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Meta-Analysis for Neurofilament Light Chain (NfL) as a Biomarker in Mouse Experimental Autoimmune Encephalomyelitis (EAE) Studies

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Disclosures

- **Bernd Kieseier**, **Sarah Tisserand**, **Pamela Ramseier**, **Uffelmann Tatjana**, **Giuseppe Locatelli**, **Bruno Cenni**, **Barbara Nuesslein-Hildesheim**, and **Marc Bigaud** are employees of Novartis

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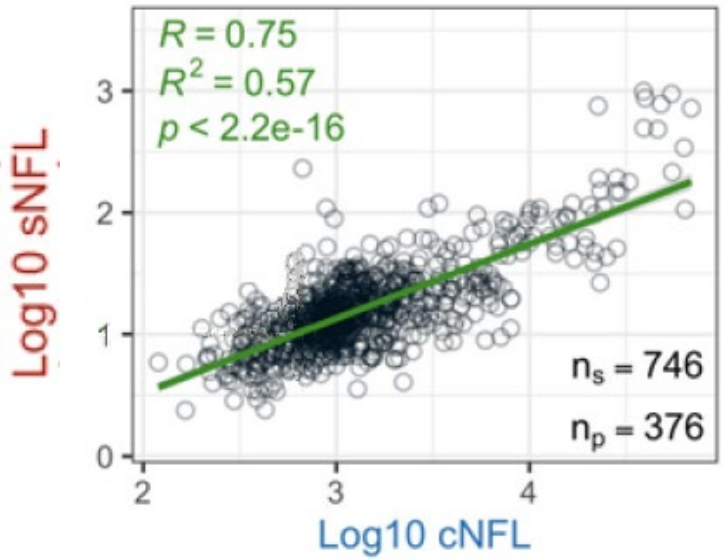
Introduction

- Neurofilament light chain (NfL): a promising prognostic biomarker of disease activity in multiple sclerosis (MS)[1,2]
- In patients with MS, the correlation between NfL levels in the cerebrospinal fluid (CSF; cNfL) and serum (sNfL)/plasma (pNfL) is well established[3,4]



("Enhancing the clinical value of serum neurofilament light chain measurement" by Kosa P et al. JCI Insight. 2023;7(15):e161415 is licensed under CC BY 4.0)

➔Main aim: assess the correlation between cNfL and pNfL in a preclinical model (experimental autoimmune encephalomyelitis [EAE] mouse)

sNfL, serum neurofilament light chain

1. Ning L et al. *PLoS One*. 2022;17(9):e0274565. **2.** Ziemssen T et al. *Front Immunol*. 2022;13:852563. **3.** Piehl F et al. *Mult Scler*. 2018;24(8):1046-1054. **4.** Kosa P et al. *JCI Insight*. 2023;7(15):e161415.

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Methods

- 25 independent mouse EAE studies (conducted *in house* within 2019-2021 for various purposes)
- All studies in C57BL/6J mice (n=238), with induction via ratMOG_{28-152} in complete Freund's adjuvant
- Main readouts:
  - Longitudinal clinical scoring (classical EAE scale)[1] up to 2 months post-disease induction (pDI)
    0 to 0.5: Normal appearance to distal limp tail
    1 to 1.5: Complete tail paralysis to hind limb weakness/grid test positive
    2 to 2.5: Unilateral partial hind limb paralysis/impaired righting reflex to bilateral partial hind limb paralysis
    3 to 3.5: Complete bilateral hind limb paralysis to fore limb weakness and complete bilateral hind limb paralysis
    4: Quadriplegia/moribund
    5: Death from EAE
  - Longitudinal plasma sampling and terminal CSF sampling to assess pNfL (n=202) and cNfL (n=176) levels (ELISA kit #10-7001, Uman Diagnostics)
- All results from control groups (Untreated and/or Vehicle-treated mice) were grouped in a single database for meta-analysis via "GraphPad Prism" and "R"

cNfL, cerebrospinal fluid neurofilament light chain; EAE, experimental autoimmune encephalomyelitis; pNfL, plasma neurofilament light chain

1. Pol S et al. *Exp Neurol*. 2019;314:82-90.

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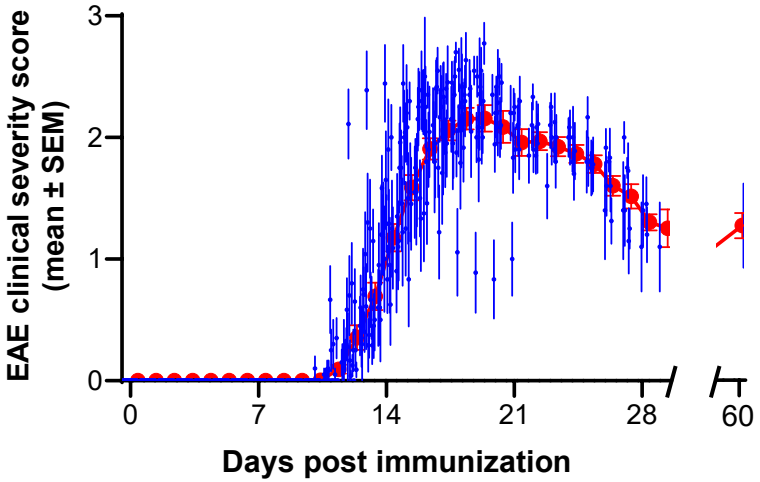
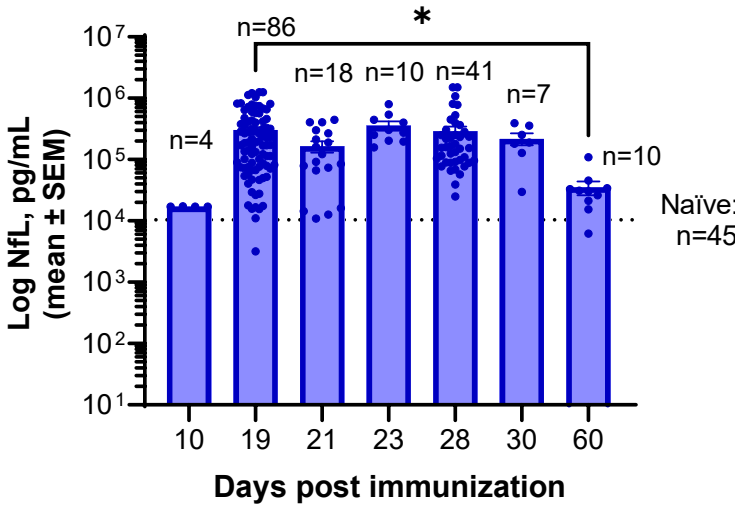
Results
Longitudinal Monitoring of EAE Scores and cNfL and pNfL Levels

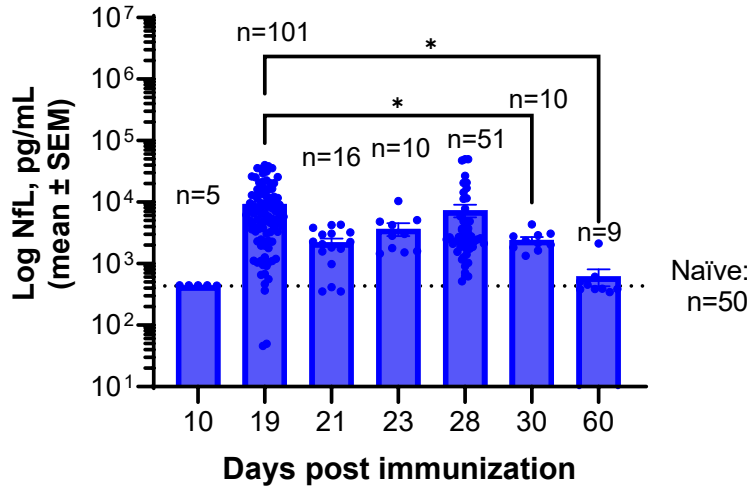
EAE Clinical Severity Scores
(25 individual studies, 238 mice in total)



Changes in cNfL Levels



Changes in pNfL Levels



- In untreated mice, EAE scores use to start increasing at 10 days pDI and peaked within 18-23 days pDI
- cNfL and pNfL levels are low in healthy controls (~10 and 0.4 ng/mL, respectively) and increase by 30-20–fold in Untreated EAE mice at peak of disease, reflecting CSF/plasma ratios of 25-35
- cNfL and pNfL levels remained elevated up to 1-month pDI and showed a ~10-fold reduction at 2 months pDI

*p=<0.05
cNfL, cerebrospinal fluid neurofilament light chain; CSF, cerebrospinal fluid; EAE, experimental autoimmune encephalomyelitis; ng/mL, nanogram per milliliter; pDI, post disease induction; pg/mL, picograms per milliliter; pNfL, plasma neurofilament light chain; SEM, standard error of mean

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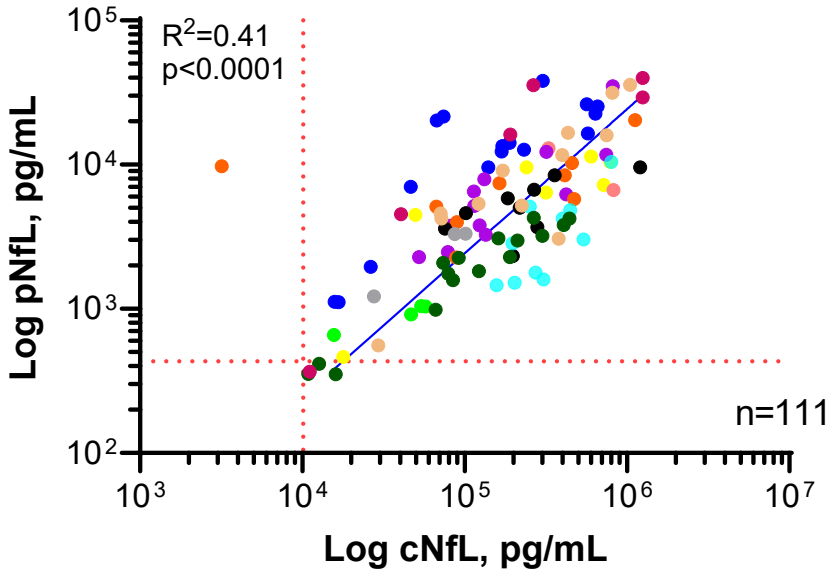
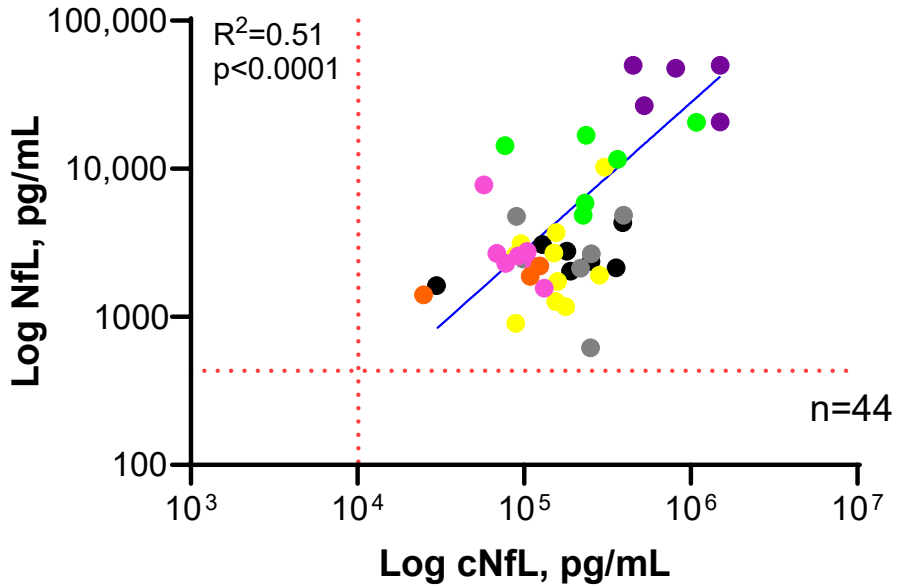
Results
Significant Correlations Between cNfL and pNfL

Correlation cNfL/pNfL at Peak of Disease (D19-23)



Correlation cNfL/pNfL at D28



➔ cNfL and pNfL were significantly correlated in untreated EAE mice at peak of disease and up to Day 28 pDI

p<0.0001 (via 2-way ANOVA)

ANOVA, analysis of variance; cNfL, cerebrospinal fluid neurofilament light chain; D, Day; EAE, experimental autoimmune encephalomyelitis; pDI, post disease induction; pg/mL, picograms per milliliter; pNfL, plasma neurofilament light chain

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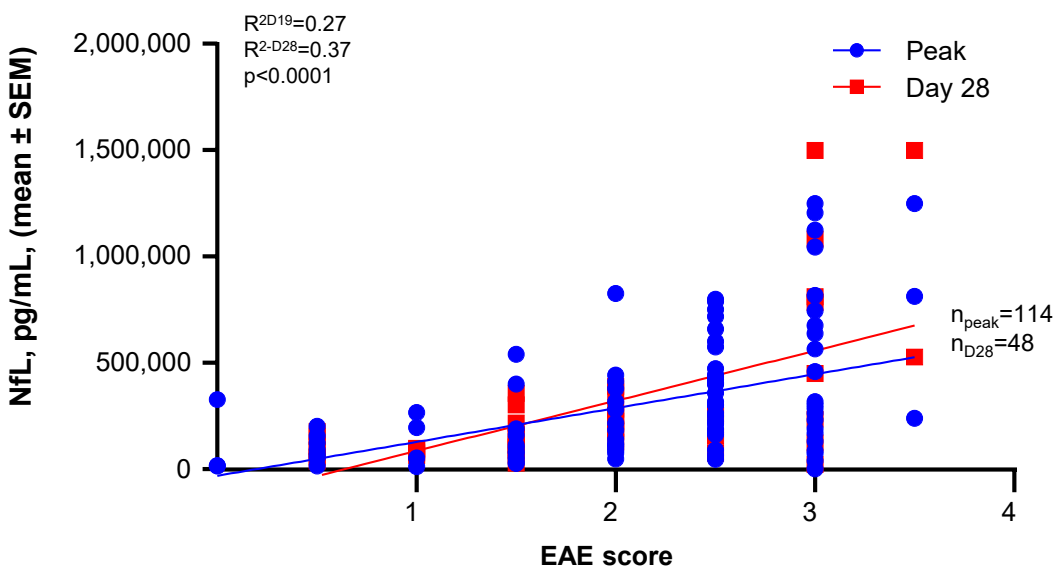
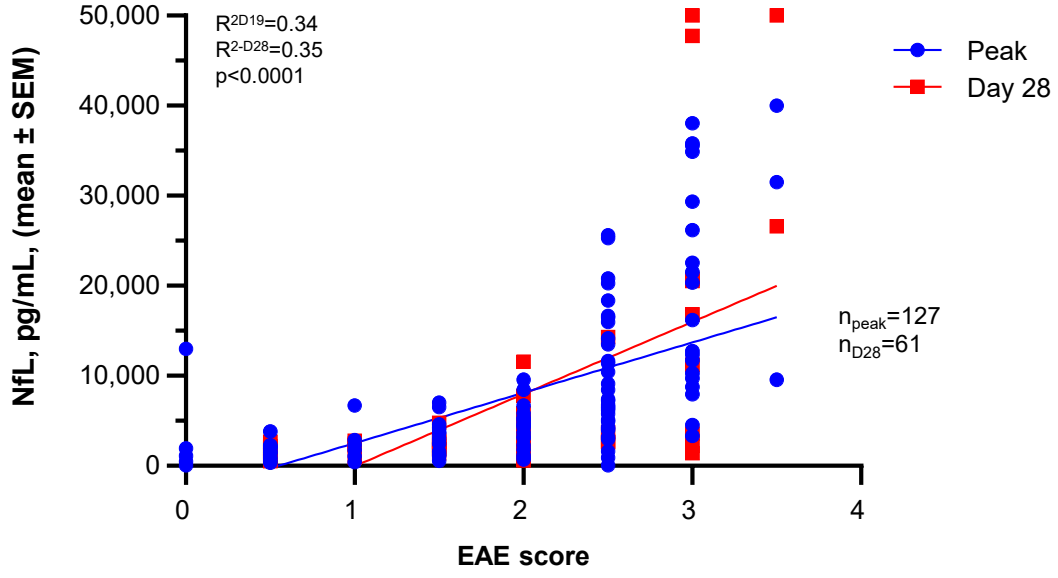
Results
Significant Correlations Between cNfL and pNfL and Clinical Scores

cNfL vs EAE Scores at Peak (D19-23) and D28



pNfL vs EAE Scores at Peak (D19-23) and D28



➔ Both cNfL or pNfL significantly correlated vs the EAE clinical scores in untreated mice at peak of disease, up to Day 28

p<0.0001 (via 2-way ANOVA)
ANOVA, analysis of variance; cNfL, cerebrospinal fluid neurofilament light chain; D, Day; EAE, experimental autoimmune encephalomyelitis; pg/mL, picograms per milliliter; pNfL, plasma neurofilament light chain; SEM, standard error of mean

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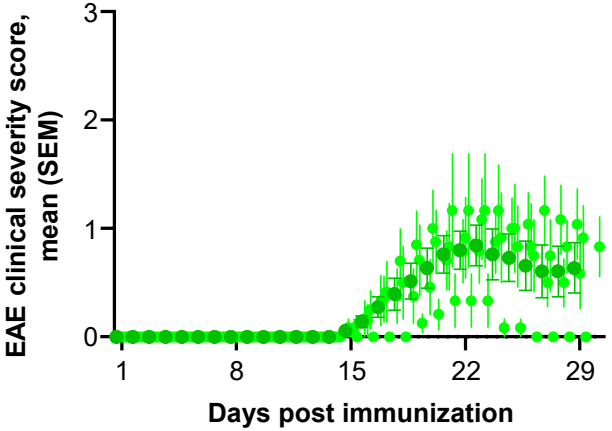
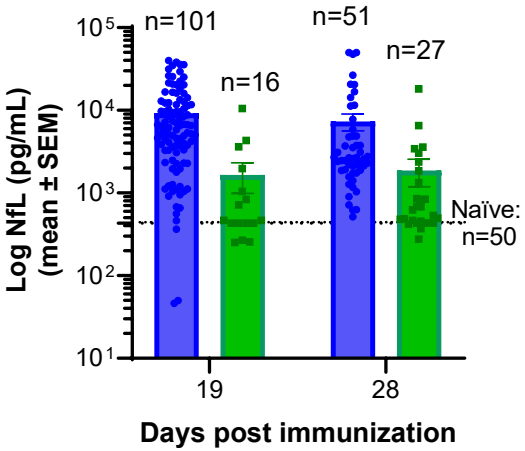
Results
Effects of Remibrutinib on cNfL and pNfL Level

Mean Curves of:
4 individual studies (46 mice)[1]

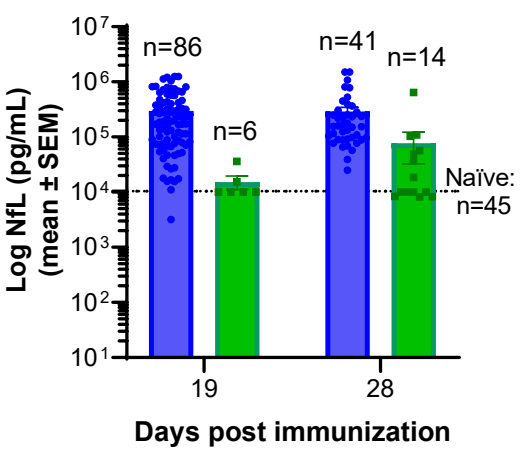


("Remibrutinib (LOU064) inhibits neuroinflammation driven by B cells and myeloid cells in preclinical models of multiple sclerosis" by Nuesslein-Hildesheim B et al. J Neuroinflammation. 2023;20(1):194 is licensed under CC BY 4.0)

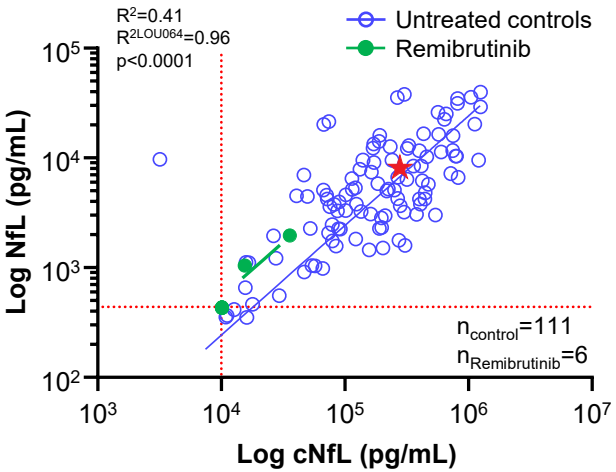
pNfL Levels
(Controls vs Remibrutinib)



cNfL Levels
(Controls vs Remibrutinib)



Correlation cNfL/pNfL
at Peak of Disease



➔ Remibrutinib, a novel, potent, and highly selective Bruton's tyrosine kinase inhibitor, achieved significant reductions in EAE scores,[1] as well as cNfL and pNfL levels

p<0.0001 (via 2-way ANOVA).
ANOVA, analysis of variance; cNfL, cerebrospinal fluid neurofilament light chain; EAE, experimental autoimmune encephalomyelitis; pg/mL, picograms per milliliter; pNfL, plasma neurofilament light chain; SEM, standard error of mean

1. Nuesslein-Hildesheim B et al. *J Neuroinflam*. 2023;20:194

Conclusions

- In EAE mice, pNfL levels are highly correlated with cNfL, consistent with observations in patients with MS
- The present meta-analysis supports the translational value of pNfL monitoring in mouse EAE studies for estimating the therapeutic potential of new disease-modifying therapies (eg, remibrutinib is currently in development for MS)
- Additional work is needed to assess the correlation between changes in pNfL levels and neuroaxonal damage in EAE mice

cNfL, cerebrospinal fluid neurofilament light chain; EAE, experimental autoimmune encephalomyelitis; MS, multiple sclerosis; pNfL, plasma neurofilament light chain

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