STATISTICAL ANALYSIS PLAN (SAP)

Determining the longitudinal stability of plasma neurofilament light chain (NfL) in individuals at-risk for symptomatic FTLD through the Neurofilament Surveillance Project (NSP)

Sponsor: The Bluefield Project to Cure Frontotemporal Dementia

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1 LIST OF ABBREVIATIONS

Abbreviation or Special Term	Explanation
AE	Adverse event
ALLFTD	ARTFL-LEFFTDS Longitudinal Frontotemporal Lobar Degeneration
ARTFL-LEFFTDS	Advancing Research and Treatment for FTLD-Longitudinal Evaluation of Familial FTLD Syndromes
BMI	Body Mass Index
CDR	Clinical Dementia Rating (CDR®) Dementia Staging Instrument
CDR®+NACC-FTLD	CDR Dementia Stage Instrument PLUS National Alzheimer's Coordinating Center (NACC) Behavior and Language Domains
CDR®+NACC-FTLD-SB	CDR®+NACC-FTLD Sum of Boxes
f-FTLD	Familial frontotemporal lobar degeneration
FTD	Frontotemporal dementia
FTLD	Frontotemporal lobar degeneration
LME	Linear mixed effects
MINT	Multilingual Naming Test
MoCA	Montreal Cognitive Assessment Test
MRI	Magnetic resonance imaging
NfL	Neurofilament light chain
NHS	Natural history study
NSP	Neurofilament Surveillance Project
ROI	Region of interest
SAP	Statistical analysis plan
TIV	Total intracranial volume
UDS3-EF	Uniform Data Set v3.0 Executive Function

2 INTRODUCTION

This statistical analysis plan (SAP) describes the analysis for the Neurofilament (NfL) Surveillance Project (NSP) in participants from families with identified pathogenic FTLD mutations who have or are at-risk for symptomatic frontotemporal lobar degeneration (FTLD), including family members who do not carry the mutation. FTLD is a heterogenous group of neurodegenerative diseases. The SAP defines the analysis population and prespecifies the statistical methods for analyzing the longitudinal NfL measurements in plasma collected through the NSP. Analyses aim to understand the stability of NfL collected every 3 months over a 36-month time period through the description and quantification of NfL measurements, as well as the correlation of NfL measurements with other outcome measures, including clinical, cognitive, and magnetic resonance imaging (MRI) measurements collected through ALLFTD.

3 STUDY DESCRIPTION

The Neurofilament Surveillance Project (NSP) is a precompetitive consortium whose goal is to prepare for pivotal clinical trials aimed at the prevention of familial FTLD (f-FTLD) by characterizing NfL levels during disease onset and progression. The NSP enrolled 342 participants who will donate blood at home every 3 months for 3 years. This study differs from other ongoing natural history studies (NHS) because of the increased frequency of NfL measurements and the investigation into the feasibility of collecting NfL samples remotely. Increased frequency of NfL measurements has the potential to improve the power to detect symptomatic disease onset, while remote NfL sample collection has the potential to improve monitoring of individuals at-risk for FTLD. The analyses set forth in this SAP will explore longitudinal NfL data collected remotely through the NSP to assess how NfL can be better leveraged to improve the treatment of FTLD.

4 OBJECTIVES AND ENDPOINTS

4.1 OBJECTIVES

We will focus on the following objectives from the study protocol.

4.1.1 NFL CHARACTERIZATION

- The primary objective of the NSP is to determine the longitudinal stability of plasma NfL measured every 3 months for 36 months in individuals at-risk for symptomatic FTLD.

- Additionally, an objective of the NSP is to assess the pre-analytic (ex. batch, assay lot number, assay run, storage time and collection time), analytic, and within subject variability associated with plasma NfL measurements on a standard, commercially available Single Molecule Array (Simoa) platform.

4.1.2 ASSOCIATION WITH CLINICAL AND NEUROIMAGING MEASURES

- An additional objective of the NSP is to evaluate ALLFTD collected clinical and magnetic resonance imaging (MRI) neuroimaging measures with plasma NfL levels in asymptomatic and symptomatic f-FTLD mutation carriers.

4.2 SCHEDULE OF ASSESSMENTS

Blood and a limited set of data will be collected from NSP participants every 3 months over the course of 3 years. Data collected include concomitant medications, interim changes in health, and adverse events.

In addition to data collected within the NSP study, the following data will be collected annually on NSP participants as part of ALLFTD, and data from the ALLFTD visits will be obtained for the NSP database.

- Medical history (history of HIV, peripheral neuropathy, traumatic brain injury, chronic kidney disease, atrial fibrillation, stroke, liver failure, renal failure)
- Vital signs (height, weight, Body Mass Index (BMI))
- Demographics (e.g., years education, age in 5 year intervals, sex)
- Clinical measures
- MRI Volumetric measures

All longitudinal data collected through NSP and specified variables collected through ALLFTD on these participants will be used for the purpose of analyses. Baseline visit will be defined as the first NSP visit. Baseline information required in analyses and for definitions of subgroups that are only available through ALLFTD visits (ex. CDR®+NACC-FTLD-Global) will be taken from the ALLFTD visit closest to the baseline NSP visit. If two consecutive ALLFTD visits are the same distance from the baseline NSP visit, baseline information will be taken from the earlier ALLFTD visit. A summary of the lag time between the ALLFTD visit used for baseline covariates and the baseline NSP visit will be included.

4.3 ENDPOINTS

Several types of endpoints will be defined to address the objectives on NfL characterization and correlation with clinical and neuroimaging measures. The primary endpoint will be used to characterize NfL, while the correlate endpoints will be used to explore the correlation of NfL with clinical and neuroimaging measures. Covariates of interest are defined below in section 4.3.4, which may be of interest when characterizing NfL and/or exploring the correlation of NfL with the correlate endpoints.

4.3.1 PRIMARY ENDPOINT

The primary endpoint of the NSP is NfL from plasma samples. NfL will be log transformed for all analyses. Data on this endpoint will be collected from baseline to 36 months in each participant within NSP.

Additional NfL levels from plasma samples collected through ALLFTD visits may be provided. Depending on data availability, these values will be included within a secondary analysis of NfL that investigates potential differences in NfL across different studies (ALLFTD vs. NSP).

4.3.2 CLINICAL CORRELATE ENDPOINTS

The relationship between NfL and several clinical measures will be explored. Primary clinical measures of interest, and their association with NfL, will be analyzed during interim analyses. One interim analysis will be conducted after each data delivery. The results for analyses with the primary clinical measures will be

included in the interim results. The relationship between NfL and the secondary clinical measures listed below will only be included in the final analysis.

- ***Primary clinical measures of interest:***
    - CDR®+NACC-FTLD-SB
    - CDR®+NACC-FTLD-SB + Motor
    - Uniform Data Set v3.0 Executive Function (UDS3-EF) Composite Score

- ***Secondary clinical measures of interest***:
    - Montreal Cognitive Assessment Test (MoCA)
    - Trail Making Test, Part A
    - Trail Making Test, Part B
    - Multilingual Naming Test (MINT)
    - Animal Fluency
    - Vegetable Fluency
    - Lexical Fluency (F&L words)

4.3.3 MRI CORRELATE ENDPOINTS

The relationship between NfL and a number of MRI outcome measures will be explored. Primary MRI measures of interest, and their association with NfL, will be analyzed during interim analyses. The results for analyses with the primary MRI measures of interest will be included in the interim results. The relationship between NfL and the secondary MRI measures of interest listed below will only be included in the final analyses. All MRI measures will use the Desikan-Killany atlas and each region of interest (ROI) will be adjusted for by total intracranial volume (TIV). MRI regions will be a bilateral composite, by default, although we will explore approaches that consider asymmetric atrophy which may reduce the associations observed with bilateral composites.

- ***Primary MRI measures of interest:***
    - Frontal lobe
    - Temporal lobe

- ***Secondary MRI measures of interest:***
    - Striatum
    - Parietal lobe
    - Thalamus
    - Putamen
    - Insula
    - Cerebellum
    - Ventricular Volume
    - Laterality index

4.3.4 COVARIATES OF INTEREST

Covariates of interest are defined, which may be used to explore or explain heterogeneity in NfL and/or the relationship of NfL with the correlate endpoints. A table of baseline covariates and time-varying covariates at baseline for the enrolled population will be provided together with baseline values of NfL (Table 1). In addition, Table 1 will include a summary of the lag time (days) between the ALLFTD visit used for baseline covariates and the baseline NSP visit.

- **Baseline covariates**
  - Sex
  - Onset Age
  - CDR®+NACC-FTLD-Global
  - Mutation status (*GRN* only, *C9orf72* only, *MAPT* only, or Control)
    - Controls defined as participants with no known mutation and CDR®+NACC-FTLD-Global=0
    - Participants with no known mutation and CDR®+NACC-FTLD-Global >0 will be classified as "Symptomatic unknown cause"
  - Family Mutation (*GRN*, *C9orf72*, *MAPT*)
  - Presence of *TMEM106B*+ mutation
  - Presence of motor symptoms based on clinical syndrome (ALS, FTD with motor neuron disease, CBS, PSP)
  - Disease Age estimated from disease progression model[1]
- **Time-varying covariates**
  - Chronological Age
  - BMI
  - Major changes in health (moderate or severe AEs experienced since last visit; specific events of interest including new diagnoses of HIV, peripheral neuropathy, traumatic brain injury, chronic kidney disease, atrial fibrillation, stroke, liver failure, renal failure, accidents involving a vehicle, AEs due to a fall, and all AEs rated a serious or severe unless related to the progression of FTD.)
  - Vaccination status (vaccine within preceding three months)
  - NfL measurement information including:
    - Batch
    - Assay lot number (Quanterix kit number)
    - Assay run
    - Storage time
    - Collection time

[1] Staffaroni AM, et al., Frontotemporal Dementia Prevention Initiative (FPI) Investigators. Temporal order of clinical and biomarker changes in familial frontotemporal dementia. Nat Med. 2022 Oct;28(10):2194-2206. doi: 10.1038/s41591-022-01942-9. Epub 2022 Sep 22. PMID: 36138153; PMCID: PMC9951811.

4.3.5 MISSING DATA

Missing data for the primary endpoint, clinical and MRI correlates, and covariates will be characterized using tables showing the proportion of missingness by visit. For the primary endpoint, clinical and MRI correlates, and time-varying covariates tables of missingness patterns across visits will be produced to enumerate the number of participants with complete data, loss to follow-up, and intermittent drop out.

Summary tables which are reported using a subgroup which contains missing values will include a 'missing' category.

As this is an observational study, complete case analysis will be used for all outcome variables (Clinical and MRI correlations) and time-varying covariates. Continuous baseline covariates, if missing, will be imputed based on the median when included as a covariate in an analysis. Missing categorical baseline covariates will be assigned to a separate "missing" category when included as a covariate. Participants with missing baseline covariate information used to define a subgroup status will be excluded from the subgroup analysis.

5 ANALYSIS POPULATION

5.1 PRIMARY ANALYSIS POPULATION

The primary analysis population is a cohort of f-FTLD participants already enrolled in ALLFTD, who are receiving quarterly home blood draws. Participants can be male or female, ages 18-85, and are members of a family with a known mutation in *C9orf72*, *GRN* or *MAPT*, including those with an individual mutation status of *GRN* only, *C9orf72* only, *MAPT* only or no known individual mutation and FTLD-CDR Global=0 (Control). Participants with 'Other' or 'Unknown' family mutation (including multiple family mutations such as '*C9orf72* and *GRN*') and those with no known mutation and CDR®+NACC-FTLD-Global >0 (Symptomatic Unknown Cause) will be excluded from the primary analysis population. All specified analyses will be conducted on this primary analysis population, unless otherwise specified.

Some analyses will be repeated for specified subgroups of interest, after they have been conducted on the primary analysis population. The subgroups of interest will be defined by:

1) Symptomatic status: Asymptomatic (Baseline CDR®+NACC-FTLD-Global =0); Pre-symptomatic (Baseline CDR®+NACC-FTLD-Global =0.5); and Symptomatic (Baseline CDR®+NACC-FTLD-Global >=1)
2) Mutation type (*C9orf72* only, *GRN* only, *MAPT* only, or Control)
3) Data collection type (ALLFTD vs NSP). Primary analyses will be conducted using NfL data collected through the NSP. Based on data availability, sensitivity analyses may also include NfL Data from ALLFTD.

6 STATISTICAL ANALYSES

6.1 CHARACTERIZATION OF ENROLLED POPULATION

The baseline demographic and clinical characteristics of the enrolled population will be summarized in a table (Table 1) with covariates of interest described in section 4.3.4 including lag time between the ALLFTD visit used for baseline covariates and the baseline NSP visit. Summary tables stratified by symptomatic status and mutation type will also be produced.

6.2 CHARACTERIZATION OF NFL

6.2.1 EXPECTED PROGRESSION

The expected longitudinal progression of NfL will be summarized using the figures, tables, and analyses outlined in the following sections. The primary goal is to understand the progression of longitudinal NfL measurements in the primary analysis population, as well as by subgroups of interest (Section 5.1).

6.2.1.1 FIGURES AND TABLES

Spaghetti plots

Spaghetti plots will be created, which show individual trajectories of:
A) log NfL values over time (months since baseline)
B) log NfL change from baseline values over time (months since baseline)

Points will be color-coded by symptomatic status. If ALLFTD NfL data is included, different plotting symbols will be used for data collection type (ALLFTD vs. NSP). Plots will be shown overall and separately based on mutation type.

Data Summaries

The log NfL values will be summarized across visits (binned in 3-month intervals) by mutation and symptomatic status in the following ways:
A) log NfL values by visit: Mean (SD), Median (1^{st} quartile, 3^{rd} quartile), Minimum, Maximum
B) log NfL change from baseline values by visit: Mean (SD), Median (1^{st} quartile, 3^{rd} quartile), Minimum, Maximum

One table will be created overall and separately for each mutation status. Within each table, columns will be provided for overall population and within each symptomatic group.

Scatterplot Matrix

A scatterplot matrix will be created to show the pairwise relationship between log NfL values at each visit:
A) log NfL values by visit (baseline vs. visit 1, baseline vs. visit 2, baseline vs. visit 3, baseline vs. visit 4, …, visit 2 vs. visit 3, visit 2 vs. visit 4, etc.)

B) log NfL baseline vs change from baseline by visit (baseline vs. change from baseline at visit 1, baseline vs. change from baseline at visit 2, baseline vs. change from baseline at visit 3, baseline vs. change from baseline at visit 4, …, change from baseline at visit 1 vs. change from baseline at visit 2, change from baseline at visit 1 vs. change from baseline at visit 3, etc.)

The scatterplot matrices will also report Pearson's correlation coefficients and Spearman's rank correlation coefficients for each pairwise comparison. These analyses will be conducted overall and separately by mutation and symptomatic status.

6.2.1.2 REPEATED MEASURES ANALYSIS OF NFL

A repeated measures analysis of NfL from baseline to 36 months will be conducted using a linear mixed effects (LME) model with subject-level random intercepts and random slopes. Time of visit will be treated as continuous. Main (fixed) effects and main effect by time interactions will be included for baseline covariates. Variable selection will be performed by exploring all $2^8 = 256$ distinct subsets of baseline covariates (from the set listed in Section 4.3.4). In addition, to adjust for the lag time between the ALLFTD visit used for baseline covariates and the baseline NSP visit, the number of days between these visits will be included as a covariate in all models with a baseline covariate from ALLFTD that can change over time (e.g., CDR®+NACC-FTLD-Global). The final set of baseline covariates will be determined using Akaike information criterion (AIC). For all baseline covariates in the final set, two-way interactions will also be explored. At the final analysis, several sensitivity analyses will be performed. To explore potential serial correlation, the first sensitivity analysis will model random variation by including a serial correlation term in addition to random effects and measurement error. To better understand the shape of progression for NfL, the second sensitivity will explore two potential functional forms of the time covariate: time categorical and cubic splines.

6.2.2 VARIABILITY

The repeated measures analysis of NfL defined within Section 6.2.1.2 will provide overall estimates of within-subject variability (measurement error and serial correlation) and across subject variability of NfL at baseline (variability in intercept) and over time (variability in slope). Within this section on NfL variability, possible additional explanations of within-subject variability (measurement error) will be explored using time-varying covariates.

The following non-biological sources of variation will be explored: collection type (NSP remote vs. ALLFTD in clinic), batch, assay lot number, assay run, storage time, and collection time.

Boxplots of baseline NfL by batch, assay lot number, and assay run will be produced to assess inter-run variation.

The following biological sources of variation will be explored: chronological age, BMI, changes in BMI, changes in health, and proximity to vaccination.

For each assessment of variation, standardized residuals from the model in Section 6.2.1.2 will be plotted vs. the value of the time-varying covariate. Additionally, the repeated measures analysis defined in Section 6.2.1.2 will be repeated with the inclusion of the time-varying covariate as a fixed effect. All single-variable models will be explored (one time-varying covariate at a time). Mean covariate effect estimates will be reported as well as reductions in residual standard error when including the covariate. Covariate variable selection will be conducted using a step-down Akaike information criterion (AIC) procedure and a final model will be reported.

In addition, standardized residuals from the final model and corresponding time differences will be used to estimate a sample variogram for the primary endpoint.

An outlier analysis will also be conducted. NfL values will be flagged as abnormal fluctuations if the absolute standardized residual from the model in Section 6.2.1.2 is larger than 3 standardized units. Summaries of biological and non-biological (Mean; SD if continuous vs. proportions if dichotomous) potential sources of variation will be provided for all NfL values flagged as abnormal fluctuations vs. those not flagged as abnormal. If a substantial number of outliers are detected (greater than 5%), sensitivity analyses will be performed with outliers removed. Outliers will also be flagged in plots.

6.3 ASSOCIATION OF NFL WITH CLINICAL AND MRI PROGRESSION

6.3.1 CORRELATION

Both cross-sectional and longitudinal correlations will be assessed individually for each clinical and MRI correlate defined in Sections 4.3.2 and 4.3.3. To explore correlations controlling for covariate effects, subject level deviations from the population mean (random intercepts) will be estimated for each participant from repeated measures models as defined in Section 6.2.1.2 replacing NfL as the outcome of interest with each potential correlate endpoint. Similarly, to assess longitudinal correlations, rates of progression (random slopes) for each endpoint will also be estimated for each participant from the repeated measures models.

For each correlate endpoint the following will be performed:

- Plot of baseline NfL values vs. baseline values of the potential correlate. Points will be colored based on mutation and different shapes will be used to denote symptomatic status. Pearson's correlation coefficients and Spearman's rank correlation coefficients will be reported by mutation and symptomatic status.

- Plot of NfL random intercepts vs. random intercepts of potential correlate. Points will be colored based on mutation and different shapes will be used to denote symptomatic status. Pearson's correlation coefficients and Spearman's rank correlation coefficients will be reported by mutation and symptomatic status.
- Plot of baseline NfL values vs. rate of progression of potential correlate. Points will be colored based on mutation and different shapes will be used to denote symptomatic status. Pearson's correlation coefficients and Spearman's rank correlation coefficients will be reported by mutation and symptomatic status.
- Plot of rate of change in NfL vs. rate of change of potential correlate. Points will be colored based on mutation and different shapes will be used to denote symptomatic status. Pearson's correlation coefficients and Spearman's rank correlation coefficients will be reported by mutation and symptomatic status.

Additionally, to explore changes in correlation between NfL and clinical and MRI correlates over time, a bivariate repeated measures analysis will be used. The bivariate model is constructed by linking the NfL and correlate repeated measures models together via a joint distribution of random effects. The joint random effects distribution is assumed to be normally distributed with mean vector (0,0,0,0) and covariance matrix estimating variance of all four random effects (NfL and correlate random intercepts and NfL and correlate random slopes) as well as all covariances. The error terms are assumed to follow a bivariate normal distribution with mean vector (0,0) and uncorrelated errors between NfL and the correlate. In particular, let the covariance of the random effects be

$$\begin{bmatrix} \sigma_{a_1}^2 & \sigma_{a_1a_2} & \sigma_{a_1b_1} & \sigma_{a_1b_2} \\ & \sigma_{a_2}^2 & \sigma_{a_2b_1} & \sigma_{a_2b_2} \\ & & \sigma_{b_1}^2 & \sigma_{b_1b_2} \\ & & & \sigma_{b_2}^2 \end{bmatrix}$$

and σ_1^2 and σ_2^2 be the variances of the residual error terms for the NfL and correlate, then the marginal correlation at time t is calculated as

$$\rho_m(t) = \frac{\sigma_{a_1a_2} + t\sigma_{a_1b_2} + t\sigma_{a_2b_1} + t^2\sigma_{b_1b_2}}{\sqrt{\sigma_{a_1}^2 + 2t\sigma_{a_1b_1} + t^2\sigma_{b_1}^2 + \sigma_1^2}\sqrt{\sigma_{a_2}^2 + 2t\sigma_{a_2b_2} + t^2\sigma_{b_2}^2 + \sigma_2^2}}$$

6.3.2 PREDICTION OF FUTURE RATES OF CLINICAL PROGRESSION

A longitudinal k-means clustering analysis will be conducted using CDR®+NACC-FTLD-SB values over time to cluster participants based on post-baseline clinical rate of progression. This generalization of the k-means algorithm will be used to assess

CDR®+NACC-FTLD-SB trajectories by shape. Baseline NfL values and changes in NfL will be summarized for each cluster overall and within each cluster by mutation status to explore NfL as a potential explanatory variable for heterogenous progression. The optimal number of clusters to be used in the clustering analysis will be explored using the elbow, average silhouette, and gap statistic methods.

In addition, clustering will be visualized with plots of CDR®+NACC-FTLD-SB over time. The following will be performed:

- Overall plot of CDR®+NACC-FTLD-SB vs. time with points and lines colored by cluster.
- Plots of CDR®+NACC-FTLD-SB vs. time stratified by tertiles of baseline NfL (bottom, middle, or upper) with points and lines colored by cluster.
- Plots of CDR®+NACC-FTLD-SB vs. time stratified by mutation type (*C9orf72* only, *GRN* only, *MAPT* only, or Control) with points and lines colored by cluster.
- Plots of CDR®+NACC-FTLD-SB vs. time stratified by tertiles of change from baseline NfL (bottom, middle, or upper) with points and lines colored by cluster.

6.3.3 PREDICTION OF SYMPTOMATIC CONVERSION

This analysis will be conducted on all participants who are asymptomatic (CDR®+NACC-FTLD-Global of 0) or pre-symptomatic (CDR®+NACC-FTLD-Global of 0.5) at baseline NSP visit. Participants will be grouped into those who are converters (increase from a baseline CDR®+NACC-FTLD-Global score < 1 to a CDR®+NACC-FTLD-Global of >=1 at any point within the time period) vs. non-converters. Conversion will be defined for three pre-specified time periods (conversion within 1 year, 2 years or 3 years). Baseline NfL values (mean, SD, median, 1st quartile, 3rd quartile, minimum, maximum) will be summarized for converters vs. non-converters at each time point by mutation status. ROC curves and corresponding AUC values will be calculated to show sensitivity and specificity of different NfL cutoffs for predicting future conversion at the different time periods overall and by mutation status. A sensitivity analysis will exclude any participants who convert and then show a CDR®+NACC-FTLD-Global score of < 1 at a subsequent timepoint.

In addition, a time to event analysis will be performed using interval-censored time to first conversion (CDR®+NACC-FTLD-Global >=1). Kaplan-Meier plots of time to conversion stratified by quintiles of baseline NfL will be produced and summaries of the probability of remaining asymptomatic or pre-symptomatic at 1 year, 2 years, and 3 years will be shown.

Depending on availability of suitable data, the association between symptomatic conversion and other functions of NfL (e.g., age-adjusted baseline NfL z-scores) may be explored by summarizing values for converters vs. non-converters.

6.4 Analysis Schedule

6.4.1 Interim Analyses

Interim analyses of available data will occur throughout the study, after each data delivery. These interim analyses will begin after the SAP is finalized. Interim analyses will contain a subset of the specified statistical analyses, including Sections 6.2.1 and Section 6.3.1 for the defined primary clinical and primary MRI potential correlate outcomes. The other specified statistical analyses will only be conducted during the final analyses. Results from interim analyses will be provided in an interim analysis report.

6.4.2 Final Analyses

The final analyses will be conducted when the last participant has had the opportunity to complete their final visit at three years. All analyses detailed in Section 6.2 and Section 6.3 will be conducted during the final analyses.

6.4.3 ANALYSIS TABLES

Here we enumerate the analyses that will be conducted and specify which analyses will be conducted at the interim vs. only at the final analysis.

Section 4.3 results in a total of 5 Tables. Section 6.2 results in a total of 35 Figures, 11 Tables, and 13 Analyses. Section 6.3 results in a total of 108 Figures, 3 Tables, and 41 Analyses.

Count	Section	Analysis Description	Population	Endpoint	Performed at Interim
Table 1 a -c (3)	**4.3.4 Baseline Demographics and Clinical Characteristics**	Summary of Enrolled population	Overall (Table 1a) and by symptomatic status (Table 1b) and mutation status (Table 1c)	Covariates	X
Table 2	**4.3.5 Missing Data**	Proportion of Missingness by visit	All data	NfL, covariates, correlates	X
Table 3		Missingness patterns across visits	All data	NfL, time-varying covariates, correlates	
Fig 1 a-j (10)	**6.2.1 Changes in NfL**	Spaghetti plots NfL	Overall (Fig 1a) and by mutation status (Fig 1b,c,d,e). Color code based on symptomatic status.	NfL	X
			Overall (Fig 1f) and by mutation status (Fig 1g,h,i,j). Color code based on symptomatic status.	Change in NfL	X
Table 4 a-j (10)		Summary Tables NfL by mutation and symp. status	Overall (Table 4a) and by mutation status (Table 4b,c,d,e). Columns for each symptomatic status and total.	NfL	X
			Overall (Table 4f) and by mutation status (Table 4g,h,i, j). Columns for each symptomatic status and total.	Change from baseline NfL	X
Fig 2 a-j (10)		Scatterplot Matrix with correlation coefficients	Overall (Fig 2a) and by mutation status (Fig 2b,c,d,e). Color code based on symptomatic status.	NfL	

			Overall (Fig 2f) and by mutation status (Fig 2g,h,i,j). Color code based on symptomatic status.	Change in NfL	
Analysis 1		LME Analysis of NfL	Overall	NfL, covariates	X
Fig 3 a-j (11)	**6.2.2 Variation of NfL**	Residual plot of NfL vs. time-varying covariate	Overall	NfL, Collection type	
				NfL, Batch	
				NfL, Assay lot number	
				NfL, Assay run	
				NfL, Storage time	
				NfL, Collection time	
				NfL, Chronological Age	
				NfL, BMI	
				NfL, Change in BMI	
				NfL, Change in Health	
				NfL, Vaccination	
Analysis 2 a-k (12)		LME Analysis of NfL covariate adjusted	Overall	NfL, Collection type	
				NfL, Batch	
				NfL, Assay lot number	
				NfL, Assay run	
				NfL, Storage time	
				NfL, Collection time	
				NfL, Chronological Age	
				NfL, BMI	
				NfL, Change in BMI	
				NfL, Change in Health	
				NfL, Vaccination	
				NfL, + all time-varying cov. w/ variable selection	
Fig 4 a-c (3)		Boxplots	Overall	Baseline NfL, Batch	
				Baseline NfL, Assay lot number	
				Baseline NfL, Assay Run	
Fig 5		Sample Variogram	Overall	NfL residuals	
Table 5		Outlier Analysis of NfL	Overall	NfL + all time-varying cov.	

Count	Section	Analysis Description	Population	Endpoint	Performed at Interim
Analysis 3 a-s (20)	**6.3.1 Correlation with NfL and Clinical + MRI Endpoints**	LME analysis of potential correlates to estimate subject-level random intercept and rate of change	Overall	CDR®+NACC-FTLD-SB	X
				CDR®+NACC-FTLD-SB + Motor	X
				UDS3-EF Composite Score	X
				Clinical Correlates Secondary (7)	
				Frontal MRI ROI	X
				Temporal MRI ROI	X
				MRI Correlates Secondary (8)	
Fig 6-9 a-s (80)		• Plot of baseline NfL vs. baseline potential correlate + correlation coef. • Plot of NfL random intercepts vs. random intercept potential correlate + correlation coefs. • Plot of baseline NfL vs. rate of progression potential correlate + correlation coef. • Plot of rate of progression NfL vs. rate of progression potential correlate + correlation coefs.	Overall	CDR®+NACC-FTLD-SB	X
				CDR®+NACC-FTLD-SB + Motor	X
				UDS3-EF Composite Score	X
				Clinical Correlates Secondary (7)	
				Frontal MRI ROI	X
				Temporal MRI ROI	X
				MRI Correlates Secondary (8)	
Analysis 4 a-s (20)		Bivariate LME Analysis of NfL and potential correlate to estimate correlations between subject-level random intercepts and rates of change and their covariance for marginal correlation	Overall	NfL, Clinical and MRI Correlates (20)	
Fig 10 a-s (20)		Plot of marginal correlation between NfL and potential correlate over time	Overall	NfL, Clinical and MRI Correlates (20)	
Analysis 5	**6.3.2 NfL Prediction of Rate of progression**	Cluster Analysis	Overall	NfL, CDR®+NACC-FTLD-SB	
Table 6		Summary of NfL (baseline and rate of change) by Cluster overall and by mutation group	Overall	NfL, CDR®+NACC-FTLD-SB	
Fig 11 a – d (4)		Plot of CDR®+NACC-FTLD-SB over time color coded by cluster overall and by baseline NfL, mutation type, and change from baseline NfL	Overall	CDR®+NACC-FTLD-SB	
Table 7	**6.3.3 NfL Prediction of Conversion**	Summary of NfL (baseline) for converters vs. non-converters at 1, 2, and 3 years by mutation	Non-symptomatic	NfL, CDR®+NACC-FTLD-Global	
Table 8*		Summary of NfL (baseline) age-adjusted z-score for	Non-symptomatic	NfL z-score, CDR®+NACC-FTLD-Global	

* *Analysis of age-adjusted NfL z-scores dependent on sufficient data availability for standardization*

		converters vs. non-converters at 1, 2, and 3 years by mutation			
Figure 12 a-c (3)		ROC curves for prediction of 1, 2, and 3 year conversion using baseline NfL by mutation	Non-symptomatic	NfL, CDR®+NACC-FTLD-Global	
Figure 13		Kaplan-Meier curves of time to conversion stratified by quintiles of baseline NfL with summarizes of probability of remaining asymptomatic or pre-symptomatic at 1 year, 2 years, and 3 years	Non-symptomatic	NfL, CDR®+NACC-FTLD-Global	