Poster 293

Plasma Neurofilament Analysis in VITALITY-ALS

Tyrell J. Simkins[1], Robert P. Bowser[2], Stuart Kupfer[1], Fady I. Malik[1], Lisa Meng[1], Stacy A. Rudnicki[1], Jenny Wei[1], Andrew A. Wolff[1], Jeremy M. Shefner[2]

[1]Cytokinetics Incorporated, South San Francisco, CA, USA;
[2]Barrow Neurological Institute, University of Arizona, and Creighton University, Phoenix, AZ, USA

BACKGROUND & OBJECTIVES

- In ALS, researchers need biomarkers that may identify people more likely to have a meaningful response to therapeutics.
- Neurofilaments are a class of intermediate filaments in neurons of both the central and peripheral nervous systems.
  - They are major constituents of the cytoskeletal structure of neurons, particularly in axons.
  - Neurofilament light chain (NfL) and phosphorylated neurofilament heavy chain (pNfH) have gained attention as potential biomarkers in several neurologic disorders, including ALS.
  - It is hypothesized that NfL and pNfH are released from the cytoplasm of injured or dying neurons, which can then be measured in either cerebrospinal fluid or plasma.

Objectives

- Measurements from the VITALITY-ALS study were used to:
  - Compare baseline NfL and pNfH values according to demographics and clinical features.
  - Assess relationships between baseline NfL or pNfH values and pre-study rate of disease progression (psRDP).
  - Evaluate NfL and pNfH values over 48 weeks.

METHODS

- VITALITY-ALS (NCT02496767) was a 48-week, randomized, double-blind, placebo-controlled Phase 3 study of *tirasemtiv*, a fast skeletal muscle troponin activator, in 565 people with ALS (pALS).[1]
  - Results found *tirasemtiv* did not show an effect on vital capacity, ALSFRS-R, or muscle strength.[1]
  - However, the dataset provides a large longitudinal cohort for analysis, with plasma samples collected every 8 weeks over 48 weeks.
- In these post hoc analyses, plasma NfL and pNfH were compared by treatment, clinical characteristics, and time using a mixed model for repeated measures adjusted for baseline values.
- Pearson correlation coefficients (r) were used to evaluate the strength of the relationship between psRDP and baseline NfL or pNfH for the overall population as well as psRDP tertiles representing fast, intermediate, and slow progressors.
  - psRDP was calculated as 48 minus baseline ALSFRS-R score divided by symptom duration in months.
- NfL was measured using the Meso Scale Discovery (MSD) Neurofilament L chain S-PLEX kit (cat #K151AKGS), and pNFH was detected using an analytically validated pNFH assay on the MSD platform developed by Iron Horse Diagnostics as previously described.[2]
- For this analysis, *P* values <0.05 were deemed significant.

RESULTS

- NfL and pNfH measurements were available for 101 participants in the placebo group and 161 in the *tirasemtiv* group (**Table 1**).
- *Tirasemtiv* had no treatment effect on NfL or pNfH values over time. Therefore, all available study samples were combined for subsequent analysis.
- Comparing NfL or pNfH values for subgroups by clinical characteristics did not identify statistically significant differences in any category (**Table 2**).
  - However, there was a moderate correlation between psRDP and baseline NfL or pNfH values (**Figure 1**).
  - When evaluated by tertiles of psRDP, the fastest progressors showed the strongest correlation (**Figure 2**).
- Over 48 weeks, NfL and pNfH both decreased (**Figure 3**).
  - For pNfH, the least squares mean difference (LSMD) first reached significance at Week 16 (*P*=0.0003); LSMD increased further with each 8-week interval (*P*<0.0001 from Week 24 onward).
  - NfL levels showed a less consistent pattern but, overall, modestly decreased over time.
  - The pattern was similar when the analysis was restricted to participants with data available to Week 48.

Table 1. Baseline demographics and disease characteristics

	Placebo (n=101)	*Tirasemtiv* (n=161)
Age, mean ± SD, years	55.7 ± 11.4	56.0 ± 9.5
Age <65 years, n (%)	76 (75.2)	130 (80.7)
Female, n (%)	33 (32.7)	48 (29.8)
% predicted SVC, mean ± SD	91 ± 16	91 ± 14
ALSFRS-R total score, mean ± SD	38.4 ± 5.1	38.6 ± 4.9
Riluzole use, n (%)	78 (77.2)	131 (81.4)
Site of onset, n (%)[a]		
Upper limb	43 (42.6)	79 (49.1)
Lower limb	41 (40.6)	62 (38.5)
Bulbar	17 (16.8)	19 (11.8)
Symptom duration, mean ± SD, months	22.9 ± 17.6	20.3 ± 11.7
Time since diagnosis, mean ± SD, months	8.4 ± 6.3	7.5 ± 5.5
Tertile of psRDP, n (%)		
Slow	35 (34.7)	49 (30.4)
Intermediate	30 (29.7)	60 (37.3)
Fast	36 (35.6)	52 (32.3)
psRDP, mean ± SD	0.57 ± 0.43	0.59 ± 0.47
ALS family history, n (%)		
Yes	2 (2.0)	14 (8.7)
Unknown	4 (4.0)	8 (5.0)

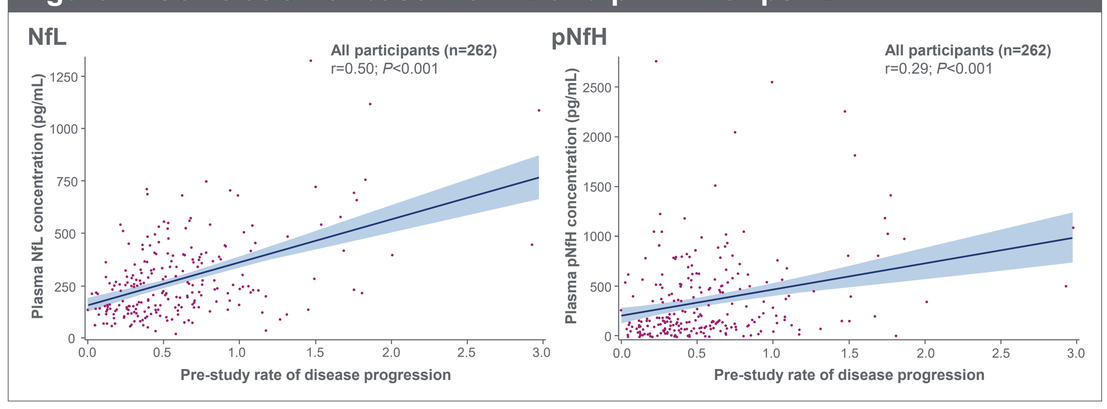
[a] One person in the *tirasemtiv* group had respiratory onset.
ALSFRS-R, ALS Functional Rating Scale-Revised; psRDP, pre-study rate of disease progression; SVC, slow vital capacity.

Table 2. Baseline NfL and pNfH levels according to clinical characteristics

	NfL, pg/mL			pNfH, pg/mL		
Site of onset	Bulbar (n=36)	Limb (n=225)		Bulbar (n=36)	Limb (n=225)	
Mean ± SD	336.8 ± 173.7	269.4 ± 188.1		404.9 ± 396.7	356.0 ± 406.4	
El Escorial criteria	Definite (n=73)	Possible / probable[a] (n=189)		Definite (n=73)	Possible / probable[a] (n=189)	
Mean ± SD	317.4 ± 222.2	263.0 ± 170.0		405.5 ± 452.3	344.4 ± 384.4	
Symptom duration, m	0–12 (n=62)	12–24 (n=118)	≥24 (n=82)	0–12 (n=62)	12–24 (n=118)	≥24 (n=82)
Mean ± SD	355.8 ± 215.5	287.6 ± 194.1	205.7 ± 115.9	461.4 ± 409.4	379.1 ± 465.8	260.3 ± 263.1

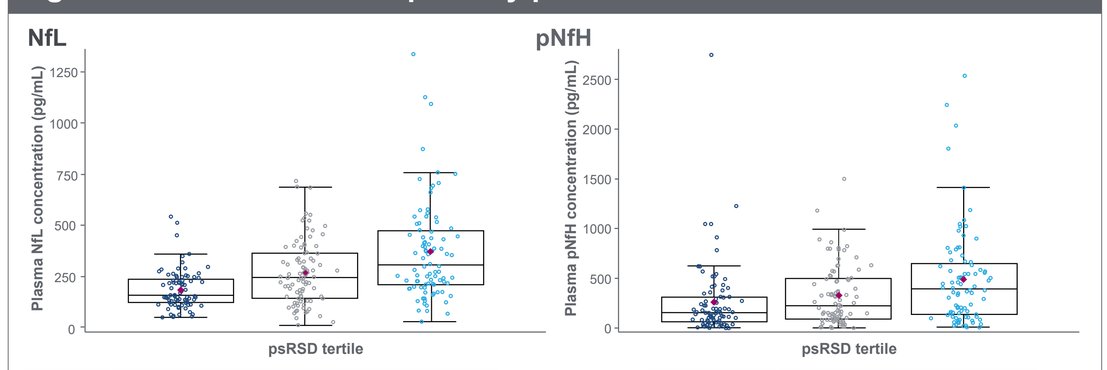
There were no statistically significant differences between groups in any category (all *P*>0.05).
[a] Includes probable lab-supported. m, months.

Figure 1. Correlation of baseline NfL and pNfH with psRDP



The solid line indicates the standardized slope of the linear regression line, and the shaded area indicates the 95% confidence intervals.
NfL, neurofilament light chain; pNfH, phosphorylated neurofilament heavy chain; psRDP, pre-study rate of disease progression; r, Pearson correlation coefficient.

Figure 2. Baseline NfL and pNfH by psRDP tertiles

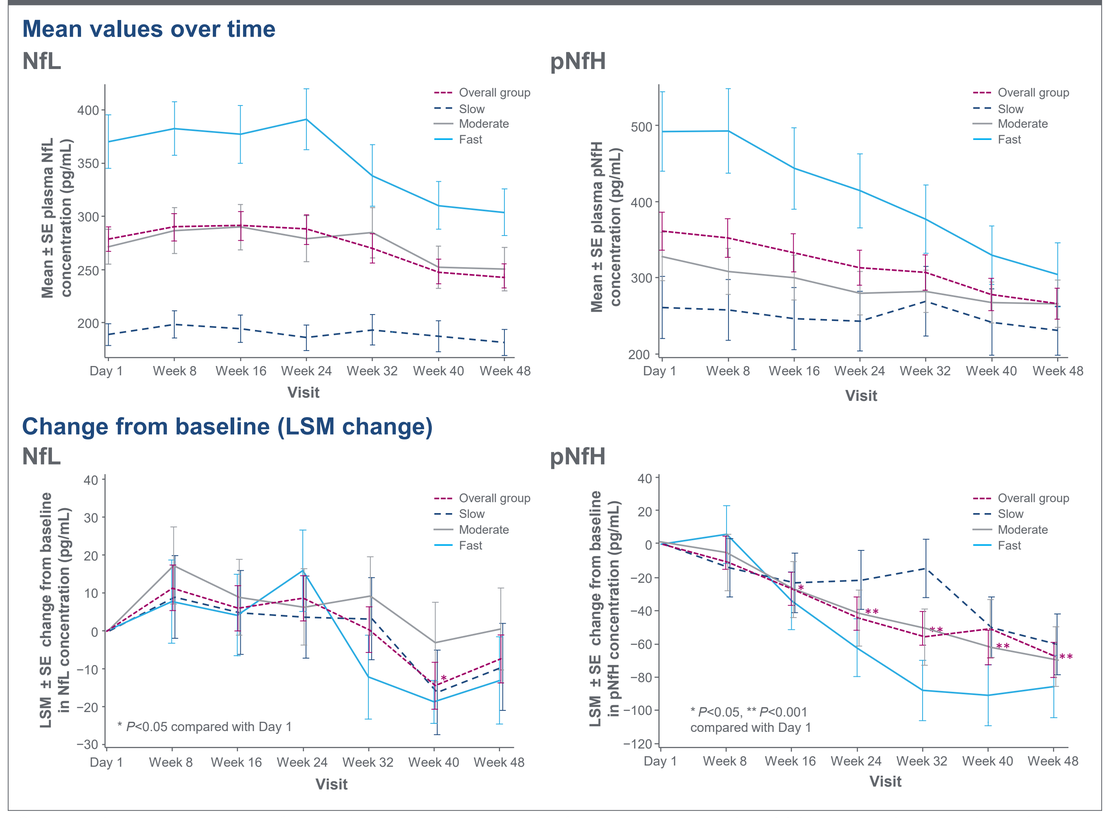


	Slow (n=84)	Intermediate (n=90)	Fast (n=88)
r	0.18	0.05	0.43
P value	0.109	0.672	<0.001

Slow (n=84)	Intermediate (n=90)	Fast (n=88)
0.10	0.01	0.24
0.370	0.890	0.027

Mean values for NfL and pNfH were not significantly different between tertiles.
In the box plots, diamonds indicate the mean. Box edges indicate the 25th to 75th percentiles (the interquartile range), horizontal lines in between the edges indicate the medians, whiskers extend to the lower and upper adjacent values. Adjacent values are the lowest and highest observations that are still inside the following limits: lower limit, Q1 minus 1.5 x (Q3 minus Q1); upper limit, Q3 plus 1.5 x (Q3 minus Q1).
NfL, neurofilament light chain; pNfH, phosphorylated neurofilament heavy chain; psRDP, pre-study rate of disease progression; r, Pearson correlation coefficient.

Figure 3. NfL and pNfH over time, for overall group and by psRDP tertiles



LSM calculated using mixed model for repeated measures was adjusted for baseline value, visit, psRDP tertile, and the interactions of baseline value-by-visit and psRDP-by-visit.
LSM, least-squares mean; psRDP, pre-study rate of disease progression.

Limitations

- This is a post hoc analysis of data obtained in an investigational drug trial.
- Data were not available for all participants and, over time, some patients died or discontinued the study; thus, later time points represent fewer patients.

CONCLUSIONS

- In the cohort of pALS participating in VITALITY-ALS, baseline neurofilament levels did not differ between subgroups with different clinical variables, including ALS diagnostic certainty and symptom duration.
  - Nevertheless, there was a moderate correlation with psRDP primarily driven by the fastest progressors.
  - NfL was more strongly correlated with psRDP with less variation observed compared with pNfH.
- Over time in this cohort of pALS, both neurofilaments measured in this analysis (NfL and pNfH) decreased.
  - The more robust decline of pNfH compared with NfL may be related to plasma stability or differential release from neurons over time.

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