Original research

Plasma neurofilament light chain levels suggest neuroaxonal stability following therapeutic remyelination in people with multiple sclerosis

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ABSTRACT

Background Chronic demyelination is a major contributor to axonal vulnerability in multiple sclerosis (MS). Therefore, remyelination could provide a potent neuroprotective strategy. The ReBUILD trial was the first study showing evidence for successful remyelination following treatment with clemastine in people with MS (pwMS) with no evidence of disease activity or progression (NEDAP). Whether remyelination was associated with neuroprotection remains unexplored.

Methods Plasma neurofilament light chain (NfL) levels were measured from ReBUILD trial's participants. Mixed linear effect models were fit for individual patients, epoch and longitudinal measurements to compare NfL concentrations between samples collected during the active and placebo treatment period.

Results NfL concentrations were 9.6% lower in samples collected during the active treatment with clemastine (n=53, geometric mean=6.33 pg/mL) compared to samples collected during treatment with placebo (n=73, 7.00 pg/mL) (B=−0.035 [−0.068 to −0.001], p=0.041). Applying age- and body mass index-standardised NfL Z-scores and percentiles revealed similar results (0.04 vs 0.35, and 27.5 vs 33.3, p=0.023 and 0.042, respectively). Higher NfL concentrations were associated with more delayed P100 latencies (B=1.33 [0.26 to 2.41], p=0.015). In addition, improvement of P100 latencies between visits was associated with a trend for lower NfL values (B=0.003 [−0.0004 to 0.007], p=0.081). Based on a Cohen's d of 0.248, a future 1:1 parallel-arm placebo-controlled study using a remyelinating agent with comparable effect as clemastine would need 202 subjects per group to achieve 80% power.

Conclusions In pwMS, treatment with the remyelinating agent clemastine was associated with a reduction of blood NfL, suggesting that neuroprotection is achievable and measurable with therapeutic remyelination.

Trial registration number NCT02040298.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Chronic demyelination is a major contributor to axonal vulnerability in multiple sclerosis. Whether remyelination was associated with neuroprotection remains unexplored.

WHAT THIS STUDY ADDS

⇒ In this work, treatment with the remyelinating agent clemastine was associated with a reduction of blood neurofilament light chain in people with multiple sclerosis, providing evidence that therapeutic remyelinating may be associated with neuroprotection.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ Our findings suggest that neuroprotection is achievable and measurable with therapeutic remyelination. A 1:1 parallel arm placebo-controlled study using a remyelinating agent with comparable effect as clemastine would need 202 subjects per group to achieve 80% power.

INTRODUCTION

Multiple sclerosis (MS) is the leading cause of non-traumatic disability in young adults.[1] While demyelination predominates during acute inflammatory events, remyelination failure is one of the main pillars of functional impairment, and disability accumulation in people with MS (pwMS).[2 3] Recent findings underpin the association between failure of remyelination and chronic neurodegeneration. Previous ex vivo and animal studies have demonstrated the neuroprotective potential of effective remyelination.[4 5] However, evidence of neuroprotection following remyelination in humans has not been assessed. This is for a few reasons, including the previous absence of clinical trials using a compound with validated remyelinating capacity and the lack of tools to document remyelination-induced neuroprotection.

Recent evidence demonstrates the potential of therapeutic remyelination. A number of pharmacological agents and at least one cell-based approach have been shown to induce remyelination in animal models of demyelination and hypomyelination.[6–9] In 2017, our group reported the first successful, double-blind, placebo-controlled remyelinating

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Table 1 Clinical characteristics and measured biomarkers in the included ReBUILD participants

	Study participants (n=34)
Baseline age in years (mean, SD)	40.04 (10.00)
Sex (Female:Male)	24:9
Baseline BMI (mean, SD)	27.88 (7.76)
Baseline DMT (count, per cent)	
Platform treatments	17 (51.5%)
High potency treatments	15 (45.5%)
Treatment naïve	1 (3%)
Baseline EDSS (mean, SD)	2.10 (1.30)
Baseline disease duration in years (mean, SD)	4.80 (3.42)
Baseline FLAIR lesions	
Count (SD)	16 (8)
Total lesion volume in mm^3 (geometric mean, SD)	3667 (10859)
NfL concentration in pg/mL	
Geometric mean, SD	6.7 (3.47)
Number of included samples	126
Mean CV%	4.8%
Geometric mean of percentiles, SD	34.1 (34.1)
Median Z-score, IQR	0.52 (−0.81 to 1.08)
Tau concentration in pg/mL	
Geometric mean, SD	2.14 (0.96)
Number of included samples	119
Mean CV%	6.6%
UCHL1 concentration in pg/mL	
Geometric mean, SD	18.64 (11.50)
Number of included samples	75
Mean CV%	12.4%
GFAP concentration in pg/mL	
Geometric mean, SD	66.46 (32.42)
Number of included samples	125
Mean CV%	5.0%
Serum creatinine in mg/mL (mean, SD)	0.78 (0.13)

Number of included samples refers to all samples with %CV below 20%.
BMI, body mass index; %CV, coefficient of variation of concentration between duplicate measures; DMT, disease-modifying treatments; EDSS, expanded disability status scale; FLAIR, fluid-attenuated inversion recovery; GFAP, glial fibrillary acidic protein; NfL, neurofilament light chain; UCH-L1, ubiquitin c-terminal hydrolase L1.

trial, ReBUILD, in MS. In the ReBUILD trial, clemastine fumarate improved the visual evoked potential (VEP) latency in pwMS with no evidence for disease activity or progression (NEDAP).[10] Nevertheless, preselected imaging-based outcome parameters failed to demonstrate a similar improvement pattern, highlighting current challenges facing the conduction of remyelination studies. In addition, the possible association of successful remyelination in pwMS with neuroprotection has never been explored.

The recent availability of ultrasensitive immunoassay technology, such as single molecule array (Simoa) technology, allows the reliable measurements of axonal and glial-derived proteins in blood. Markers of neuroaxonal injury, such as neurofilament light chain (NfL), are elevated in pwMS compared with controls and correlate to various clinical and imaging disease activity and progression metrics.[11–16] We, therefore, postulated that remyelination-associated neuroprotection would be associated with a reduction of blood NfL levels. To that end, we measured blood concentrations of NfL, as well as tau, and ubiquitin c-terminal hydrolase L1 (UCH-L1) and the astrocyte activation marker, glial fibrillary acidic protein (GFAP), which are all part of a Simoa multiplex assay from the ReBuild study's subjects' samples.

METHODS

Study design

The ReBUILD trial (NCT02040298)[10] was a double-blind, randomised, placebo-controlled, within-groups comparison trial, including 50 patients with stable relapsing-remitting MS treated at the University of California, San Francisco (San Francisco, CA, USA). Participants with a history of glucocorticoid use within 30 days before screening, any clinical or radiological activity in the 90 days, or changes in disease-modifying treatments (DMTs) in the last 180 days were excluded. Participants were randomised into two groups; the first group (G1) received daily clemastine fumarate for the first 90 days (Epoch 1 [E1]), followed by placebo for 60 days (Epoch 2 [E2]). In group 2 (G2), patients were initially treated with placebo for 90 days, followed by the active substance for 60 days. VEP were conducted at each visit, including the screening visit.

Plasma processing and biomarker measurements

BD Vacutainer ACD tubes were collected from a subset of participants, who additionally consented to longitudinal blood sample collection at each study visit (baseline, month 1, month 3, month 5). Processed plasma was stored at the local biobank at −80°C. Biomarker concentrations were measured using the Neurology 4-Plex B (Quanterix Corporation, Lexington, MA, USA) on an HD-X analyser by a lab technician blinded to the clinical data and group assignment of the included subjects. A multiplex kit was selected to measure NfL levels, as it requires lower sample volume than the NF-light advantage kit from the same commercial vendor. All samples were measured in duplicates, and only samples showing a coefficient of variation (%CV) less than 20% were included in the analysis. The age and body mass index (BMI) adjusted NfL percentiles, and Z-scores were calculated based on a large reference database with 4532 serum samples from control persons.[17] To calculate the adjusted percentiles and Z-scores, the following validated equation was applied to convert the plasma NfL concentrations to corresponding serum levels: serum NfL (pg/mL) = $-0{\cdot}33+1{\cdot}11\times$pNfL (pg/mL), which was calculated from 299 paired serum and plasma samples.[17]

Magnetic resonance imaging

Total white matter lesion burden was assessed on the FLAIR and T1-weighted 3D MPRAGE images at each study visit using Samseg.[18] The detailed MRI protocol is provided elsewhere.[10 19]

Statistical analysis

We explored the association between serial biomarker blood concentrations (dependent variable), clemastine treatment status, serial P100 latency, serial delta-P100 (fixed effects) at each visit with a mixed effect model (MLM), including a random effect for individual subjects. The active treatment group constitute of samples G1-E1 and G2-E2, while samples from G1-E2 and G2-E1 are assigned to the placebo treatment. Considering the study design (randomised case-crossover trial), the within-subject comparison over a short duration (150 days), we did not adjust for additional covariates in the primary analysis. Yet, in secondary analyses, a series of MLM models were fit, including relevant covariates (age, sex, disease duration, Expanded Disability Status Scale (EDSS), treatment effect, serum creatinine, and FLAIR lesion load). In addition, in a secondary model assuming a carryover effect of clemastine fumarate, NfL levels

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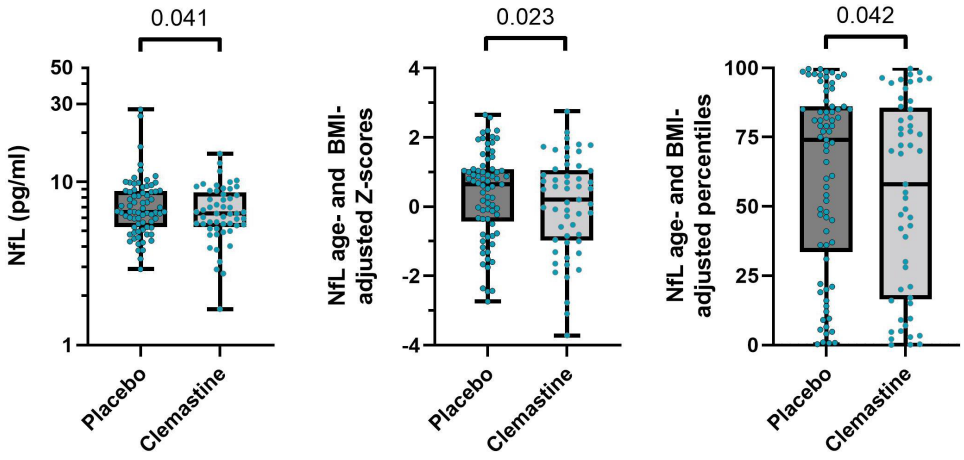

Figure 1 Neurofilament light chain (NfL) levels were lower during the treatment with clemastine. Plasma NfL concentrations (right), age- and BMI-adjusted Z-scores (middle), and percentiles (left). P value reported from a mixed linear model accounting for longitudinal biomarker measurements, treatment status (clemastine=53, placebo=73) and subject-ID (as random factor). Boxes showing median, and IQR, upper and lower bars indicate maximum and minimum, respectively.

were compared between the treated group (G1-E1, G1-E2, and G2-E2), and the placebo samples (G2-E1). Comparison of the MLM models was adjudicated with the Akaike information criterion (AIC). Given the limited sample size in both groups, we applied a conservative covariance structure, compound symmetry, that requires two parameters (a common SD

Table 2 Statistical analysis of the association between neurofilament light chain (NfL) levels and various outcome parameters

Linear mixed model terms	Unstandardised beta (B)	95% CI (lower end to higher end)	Akaike information criterion*	P value
Association between NfL and active treatment with clemastine				
NfL†~active treatment (yes/no(ref))+epoch	−0.035	−0.068 to −0.001	−164.392	0.041
+ Age	−0.035	−0.069 to −0.001	−162.397	0.041
+ Sex	−0.035	−0.069 to −0.001	−166.012	0.042
+ *BMI†*	*−0.034*	*−0.068 to −0.0006*	*−170.768*	*0.046*
+ EDSS	−0.025	−0.073 to 0.023	−105.365	0.294
+ Disease duration	−0.034	−0.068 to 0.0006	−156.595	0.055
+ DMT category	−0.036	−0.069 to −0.002	−164.628	0.039
+ Creatinine†	−0.035	−0.070 to −0.0006	−157.370	0.046
+ FLAIR lesion load†	−0.030	−0.078 to 0.019	−101.248	0.225
Association between NfL and mean P100 of both eyes				
NfL†~P100 latency†	1.33	0.261 to 2.407	−167.965	0.015
+ Age	1.33	0.262 to 2.408	−165.966	0.015
+ Sex	1.41	0.378 to 2.433	−170.697	0.008
+ *BMI†*	*1.36*	*0.369 to 2.351*	*−175.558*	*0.008*
+ EDSS	1.32	0.210 to 2.429	−110.449	0.021
+ Disease duration	1.37	0.279 to 2.463	−160.717	0.015
+ DMT category	1.33	0.279 to 2.378	−168.281	0.014
+ Creatinine†	1.41	0.331 to 2.494	−161.697	0.011
+ FLAIR lesion load†	1.24	0.128 to 2.353	−105.272	0.029
Association between NfL and changes in P100 of both eyes between visits				
NfL†~delta-P100	0.003	−0.0004 to 0.007	−165.266	0.081
+ Age	0.003	−0.0004 to 0.007	−163.281	0.081
+ Sex	0.003	−0.0005 to 0.007	−166.811	0.086
+ *BMI†*	*0.003*	*−0.0008 to 0.007*	*−171.137*	*0.121*
+ EDSS	0.004	−0.0008 to 0.009	−107.914	0.100
+ Disease duration	0.003	−0.0004 to 0.007	−157.955	0.080
+ DMT category	0.003	−0.0004 to 0.069	−165.644	0.080
+ Creatinine†	0.004	0.00004 to 0.008	−159.325	0.047
+ FLAIR lesion load†	0.005	−0.0006 to 0.010	−103.609	0.083

High potency treatments in the ReBUILD study were natalizumab, rituximab and fingolimod. Platform treatments included Interferon-beta, glatiramer acetate and dimethyl fumarate. *Italic* highlights models with the lowest AIC values.
*Lower values indicate better goodness-of-fit.
†Log-transformed.
BMI, body mass index; DMT, disease-modifying treatments; EDSS, expanded disabiliy status scale; FLAIR, fluid-attenuated inversion recovery; NfL, neurofilament light chain.

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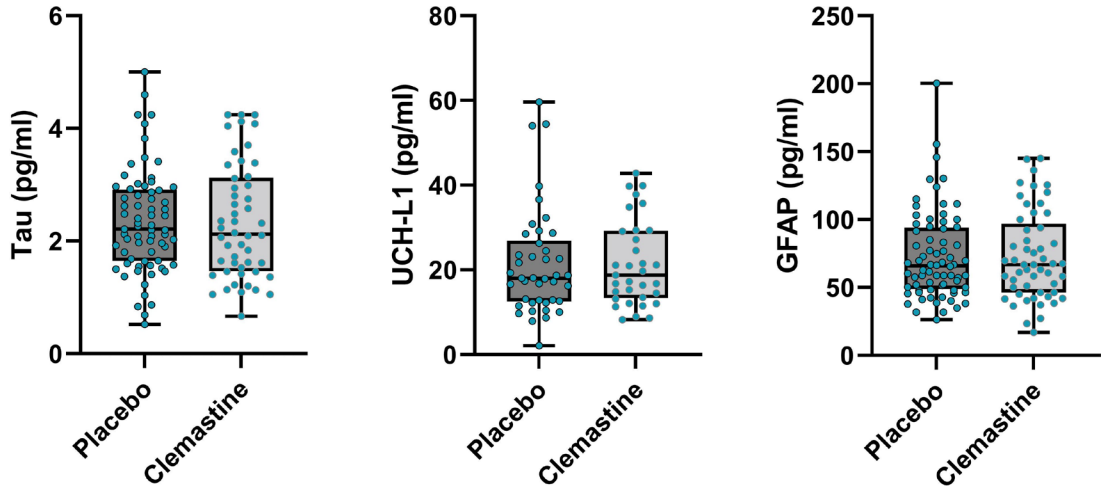

Figure 2 Levels of measured biomarkers from ReBUILD samples. UCH-L1, ubiquitin c-terminal hydrolase L1; GFAP, glial fibrillary acidic protein. P value reported from a mixed linear model accounting for longitudinal biomarker measurements (n=119, 75, and 125 samples for Tau, UCH-L1 and GFAP, respectively), treatment status, and subject-ID (random factor). Boxes showing median, and IQR, upper and lower bars indicate maximum and minimum, respectively.

parameter and a common inter-time-point correlation parameter). Log-transformation was performed for variables showing skewed distribution. The evolution of clinical parameters and lesion load over the study duration was evaluated by analysis of variance (ANOVA), comparing mean values between visits. We report p values for those hypothesis-driven analyses without adjustment for multiple testing, as all comparisons of interest were prespecified. Mean difference (Cohen's d) was calculated using log-transformed values. The analyses were performed using IBM SPSS software V.28 . Sample size calculations were conducted on G*Power V.3.1.9.7.[20]

RESULTS

We included 126 available plasma samples from 34 patients (33 samples from baseline and month 3, 32 from month 1, 28 from month 5). The clinical characteristics and biomarker measures are included in table 1. All the included participants, but one, were treated with a DMT at least 6 months before and during the whole trial period (most commonly fingolimod, glatiramer acetate, dimethyl fumarate, and natalizumab [n=7 each]).

NfL levels were associated with BMI (unstandardised beta (B)=−0.68, 95% CI −1.13 to 0.225, p=0.004), but not age (0.0002 [−0.006 to 0.007], p=0.950), EDSS (−0.008 [−0.04 to 0.02], p=0.588), disease duration (−0.007 [−0.02 to 0.01], p=0.395), FLAIR lesion volume (0.01 [−0.06 to 0.09], p=0.718) or DMT category (0.07 [−0.03 to 0.17], p=0.096). A trend for association has been found with sex (0.12 [−0.004 to 0.024], p=0.058). There was no significant change in BMI (ANOVA p=0.996), serum creatinine (ANOVA p=0.814), log-FLAIR lesion load (ANOVA p=0.786) between the study visits. Most importantly, no cases of disease activity (MRI activity, or clinical relapses), EDSS progression, or switch of DMT were documented during the trial period.

NfL concentrations were 9.6% lower during the active treatment with clemastine (n=53, geometric mean=6.33 pg/mL) compared with samples from untreated subjects (n=73, geometric mean=7.00 pg/mL) (B=−0.035, p=0.041). Applying the age- and BMI-standardised NfL Z-scores and percentiles revealed similar results (0.04 vs 0.35, and 27.5 vs 33.3, p=0.023 and 0.042, respectively) (figure 1, online supplemental

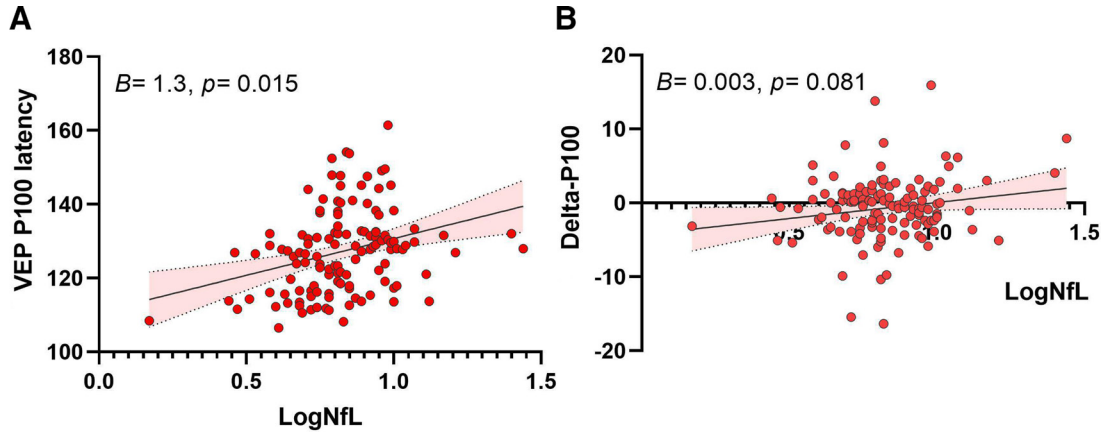

Figure 3 Plasma neurofilament light chain (NfL) concentrations correlate to visual evoked potentials (VEP) dynamics. Log-NfL concentrations correlated positively with P100 latencies in milliseconds (A) and showed a trend for inverse correlation with changes of P100 latencies (Delta-P100) between longitudinal visits (B) in a mixed linear effect model accounting for longitudinal measures (n=126).

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figure S1). Assuming a carryover effect for clemastine, there was a trend (p=0.094) for lower NfL concentrations (n=67, geometric mean=6.54 pg/mL) compared with placebo (n=59, 6.91 pg/mL).

A sensitivity analysis that adjusts for additional covariates did not impact the statistically significant association, when the models showing the lowest AIC (ie, goodness-of-fit) were selected (table 2). Tau (geometric mean=2.11 vs 2.33, p=0.809), UCH-L1 (19.19 vs 18.21, p=0.404), and GFAP (66.00 vs 66.8, p=0.924) concentrations did not differ during active treatment compared with placebo (figure 2).

To support the assumption of an association between lower levels of NfL during treatment with clemastine and remyelination, we evaluated the correlation between the visual outcome metrics (P100, delta-P100) and NfL levels. Higher NfL concentrations were associated with more delayed P100 latencies (B=1.33 [0.26 to 2.41], p=0.015) (table 2, figure 3A). In addition, improvement of P100 latencies between visits was associated with a trend for lower NfL values (B=0.003 [−0.0004 to 0.007], p=0.081) (table 2, figure 3B). Samples collected from participants with significant VEP P100 improvement (n=5, improvement of ≥6 ms during active treatment) had lower NfL values (geometric mean=6.1 pg/mL), compared with samples from participants with any improvement (n=29, improvement of between 0 and 6 ms, geometric mean=6.8 pg/mL) and those with no improvement (n=19, 7.1 pg/mL), the difference was not statistically significant (p=0.894). None of the other biomarkers showed any statistically significant association with P100 or delta-P100.

DISCUSSION

Our results provide evidence that treatment with a remyelinating agent may be associated with neuroprotection in pwMS. In the ReBUILD study, treatment with clemastine, a remyelinating agent without immunomodulatory effect,[4] was associated with a 9.6% reduction of NfL levels in plasma. Of note, this association between NfL and treatment with clemastine was achieved in an exceptionally well-selected cohort with NEDAP in the last 3 months before as well as during the study.

Our findings provide evidence for a possible new outcome parameter in remyelination trials. The reported effect size for the difference in NfL levels between treatment groups in the ReBUILD trial (Cohen's d=0.248 in independent sample t-test) might guide future trials that assess remyelination-induced neuroprotection. In contrast to the case-crossover design of the ReBUILD trial, a 1:1 parallel arm placebo-controlled study using a remyelinating agent with comparable effect as clemastine would need 202 subjects per group to achieve 80% power. Agents with a more substantial remyelinating effect than clemastine or a more extended treatment duration might require a smaller sample size. The capacity to detect the observed effect in this trial was significantly enhanced by the within groups comparison in a crossover/delayed treatment trial rather than between groups statistical comparison . Yet, the small number of samples might have precluded the accurate estimation of the strength of the correlation between NfL and changes in P100 and the assessment of the magnitude of NfL dynamics assuming a carryover effect of clemastine. In line with previous results from animal models of remyelination,[4] we expect a steeper decrease in NfL concentrations if a remyelination agent was initiated during acute relapses. Thus, a smaller sample size would be needed to detect the effect.

In the ReBUILD study participants, we found a significant correlation between chronic demyelination (ie, chronic VEP delay) and neuroaxonal damage (here, NfL levels). This association adds to the recent, accumulating evidence that permanent demyelination is a considerable driver of neurodegeneration. Indeed, numerous investigations of brain tissue from pwMS showed accelerated pathology in chronically demyelinated axons through mitochondrial dysfunction, enhancement of oxidative injury, energy failure and altered calcium homoeostasis.[21] Moreover, more recent studies, both in non-human primate models and pwMS, demonstrated an association between chronic VEP delays and longitudinal retinal neuronal loss.[22 23] Altogether, all those findings affirm the relevance of monitoring, preventing and treating myelin injury for neuroaxonal health in pwMS.

The considerably low concentrations of NfL found in the ReBUILD trial, compared with other studies, could be explained by the strict inclusion criteria, which preferentially selected younger patients with stable disease and treated with DMT. In addition, plasma was processed from tubes using citrate as additive/anticoagulant, which has been recently found to be associated with significantly lower NfL values (~20%) compared with the more standard EDTA plasma.[24] Therefore, caution is warranted when comparing the absolute NfL concentrations, Z-scores and percentiles with previous studies in MS.

While we were able to demonstrate a reduction of NfL levels following remyelinati, no such difference was identified for other neuroaxonal markers, in line with existing evidence of limited application of blood tau and UCH-L1 in MS compared with NfL.[25] Similarly, GFAP levels remained stable over the trial period. A possible explanation could be the lack of clemastine's effect on astrocytes.[26]

A limitation of our study is the relatively limited number of participants, as samples were not available for all ReBUILD participants. In addition, none of ReBUILD subjects suffered from activity or progression during the study, which might limit the generalisation of the findings. Beyond that, the short follow-up period did not allow for evaluating the clemastine cessation's effect on NfL levels.

In summary, our study uses the unique cohort of the only successful remyelination phase-II trial reported in MS to provide evidence that remyelination-induced neuroprotection could be achievable, and could be evaluated using an easily accessible, blood-based neuroaxonal marker.

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Competing interests None declared.

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Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available upon reasonable request.

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