Plasma Neurofilament Light Chain and Glial Fibrillary Acidic Protein Levels are Prognostic of Disability Worsening: A Biosignature That Helps in Differentiating Active From Non-active SPMS

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Disclosures

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David Leppert was the Therapeutic Area Head at Novartis, Neuroscience Development Unit, until January 2019. He is Chief Medical Officer of GeNeuro. He has received personal compensation for consulting and speaking, and travel reimbursement from Quanterix, Orion, Novartis, Roche, and Sanofi.

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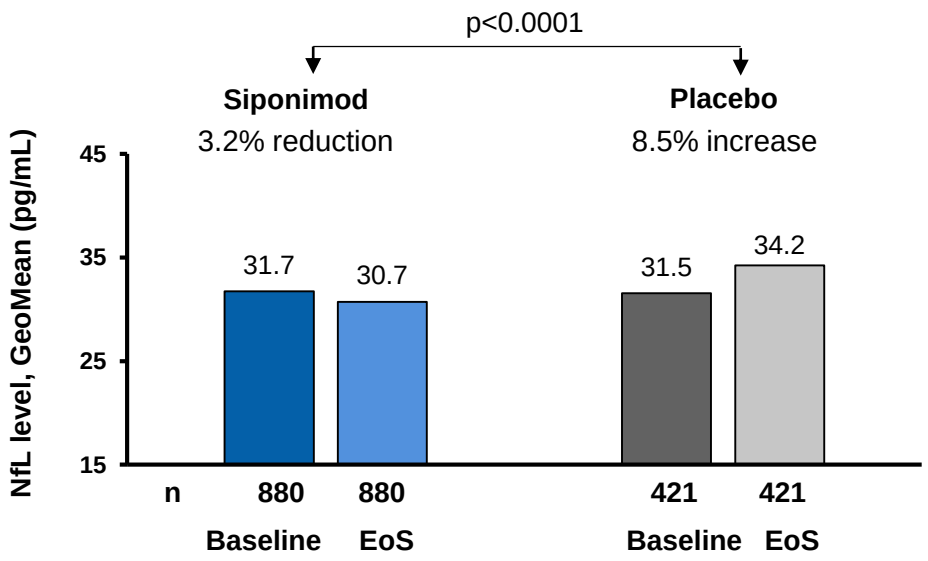
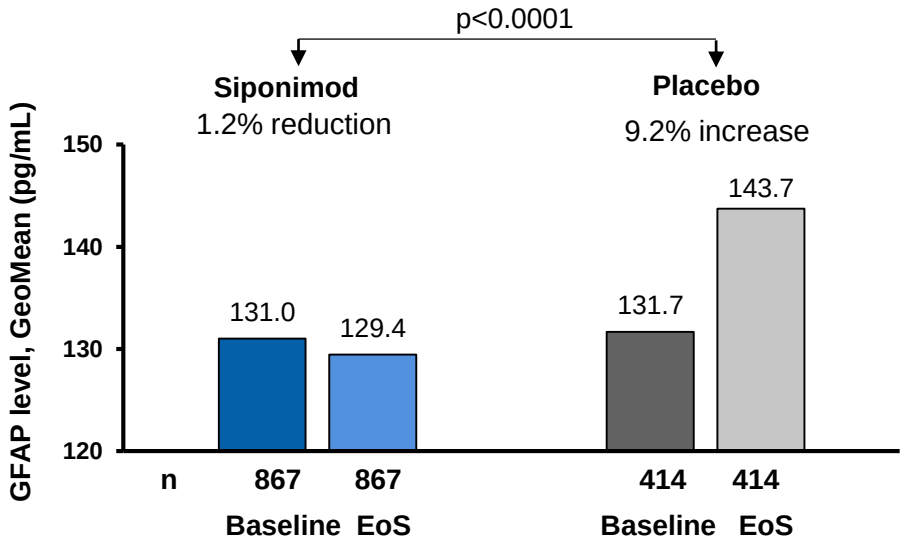
Background (1/2)

- Baseline pNfL and pGFAP levels correlate with disease activity and showed utility as markers of disability and treatment response in SPMS in the Phase 3 EXPAND trial[1,2]
  - Siponimod (vs placebo) significantly reduced pNfL levels[3] and pGFAP levels[2]
- Both GFAP and NfL showed a strong correlation between CSF and serum levels[4]

Change in NfL levels[1,3]



Change in GFAP levels[2]



CSF, cerebrospinal fluid; EoS, end of study; GeoMean, geometric mean; GFAP, glial fibrillary acidic protein; n, number of patients; NfL, neurofilament light chain; pGFAP, plasma glial fibrillary acidic protein; pNfL, plasma neurofilament light chain; SPMS, secondary progressive multiple sclerosis

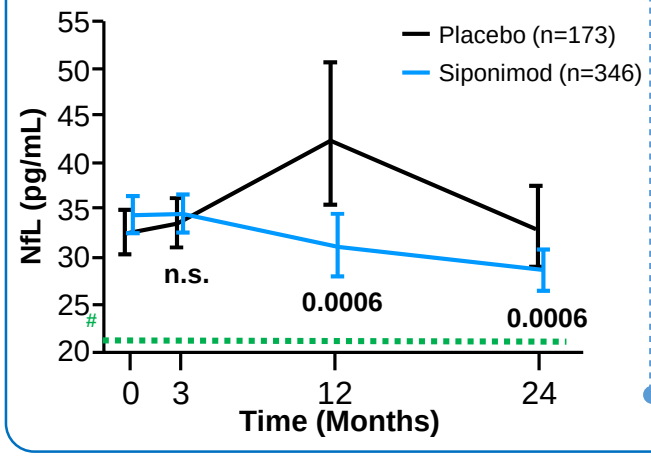
1. Kuhle J et al, Presented at *AAN* 2018. S8.006; 2. Kuhle J et al. Presented at *AAN* 2020. S10.006 3. Kuhle J et al. Presented at *ACTRIMS* 2021. P017; 4. Watanabe M. et al *Neurology*. 2019;93:e1299-e1311.

Background (2/2)

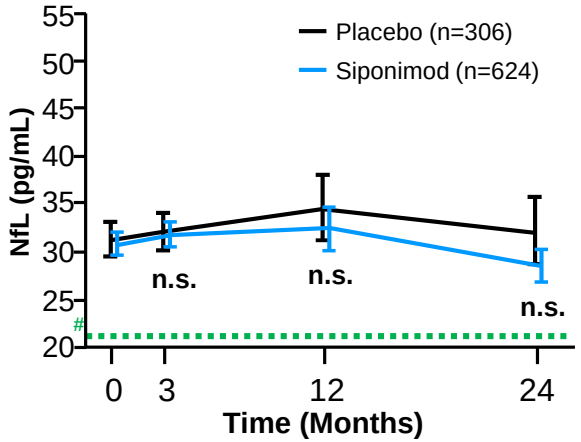
- In the MS disease course, pNfL was shown to be a treatment response marker mainly in patients with relapses[1]; whereas pGFAP was responsive to treatment effects in both patients with/without relapses[2]
- Patients with high pGFAP levels at baseline were at a higher risk of disability worsening (reaching EDSS 7.0, i.e. being wheelchair restricted)[3]
- Combining information from both blood biomarkers may enable better identification of patients at risk of progression

Change in blood NfL levels over time[1]

Patients with pre-study relapses

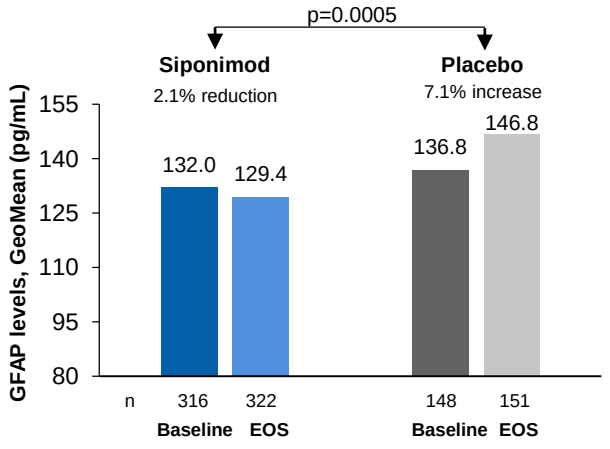


Patients without pre-study relapses

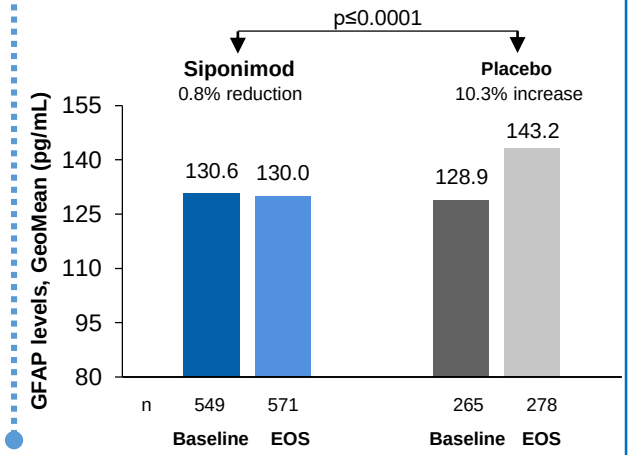


Change in blood GFAP levels over time[3]

Patients with pre-study relapses (N=518)



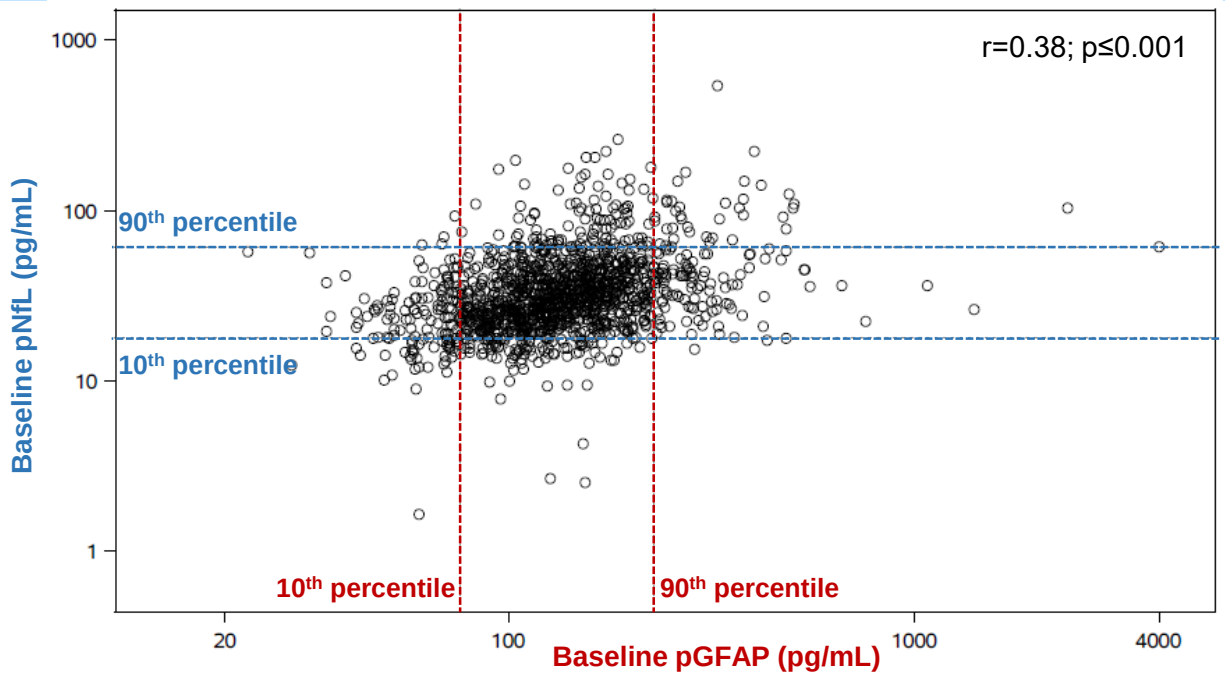
Patients without pre-study relapses (N=928)



#The green dotted line marks an estimate of the NfL level of healthy controls of similar age[4]; EDSS, expanded disability status scale; EoS, end of study; GeoMean, geometric mean; GFAP, glial fibrillary acidic protein; MS, multiple sclerosis; n, number of patients; NfL, neurofilament light chain; n.s., non-significant; pGFAP, plasma glial fibrillary acidic protein; pNfL, plasma neurofilament light chain

1. Kuhle J et al, et al. presented at *AAN* 2018. S8.006; 2. Kuhle J et al. Presented at *ACTRIMS-ECTRIMS* 2020. FC04.03; 3. Kuhle J et al. Presented at *AAN* 2020. S10.006; 4. Disanto G, et al. *Ann Neurol* 2017;81:857–870.

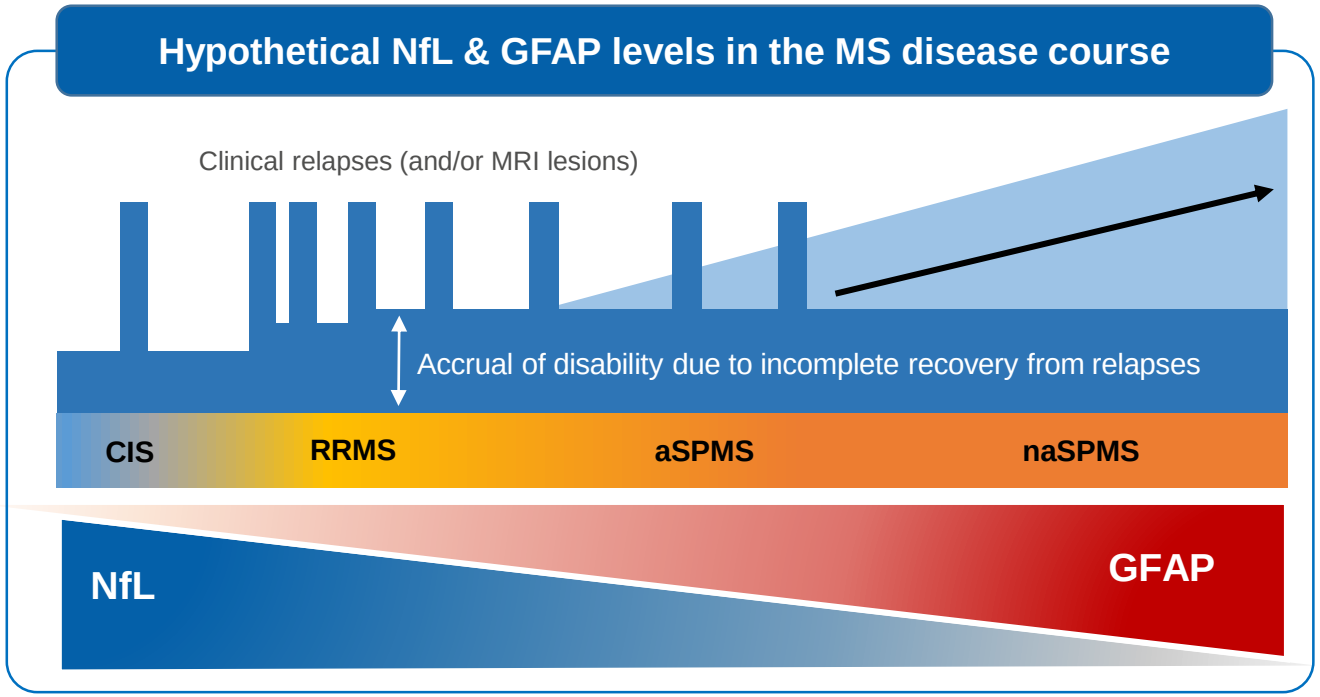
Correlation Between pNfL and pGFAP in SPMS (EXPAND Trial)



- **Despite correlation, there are patients with high pNfL but low pGFAP or high pGFAP but low pNfL**
- **Patients with baseline pNfL values at the 10th percentile spread in their baseline pGFAP values wider than from the 10th to the 90th pGFAP percentile. Likewise, patients with baseline pGFAP values at the 10th percentile vary similarly strongly in their pNfL values**
- **pNfL and pGFAP may provide non-overlapping and complementary information, being potential candidates for a "biosignature"**

pGFAP, plasma glial fibrillary acidic protein; pNfL, plasma neurofilament light chain; SPMS, secondary progressive multiple sclerosis

Objective



To explore the value of pNfL+pGFAP as prognostic markers of disability worsening in SPMS

aSPMS, active secondary progressive multiple sclerosis; CIS, clinically isolated syndrome; GFAP, glial fibrillary acidic protein; MRI, magnetic resonance imaging; naSPMS, non-active secondary progressive multiple sclerosis; NfL, neurofilament light chain; pGFAP, plasma glial fibrillary acidic protein; pNfL, plasma neurofilament light chain; RRMS, relapsing-remitting multiple sclerosis; SPMS, secondary progressive multiple sclerosis

Methods

A post hoc analysis from the Phase 3 EXPAND study (core+extension[a]) in SPMS patients

Assessments

- pNfL and pGFAP levels at baseline were measured in EDTA plasma samples (1369/1651 patients) using SIMOA technology
- pNfL: low (<30 pg/mL) versus high (≥30 pg/mL)
- pGFAP: low (<130 pg/mL) versus high (≥130 pg/mL)

Outcomes

- The relationship between the levels of pNfL and pGFAP and below outcomes was assessed in a regression model with baseline pNfL and pGFAP categories as a covariable
  - Time to 3mCDW based on EDSS
  - Time to 1-point sustained EDSS worsening
  - Time to sustained EDSS 7.0

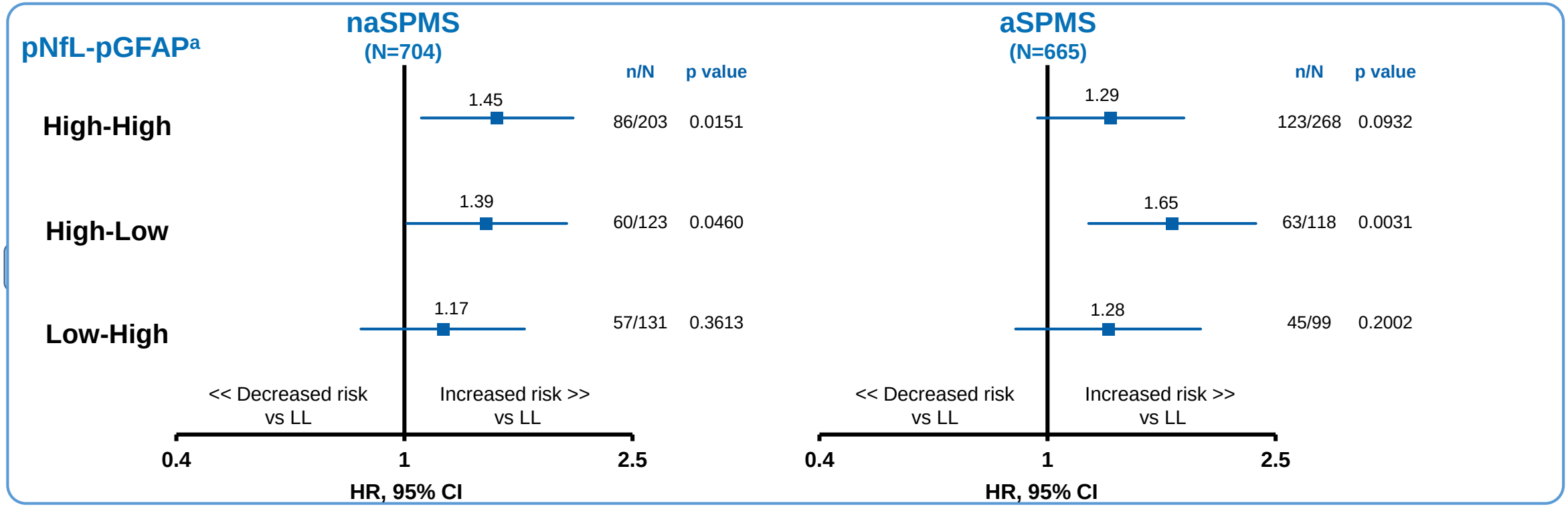
Statistical analysis

- In order to evaluate the value of pNfL and pGFAP under different disease conditions, analyses were conducted separately for the naSPMS[b] and aSPMS[c] patient groups
- The Cox regression model was adjusted for treatment, age, sex, disease duration, and EDSS score at baseline

[a]Ongoing, data cut-off: 06 Apr 2019; [b]Patients without relapses ≤24 months prior to study entry and no gadolinium-enhancing T1 lesions at baseline; [c]Patients with relapses ≤24 months prior to study entry and/or gadolinium-enhancing T1 lesions at baseline; 3mCDW, 3-month confirmed disability worsening; aSPMS, active secondary progressive multiple sclerosis; EDSS, Expanded Disability Status Scale; EDTA, ethylenediaminetetraacetic acid; naSPMS, non-active secondary progressive multiple sclerosis; pGFAP, glial fibrillary acidic protein; pNfL, plasma neurofilament light chain; SIMOA, Single Molecule Array; SPMS, secondary progressive multiple sclerosis

Association Between pNfL and pGFAP and Time to 3mCDW in Patients with naSPMS and aSPMS

Risk of disability worsening associated with signatures of high baseline pNfL and/or high baseline pGFAP vs. the Low-Low (LL) signature



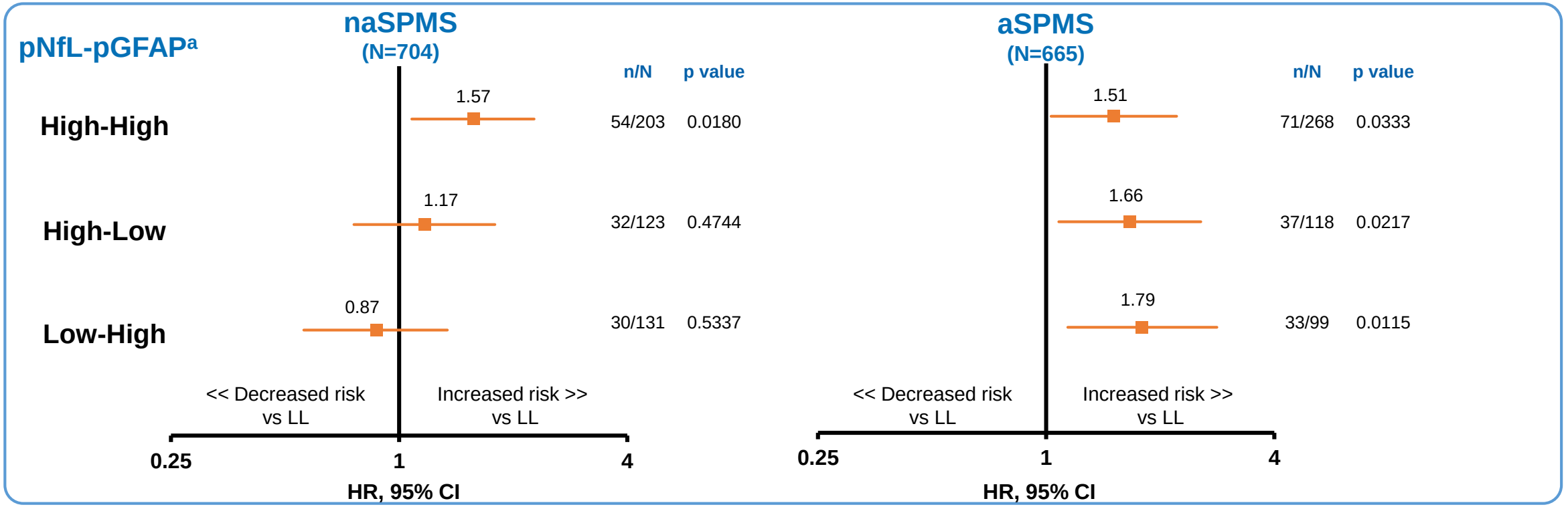
- **In naSPMS, the signature high NfL+high GFAP (High-High) reached significance in the prognosis of 3mCDW**
- **In aSPMS, the signature high NfL+low GFAP (High-Low) had a higher HR than the signature High-High or Low-High**

[a]Baseline pNfL: low (<30 pg/mL) versus high (≥30 pg/mL); pGFAP: low (<130 pg/mL) versus high (≥130 pg/mL); the Cox regression model was adjusted for treatment, age, sex, disease duration, and EDSS score at baseline

3mCDW, 3-month confirmed disability worsening; aSPMS, active secondary progressive multiple sclerosis; CI, confidence interval; HR, hazard ratio; naSPMS, non-active secondary progressive multiple sclerosis; pGFAP, plasma glial fibrillary acidic protein; pNfL, plasma neurofilament light chain

Association Between the pNfL-pGFAP Biosignature and Time to 1-point Sustained EDSS Worsening in Patients with naSPMS and aSPMS

Risk of disability worsening associated with signatures of high baseline pNfL and/or high baseline pGFAP vs. the Low-Low (LL) signature



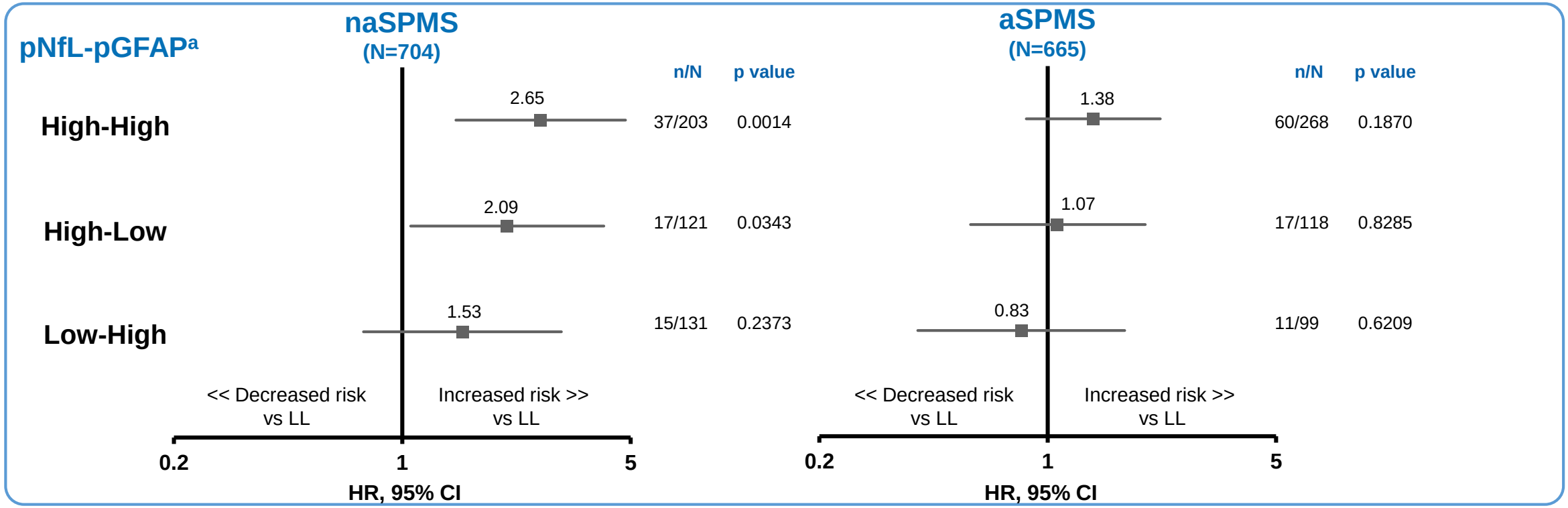
- **In naSPMS patients, the high pNfL+high pGFAP (High-High) signature was the signature with the highest risk of 1-point sustained EDSS worsening**
- **In aSPMS patients, the High-High signature was associated with a lower HR compared with the Low-High signature**

[a]Baseline pNfL: low (<30 pg/mL) versus high (≥30 pg/mL); pGFAP: low (<130 pg/mL) versus high (≥130 pg/mL); the Cox regression model was adjusted for treatment, age, sex, disease duration, and EDSS score at baseline

aSPMS, active secondary progressive multiple sclerosis; CI, confidence interval; EDSS, Expanded Disability Status Scale; HR, hazard ratio; naSPMS, non-active secondary progressive multiple sclerosis; pGFAP, plasma glial fibrillary acidic protein; pNfL, plasma neurofilament light chain

Association Between the pNfL-pGFAP Biosignature and Time to Sustained EDSS 7.0 in Patients with naSPMS and aSPMS

Risk of disability worsening associated with signatures of high baseline pNfL and/or high baseline pGFAP vs. the Low-Low (LL) signature



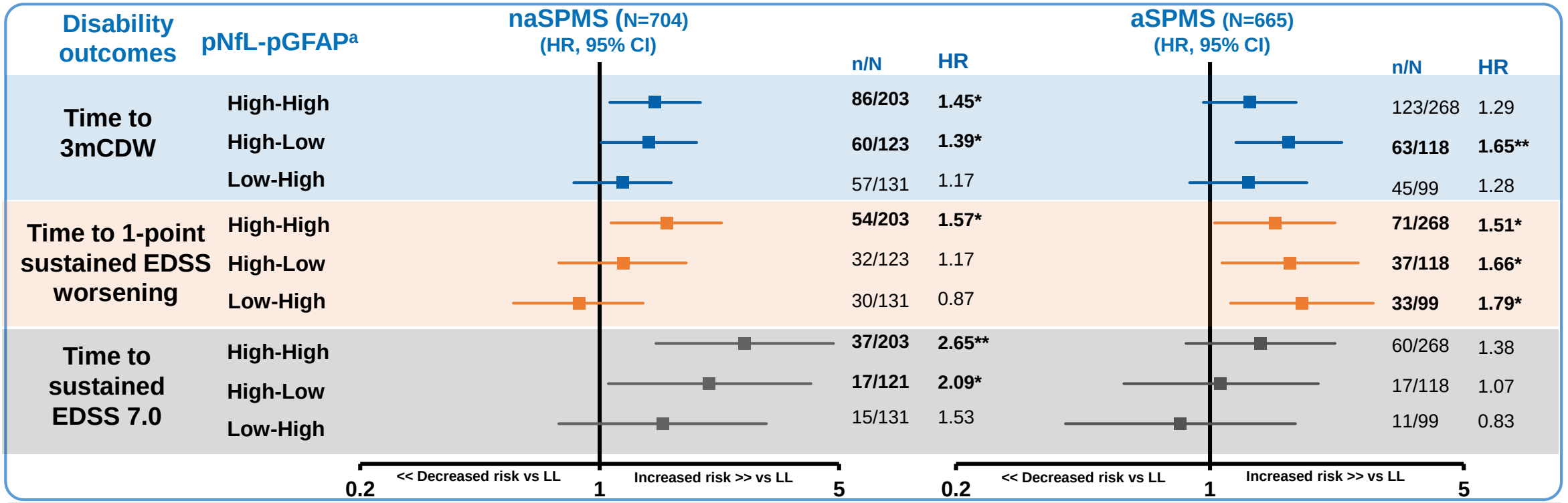
In both naSPMS and aSPMS patients, the high pNfL+high pGFAP (High-High) signature was associated with the highest risk of worsening towards EDSS 7.0 compared with the other signatures

[a]Baseline pNfL: low (<30 pg/mL) versus high (≥30 pg/mL); pGFAP: low (<130 pg/mL) versus high (≥130 pg/mL); the Cox regression model was adjusted for treatment, age, sex, disease duration, and EDSS score at baseline

aSPMS, active secondary progressive multiple sclerosis; CI, confidence interval; EDSS, Expanded Disability Status Scale; HR, hazard ratio; naSPMS, non-active secondary progressive multiple sclerosis; pGFAP, plasma glial fibrillary acidic protein; pNfL, plasma neurofilament light chain

pNfL-pGFAP Biosignatures and the Risk of Disability Worsening in Patients with naSPMS and aSPMS

Risk of disability worsening associated with signatures of high baseline pNfL and/or high baseline pGFAP vs. the Low-Low (LL) signature



- **The signature high pNfL in combination with high pGFAP (High-High) was most consistently associated with higher risk of disability worsening in naSPMS**
- **The added value of the High-High signature was less apparent in aSPMS (no consistent trend across disability outcomes); high pNfL seems to be more important than high pGFAP in naSPMS**

[a]Baseline pNfL: low (<30 pg/mL) versus high (≥30 pg/mL); pGFAP: low (<130 pg/mL) versus high (≥130 pg/mL); *Statistical significance at *P*<0.05;**Statistical significance at *P*<0.01; the Cox regression model was adjusted for treatment, age, sex, disease duration, and EDSS score at baseline

3mCDW, 3-month confirmed disability worsening; aSPMS, active secondary progressive multiple sclerosis; CI, confidence interval; EDSS, Expanded Disability Status Scale; HR, hazard ratio; N, number of patients included in the analysis, n, number of patients with event; naSPMS, non-active secondary progressive multiple sclerosis; pGFAP, plasma glial fibrillary acidic protein; pNfL, plasma neurofilament light chain

Conclusions

- pNfL and pGFAP levels are significantly but only weakly correlated in SPMS patients and may harbor complementary information (“biosignature” composed of both biomarkers)
- High baseline levels of both pNfL and pGFAP were most consistently associated with higher risk of worse disability outcomes in naSPMS
- In this analysis, the prognostic value of baseline levels of these two markers individually or in combination was weaker in the aSPMS population. Further analysis of the data, investigating other cut-offs and taking into account potential differential and time dependent treatment effects are ongoing
- This is a post hoc analysis and the study was not powered to achieve clinically relevant effects with statistical significance; no adjustments for multiple comparisons have been applied
- Validation of this biosignature in real-world practice and in other progressive forms MS is warranted

aSPMS, active secondary progressive multiple sclerosis; MS, multiple sclerosis; naSPMS, non-active secondary progressive multiple sclerosis; pGFAP, plasma glial fibrillary acidic protein; pNfL, plasma neurofilament light chain; SPMS, secondary progressive multiple sclerosis