) on Month 24 relapses, PBVC, and disability worsening that could be attributed to the effects on NfL and active lesions at Month 6 was calculated according to Lin, Fleming and De Gruttola (Table S1).²⁵ For a perfect surrogate marker, the PTE would be 100%, indicating that the marker mediates the full effect of the treatment; a PTE equal to 0% would reflect the absence of surrogacy.

Finally, we estimated the sample size needed for a Phase 2 study based on an evaluation of 6-month log-transformed NfL concentrations, assuming different treatment effect sizes.

Data availability

The current analysis included data from patients who had provided blood samples during a Phase 3 clinical trial (FREEDOMS). Any data not provided in the article, including statistical analyses, assumptions and de-identified NfL levels may be shared at the request of other investigators.

Results

Baseline characteristics of the 246 patients included in this study are shown in Table 1 and are well comparable to the whole FREEDOMS population,¹⁷ with no significant differences detected. At baseline, the mean (SD) NfL levels was 39.0 (43.8) pg/mL in the placebo and 40.0 (46.7) pg/mL in the fingolimod arm. After 6 months, mean (SD) NfL levels were 31.9 (23.8) pg/mL in the placebo and 23.1 (23.6) pg/mL in the fingolimod arm (Fig. 1, values on a log-scale). The median percentage decrease in NfL levels in the fingolimod arm (-33.5%) was significantly higher than the decrease in NfL in the placebo arm (-5.4% , $P < 0.001$).

The 6-month NfL level was associated with the following disease variables at Month 24 (Table 2 and Fig. 2): number of relapses ($r = 0.25$, $P < 0.001$), cumulative risk of 6-month CDW (hazard ratio [HR] = 1.83, $P = 0.012$; the HR indicating the increase in risk of CDW for 10 pg/mL increase in NfL at Month 6; HR = 2.08, $P = 0.023$ when comparing patients with 6-month NfL higher or lower than 30 pg/mL), cumulative number of active lesions ($r = 0.46$, $P < 0.001$), and PBVC ($r = -0.41$, $P < 0.001$). Similar correlation levels were observed between 6-month active lesions and number of relapses ($r = 0.23$, $P < 0.001$), cumulative risk of 6-month CDW (HR = 1.03, $P = 0.012$; HR indicating the increase in hazard of CDW for each additional active lesions on the 6-month scan) and PBVC ($r = -0.30$, $P < 0.001$) at Month 24. Only the correlation with 24-month active lesions was higher for 6-month active lesions ($r = 0.78$, $P < 0.001$) than for 6-month NfL. Interestingly, changes in NfL levels (both absolute and percentage) were, at best, very weakly associated with all disease activity endpoints at Month 24 (Table 2). In the three separate multivariate models testing simultaneous impact of NfL and active T2 lesions at 6 months on relapses, PBVC, and disability worsening at Month 24 (as the dependent variables), respectively, NfL appeared to be a better predictor for these outcomes than active lesions (Table 3).

The PTE (95% CI) on 24-month relapses explained by 6-month NfL was 25% (8–60%); 6-month active lesions explained the same PTE on relapses (28%, 11–66%). The PTE on 24-month PBVC explained by 6-month NfL was 60% (32–132%), while the PTE on PBVC explained by 6-month active T2 lesions was 45% (18–115%). The PTE explained by active lesions and NfL at Month 6 on the risk of disability worsening over 2 years was 16% (0–56%) and 8% (0–32%), respectively.

Table 4 reports the estimated sample sizes for a Phase 2 clinical trial with blood NfL as the primary endpoint measured at baseline and Month 6 based on different assumptions about the treatment effect. For a standard Phase 2 study, with 90% power and a 5% significance level, 38 patients per arm would be needed to detect a reduction of NfL levels by 35% at 6 months (the percentage obtained for fingolimod vs. placebo in our study was 33.5%). For a treatment effect of 30% or lower, the estimated sample sizes range from 54 to 143 patients per arm, e.g., close to the typical sample sizes of Phase 2 trials in RRMS with MRI lesions as the primary endpoint.

Discussion

In this study, our focus was to assess the potential of blood NfL as a primary endpoint in Phase 2 clinical trials in RRMS, extrapolating from blood NfL measurements and clinical and MRI data obtained in FREEDOMS¹⁷, a

Table 1. Baseline characteristics*.

Baseline variables	NfL subset		FREEDOMS study	
	Placebo	Fingolimod	Placebo	Fingolimod
N	114	132	418	425
Age, years, mean (range)	38 (19–54)	37 (19–54)	37 (18–55)	37 (18–55)
EDSS score, median (range)	2 (0–5.5)	2.25 (0–5.5)	2 (0–5.5)	2 (0–5.5)
Duration of disease, years, median (range)	7.0 (0.4–23)	6.7 (0.5–29.2)	7.0 (0–32)	6.6 (0–35)
Sex, female, %	75	69	71	70
T2LV, mm ³ , median (range)	3237 (0–32011)	3303 (34–43750)	3416 (0–37148)	3303 (0–47148)
NBV, cm ³ , mean (SD)	1504 (85)	1524 (82)	1512 (85)	1521 (83)
NfL, pg/mL				
Mean (SD)	39.0 (43.8)	40.0 (46.7)		
Median (range)	25.9 (8.6–379.8)	27.7 (8.4–419.4)		

EDSS, Expanded Disability Status Scale; NBV, normalized brain volume; NfL, neurofilament light chain; SD, standard deviation; T2LV, T2 lesion volume.

*No significant differences in any of the baseline characteristics were detected between the group of patients who had NfL assessed versus the other patients included in the FREEDOMS study.

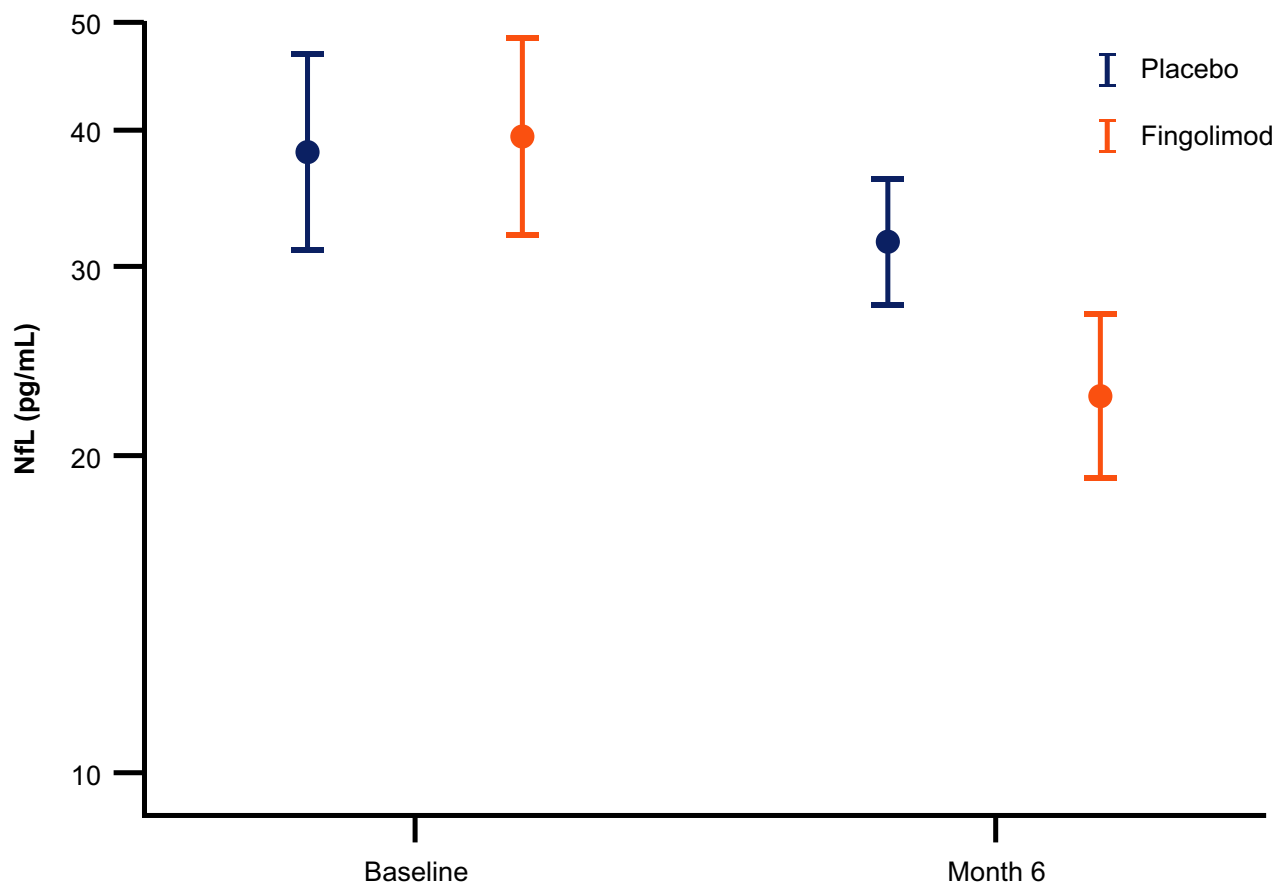


Figure 1. Mean NfL levels (log-transformed) at baseline and Month 6 in patients treated with placebo (blue) and fingolimod (orange). NfL, neurofilament light chain.

2-year placebo-controlled Phase 3 trial of fingolimod in patients with RRMS. Phase 2 studies are key to the development of new drugs and need outcomes that allow

detection of a treatment effect in a short time and on a small number of subjects. Therefore, primary outcomes of Phase 2 studies must be sensitive to change but also

reliable and meaningful e.g., related to and predictive of other established and clinically relevant outcomes.

Our post hoc analysis provides several lines of evidence that blood NfL measurements compare favorably with established MRI-based outcomes of phase 2 studies in relapsing MS: blood NfL levels at 6 months were sensitive to treatment effects showing significantly lower levels in

those treated with fingolimod; blood NfL at Month 6 correlated with other established clinical and MRI-based outcomes cross-sectional and – more importantly – independently predicted these clinical and MRI-based outcomes at month 24, and mediated part of the net effect of the treatment on the relevant clinical endpoints. Taking into consideration the above findings, blood NfL

Table 2. Spearman correlations of NfL levels (and change from baseline) and number of active T2 lesions at month 6 with 24-month new or enlarging T2 lesions, PBVC and relapses.

6-month variables	24-month variables ^r (P value)		
	New or enlarging T2 lesions	Percent brain volume change	Number of relapses
NfL	0.46 (<0.001)	−0.41 (<0.001)	0.25 (<0.001)
NfL absolute change	0.08 (0.25)	0.16 (0.02)	−0.04 (0.52)
NfL percentage change	0.13 (0.05)	0.09 (0.22)	0.00 (0.99)
Active T2 lesions	0.78 (<0.001)	−0.30 (<0.001)	0.23 (<0.001)

NfL, neurofilament light chain; PBVC, percentage brain volume change; *r*, correlation coefficient.

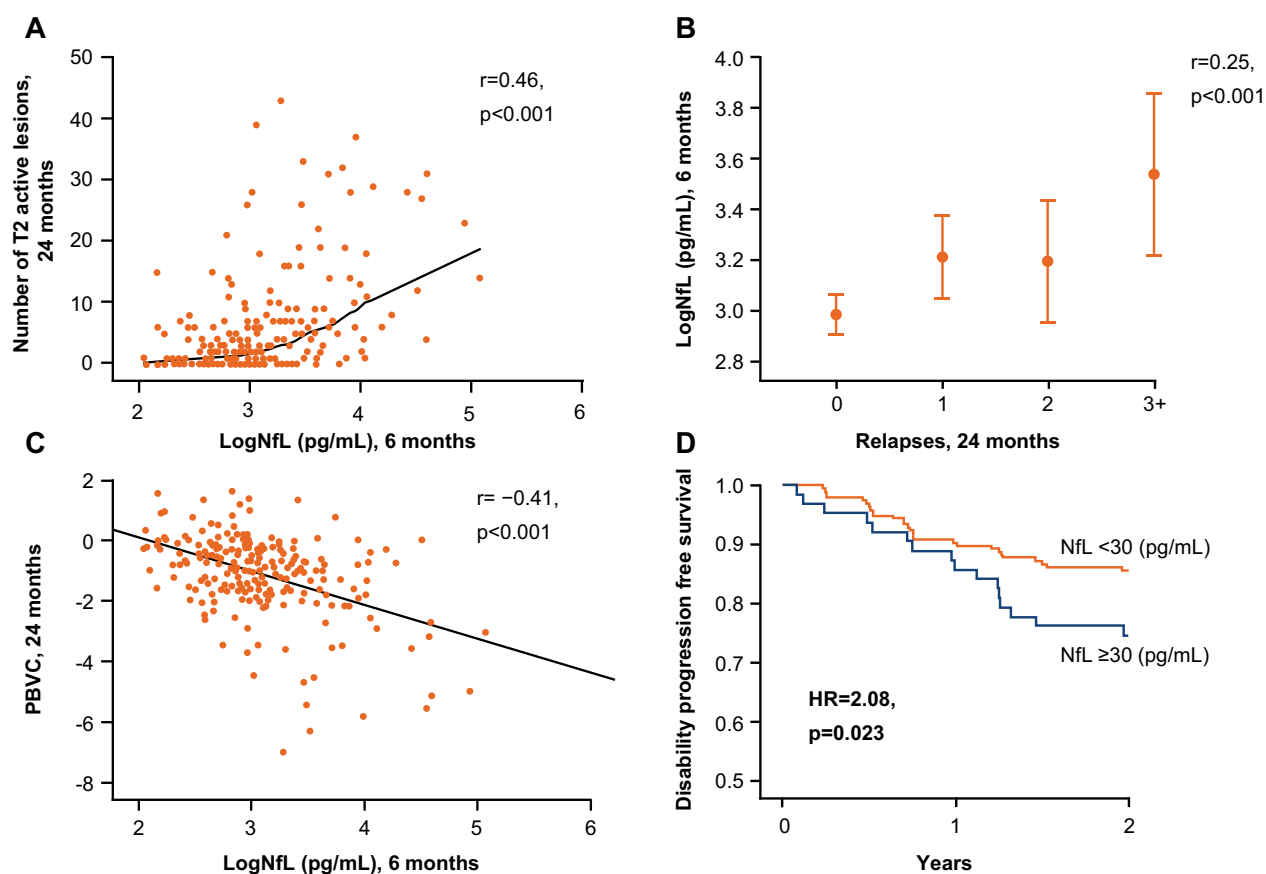


Figure 2. Spearman Correlations of 6-month log-transformed NfL levels with 24-month disease outcomes: Number of new or enlarging T2 lesions at Month 24 (A), number of relapses experienced over 24 months (B), Percent Brain Volume Change over 24 months (C) and the risk of 6-month confirmed disability worsening (D). HR, hazard ratio; NfL, neurofilament light chain; PBVC, percentage brain volume change; *r*, correlation coefficient. The HR reported in the figure refers to the Cox model run with neurofilament light chain included as a binary variable as explained in the legend.

Table 3. Multivariate models for assessing the predictive value of 6-month measures (neurofilament light chain and new or enlarging T2 lesions) on 24 month outcomes.

Effect of 6 month-NfL and new or enlarging T2 lesions on 24-month relapses (Poisson model)					
Variable	Unit of measure	Relative risk	95% confidence interval		P value
NfL	Log scale	1.75	1.36	2.26	<0.001
T2 active lesions	Number	1.01	0.99	1.02	0.31
Effect of 6-month NfL and new or enlarging T2 lesions on 24 month-PBVC (ANOVA model)					
Variable	Unit of measure	beta	95% confidence interval		P value
NfL	Log scale	-1.09	-1.41	-0.76	<0.001
T2 active lesions	Number	-0.01	-0.04	0.14	0.38
Effect of 6 month-NfL and active T2 lesions on 24-month disability worsening confirmed at 6 months (Cox model)					
Variable	Unit of measure	Hazard ratio	95% confidence interval		P value
NfL	Log scale	1.64	0.98	2.75	0.06
T2 active lesions	Number	1.02	0.99	1.04	0.21

ANOVA, Analysis of variance; NfL, neurofilament light chain; PBVC, percentage brain volume change.

Table 4. Sample size needed for a Phase 2 clinical trial with NfL levels measured at Month 6 as the primary endpoint (90% power, 5% significance level).

Number of subjects per arm	
Treatment effect*	Sample size (per arm)
20%	143
25%	83
30%	54
35%	38
40%	28

NfL, neurofilament light chain.

*Treatment effect is expressed as a percentage reduction of 6-month NfL levels in the experimental versus control arm.

qualifies as a good biomarker to be used as a primary endpoint in phase 2 trials.

In our study, we found no or weaker correlations of NfL changes (absolute and/or percentage) at 6 months with 24-month outcomes. There are contradictory reports on the association of changes in NfL with disease outcome. In a randomized controlled study, Kuhle et al. found that over 24 months, changes in NfL levels positively correlated with changes in EDSS score and enhancing lesions, but not with brain atrophy or T2 lesion volume.¹¹ Blood NfL levels also did not correlate with disease duration.^{10,24} A clear understanding of NfL homeostasis and clearing mechanisms and how NfL levels in blood relate to the progressive changes in MS disease pathology does not exist.

However, a number of studies have shown that blood NfL levels are significantly higher in patients with MS and

were reduced after treatment with fingolimod,²⁶ natalizumab²⁷, and rituximab²⁸ in CSF but also in several observational studies in the blood.^{10,12,14,16} NfL levels at baseline correlate with clinical (such as a recent relapse and an EDSS score) as well as MRI-related measures (such as T2 lesion volume and number of gadolinium-enhancing lesions and brain volume loss).^{10,29} High NfL levels in blood were also associated with higher risk of future relapses and EDSS worsening.^{10,16} Blood NfL levels predicted disability worsening after up to 8 years and lesion load and atrophy after 10 years in patients with MS.^{29,30} Notably, NfL predicted future brain and spinal cord atrophy.^{13,16} The PTE measures provide a quantitative description of the level of surrogacy. Our results show that NfL is a suitable surrogate for the clinical and MRI outcomes and not inferior to MRI lesions as a surrogate of these outcomes. NfL levels in blood tended to be better predictors of effects on BVL than active lesions in our study. This finding is particularly important as BVL is a comprehensive marker of both focal and diffuse damage and is predictive of future disability worsening and disease progression on the group level.

The findings from our study have additional relevance. Up to now, phase 2 clinical studies have used imaging endpoints that relates to acute disease activity (such as Gd + lesions and relapses) to assess the effect of candidate treatments within the study duration. These MRI lesion-based endpoints have shown a correlation with relapses in both short-term (6–9 months, typical duration of Phase 2 trials) and mid- to long-term (12–24 months, typical duration of Phase 3 trials) follow-up durations and relate only indirectly to disability worsening. The experimental setting

of our study is a simulation of Phase 2 trial, based on data from a Phase 3 trial. This allowed us to test endpoints that are more relevant to long-term disability outcomes such as, 6-month CDW and brain volume loss (BVL). Therefore, our study provides evidence for a new phase 2 paradigm with endpoints that relate more closely to disability not only within the 6 months of study period but provides useful prognostic information beyond the study duration. Monitoring MRI lesions is useful in clinical practice to determine disease activity, but they provide retrospective aspects of the disease. Also, it is not feasible to repeat cranial MRI scans and even more difficult spinal MRI scans frequently in routine practice because of the complexity and cost. The temporal association with active disease and the corresponding clinical manifestation is therefore frequently lacking. Measurement of NfL in blood is minimally invasive, can be done at high frequency, is less burdensome to patients, and correlates well with relevant clinical outcomes in RRMS. Therefore, NfL levels are a promising biomarker candidate to be used in future short-term proof of concept studies in relapsing MS. Future studies will investigate their potential to facilitate and inform trials in progressive MS.

The post hoc nature of this analysis is a limitation of the study but the setting of a large Phase 3 trial provides the opportunity to evaluate the prognostic qualities of NfL in a well-characterized sample of typical relapsing MS patients. However, our results may not be generalizable to other MS phenotypes or to patients in routine clinics who may present with comorbidities and would have been otherwise excluded from controlled clinical studies. Whether the role of NfL as a correlate of clinical outcomes is independent, or at least complementary, to inflammatory MRI activity should be further investigated. Additionally, future studies need to directly compare the added value of frequent, e.g., monthly NfL and imaging assessments. This would be particularly important if we take into account that measuring NfL blood levels would be much easier and cost efficient than frequent MRI scans.

In summary, our results show that levels of NfL in blood measured at 6 months have the necessary properties to qualify as an endpoint for Phase 2 studies in RRMS, with the potential to provide comprehensive information on neuroaxonal integrity, irrespective of its specific cause, inflammatory or degenerative, on a trial level.

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Author Contributions

MPS contributed to the conception and design of the study, statistical analysis and drafting of manuscript outline and critical revision of subsequent drafts of the manuscript and is responsible for the overall content of the manuscript. DAH contributed to the conception and design of the study, statistical analysis and interpretation of data and critical revision of the manuscript. HK contributed to the conception of the study, interpretation of data and critical revision of the manuscript. DL contributed to the conception and design of the study, acquisition, analysis and interpretation of data, and critical revision of the manuscript. UK contributed to the literature search, manuscript drafting, revising, and editing. CB contributed to the acquisition and interpretation of data and critical revision of the manuscript. LK, as the Principal Investigator of FREEDOMS trial, was responsible for supervising the trial. He also contributed to the interpretation of data and critical review of manuscript. DT contributed to the conception, design and execution of the study, interpretation of data, and critical revision of the manuscript. JK contributed to the conception of the study, acquisition, analysis and interpretation of data, and critical revision of the manuscript.

Conflicts of Interest

The study sponsor (Novartis Pharma AG) participated in the design and conduct, data collection, data management, data analysis, and interpretation of the original Phase 3 studies and in the preparation, review, and approval of this paper. The measurement of NfL levels was performed (by fully blinded staff) at the University Hospital, Basel, Switzerland, and the data were provided to the sponsor. The statistical analysis for this paper was performed by Prof. Maria Pia Sormani. Maria Pia Sormani received compensation for serving on scientific advisory boards from Teva, Genzyme, Novartis, Roche, and Vertex; funding for travel or speaker honoraria from Merck Serono, Teva, Genzyme, Novartis, Biogen, and Roche; consultancy from Merck Serono, Biogen, Teva, Genzyme, Roche, GeNeuro, Medday and Novartis; speakers' bureaus from Teva, Merck Serono, Biogen, Novartis, and Genzyme. Dieter A. Haering is an employee of Novartis Pharma AG. Harald Kropshofer is an employee of Novartis Pharma AG. David Leppert is an employee of Novartis Pharma AG. Uma Kundu is an employee of Novartis Healthcare Pvt. Ltd. Christian Barro received travel support from Teva and Novartis. Ludwig Kappos' institution (University Hospital Basel) has received in the last 3 years and used exclusively for research support: steering committee, advisory board, and consultancy fees from Actelion, Addex, Bayer HealthCare, Biogen Idec, Biotica, Genzyme,

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. PTE estimation from the regression models