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ORIGINAL ARTICLE

# Plasma neurofilament light chain concentrations as a biomarker of clinical and radiologic outcomes in relapsing multiple sclerosis: Post hoc analysis of Phase 3 ozanimod trials

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## Abstract

**Background and purpose:** We investigated plasma neurofilament light chain concentration (pNfL) as a biomarker for neuroaxonal damage and disease activity using data from Phase 3 trials of ozanimod in relapsing multiple sclerosis (RMS).

**Methods:** pNfL was measured before and after ozanimod 0.46 mg or 0.92 mg daily or interferon β-1a 30 μg weekly in the randomized, double-blind SUNBEAM and RADIANCE trials. In these post hoc analyses, we investigated relationships between pNfL (at baseline and median percentage change from baseline to Month 12 [SUNBEAM] or 24 [RADIANCE]) and clinical and magnetic resonance imaging outcomes.

**Results:** Median (Q1, Q3) baseline pNfL, available in 1244 of 1346 SUNBEAM participants, was 14.70 (10.16, 23.26) pg/ml and in 1109 of 1313 RADIANCE participants was 13.35 (9.42, 20.41) pg/ml. Baseline gadolinium-enhancing (GdE) and T2 lesion counts increased and brain volume decreased with increasing baseline pNfL. Baseline pNfL was higher in those with versus without on-treatment relapse. Median percentage reduction in pNfL at 12 months in SUNBEAM ($n = 1238$) and 24 months in RADIANCE ($n = 1088$) was greater for ozanimod (20%–27%) than interferon β-1a (13%–16%; $p < 0.01$). Greater pNfL reduction was associated with fewer GdE lesions, fewer new/enlarging T2 lesions per scan, less loss of brain volume, lower annualized relapse rate (ARR), and no evidence of disease activity. The following models predicted ARR: $0.5111 + 0.0116 \times \Delta\text{NfL}$ at 12 months (SUNBEAM) and $0.4079 + 0.0088 \times \Delta\text{NfL}$ at 24 months (RADIANCE).

**Conclusions:** pNfL was associated with clinical and radiologic measures of disease and treatment effects in RMS, supporting its use as a biomarker.

KEYWORDS
blood biomarkers, multiple sclerosis, neurofilament light, relapse, treatment outcome

## INTRODUCTION

Neurofilament light chain is a structural component of the neuron and axon cytoskeleton. Neurofilament light chain is released into the cerebrospinal fluid (CSF) and bloodstream after neuronal injury and degeneration in various neurodegenerative disorders, including multiple sclerosis (MS), amyotrophic lateral sclerosis, Alzheimer disease, Guillain–Barré syndrome, and Huntington disease [1–7].

Blood (serum and plasma) and CSF neurofilament light chain concentrations (NfLs) correlate with each other [1,8,9] and with MS disease activity, including relapse rate, disability worsening, magnetic resonance imaging (MRI) activity, and brain volume loss [1,2,10–16]. Several studies demonstrated that disease-modifying therapies (DMTs) reduce NfL in CSF and blood [9,11,13,17–20], and some reported correlations between reductions in NfL during DMT use and fewer new/enlarging T2 lesions and/or gadolinium-enhancing (GdE) lesions on MRI [11,12,19,20]. Based on these findings, NfL was proposed as a biomarker for neurologic damage and disease activity in relapsing MS (RMS) [1,2,8,11,14]. Here, NfL was evaluated as a biomarker in RMS using data from two Phase 3 trials of ozanimod, an oral sphingosine 1-phosphate receptor 1 and 5 modulator that reduces lymphocyte migration into the central nervous system [21] and has proven efficacy in MS [22,23]. Post hoc exploratory analyses evaluated the effect of ozanimod versus interferon (IFN) β-1a on plasma NfL (pNfL) in patients with RMS, as well as the relationships between pNfL (baseline and median percentage change during treatment), and baseline and on-treatment clinical and radiologic outcomes.

## METHODS

### Phase 3 studies

As previously reported, SUNBEAM (clinicaltrials.gov identifier: NCT02294058) and RADIANCE (clinicaltrials.gov identifier: NCT02047734) were multicenter, randomized, double-blind, double-dummy, active-controlled, Phase 3 trials of ozanimod 0.92 and 0.46 mg (equivalent to ozanimod HCl 1 and 0.5 mg, respectively) compared with intramuscular IFN β-1a 30 μg weekly in patients with RMS [22,23]. The primary efficacy endpoint in the Phase 3 trials was annualized relapse rate (ARR).

Brain MRI scans were performed at baseline, Month 6, and Month 12 in SUNBEAM, and at baseline, Month 12, and Month 24 in RADIANCE [22,23]. An independent MRI analysis center (NeuroRx, Montreal, Quebec, Canada) with no knowledge of treatment assignment or outcomes assessed and scored all MRI scans [22,23]. Whole brain volume and cortical gray matter volume at baseline were measured using SienaX, and thalamic volume was measured using ThalamicVolume software [22,23]. Percentage change in whole brain volume was established using SIENA in Phase 3 RADIANCE and Jacobian atrophy software using longitudinal Jacobian integration for whole brain volume in SUNBEAM [22,23]. Percentage change in cortical gray matter and thalamic volumes was calculated with Jacobian atrophy software using longitudinal Jacobian integration in both trials [22,23].

### Ethical considerations

The institutional review board or ethics committee at each site approved the protocol and informed consent (Table S1). All participants provided written informed consent, and the trials conformed with the World Medical Association Declaration of Helsinki. Funding for the trials was provided by Celgene International II.

### Study procedures and outcomes

In this exploratory, post hoc analysis, pNfL was measured in heparinized plasma samples obtained at baseline and Month 12 in both trials, and at Month 24 in RADIANCE. Specimens were sent to Quanterix Corporation (Lexington, MA) for analysis. The Simoa NF-light Advantage Kit, which is a two-step, digital, immunoassay, quantified total pNfL using the Simoa HD-1 Analyzer and Single Molecule Array (Simoa) technology [24]. The precision and sensitivity of the Simoa immunoassay at the subfemtomolar level in serum samples have been established previously [25]. The lower limit of detection was 0.152 pg/ml, the lower limit of quantification was 0.696 pg/ml, and the average coefficient of variation was 3.4% in SUNBEAM and 4.0% in RADIANCE.

Relationships between baseline pNfL and number of T2 and GdE brain lesions at baseline; whole brain, cortical gray matter, and thalamic volumes at baseline; and number of relapses during ozanimod treatment were evaluated. In addition, the probability of having one or more relapses during treatment with ozanimod versus IFN β-1a was assessed according to baseline pNfL. The median pNfL value and median percentage change in pNfL from baseline to Month 12 (SUNBEAM) and Month 24 (RADIANCE) were determined and analyzed by treatment group. Relationships between ARR and number of GdE lesions over the study period, and between ARR and new/enlarging T2 lesions per scan were evaluated. In addition, how the median percentage change in pNfL from baseline related to ARR, number of GdE lesions, number of new/enlarging T2 lesions, changes in brain volume (whole brain, cortical gray matter, and thalamic volume), and no evidence of disease activity (NEDA-3) at Month 12 in SUNBEAM and Month 24 in RADIANCE were assessed. NEDA-3 was defined as no relapses, no Expanded Disability Status Scale progression, no new/enlarging T2 lesions, and no GdE lesions. Finally, models to predict the number of relapses over a 12-month (SUNBEAM) or 24-month (RADIANCE) period based on median percentage change in pNfL from baseline were developed. Medians were used, rather than arithmetic or geometric means, due to the presence of outliers and the skewness of the data.

## Statistics

This exploratory, post hoc analysis was hypothesis-generating. All fitted models were descriptive and exploratory, and there was no adjustment for multiplicity of testing. The relationships between baseline T2 and GdE lesion counts and log(baseline pNfL) were explored with Poisson generalized linear models, with log(baseline pNfL) as the predictor. The relationship between baseline brain volume (whole brain, cortical gray matter, and thalamic volume) and log(baseline pNfL) was explored in each study via robust linear regression analysis using MM estimation with bisquare weight functions and 85% Gaussian efficiency [26] with log(baseline pNfL) as the predictor. The relationship between risk of one or more on-treatment relapses and log(baseline pNfL) was explored via logistic regression model and contained terms for log(baseline pNfL) and treatment group. Robust linear models were used to investigate between-treatment differences in postbaseline (Month 12, SUNBEAM; Month 24, RADIANCE) pNfL. Baseline and postbaseline pNfLs were log-transformed, and MM estimation with bisquare weight functions and 85% Gaussian efficiency was used [26].

The number of T2 lesions per scan was estimated by negative binomial model adjusted for treatment group, with an offset for number of scans. Median T2 lesions per scan in this analysis differ from the median values in the primary publications, because this analysis did not adjust for baseline GdE count, as it is related to pNfL.

To examine the relationships at the study level, between median percentage change in pNfL from baseline and clinical and radiologic outcomes, we arranged the data such that relevant measures were in a single row for each patient, used stratified bootstrap sampling to resample rows by treatment group (thus retaining the relationships between columns), computed summary statistics, and then repeated this algorithm 1000 times. The bootstrap procedure allowed us to estimate the treatment effects on pNfL, ARR, MRI outcomes, and NEDA-3 as if the studies had been run many independent times, and, therefore, allowed us to examine the relationships between the outcomes in each treatment group across hypothetical repetitions of the studies.

A simple least squares regression was used to predict ARR as the response and median percentage change from baseline pNfL (ΔNfL) as the explanatory variable. The model coefficients can be used to predict the number of relapses seen in a future study given a known median reduction in pNfL, and the residual standard error can be used to produce a prediction interval, within which a future observation will fall for such an estimate.

For each relationship, participants who were missing data for one or both of the relevant outcomes were excluded, such that only those with paired data were analyzed. For example, if a participant had data available for GdE lesions and relapse, but not NfL, that participant was included in analyses of the relationship between GdE and relapse, but not in analyses of the relationship between NfL and either GdE lesions or relapse.

R version 4.0.2 (2020-06-22; R Core Team 2020) was used for all analyses. The Tidyverse suite of packages [27] was used for various aspects of data restructuring and production of graphs. The mgcv package [28] was used for negative binomial modeling [29].

**TABLE 1** Baseline demographics and disease characteristics among participants in SUNBEAM and RADIANCE with baseline plasma neurofilament light chain assessments

| Characteristic | SUNBEAM, *N* = 1244 | RADIANCE, *N* = 1109 |
|---|---|---|
| Age, years, mean (SD) | 35.7 (9.3) | 35.6 (8.9) |
| Sex, % | | |
| Male | 34 | 33 |
| Female | 66 | 67 |
| Race, % | | |
| White | 99.6 | 99.3 |
| Other | 0.4 | 0.7 |
| Number of GdE lesions, %[a] | | |
| 0 | 54 | 58 |
| 1 | 18 | 15 |
| 2 | 9 | 7 |
| 3–5 | 10 | 12 |
| >5 | 9 | 9 |
| Number of T2 lesions, %[a] | | |
| 0–10 | 7 | 6 |
| 11–20 | 11 | 13 |
| 21–50 | 38 | 44 |
| 51–75 | 19 | 19 |
| >75 | 24 | 18 |

Abbreviations: GdE, gadolinium-enhancing; SD, standard deviation.
[a]Percentages may not add up to 100% due to rounding.

# RESULTS

## Disposition and baseline demographics

pNfL was available at baseline for 1244 of 1346 (92.4%) SUNBEAM study [22] participants and for 1109 of 1313 (84.5%) RADIANCE study [23] participants. A total of 1238 (92.0%) and 1088 (82.9%) participants had paired pNfL at baseline and Month 12 (SUNBEAM) or Month 24 (RADIANCE), respectively.

Demographics and baseline disease characteristics, including categorical distribution of T2 and GdE lesion counts, were similar for participants with pNfL at baseline in both studies (Table 1). Most participants were white women with a mean age of approximately 36 years.

## pNfL at baseline and after treatment

Baseline pNfL was similar in both studies: median (Q1, Q3) baseline pNfL was 14.70 (10.16, 23.26) pg/ml in SUNBEAM and 13.35 (9.42,

20.41) pg/ml in RADIANCE. At Month 12 in SUNBEAM, the median percentage change in pNfL was −13.4% with IFN β-1a, −22.8% with ozanimod 0.46 mg ($p = 0.0003$ vs IFN β-1a, derived based on approximate asymptotic *z*-tests), and −26.9% with ozanimod 0.92 mg ($p < 0.0001$ vs IFN β-1a; Table S2). Similar results were obtained at Month 24 in the RADIANCE trial, with median pNfL reductions of −15.5%, −19.7% ($p = 0.0024$ vs IFN β-1a), and −23.5% ($p = 0.0001$ vs IFN β-1a), respectively (Table S2).

## Relationships between pNfL and MRI metrics

Baseline counts of GdE lesions were higher with higher baseline pNfL ($p < 0.0001$; Figure 1A, Table 2). Treatment groups with fewer GdE lesions over the study period had a greater median percentage reduction in pNfL (Figure 1B).

Baseline counts of T2 lesions were higher with higher baseline pNfL in both studies ($p < 0.0001$; Figure 1C, Table 2). Groups with fewer new/enlarging T2 lesions per scan also had a greater median percentage reduction in pNfL (Figure 1D).

Whole brain volume, cortical gray matter volume, and thalamic volume at baseline were smaller in participants who had higher baseline pNfL ($p < 0.05$ for all measures; Table 2; Figure S1A–C). During treatment, participants with a greater median percentage reduction from baseline in pNfL exhibited less loss of whole brain volume, cortical gray matter volume, and thalamic volume (Figure S1D–F). Greater differences between IFN β-1a and ozanimod were observed in cortical gray matter volume and thalamic volume compared with whole brain volume.

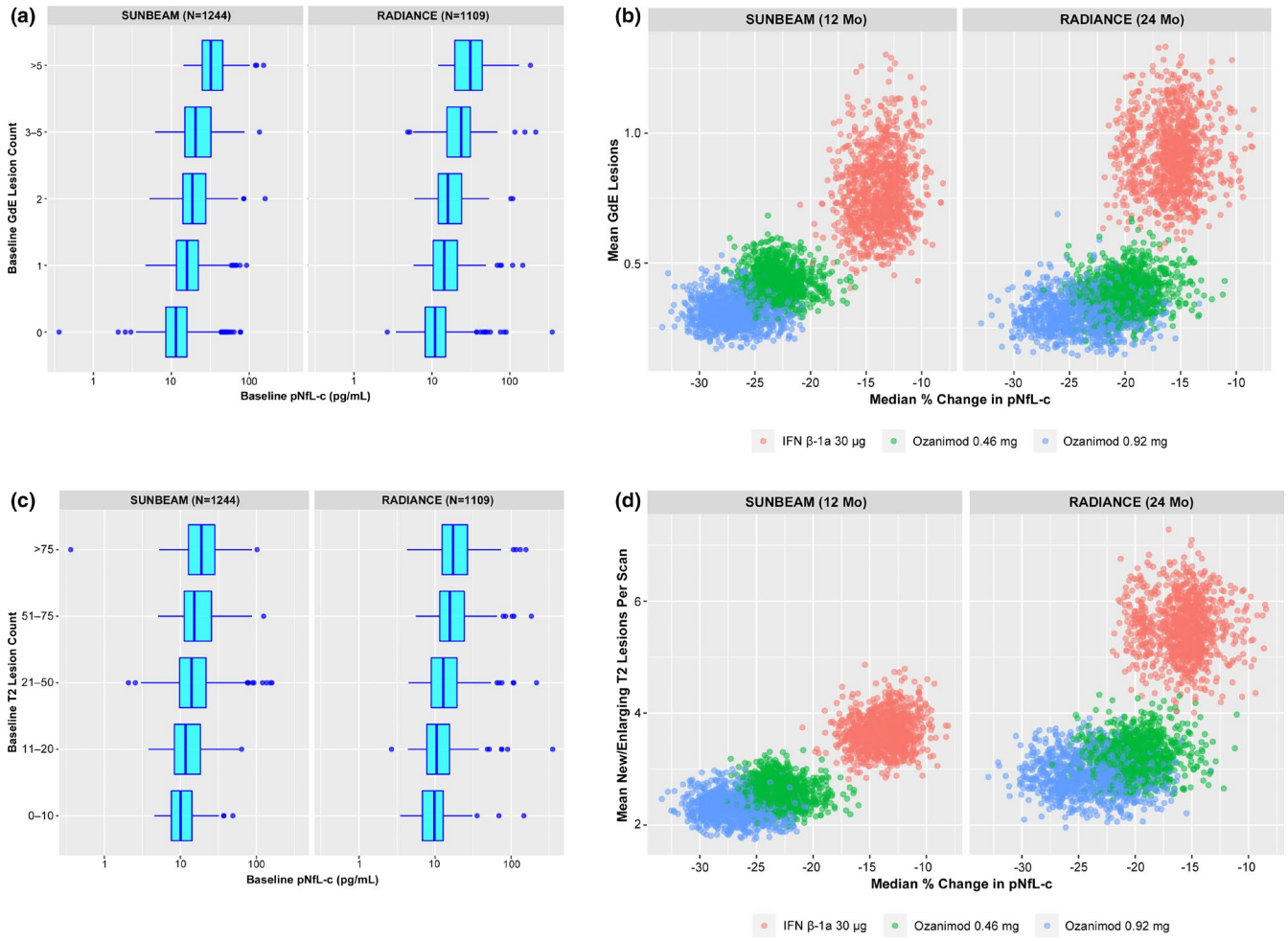



**FIGURE 1** Relationships between brain lesions and plasma neurofilament light chain concentration (pNfL). (a) Baseline pNfL and gadolinium-enhancing (GdE) lesion counts at baseline. (b) On-treatment pNfL reduction and adjusted mean numbers of GdE lesions over the study period (SUNBEAM, 12 months; RADIANCE, 24 months). (c) Baseline pNfL and T2 lesion counts at baseline. (d) On-treatment pNfL reduction and adjusted mean numbers of new/enlarging T2 lesions per scan. (a, c) The relationship between baseline GdE and T2 brain lesion counts and baseline pNfL was explored via Poisson generalized linear models, with log(baseline pNfL) as the predictor. (b, d) Adjusted mean numbers of GdE lesions and new/enlarging T2 lesions per scan were estimated from a negative binomial regression model adjusted for treatment group, with an offset for number of scans. Relationship between median percentage change from baseline in pNfL and lesion counts was based on bootstrap sampling. Individual dots on each plot represent the individual simulations from the bootstrap procedure. IFN, interferon [Colour figure can be viewed at wileyonlinelibrary.com]

**TABLE 2** Relationship between imaging parameters and baseline plasma neurofilament light chain concentrations[a]

| | SUNBEAM | | | RADIANCE | | |
|---|---|---|---|---|---|---|
| | Intercept (SE) | Slope (SE) | *p* | Intercept (SE) | Slope (SE) | *p* |
| Brain lesion counts at baseline | | | | | | |
| GdE lesions[b] | −3.37 (0.10) | 1.28 (0.03) | <0.0001 | −2.62 (0.09) | 1.06 (0.03) | <0.0001 |
| T2 lesions[c] | 3.22 (0.02) | 0.27 (0.01) | <0.0001 | 3.17 (0.02) | 0.26 (0.01) | <0.0001 |
| Brain volume at baseline | | | | | | |
| Whole brain volume | 1491.01 (10.58) | −14.63 (3.74) | <0.0001 | 1475.77 (10.89) | −9.70 (3.95) | 0.0141 |
| Cortical gray matter volume | 550.12 (5.89) | −8.81 (2.09) | <0.0001 | 547.00 (5.72) | −5.94 (2.08) | 0.0042 |
| Thalamic volume | 17.23 (0.27) | −0.68 (0.09) | <0.0001 | 17.04 (0.26) | −0.50 (0.09) | <0.0001 |

Abbreviations: GdE, gadolinium-enhancing; SE, standard error.

[a]Based on log(baseline plasma neurofilament light chain concentration).

[b]Indicative of ongoing disease activity.

[c]Indicative of cumulative disease (magnetic resonance imaging lesion) burden.

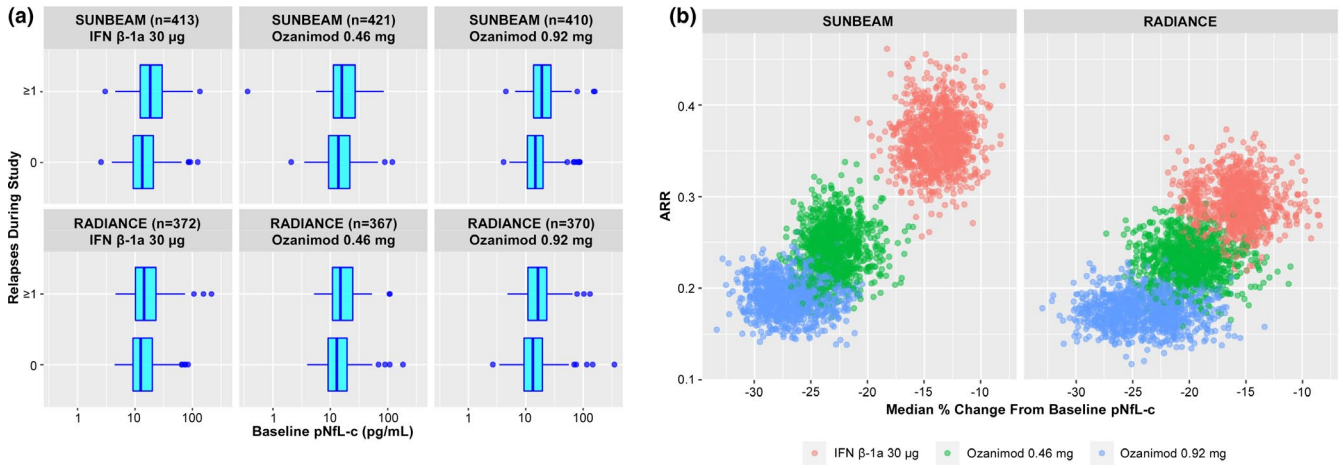



**FIGURE 2** Relationships between relapse and (a) baseline plasma neurofilament light chain concentration (pNfL) and (b) change in pNfL on-treatment. (a) The relationship between risk of one or more relapses during treatment and log(baseline pNfL) was explored via logistic regression model. (b) Relationship between adjusted annualized relapse rate (ARR) over the treatment period (SUNBEAM, 12 months; RADIANCE, 24 months) and median percentage change from baseline in pNfL based on bootstrap sampling. ARR was based on a Poisson generalized linear regression model as a function of treatment group, with an offset for duration. Individual dots on each plot represent the individual simulations from the bootstrap procedure. IFN, interferon [Colour figure can be viewed at wileyonlinelibrary.com]

## Relationship between MRI lesions and ARR

Treatment groups with lower ARR had fewer GdE lesions at Months 12 and 24 (Figure S2A). Groups with lower ARR also had fewer new/enlarging T2 lesions per scan over 12 and 24 months (Figure S2B).

## Relationship between pNfL and relapse

A trend was observed suggesting that baseline pNfL was higher in those who relapsed during treatment compared with those who did not relapse (Figure 2A). Treatment groups with a greater median percentage reduction from baseline in pNfL during treatment had lower adjusted ARR (Figure 2B).

## Relationship between pNfL and NEDA-3

NEDA-3 rates at Month 12 in SUNBEAM were 22.5% with IFN β-1a, 25.9% with ozanimod 0.46 mg, and 26.8% with ozanimod 0.92 mg. Corresponding NEDA-3 rates at Month 24 in RADIANCE were 12.9%, 17.3%, and 18.2%. In both studies, NEDA-3 was associated with larger reductions in pNfL during treatment (Figure S3).

## Predicting relapse based on change in pNfL

Probability of having one or more relapses in the next 12 months (SUNBEAM) or 24 months (RADIANCE) increased with increasing baseline pNfL and was numerically lower with ozanimod 0.92 mg than with IFN β-1a (Table 3).

**TABLE 3** Probability of one or more relapses in the next 12 months (SUNBEAM) or 24 months (RADIANCE) based on baseline pNfL

| | SUNBEAM Probability (95% CI) of ≥1 relapse in next 12 months | | | RADIANCE probability (95% CI) of ≥1 relapse in next 24 months | | |
|---|---|---|---|---|---|---|
| Baseline pNfL, pg/ml | IFN β-1a | Ozanimod 0.46 mg | Ozanimod 0.92 mg | IFN β-1a | Ozanimod 0.46 mg | Ozanimod 0.92 mg |
| 1 | 0.07 (0.04–0.13) | 0.05 (0.03–0.09) | 0.04 (0.02–0.07) | 0.16 (0.10–0.26) | 0.13 (0.07–0.21) | 0.11 (0.06–0.18) |
| 5 | 0.17 (0.13–0.22) | 0.11 (0.08–0.15) | 0.10 (0.07–0.14) | 0.26 (0.20–0.32) | 0.20 (0.15–0.26) | 0.17 (0.13–0.23) |
| 10 | 0.24 (0.19–0.28) | 0.16 (0.13–0.20) | 0.14 (0.11–0.18) | 0.31 (0.26–0.36) | 0.24 (0.20–0.29) | 0.21 (0.17–0.26) |
| 20 | 0.32 (0.27–0.37) | 0.23 (0.19–0.27) | 0.20 (0.16–0.25) | 0.36 (0.31–0.41) | 0.29 (0.24–0.34) | 0.25 (0.21–0.30) |
| 50 | 0.45 (0.37–0.52) | 0.34 (0.27–0.42) | 0.30 (0.24–0.38) | 0.44 (0.36–0.52) | 0.36 (0.29–0.44) | 0.32 (0.25–0.39) |
| 100 | 0.55 (0.45–0.65) | 0.44 (0.33–0.55) | 0.40 (0.30–0.51) | 0.50 (0.39–0.61) | 0.42 (0.32–0.53) | 0.37 (0.27–0.48) |

Abbreviations: CI, confidence interval; IFN, interferon; pNfL, plasma neurofilament light chain concentration.

The following model was developed to predict relapse based on change in pNfL using 95% prediction intervals:

$$\text{SUNBEAM: ARR} = 0.5111 + 0.0116 \times \Delta\text{NfL at 12 months; residual standard error} = 0.035$$

$$\text{RADIANCE: ARR} = 0.4079 + 0.0088 \times \Delta\text{NfL at 24 months; residual standard error} = 0.037$$

Predictive modeling showed that the median percentage change from baseline pNfL appears to be approximately linearly related to ARR (Figure 3). The modeling estimated that a 25% reduction in pNfL (similar to that observed with ozanimod 0.92 mg in SUNBEAM and RADIANCE) predicts an ARR (standard error [SE]) of 0.22 (0.04) and 0.19 (0.04) based on SUNBEAM and RADIANCE, respectively. A 13% pNfL reduction (similar to that observed with IFN β-1a in SUNBEAM and RADIANCE) predicts an ARR (SE) of 0.36 (0.04) and 0.29 (0.04), respectively.

## DISCUSSION

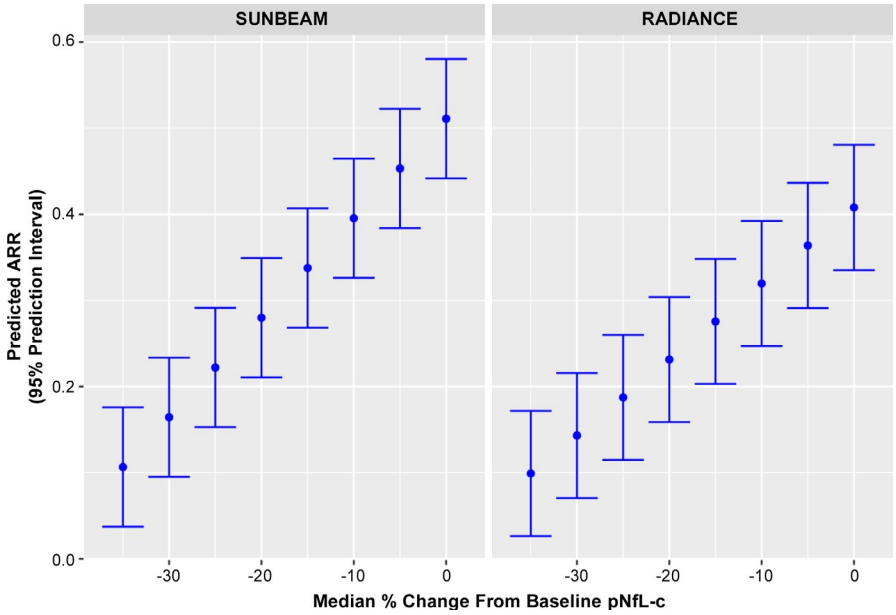
This exploratory, post hoc analysis of the Phase 3 SUNBEAM and RADIANCE trials of ozanimod supports further evaluation of pNfL as a biomarker to monitor and predict disease activity and neurologic damage in patients with RMS and is the first publication describing the impact of ozanimod on pNfL. In SUNBEAM and RADIANCE participants, pNfL was reduced by treatment with ozanimod to a greater extent than IFN β-1a. At the study level, relationships were found between treatment-related reduction in pNfL and reductions in clinical relapse and radiologic disease activity (GdE and new/enlarging T2 lesion counts). Furthermore, median change in pNfL was related to magnitude of brain volume loss and NEDA-3 across the study population.

These results add to literature providing support for pNfL as a biomarker for RMS disease activity and treatment response. Reductions of NfL in CSF and blood were observed following treatment with IFN β-1a [11,12], natalizumab [17,18,30,31], rituximab [32,33], cladribine [34], dimethyl fumarate [35], alemtuzumab [36], and fingolimod [9,13,19,20,37]. These reductions in NfL corresponded to reductions in MS activity. For example, pNfL was measured over a 1-year or 2-year period in 589 patients with relapsing–remitting MS enrolled in the Phase 3, double-blind, randomized, controlled fingolimod studies (TRANSFORMS and FREEDOMS) [13]. High baseline pNfL was associated with presence of GdE lesions at baseline ($p < 0.0001$) and predicted an increased number of new/enlarging T2 lesions ($p = 0.0006$), higher ARR ($p < 0.0001$), and greater rate of brain volume loss ($p < 0.0001$) over 24 months in FREEDOMS [13]. Patients with new/enlarging T2 lesions during the studies had higher pNfL at the end of treatment. In addition, fingolimod reduced pNfL to a greater extent than IFN β-1a [13]; thus, both fingolimod and ozanimod produce greater reductions in pNfL than IFN β-1a.

Other studies also suggest differential effects of DMTs on pNfL. Two large studies found that patients receiving higher efficacy DMTs (e.g., natalizumab, ocrelizumab, alemtuzumab, fingolimod, dimethyl fumarate, mitoxantrone) had a larger relative decrease in NfL compared with no DMT, IFNs, or glatiramer acetate [14,38]. In addition, a study of 1139 patients with RMS who were initiating newer DMTs and had pNfL over two time points found the largest NfL reduction in the alemtuzumab-treated group and the least reduction in the teriflunomide treated-group, consistent with differences in clinical efficacy [39]. Thus, NfL in serum/plasma or CSF is a therapeutic response biomarker in RMS that may be related to anti-inflammatory activity, and a consequent prevention of neurologic damage, by DMTs.

Currently, due to lack of an accepted biomarker, serial MRI scans are standard of care to assess MS disease activity and treatment response despite their high cost [15,40]. Compared with MRI, pNfL has some advantages for monitoring for subclinical disease activity, including lower costs, accessibility, and a single assessment that includes both spinal cord and brain pathology [16]. CSF and serum NfL (sNfL) were previously found to have similar long-term predictive value as MRI measures, suggesting complementary use [12]. In a study of 94 MS patients, sNfL was significantly higher (estimated 35% increase) within 90 days of a GdE-positive lesion [41]. In addition, significantly elevated sNfL was observed within 3 months of clinical relapse only when associated with a GdE lesion. The present study found similar relationships of pNfL with clinical and radiologic disease activity, although relationships with MRI endpoints were assessed only at the study level.

A recent analysis of Phase 2 and 3 fingolimod studies and their extensions concluded that longitudinal measurements of NfL over 12 or 24 months add prognostic value for 10-year disability outcomes




| Study | Median ΔNfL-c | ARR | SE |
|---|---|---|---|
| SUNBEAM (12 Mo) | −35 | 0.11 | 0.04 |
| | −25[a] | 0.22 | 0.04 |
| | −13[b] | 0.36 | 0.04 |
| | 0 | 0.51 | 0.04 |
| RADIANCE (24 Mo) | −35 | 0.10 | 0.04 |
| | −25[a] | 0.19 | 0.04 |
| | −13[b] | 0.29 | 0.04 |
| | 0 | 0.40 | 0.04 |

**FIGURE 3** Prediction of relapse based on plasma neurofilament light chain concentration (pNfL). Prediction of annualized relapse rate (ARR) is based on median percentage change from baseline pNfL. The model is based on a least squares regression model with ARR as the response and median percentage change from baseline NfL as the explanatory variable. A 25% reduction in plasma NfL was similar to that observed in participants treated with ozanimod 0.92 mg in SUNBEAM and RADIANCE. A 13% reduction in plasma NfL was similar to that observed in participants treated with interferon β-1a in SUNBEAM and RADIANCE. [a]A 25% reduction in plasma NfL was similar to that observed in participants treated with ozanimod 0.92 mg in SUNBEAM and RADIANCE. [b]A 13% reduction in plasma NfL was similar to that observed in participants treated with IFN β-1a in SUNBEAM and RADIANCE. [Colour figure can be viewed at wileyonlinelibrary.com]

when used in combination with both clinical measures and conventional MRI [42]. The frequency of sampling sufficient for prediction of relapsing activity has not been established but may need to be more frequent than annually [38].

This analysis has a number of strengths. The two prospective Phase 3 clinical trials included large cohorts of patients treated for 1 and 2 years, an active comparator, and clinical relapse as the primary outcome. In addition, the highly sensitive Simoa assay was used to quantify pNfL [43]. Use of a robust regression and median versus means was intended to reduce the undue influence of outliers in the data. One key limitation is the exploratory, post hoc nature of this analysis. Although a bootstrap procedure was used to estimate the study-level relationship between the changes from baseline in pNfL and on-treatment outcomes, two studies are too few to estimate

between-study variances in this relationship. Also, the two studies were of different durations and included only three treatment groups and two drugs. The effect of confounding factors known to affect NfL in plasma or serum (e.g., neurologic comorbidities [4–7], age [44]) was not determined, and may have limited detection of the full range of associations and predictive values.

## CONCLUSIONS

These findings support the potential use of pNfL to characterize RMS at a specific time point and to serve as a biomarker to monitor and predict disease activity and treatment response. Ozanimod caused dose-dependent reductions in pNfL from baseline compared with IFN β-1a, and these reductions were related to ARR, number of MRI brain lesions, and rate of brain volume loss at the study level. These findings, coupled with the primary analyses from the ozanimod Phase 3 trials, support the use of pNfL as a biomarker in RMS.

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### CONFLICT OF INTEREST

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### AUTHOR CONTRIBUTIONS

**Sarah Harris:** Data curation (equal), writing–original draft (equal), writing–review & editing (equal). **Giancarlo Comi:** Conceptualization (equal), data curation (equal), supervision (equal), writing–original draft (equal), writing–review & editing (equal). **Bruce A. C. Cree:** Conceptualization (equal), data curation (equal), supervision (equal), writing–original draft (equal), writing–review & editing (equal). **Douglas L. Arnold:** Conceptualization (equal), data curation (equal), supervision (equal), writing–original draft (equal), writing–review & editing (equal). **Lawrence Steinman:** Conceptualization (equal), data curation (equal), supervision (equal), writing–original draft (equal), writing–review & editing (equal). **James K. Sheffield:** Data curation (equal), writing–original draft (equal), writing–review & editing (equal). **Harry Southworth:** Data curation (equal), formal analysis (equal), validation (equal), writing–original draft (equal), writing–review & editing (equal). **Ludwig Kappos:** Conceptualization (equal), data curation (equal), supervision (equal), writing–original draft (equal), writing–review & editing (equal). **Jeffrey A. Cohen:** Conceptualization (equal), data curation (equal), supervision (equal), writing–original draft (equal), writing–review & editing (equal).

### DATA AVAILABILITY STATEMENT

**Bristol Myers Squibb policy on data sharing may be found at** https://www.bms.com/researchers-and-partners/independent-research/data-sharing-request-process.html

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## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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