Statistical Analysis Plan

Title: A double-blind, placebo-controlled, exploratory randomised clinical trial to assess the safety and efficacy of IFB-088 plus riluzole 100 mg vs placebo plus riluzole 100 mg in patients with bulbar-onset amyotrophic lateral sclerosis Protocol Number: P288ALS

Protocol Version: Version 4.1 (Final), 24 May 2024

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STATISTICAL ANALYSIS PLAN		
InFlectis BioScience	Sponsor:	InFlectis BioScience
	Protocol Number:	P288ALS
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REVIEW / APPROVAL SIGNATURES

Plan Author

Anne Bijon, Statistician, Keyrus Life Science

Signature: 12-Nov-2024 | 3:04 PM CET

Signé par :
Anne BIJON
Nom du signataire : Anne BIJON
Motif de la signature : J'ai rédigé ce document
Heure de signature : 12-Nov-2024 | 3:04 PM CET
C0F4702EB1FE4082BB18F1B3DE2152C9

Plan Approver, Sponsor Clinician

Anne Visbecq, MD, Senior Medical Director

Signature: 12-Nov-2024 | 4:55 PM CET

Signé par :
Anne Visbecq
Nom du signataire : Anne Visbecq
Motif de la signature : J'approuve ce document
Heure de signature : 12-Nov-2024 | 4:55 PM CET
6EB7F18C1A9147F28D0F7F29974AA2C8

Plan Approver, Sponsor Statistician

Prof. Jean-Marie Grouin, Ph.D Statistics, University of Rouen, France

Signature: 13-Nov-2024 | 12:48 PM CET

Signé par :
Jean-Marie Grouin
Nom du signataire : Jean-Marie Grouin
Motif de la signature : J'approuve ce document
Heure de signature : 13-Nov-2024 | 12:48 PM CET
E423F4775807412F9D5A4462F43AA0ED

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LIST OF ABBREVIATIONS

Abbreviation	Definition
ABG	Arterial Blood Gases
ALQ	Above Limit of Quantification
AE	Adverse event
ALS	Amyotrophic Lateral Sclerosis
ALSFRS-R	Revised Amyotrophic Lateral Sclerosis Functional Rating scale
ANCOVA	ANalysis of COVAariance
BLQ	Below Limit of Quantification
BNP	B type natriuretic peptide
BMI	Body Mass Index
CBEU	Cytobacteriological Examination of Urine
CI	Confidence Interval
CS	Clinically significant
DBL	Data Base Lock
ECG	Electrocardiogram
eCRF	Electronic Case Report Form
FAS	Full Analysis Set
GFR	Glomerular Filtration Rate
IMP	Investigational Medical Product
LLN	Lower Limit of Normal reference
LLOQ	Lower Limit of Quantification
MCMC	Markov Chain Monte Carlo
MITOS	Amyotrophic Lateral Sclerosis-Milano-Torino staging system
NCS	Non-Clinically Significant
NFL	Neurofilament Light Chain
QoL	Quality Of Life
PK	Pharmacokinetics
PPS	Per Protocol Set

SAP	Statistical Analysis Plan
SD	Standard Deviation
SE	Standard Error
SS	Safety Set
SVC	Slow Vital Capacity
TAESI	Treatment Adverse Event of Specific Interest
TEAE	Treatment Emergent Adverse Event
TFL	Table, Figure, Listing
RS	Randomized Set
ULN	Upper Limit of Normal reference
ULOQ	Upper Limit of Quantification

VERSION HISTORY

Version	Version Date	Changes	Author
1.0			

1 INTRODUCTION

It has been demonstrated that targeting the unfolded protein response (UPR) pathway, in particular the phosphatase complex protein phosphatase 1 regulatory subunit 15A/protein phosphatase 1c (PPP1R15A/PP1c) could be beneficial to ALS patients. IFB-088 is a PPP1R15A/PP1c phosphatase complex inhibitor that has demonstrated activity in the so far best studied animal model of ALS, i.e. transgenic rodents overexpressing the gene encoding superoxide dismutase 1 (SOD-1). Similarly, another PPP1R15A/PP1c phosphatase complex inhibitor, guanabenz, also demonstrated benefits in ALS animal models. The 2 molecules have the same pharmacological target but guanabenz also exhibits α2 adrenergic activity responsible for lowering blood pressure. IFB-088 has been shown to be devoid of this α2 adrenergic activity and demonstrated a good safety profile in a phase I study in healthy volunteers as no toxicity signal has been detected at any doses evaluated, including single daily doses up to 60 mg and repeated daily doses up to 50 mg for 14 days. Guanabenz has been investigated in an exploratory phase II study in ALS patients. It showed encouraging results, especially in patients with bulbar-onset ALS. The incidence of hypotension in this study discouraged further development in ALS. Since a similar efficacy is expected for IFB-088 without hypotensive effects, an exploratory study has been designed to assess the safety and the efficacy of IFB-088 in patients with bulbar-onset ALS. P288ALS is a double-blind, placebo-controlled, exploratory randomised clinical trial to assess the safety and efficacy of IFB-088 plus riluzole 100 mg vs placebo plus riluzole 100 mg in patients with bulbar-onset amyotrophic lateral sclerosis.

2 STUDY OBJECTIVES, ENDPOINTS, AND ESTIMANDS

2.1 Objectives

2.1.1 Primary Objective

The primary objective is to assess the safety of IFB-088 50 mg/day in patients with bulbar-onset ALS.

2.1.2 Secondary Objectives

The secondary objectives are:

- To assess the efficacy of IFB-088 50 mg/day plus riluzole 100 mg/day versus placebo plus riluzole 100 mg/day over a 6-month period in patients with bulbar-onset ALS
- To determine pharmacokinetics (PK) parameters of IFB-088
- To investigate the effects of IFB-088 on ALS potential biomarkers
- To investigate quality of life (QoL).

2.2 Endpoints

2.2.1 Safety Endpoints

- Adverse Event (AE) and Treatment Emergent Adverse Event (TEAE)
- Laboratories parameters
- Vital signs
- ECG parameters

2.2.2 Efficacy Endpoints

- Change in Revised ALS functional rating scale (ALSFRS-R) score from baseline to month 3 and to month 6,
- Worsening according to ALS-Milano-Torino staging system (MITOS) score, i.e. progression to a higher stage at 6 months compared to the baseline,
- Change in King's College score from baseline to month 6,
- Assessment of respiratory function (slow vital capacity [SVC], sniff test [optional], arterial blood gases [ABG]): exploratory endpoint.
- Evaluation of nutritional status and body composition by bioelectrical impedance: exploratory endpoint

2.3 Estimands

Table 1. Change in ALSFRS-R from baseline to month 6 (V4)

Objective: To estimate the treatment effect (IFB-088 vs. placebo), on the Change in ALSFRS-R assessed at Month 6.
Estimand: Treatment-Policy Strategy
Treatment: IFB-088 or matching placebo

ESTIMAND	ANALYSIS
Target population	**Analysis set**
Patients with bulbar-onset amyotrophic lateral sclerosis	Full Analysis Set (FAS) defined as all randomized subjects who received at least one dose of study treatment (even if discontinued prematurely) with non-missing baseline ALSFRS-R value.
Variable	**Outcome measure**
Change in ALSFRS-R assessed at Month 6	Mean change
Handling of intercurrent events	**Handling of missing data**
• Death related to disease progression • Death not related to disease progression. • Drop-out Treatment policy will be applied.	• Estimand #1 (primary estimand): Missing data will be handled depending on the type of the intercurrent event (i.e.). - Missing data due to deaths: a) due to disease progression: the ALSFRS-R score at month 6 will be imputed by the worst value (i.e. 0) b) other reason (sudden death included): - if the ALSFRS-R score is not missing at Month 3, the ALSFRS-R score at month 6 will be imputed by linear extrapolation given baseline and Month 3 ALSFRS-R score available values. Note: in the linear extrapolation at month 6, the timepoint of ALSFRS assessment considered at V3 will be the exact calendar date of V3 assessment. Calendar time of V4 will be considered as 180 days. - if the ALSFRS-R score is missing at Month 3, the ALSFRS-R score at month 6 will be imputed by linear extrapolation based on the predicted value in the linear regression model including terms for intercept and baseline ALSFRS-R score estimated within the treatment group to

	which the patient is randomized. Imputed values will be rounded to the nearest integer. Imputed value higher than 48 will be replaced by 48 and imputed value lower than 0 will be replaced by 0. - Missing data due to drop-outs or withdrawal will be imputed with the same approach used for handling missing data due to deaths for reasons <u>not related</u> to disease progression. Imputed values will be rounded to the nearest integer. Imputed value higher than 48 will be replaced by 48 and imputed value lower than 0 will be replaced by 0. • Estimand #2: Missing data will be handled depending on the type of the intercurrent event (i.e.). - Missing data due to deaths, whatever the cause: the ALSFRS-R score at month 6 will be imputed by the worst value (i.e. 0). - Missing data due to drop-outs or withdrawal will be imputed with the same approach as for Estimand #1 used for handling missing data due to deaths <u>not related</u> to disease progression. Imputed values will be rounded to the nearest integer. Imputed value higher than 48 will be replaced by 48 and imputed value lower than 0 will be replaced by 0. • Estimand #3: Missing data will be handled depending on the type of the intercurrent event (i.e.). -Missing data due to death whatever the cause: the ALSFRS-R score at month 6

	will be imputed by the worst value (i.e. 0). -Missing data due to drop-outs or withdrawal will be replaced by multiple imputation assuming Missing at random and monotone regression. Note: model for multiple imputation will be done without patient who died before V4. Imputed values will be rounded to the nearest integer.
Population-level summary measure	**Analysis approach**
Difference in LS mean changes between IFB-088 and placebo	An analysis of covariance (ANCOVA) model of change from baseline in ALSFRS-R Score to month 6 will be fitted. Adjusted LS means and 95% confidence interval for each treatment group and the difference in LS means along with 95% confidence interval and p-value will be estimated in this ANCOVA model. Estimand # 1 will be considered primarily. Sensitivity analyses considering Estimands # 2 and # 3. Supportive analysis will be performed on the PPS, considering Estimand # 1
Hodges-Lehmann estimate of the difference between IFB-088 and placebo	A non-parametric analysis on the FAS will be performed considering Estimand #1. The Hodges-Lehmann estimate along with the 95% CI and Wilcoxon p-value will be provided.

Table 2: Change in MITOS stage from baseline to month 6 (V4)

Objective: To estimate the treatment effect (IFB-088 vs. placebo), on the change in MITOS stage from baseline to Month 6 (V4).
Estimand: Treatment-Policy Strategy
Treatment: IFB-088 or matching placebo

ESTIMAND	ANALYSIS
Target population	**Analysis set**
Patients with bulbar-onset amyotrophic lateral sclerosis	Full Analysis Set (FAS) defined as all randomized subjects who received at least one dose of study treatment (even if discontinued prematurely) with non-missing baseline value.
Variable	**Outcome measure**
Binary endpoint. Progression is defined as a MITOS stage higher at month than at baseline	Percentage of patients who did not progress to a higher MITOS stage at 6 months compared to baseline.
Handling of intercurrent events	**Handling of missing data**
• All -cause deaths • Drop-out Treatment policy will be applied.	Estimand #1 Missing data at month 6 for whichever reason will be considered as progression.
Population-level summary measure	**Analysis approach**
Difference in non-progression rates at month 6 between IFB-088 and placebo Non-progression rate ratio at month 6 between IFB-088 and placebo .	Difference in rates of non-progressors at month 6 (according to change in MITOS stage) between IFB-088 vs placebo along with 95% CIs estimated according to the binomial exact method. Ratio of rates of non-progressors at month 6 (according to change in MITOS stage) between IFB-088 vs placebo along with 95% CIs estimated according to the binomial exact method. Supportive analysis will be performed on the PPS

Table 3: Change in King's College stage from baseline to month 6 (V4)

Objective: To estimate the treatment effect (IFB-088 vs. placebo), on the change in King's College stage from baseline to Month 6 (V4).	
Estimand: Treatment-Policy Strategy	
Treatment: IFB-088 or matching placebo	
ESTIMAND	ANALYSIS

Target population	**Analysis set**
Patients with bulbar-onset amyotrophic lateral sclerosis	Full Analysis Set (FAS) defined as all randomized subjects who received at least some dose (even if discontinued prematurely) with no missing value at baseline.
Variable	**Outcome measure**
Binary endpoint. Progression is defined as a King's College stage higher at month 6 than at baseline	Percentage of patients who did not progress to a higher King's College stage at 6 months compared to baseline
Handling of intercurrent events	**Handling of missing data**
• All -cause deaths • Drop-out Treatment policy will be applied.	Estimand #1 Missing data at month 6 for whichever reason will be considered as progression.
Population-level summary measure	**Analysis approach**
Difference in non-progression rates at month 6 between IFB-088 and placebo Non-progression rate ratio at month 6 between IFB-088 and placebo	Difference in rates of non-progressors at month 6 between IFB-088 vs placebo according to King's College along with 95% CIs estimated according to the binomial exact method. Ratio of rates of non-progressors at month 6 between IFB-088 vs placebo according to King's College along with 95% CIs estimated according to the binomial exact method Supportive analysis will be performed on the PPS

3 SAMPLE SIZE

The minimum required sample size for this study has been estimated at 42 evaluable patients. Since it is an exploratory safety study, it has not been based on a formal sample size calculation. However, with regard to the primary safety endpoint, it is worth noting that 28 patients enrolled in the IFB-088 arm will be sufficient to observe at least one SAE which incidence is greater than or equal to 5% (10% resp.) with a probability of at least 76.2% (94.8% respectively). In addition, 42 patients are also considered sufficient to observe some numerical efficacy signal on some key parameters. Assuming a 15% drop-out rate, 50 patients were to be randomised.

4 RANDOMIZATION

All participants were centrally randomised in a 2:1 allocation ratio to receive either IFB-088 + riluzole or placebo + riluzole, respectively. No stratification factor was considered. The randomisation was performed through an interactive web response system (IWRS) integrated into the eCRF. Before the study is initiated, the log in information and directions for the IWRS was provided to each site. Once a set of medical characteristics is entered, the IWRS immediately assigned a patient to one of the 2 arms. After the randomisation visit, the investigator automatically received a confirmation by email with the treatment number allocated to the patient.

This is a double-blinded study. Neither participants, nor investigators, nor trial staff, nor the sponsor study team was aware of treatment assignments prior to the database lock at the conclusion of the study.

5 PLANNED ANALYSES

The Statistical Analysis Plan (SAP) and Table, Figure, Listing (TFL) Shells (and any amendments) must be approved prior to breaking of the blind for the analysis. If post final database lock (DBL) additional statistical analyses or changes to the statistical analysis are required, then those will be documented in a post DBL SAP Addendum.

5.1 Analysis Sets

5.1.1 Screened Set

The Screened Set will include all patients that were screened, regardless of whether they were randomized and/or treated or not.

5.1.2 Randomized Set

The Randomized Set (RS) will include all patients as randomized.

5.1.3 Full Analysis Set

The Full Analysis Set (FAS) is a subset of the RS including all as-randomized subjects who received at least one dose of the IMPs.

The FAS will be considered as the primary set for all efficacy analyses.

5.1.4 Per-Protocol Analysis Set (PPS)

The Per-Protocol Analysis Set (PPS) will include the patients from the FAS with adequate study medication compliance, defined as intake of at least 80% of the planned total dose and having no major protocol violations which could affect the assessment of efficacy. Before database lock, potential patient exclusions from PPS will be reviewed by the Sponsor and documented in a patient evaluability document.

5.1.5 Safety Set (SS)

The Safety Analysis Set includes all patients who received at least one dose of study treatment, even if discontinued prematurely. Patients who received the wrong treatment will be analyzed as actually treated for safety analyses. This analysis set will be used for all safety analyses.

5.2 Derived Data

This section describes the derivations required for statistical analysis. Unless otherwise stated, variables derived in the source data will not be re-calculated.

5.2.1 Baseline (all parameters except ALSFRS-R)

Baseline is defined as the last available value before or on the same day as first treatment intake, i.e. value at inclusion (V0) or value at screening visit if no value is available at inclusion.

5.2.2 ALSFRS-R Baseline

ALSFRS-R baseline is defined as

- The screening value OR
- The V0 value if V0 is performed > 15 days after the screening visit and available before or on the same day of first treatment intake

If ALSFRS-R was evaluated at V0 while V0 was performed 15 days or less than 15 days after screening, the value at screening will be considered as baseline value.

5.2.3 Change from Baseline

Absolute change from baseline for any variable at a given visit will be calculated by subtracting the baseline value of that variable from the value of the variable at the given visit, eg for Visit 4 at month 6:

*Change from **baseline** to month 6 = month 6 value – baseline value*

If there is no baseline value, then the absolute change from baseline will be set to missing.

5.2.4 Relative change from Baseline

Percent relative change from baseline for continuous variables at a given visit will be calculated by subtracting the baseline value of that variable from the value of the variable at the given visit and then dividing by the baseline value, all multiplied by 100 e.g. for month 6:

*Relative change from **baseline** to month 6 = 100*(month 6 value – baseline value)/ baseline value*

If there is no baseline value, then the relative change from baseline will be set to missing.

5.2.5 Study visit

If a premature discontinuation visit occurs between [83-99] days after V0 then consider this visit as V3 visit.

If a premature discontinuation visit occurs between [170-194] days after V0 then consider this visit as V4 visit.

5.2.6 Conventions for Missing and Partial Dates

Imputation rules for missing start date of treatment:

- If start date of treatment, defined as study first drug intake corresponding to the date of the baseline (V0) visit, is missing the date of randomization is taken.

Imputation rules for partial or missing start dates for AE/medication:

- If the month and year are present, impute the day by the first of that month.
- If the month and year are the same as those of the date of first dose, impute the day with the day of first intake of treatment.

- If only the year is present, impute by January 1st of that year.
- If the year is the same as the year of the date of first dose, impute by the day of first intake of treatment.

- If the start date is entirely missing, impute by the day of first intake of treatment.
- If after imputation AE/medication start date is posterior to AE/medication end date, then imputed start date should be replaced by AE/medication end date.

Imputation rules for partial or missing stop dates for AE and medication:

- If the month and year are present, impute the day by the last day of that month.
- If only the year is present, impute by December 31st of that year.
- If the stop date is entirely missing, assume the event or medication is ongoing except if patient is dead impute by the date of death

Imputation rules for partial date of ALS diagnosis/ date of first signs/symptoms:

- If the month and year are present, impute the day by the 1st of that month.

5.2.7 Completion of visits

A visit will be considered as having been completed if a visit date is reported for this visit.

5.2.8 Treatment status

The treatment status will be considered as:

- “Completed” if the patient has completed the V4 visit without having permanently stopped the treatment before the V4 visit.
- “Discontinued” if the patient has permanently discontinued the treatment before the V4 visit.

5.2.9 Study status

The study status will be considered as:

- “Completed” if the box “Yes” for the question “Was the study completed?” in the End of Study form of the eCRF is ticked.
- “Discontinued” if the box “No” for the question “Was the study completed?” in the End of Study form of the eCRF is ticked.

5.2.10 Last IMP intake

Date of last IMP intake will be the date of V4 for patients with "treatment status" at "completed".

Date of last IMP intake will be end of study date for patients with "treatment status" as "discontinued".

5.2.11 Treatment duration

The duration of treatment (days) will be derived as follows: (Date of last visit on treatment administration – date of last IMP intake + 1).

5.2.12 Study duration

The duration of study (days) will be derived as follows: (End of study date – date of screening visit +1).

5.2.13 Amyotrophic lateral sclerosis history

Time from ALS diagnosis to screening (in months) will be derived as: (date of screening visit – date of ALS diagnosis + 1)/30,4375.

Time from onset date of first ALS symptoms to screening (in months) will be derived as: (date of screening visit – date of onset of first ALS symptoms + 1)/30,4375.

5.2.14 Compliance

Compliance will only be assessed for patient present at V4 over the treatment period until V4 and defined as:

(Number of pills taken / Number of theoretical pills to be taken) * 100

Total number of pills dispensed = Total number of dispensed bottles (sum of all bottles dispensed during the study) * 65

Number of pills taken = total number of pills dispensed - number of remaining pills at V4

Number of theoretical pills to be taken = Date of V4 – Date of V0 (in days) * 2

Compliance (Yes/No): a global compliance higher than 80% is considered as a good compliance.

5.2.15 Treatment emergent adverse event

TEAE is defined as an adverse event occurring (or existing symptoms worsened) on or after the day of first dose intake of study product and up to study end.

5.2.16 Treatment emergent adverse event related to study drug

TEAE related to study treatment is defined as an event:

- occurring on or after the day of first dose intake of study treatment and up to study end AND
- having a relationship to study treatment equals to “Unlikely”, “Possibly”, “Probably”, “Definitely”, or with a missing relationship.

5.2.17 Non-treatment emergent adverse event

Non-treatment emergent adverse event corresponds to AE that occurs before the study intervention administration (and not worsen). An AE that will not qualify as a TEAE will be considered as a Non-Treatment Emergent Adverse Event (Non-TEAE).

5.2.18 Serious adverse event

SAE corresponds to AE considered as serious or AE with a missing or unknown seriousness. Seriousness of AE will be analyzed according to the investigator judgement.

5.2.19 Adverse event of special interest

The following adverse events will be considered as AESIs (as per study protocol):

- Renal toxicities.
- Symptomatic hypotension grade 2 (requiring non-urgent medical intervention) or above according to common terminology criteria for adverse events (CTCAE) version 5.0.
- Hypertriglyceridaemia: A cut-off value is set at triglycerides > 5.7 mmol/L (5.0 g/L) with at least 20% increase compared to baseline value.

The AESIs will be evaluated using the safety data collected during the study, no specific identification of AESI using pre-defined MedDRA preferred term code will be formally performed on the AE data. Of note, the preferred term code “10021097” (preferred term=”Hypotension”) and “10020869” (preferred term=” Hypertriglyceridaemia”) could be used to identify those AESIs from the AE summary. For the triglycerides parameter, a specific check of the result could also be performed using the quantitative results to identify potential HTG.

5.2.20 Concomitant medication

A concomitant medication corresponds to any medication reported in the “PREVIOUS AND CONCOMITANT MEDICATIONS” form of the eCRF with a {medication start date ≤ first treatment intake date and medication end date ≥ first treatment intake date or ongoing} or with a {first treatment intake date ≤ medication start date} (after application of missing dates replacement rules, see section 7.4).

5.2.21 Efficacy endpoints

- ALSFRS-R subscore:

Three subscores will be considered as follows:

- Bulbar subscore: sum of items 1-3
- Motor subcore: sum of items 4-9
- Respiratory subcore: sum of items 10-12

Subscore will be considered as missing if an item is missing.

Note: for motor subscore, item 5 will be considered at missing if item 5a and 5b are missing.

- Worsening (i.e. progression to a higher stage compared to baseline defined by ALS-MITOS score):

Non progression at 6 months (V4) and 3 (V3) months will be derived as follows:

- 0 if ALS-MITOS score is higher at 6 months (or 3 months) compared to ALS-MITOS score at baseline
- 1 if ALS-MITOS score is lower or equal at 6 months (or 3 months) compared to ALS-MITOS score at baseline

- Change in King's College score

Non progression at 6 months and 3 months will be derived as follows:

- 0 if King's College score is higher at 6 months (or 3 months) compared to baseline
- 1 if King's College score is lower or equal at 6 months (or 3 months) compared to baseline

- SVC: percentage of the predicted value for age and sex (%)

Percentage of the predicted value will be calculated according to the formula of predicted FVC in 'Multi-ethnic reference values for spirometry for the 3–95-yr age range: the global lung function 2012 equations' article of Quanjer.

% of the predicted value = (SVC/ M_predicted)*100

Where

For man:

M_predicted = exp(-11.2281 + 2.4135*ln(Height in cm) + 0.0865*ln(Age) + Mspline)

M_spline = 0.3298 -1.1230*(Age/100) + 2.8110*(Age/100)^2 -5.4811*(Age/100)^3 + 3.5964*(Age/100)^4 -0.5884*(Age/100)^5

For woman:

M = exp(-10.4030 + 2.2633*ln(Height in cm) +0.0234*ln(Age) + Mspline)

Mspline = 0.0745 +0.6006*(Age/100) -1.0684*(Age/100)^2 -1.1308*(Age/100)^3 + 0.9730*(Age/100)^4 + 0.0643*(Age/100)^5

For example, 40 years white man, height 185 cm, SVC of 5L:

%predicted value = (5/5.79)*100 = 86 %

For example, 35 years white woman, height 170 cm, SVC of 2.5L:

%predicted value = (2.5/4.16)*100 = 60 %

5.2.22 Laboratories data

- Triglycerides will be categorized as:
  - ≤1.71 mmol/L (≤150 mg/dL, ≤1.5 g/L, ≤171 umol/L),
  -]1.71;.3.42] mmol/L (]150;300] mg/dL,]1.5;3] g/L,]171;.342] umol/L),
  -]3.42;5.7] mmol/L (]300;500] mg/dL,]3;5] g/L,]342;570] umol/L),
  -]5.7;11.4] mmol/L (]500;1000] mg/dL,]5;10] g/L,]570;1140] umol/L)
  - >11.4 mmol/L (>1000 g/L, >10 g/L, >1140 umol/L)

- Glomerular Filtration Rate will be categorized as:
  - <15 ; [15;29] ;]29;44] ;]44;59] ;]59;90[; ≥90 mL/min/1.73 m2

5.2.23 Subgroup

Five subgroups will be studied:

- Baseline NFL (neurofilament light chain): ≤ median vs > median defined according to the median of baseline NFL of the FAS.

- Country: France vs Italy

- Sex: Man vs Woman

- ALSFRS-R progression rate (point/month) (from symptoms onset to screening visit): ≤ median vs > median according to the median of ALSFRS-R progression rate of the FAS.

- Time from onset date of first ALS symptoms to screening: ≤ median vs > median according to the median of time from onset date of first ALS symptoms to screening of the FAS.

5.3 Conventions

All data listings, summaries, figures and statistical analyses will be generated using SAS version 9.4 or higher**Erreur ! Source du renvoi introuvable.**.

Summaries will be presented by treatment group or overall. Treatment group labels will be displayed as follows:

IFB-088	Placebo
(N=XX)	(N=XX)

Listings will be sorted in the following order: treatment group, patient, visit and parameter unless otherwise stated. All data will be listed, and screen failures will be displayed after randomized and/or treated patients in applicable listings (e.g. patient disposition).

Continuous variables will be summarized by the number of non-missing observations, mean, median, standard deviation (SD), standard error (SE), first and third quartile, minimum and maximum.

Categorical variables will be summarized by presenting the frequency and percent. Percentages will be based on the number of subjects in the column header unless otherwise specified in the footnote. For each variable, all categories will be shown. Zero frequencies (but not the percent) within a category will be presented.

Means, medians, and percentiles will be displayed to one more decimal place than the data**,** dispersion statistics (e.g., SD) will have two more decimal places, and the minimum and maximum will be displayed to the same number of decimal places as reported in the raw data. Percentages will be displayed with one decimal place.

P-values will be quoted to 4 decimal places consistent with SAS PVALUE*w.d* format set to PVALUE6.4. P-values < 0.0001 will be presented as p<0.0001.

5.4 Patient Disposition

Subject disposition will be summarized as follows:

- The number of patients who failed screening and the reasons for failure will be tabulated for the Screened Set.
- The number of patients who were screened, randomized, treated, randomized or treated (i.e. enrolled) and who are in each analysis set will be summarized by treatment group and overall for the Screened set.
- The number of patients completing the study and those prematurely discontinuing the study as well as the reasons for discontinuation will be tabulated by treatment group and overall.
- The number of patients present at each scheduled visit will be summarized by treatment group.
- The reasons for exclusion from the PPS will be summarized by treatment group and overall.
- Study duration (days) will be summarized by treatment group and overall in RS.
- Treatment duration (days) will be summarized by treatment group and overall in RS.

Early termination (ET) assessments as well as V3 bis will only be listed and not included in by-visit summary displays.

All patients disposition data will also be listed.

5.5 Protocol Deviations

Protocol deviations will be summarized by classification (minor, major) and category (e.g. study procedure, etc.). A listing of protocol deviations will be provided within Appendix 16.2 of the CSR.

5.6 Baseline description

The baseline description will be performed on the Randomized Set.

Demographic characteristics parameters will be described at screening by treatment group and overall:

- Sex,
- Age (years),

- Race,
- BMI
- Smoking habits,
- Temperature

5.7 Amyotrophic lateral sclerosis history

The following history of amyotrophic lateral sclerosis will be presented on the Randomization Set by treatment group and overall:

- Time from onset date of first ALS symptoms to screening (months),
- Progression rate at screening
- Time from ALS diagnosis to screening (months),
- Weight at first ALS symptoms onset (kg),
- Weight variation between first ALS symptoms onset and screening (kg) (calculated in the eCRF),
- Type of ALS (Familial, Sporadic, Unknown).

5.8 Medical History

The following parameters will be described on the Randomization Set by treatment group and overall at screened visit:

- Medical history
- Planned procedures
- Active psychiatric illnesses (y/n)
- Suicidal risk (y/n)

5.9 Concomitant Medications

Medications will be coded using the WHO Drug Classifications (Global version B3, or later).

The number and percentage of patients with at least one concomitant medication will be described by Medication Class (ATC Level 4) and Medication Name overall in the Randomized Set. In case a same patient reported the same medication (i.e. same medication as defined according to WHO Drug classification) several times during the study, only one occurrence will be reported. This table will be sorted by decreasing percentage of Medication Class (ATC Level 4) and Medication Name within Medication Class (ATC Level 4) for the overall column.

5.10 Treatment Compliance

The compliance will be described at V4 by treatment group and overall on the Randomized Set.

5.11 Efficacy Analyses

5.11.1 General Considerations

The final version of the SAP will be finalized prior to the unblinding of data and database lock.

Analyses on Randomized Set and Full Analysis Set will be performed on randomized treatment.

Analyses on Per Protocol Set and Safety Set will be performed on actual treatment received.

5.11.1.1 Nominal alpha level of significance

As the trial is a safety trial and an estimation efficacy study and as it is not powered for efficacy endpoints, all tests performed on efficacy assessments were planned to be conducted at a 2-sided nominal alpha level of 0.20. 2- sided 95% confidence intervals will be provided.

5.11.1.2 Multiplicity

As it is an efficacy estimation trial, there will be no adjustment for multiplicity.

5.11.1.3 Missing data handling

Handling missing data is specified in the analysis of each endpoint.

Missing data will not be replaced for descriptive analyses.

5.11.2 Analysis of Change in ALSFRS-R

5.11.2.1 Expressions

This parameter will be expressed in terms of:

- Value at baseline and at each planned visit (baseline (V0), V3, and V4).
- Change from baseline to each post baseline visit.
- Change from V3 to V4.

For ALSFRS-R subscores and ALSFRS-R total score.

5.11.2.2 Change in ALSFRS-R total score from baseline to Month 6 (V4)

5.11.2.2.1 Primary analysis

The primary analysis of the Change in ALSFRS-R total score from baseline to Month 6 will be conducted on the FAS patients with a non-missing baseline ALSFRS-R total score as described in Table 1**Erreur ! Source du renvoi introuvable.**.

Missing data will be handled as described in Table 1 for estimand #1.

The change in ALSFRS-R score from baseline to Month 6 will be analyzed using an analysis of covariance (ANCOVA) model including treatment (IFB-088 vs placebo), and baseline ALSFRS-R score as continuous covariates. LS mean change with SE and 95% CI by treatment group and the difference in the LS means between 088 and placebo along with SE and 95%CI and p-value will be provided for estimand #1.

Residuals normality will be checked. Homogeneity of slopes across treatment groups will be investigated in an ANCOVA model including an additional term for treatment by baseline ALSFRS-R interaction.

5.11.2.2.2 Sensitivity analyses

Sensitivity analysis #1 considering estimand #2:

The same analysis proposed for the primary analysis will be provided for estimand #2

Sensitivity analysis #2 considering estimand #3:

The same analysis proposed for the primary analysis will be provided for estimand #3.

Missing data due to death whatever the cause: the ALSFRS-R score at month 6 will be imputed by the worst value (i.e. 0).

For the MAR multiple imputation part, the model used for imputation will be done without patients who died before V4.

First step (if necessary):

If the missing pattern is not monotone (missing values at month 3 and not at month 6), a first step will use the Markov Chain Monte Carlo (MCMC) method to impute data only partially to obtain a dataset with monotone missingness as proposed by O'Kelly, Michael; Ratitch, Bohdana. Clinical Trials with Missing Data: A Guide for Practitioners (Statistics in Practice), Wiley.

This first step assumes data are missing at random to impute intermittent (non- monotone) missing data using MCMC method with single chain, non-informative prior, 200 burn-in iterations, 50 iterations between imputations in a chain and Expectation-Maximization algorithm (using mcmc chain=single impute=monotone prior = jeffreys options) and seed of xxxxx.

The imputation model will be fitted **by treatment** and will include the following terms in this specific order:

- ALSFRS total scores in order: at baseline, Month 3 and Month 6

Note: imputed values will be rounded to the nearest integer.

Second step:

If step 1 is necessary, the output data set from Imputation Model Step 1 will be the input for Step 2 using Monotone Regression. The second imputation model will generate one imputation for each of previous multiply imputed datasets of MI1 (i.e. by _Imputation_) and a seed of yyyyy. The model will include only the following variables:

- Randomized Treatment (as a classification variable)
- ALSFRS total scores in order: at baseline, Month 3 and Month 6

If step 1 is not necessary, the imputation model will use monotone regression and generate 50 imputations and a seed of 293654. The model will include only the following variables:

- Randomized Treatment (as a classification variable)
- ALSFRS total scores in order: at baseline, Month 3 and Month 6

Note: imputed values will be rounded to the nearest integer. Imputed value higher than 48 will be replaced by 48 and imputed value lower than 0 will be replaced by 0.

Third step:

50 estimates of treatment LS means and LS means differences with standard errors from ANCOVA model of the multiply imputed datasets will be pooled using PROC MIANALYZE to produce an overall estimate (the mean of the 50 estimates) and a standard error (based on Rubin's formula) with associated 95% confidence interval and p-value. Pooled adjusted LS means of change from baseline ALSFRS-R score with standard error and 95% confidence interval will be presented for each treatment group and the pooled estimated LS mean difference between Active and Placebo will be presented with standard error and 2-sided p-value.

5.11.2.2.3 Supportive analysis

The same analysis proposed for estimands #1 will be repeated on PPS.

5.11.2.2.4 Additional non-parametric analysis

A non-parametric analysis will be performed considering Estimand #1 as described in Table 1. The Hodges-Lehmann estimate of the difference between IFB-088 and placebo along with 95%CI and Wilcoxon p-value will be provided.

5.11.2.2.5 Descriptive analysis

In addition, a descriptive summary of observed ALSFRS-R Score values at baseline, Month 3 and Month 6 as well as their change from baseline to month 3 and to month 6 will also be provided.

5.11.2.2.6 Subgroup Analysis

Subgroup analyses will be performed considering Estimand #1 only, based on an ANCOVA model including terms for treatment, subgroup, treatment by subgroup interaction and baseline ALSFRS-R score. Treatment effect with 95% CI and p-value will be estimated by subgroup within this model. P-value of the test for interaction will also be provided.

Subgroups are as follows.

- Baseline NFL (neurofilament light chain): ≤ median vs > median
- Country: France vs Italy
- Sex: Man vs Woman
- ALSFRS-R progression rate (point/month) (from symptoms onset to screening visit): ≤ median vs > median
- Time from onset date of first ALS symptoms to screening: ≤ median vs > median

A forest plot will display the treatment effects by subgroup with 95% CI and p-values and treatment by subgroup interaction p-value

5.11.2.3 Change in ALSFRS-R total score from baseline to Month 3 (V3)

The analysis will be performed considering estimand #1 at month 3 on FAS .

The same analysis proposed for the change in ALSFRS-R score from baseline to Month 6 will be proposed at month 3.

Missing ALSFRS-R score at Month 3 will be imputed with the same method considered for estimand #1 definition at month 6, i.e. as follows:

Missing data will be handled depending on the type of the intercurrent event (i.e.).

- Missing data due to deaths occurring prior to Month 3:
  a) due to disease progression, the ALSFRS-R score at month 3 will be imputed by the worst value (i.e. 0)
  b) other reason (sudden death included): the missing ALSFRS-R score at month 3 will be imputed by linear extrapolation based on the predicted value in the linear regression model including terms for intercept and baseline ALSFRS-R score estimated within the treatment group to which the patient is randomized. Imputed values will be rounded to the nearest integer.
  Calendar time of V3 will be considered as 90 days.

- Missing data due to drop-outs or withdrawal occurring prior to Month 3 will be imputed with the same approach used for handling missing data due to deaths for other reasons (i.e. other than disease progression).

Note: Imputed values will be rounded to the nearest integer. Imputed value higher than 48 will be replaced by 48 and imputed value lower than 0 will be replaced by 0.

The additional non-parametric analysis will also be performed considering Estimand #1 only.

5.11.2.4 Change in ALSFRS-R Subscores from baseline to Month 6 (V4)

The ALSFRS-R consists of 3 domains (bulbar, motor and respiratory)

The 3 subscores will be considered as follows:

- Bulbar subscore: sum of items 1-3
- Motor subcore : sum of items 4-9
- Respiratory subcore: sum of items 10-12

The analysis of each subscore will be performed considering estimand #1 at month 6 on the FAS. Missing subscore will be imputed with the same method considered for estimand #1 definition of the ALSFRS-R total score at month 6, i.e., as follows:

Missing data will be handled depending on the type of the intercurrent event (i.e.).

- Missing data due to deaths:
  a) due to disease progression, the ALSFRS-R subscore at month 6 will be imputed by the worst value (i.e. 0)
  b) other reason (sudden death included):
  - if the ALSFRS-R subscore is not missing at Month 3, the ALSFRS-R subscore at month 6 will be imputed by linear extrapolation given baseline and Month 3 ALSFRS-R score available values. Note: in the linear extrapolation at month 6, the timepoint of ALSFRS assessment considered at V3 will be the exact calendar date of V3 assessment. Calendar time of V4 will be considered as 180 days. Imputed values will be rounded to the nearest integer.
  - if the ALSFRS-R subscore is missing at Month3, the ALSFRS-R subscore at month 6 will be imputed by linear extrapolation based on the predicted value in the linear regression model including terms for intercept and baseline ALSFRS-R subscore estimated within the treatment group to which the patient is randomized. Imputed values will be rounded to the nearest integer.

- Missing data due to drop-outs or withdrawal will be imputed with the same approach used for handling missing data due to deaths for other reasons (i.e. other than disease progression). Imputed values will be rounded to the nearest integer.

Note: Imputed value lower than 0 will be replaced by 0.

For bulbar subscore: imputed value higher than 12 will be replaced by 12.

For motor subscore: imputed value higher than 24 will be replaced by 24.

For respiratory subscore: imputed value higher than 12 will be replaced by 12.

The analysis of each subscore will be performed in an ANCOVA model including terms for treatment and baseline ALSFRS-R subscore in FAS. The additional non-parametric analysis will also be performed in FAS.

5.11.2.4.1 Subgroup Analysis

Subgroup analyses of each ALSFRS-R subscore will be performed considering Estimand #1 only, based on an ANCOVA model including terms for treatment, subgroup, treatment by subgroup interaction and baseline ALSFRS-R subscore in FAS. Treatment effect with 95% CI and p-value will be estimated by subgroup within this model. P-value of the test for interaction will also be provided.

Subgroups are as follows.

- Baseline NFL (neurofilament light chain): ≤ median vs > median
- Country: France vs Italy
- Sex: Man vs Woman
- ALSFRS-R progression rate (point/month) (from symptoms onset to screening visit): ≤ median vs > median
- Time from onset date of first ALS symptoms to screening: ≤ median vs > median

A forest plot will display the treatment effects by subgroup with 95% CI p-values and treatment by subgroup interaction p-value.

5.11.3 Analysis of change in ALS-Milano-Torino staging system (MITOS)

5.11.3.1 Expressions

This parameter will be expressed in terms of:

- Value at baseline and at each planned visit (V3, and V4).
- Change from baseline to each post baseline visit.
- Change from V3 to V4.

5.11.3.2 Change in MITOS Stage from baseline to Month 6 (V4)

5.11.3.2.1 Primary analysis

The primary analysis of the Change in MITOS stage from baseline to Month 6 will be conducted on the FAS as described in Table 2.

The Change in MITOS stage will be analyzed as a binary endpoint defined as the progression (versus non-progression) of the MITOS stage to a higher stage at 6 months compared to baseline. Hence, subjects who did not progress to a higher stage at 6 months compared to baseline stage are "non-progressors". "Non-progressors" will be modelled in the analysis.

Missing data at month 6 whichever reason (death or drop-out prior to month 6) will be imputed as progressors (i.e. with a higher stage at month 6 compared to baseline stage).

The difference in rates of "non-progressors" at month 6 between IFB-088 vs placebo along with 95% CIs and p-value will be estimated according to the binomial exact method.

5.11.3.2.2 Supportive analysis

The same analysis will be repeated on the PPS.

5.11.3.2.3 Descriptive analysis

In addition, a descriptive summary of observed MITOS stage values at baseline, Month 3 and Month 6 as well as their change from baseline to month 3 and to month 6 will also be provided.

5.11.4 Change in King's College stage

5.11.4.1 Expressions

This parameter will be expressed in terms of:

- Value at baseline and at each planned visit (V3, and V4).
- Change from baseline to each post baseline visit.
- Change from V3 to V4.

5.11.4.2 Change in King's College stage at Month 6 (V4)

5.11.4.2.1 Primary analysis

The primary analysis of the Change in KCL stage from baseline to Month 6 will be conducted on the FAS as described in Table 3**Erreur ! Source du renvoi introuvable.**. The Change in KCL stage will be analyzed as a binary endpoint defined as the progression (versus non-progression) of the KCL stage to a higher stage at 6 months compared to baseline. Hence, subjects who did not progress to a higher stage at 6 months compared to baseline stage are "non-progressors". "Non-progressors" will be modelled in the analysis.
Missing data at month 6 whichever reason (death or drop-out prior to month 6) will be imputed as progressors (i.e. with a higher stage at month 6 compared to baseline stage).

The difference in rates of "non-progressors" at month 6 between IFB-088 vs placebo along with 95% CIs and p-value will be estimated according to the binomial exact method.

The ratio of rates of "non-progressors" at month 6 between IFB-088 vs placebo along with 95% CIs and p-value will be estimated according to the binomial exact method.

5.11.4.2.2 Supportive analysis

The same analysis will be repeated on the PPS.

5.11.4.2.3 Descriptive analysis

In addition, a descriptive summary of observed King's College stage values at baseline, Month 3 and Month 6 as well as their change from baseline to month 3 and to month 6 will also be provided.

5.11.5 Analysis of biomarkers

Analyses will be done on the FAS.

5.11.5.1 Parameters

- TDP-43 plasmatic concentration
- Neurofilament (NfL) light chain
- NfL heavy chain plasmatic concentration
- Growth differentiation factor 15
- Interleukin IL-6
- Tumour necrosis factor-α
- Interferon γ
- IL-1β

- IL-8
- IL-10
- Monocyte chemoattractant protein-1
- Nerve growth factor
- Brain-derived neurotrophic factor
- Vascular endothelial growth factor
- Transforming growth factor beta 1
- Transforming growth factor beta 2
- Transforming growth factor beta 3
- 8-hydroxy 2 deoxyguanosine
- Fibroblast Growth Factor 21
- NGFR/p75ECD
- Neopterin
- Creatinine

5.11.5.2 Expressions

These parameters will be expressed in terms of:

- Log10 value at baseline, V3 at V4.
- Change from baseline to V3 of log10 value.
- Change from baseline to V4 of log10 value.

Note: TDP-43 plasmatic concentration, Neurofilament (NfL) light chain and NfL heavy chain plasmatic concentration parameters are not collected at V3.

Values “above limit of quantification’ (ALQ) will be imputed by ULOQ and values “below limit quantification” (BLQ) will be imputed by LLOQ.

5.11.5.2.1 Analysis of NFL

NFL missing values will not be imputed.

As NFL distribution might be rather skewed with extreme values, NFL assessed at baseline and at month 6 will be log10 transformed. A descriptive analysis will be performed on the log10-scale and then back-transformed: geometrical means and their 95% Cis will be provided.

NFL (log10 transformed) at month 6 will be analyzed in an ANCOVA model adjusting for treatment and baseline NFL (log10 transformed). Adjusted means and the difference in adjusted means will be estimated in this model and then back transformed into the original scale. Adjusted mean NFL at month 6 in each treatment is interpreted as a geometric mean (adjusted for baseline

NFL). The back transformed adjusted treatment difference is interpreted as the ratio of adjusted geometric means between treatments.

A descriptive analysis will only be performed on the log10-scale and then back-transformed: geometrical means and their 95% CIs will be also provided at 3 months.

5.11.5.2.2 Analysis of TDP-43

TDP-43 will be analyzed with the same approach as for NFL on the FAS.

5.11.5.2.3 Other biomarkers.

A descriptive analysis will only be performed on the log10-scale and then back-transformed: geometrical means and their 95% Cis will be provided on the FAS.

5.11.6 Change in Bioelectrical impedance

5.11.6.1.1 Parameters

Bioelectrical parameters are:

- Phase angle,
- Z200/Z5 impedance ratio,
- Skeletal muscle mass,
- Dry mass
- Dry mass without fat
- Mineral bone content
- ECM/BCM ratio ECM
- Energy expenditure

5.11.6.1.2 Expressions

These parameters will be expressed in terms of:

- Value at baseline and at each planned visit (baseline (V0), V3, and V4).
- Change from baseline to each post baseline visit.

5.11.6.1.3 Analysis

A descriptive analysis will be provided only at each visit by treatment group on FAS.

5.11.7 Change in ALSAQ-40 at Month 6 (V4)

5.11.7.1.1 Expressions

These parameters will be expressed in terms of:

- Value at baseline and at V4.
- Change from baseline to V4.

***5.11.7.1.2** Analysis*

A descriptive analysis will be provided only on the FAS.

5.11.8 Respiratory function

5.11.8.1.1 Parameters

Respiratory parameters are:

- arterial blood gases [ABG]:
  - PaCO2 mmHG
  - PaO2 mmHG
  - HCO3 mEq/L
  - SO2 %
  - Base excess mmol/L
- slow vital capacity [SVC] :
- % of the predicted value for age and sex (%) recalculated

5.11.8.1.2 Expressions

These parameters will be expressed in terms of:

- Value at baseline and at each planned visit (baseline (V0), V3, and V4).

Change from baseline to each post baseline visit.

5.11.8.1.3 Analyses

Analyses will be performed on the FAS.

<u>For arterial blood gases:</u>

Descriptive analyses only will be done for parameters assessed at each visit and for change from baseline to V3 and V4.

<u>For the percentage of the predicted value for age and sex of SVC recalculated:</u>

The change from baseline to Month 6 will be analyzed in an ANCOVA model including terms for treatment and baseline value.

Missing data will be imputed according to strategy of estimand 1:

Missing data will be handled depending on the type of the intercurrent event (i.e.).

- Missing data due to deaths:
  a) due to disease progression, the % of predicted value at month 6 will be imputed by the worst value (i.e. 0)
  b) other reason (sudden death included):
  - if the % of predicted value is not missing at Month 3, the % of predicted value at month 6 will be imputed by linear extrapolation given baseline and Month 3 ALSFRS-R score available values.
  - if the % of predicted value is missing at Month3, the % of predicted value at month 6 will be imputed by linear extrapolation based on the predicted value in the linear regression model including terms for intercept and baseline % of predicted value estimated within the treatment group to which the patient is randomized.

- Missing data due to drop-outs or withdrawal will be imputed with the same approach used for handling missing data due to deaths for other reasons (i.e. other than disease progression).

Imputed value lower than 0 will be replaced by 0.

Descriptive analyses will also be done at each visit and for changes from baseline to V3 and V4.

5.12 Safety Analyses

The safety analyses will be presented by the treatment received for the Safety Set.

5.12.1 Adverse Events

Adverse events will be coded by Primary System Organ Class (SOC) and Preferred Term (PT) using MedDRA dictionary.

5.12.1.1 Summary of treatment emergent adverse events

A summary of the number of treatment emergent adverse events, number and percentage of patients with at least one AE will be presented, including:

- AE

- TEAE
- TEAE related to study drug
- Serious TEAE
- Serious TEAE related to study drug

- Fatal TEAE
- Fatal TEAE related to study drug

- Grade 1 (mild) TEAE
- Grade 2 (moderate) TEAE
- Grade ≥3 (severe) TEAE

- Grade ≥3 TEAE related to study drug
- Grade ≥3 and serious TEAE
- Grade ≥3 and serious TEAE related to study drug

- TEAE possibly related to Riluzole

- TEAE leading to temporary discontinuation of study drug
- TEAE leading to definitive discontinuation of study drug
- TEAE leading to premature withdrawal from the study

5.12.1.2 Treatment emergent adverse events

The number of patients with at least one TEAEs and the corresponding number of events will be described by System Organ Class and Preferred Term overall in the safety population. In case a same patient presented the same AE (i.e. same event as defined according to SOC and PT) several times during the study, only one occurrence will be reported, but the total number of events will be counted. This table will be sorted by decreasing percentage of SOC and PT within SOC for the overall column.

The following listings will be edited in order to complete the AE analyses:

- All TEAE reported along the study
- TEAE related to study drug,
- Serious TEAE,
- Grade ≥3 (severe) TEAE,

- TAESIs

For each listing of AE, the following items will be included: Patient identifier, Country, Age (years), Sex, Start date of treatment - Reported term, MedDRA primary SOC, MedDRA preferred term, Start/end datetime of AE, Seriousness, Seriousness criteria, Relationship to study drug, Intensity, Outcome, Action taken regarding investigational drug, Corrective treatment, and if it is an AE of special interest.

In addition, for specific event of interest, a listing of patients with triglycerides > 5.7 mmol/L with at least 20% increase compared to baseline value will be provided. Triglycerides and Glomerular Filtration Rate will be described by classes defined in section 5.2.

5.12.2 Deaths

A listing of fatal adverse events will be provided including the same items as for AE listing described above, with in addition the primary reason for death (if available) and the date of death.

5.12.3 Laboratory Data

5.12.3.1 Parameters

5.12.3.1.1 Haematology

Haematology parameters are:

- Erythrocytes
- Hemoglobin
- Hematocrit
- White blood cells, total
- Neutrophils
- Lymphocytes
- Monocytes
- Eosinophils
- Basophils
- Platelets

5.12.3.1.2 Biochemistry

Biochemistry parameters are:

- Glucose
- Total cholesterol
- HDL cholesterol
- LDL cholesterol
- Triglycerides
- C Reactive Protein
- Troponin T
- Creatine Kinase
- Uric Acid

Only at V3bis visit:

- Prothrombin time
- D-Dimers
- INR
- B type natriuretic peptide (BNP)

5.12.3.1.3 Renal function (blood)

Renal function parameters are:

- Creatinine
- Blood Urea Nitrogen
- Glomerular Filtration Rate, Estimated
- Protein

5.12.3.1.4 Liver function

Liver function parameters are:

- Albumin
- Bilirubin
- Direct Bilirubin
- Alkaline Phosphatase
- Aspartate Aminotransferase
- Alanine Aminotransferase
- Gamma Glutamyl Transferase
- Lactate Dehydrogenase

5.12.3.1.5 Electrolytes

Electrolytes parameters are:

- Sodium
- Potassium
- Chloride
- Bicarbonate
- Calcium
- Magnesium
- Phosphate

5.12.3.1.6 Urinalysis

Urinalysis parameters are:

Urine dipstick:

- Blood
- Leukocytes
- Glucose
- Protein
- pH

Quantitative urinary measurements:

- Beta-2 Microglobuline
- Albumin
- Creatinine
- Albumin-to-creatinine ratio
- Protein (mg/dL)

Cytobacteriological examination of urine (CBEU):

- Parameter collected during CBEU.

5.12.3.1.7 24H protein test

For patient with protein >= 30 mg/dL:

24h protein parameters are:

- Urine volume (mL)
- Amount of protein (mg/day)

- Albumine (mg/day)
- Creatinine (mg/day)
- Protein/Creatinine (RATIO)
- Investigator's overall assessment

5.12.3.1.8 Crystalluria

Only a listing of crystalluria will be done.

5.12.3.2 Expressions

For investigator's overall assessment of 24h protein test and urinalysis parameters, values will be expressed in class (Normal, Abnormal clinically significant (CS), Abnormal non clinically significant (NCS)) at baseline and at each post-baseline visit (baseline (V0), V1, V2, V3, V3bis, V4 and V-FU visit).

For hematology, biochemistry, renal function, liver function and electrolytes parameters, values will be expressed as following:

- in class according to reference ranges at each visit (Low, In range or High) at baseline and at each post-baseline visit (baseline (V0), V1, V2, V3, V4 and V-FU visit)
- quantitatively with value at baseline, at each post baseline visit, change from baseline to each post baseline visit and relative change from baseline to each post baseline visit for listing value

Note:

- for creatinine and Glomerular Filtration Rate, other visits are performed: V2 + 1 month, V3 + 1 month, V3 + 2 months
- And for urine dipstick: V0 + 1 week

5.12.3.3 Analysis

Clinically significant abnormalities in laboratory values across the whole study will be summarized and listed.

<u>For urinalysis parameters:</u>

Shift tables in relation with normal, abnormal CS and abnormal NCS value from baseline to V3 and V4 will be presented for each parameter.

Urinalysis data will also be listed separately for each parameter.

<u>For 24h protein test parameters:</u>

Shift tables in relation with normal, abnormal CS and abnormal NCS value from baseline to V3 and V4 will be presented for the investigator's overall assessment.

A listing will also be presented for patients with at least one abnormal clinically significant of 24h protein test during the study.

For hematology, biochemistry, renal function, liver function and electrolytes parameters:

Each measurement (continuous data) will be categorized as below, within, or above normal range, based on ranges supplied by the laboratory used. Shift tables in relation to the normal range from Baseline to V3 and V4 will be presented for each parameter.

Laboratory data will also be listed separately for each parameter (indicating abnormal values at each applicable follow-up time point). Quantitative value at each visit with the change from baseline and relative change from baseline will be also presented in the listing.

Table 4: Specific rules for ULN and LLN by parameters

Parameter	Units	Below normal range	In normal range	Above normal range
Glomerular Filtration Rate	ml/min/1,73 m^2	<90	≥90	NA
Basophils	10^3/mm^3 ou 10^3/uL ou 10^9/L ou G/L	NA	<0.1	≥0.1
	%	Not analysable (will be converted by data management)		
Eosinophils	10^3/mm^3 ou 10^3/uL ou 10^9/L ou G/L	NA	<0.4	≥0.4
	%	Not analysable (will be converted by data management)		
Erythrocytes	10^12/L, 10^6/mm^3, 10^6/uL, T/L	<3.8	[3.8 ;5.5]	>5.5
Hemoglobin	g/dL	<12	[12 ;16]	>16
	g/L	<120	[120 ;160]	>160
Lymphocytes	10^3/mm^3 ou 10^3/uL ou 10^9/L ou G/L	<1,5	[1,500 ; 4]	>4
	%	Not analysable (will be converted by data management)		
Monocytes	10^3/mm^3 ou 10^3/uL ou 10^9/L ou G/L	<0.5	[0.5 ; 0.8]	>0.8
	%	Not analysable (will be converted by data management)		
Neutrophils	10^3/mm^3 ou 10^3/uL ou 10^9/L ou G/L	<1.5	[1.5 ; 7]	>7
	cells/mm^3	<1500	1500 et 7000	>7000

	%	Not analysable (will be converted by data management)		
White blood cells, total	1000/uL, 10^3/mm^3, 10^3/uL, 10^9/L et G/L	<4.5	[4.5 ; 11]	>11
Glucose	mg/dl	<70	[70 ; 110]	>110
	mmol/L	<4	[4 ; 6.1]	>6.1
	umol/L	<400	[400 ; 610]	>610
HDL cholesterol	g/L	<0.4	≥ 0,4	NA
	mg/dl	<40	≥ 40	NA
	mmol/L	<1.04	≥ 1.04	NA
LDL cholesterol	mg/dL	NA	<160	≥160
	mmol/L	NA	<4.1	≥4.1
Total Cholesterol	mg/dL	NA	<200	≥200
	mmmol/L	NA	<5	≥5
Triglycerides	g/L	NA	≤1.5	>1.5
	mg/dL	NA	≤150	>150
	mmol/L	NA	<1.71	>1.71
	umol/L	NA	<=171	>171
Alanine Aminotransferase	All units	NA	<ULN	>ULN
Aspartate Aminotransferase	All units	NA	≤ULN	>ULN
D-Dimers	All units	NA	≤ULN	>ULN
Direct Bilirubin	All units	NA	≤ULN	>ULN
Gamma Glutamyl Transferase	All units	NA	≤ULN	>ULN
Other parameters	All units	<LLN	[LLN ; ULN]	>ULN

If LLN is missing then all values below the ULN will be considered as in reference range.
If ULN is missing then all values above the LLN will be considered as in reference range.

If a value is <xx then:

- If the LLN = 0 or missing or NA then consider the value as in reference range
- Else consider the value as low reference range

If a value is >xx then:

- If the ULN is missing or NA then consider the value as in reference range
- Else consider the value as upper reference range

For Glomerular Filtration Rate:
If a value is >xx with xx<90 then consider value as below reference range
If a value is >xx with xx>=90 then consider value as in reference range
If a value is <xx then consider value as below reference range

Note: for all listing value of premature discontinuation and V3bis visit will be also presented when applicable.

5.12.4 Vital Signs

5.12.4.1 Parameters

Vital signs parameters are:

- BMI (kg/m²),
- Heart Rate (beats/min) in supine and standing positions,
- Systolic Blood Pressure (mmHg) in supine and standing positions,
- Diastolic Blood Pressure (mmHg) in supine and standing positions.
- Investigator's overall assessment.

5.12.4.2 Expressions

These parameters will be expressed in terms of:

- Value at baseline and at each planned visit (baseline (V0), V1, V2, V3, V3bis, V4 and V-FU visit).
- Change from baseline to each post-baseline value.
- Investigator's overall assessment (normal, abnormal NCS, abnormal CS) at each visit

5.12.4.3 Analysis

Descriptive statistics for observed values and changes from Baseline in the following vital signs will be presented by treatment group and visit.

All vital signs data will also be listed. Value of premature discontinuation and V3 bis visit will be also presented when applicable.

5.12.5 Electrocardiogram Data

5.12.5.1 Parameters

ECG parameters are:

- Heart Rate (beats/min),
- RR interval (ms),
- PR interval (ms),
- QRS interval (ms),
- QT interval (ms),
- QTcF (ms).

- Investigator's overall assessment.

A transthoracic cardiac echocardiography is performed at V3bis visit.

5.12.5.2 Expressions

For ECG parameters will be expressed in terms of:

- Value at baseline and at each planned visit (baseline (V0), V1, V2, V3, V3bis, V4 and V-FU visit).
- Change from baseline to each post-baseline value.
- Investigator's overall assessment (normal, abnormal NCS, abnormal CS)

Investigator's overall assessment (normal, abnormal NCS, abnormal CS) and conclusion of transthoracic cardiac echocardiography at V3bis will be listed.

5.12.5.3 Analysis

The following analyses will be performed:

- Description of actual value at each visit and change from baseline at each visit.
- Shift tables in relation to the overall interpretation (Normal, Abnormal Not Clinically Significant (NCS), and Abnormal Clinically Significant (CS)) from Baseline to each applicable visit will be presented by treatment group.
- All ECG data will be listed, with a separate listing provided that includes all parameters for timepoints where an abnormal finding was noted. Value of discontinuation visit and V3 bis visit will be also presented when applicable.

Investigator's overall assessment and conclusion of transthoracic cardiac echocardiography at V3bis will be listed.

5.12.6 Physical Examination

5.12.6.1 Parameters

The following physical examination will be described at screening, baseline (V0), V1, V2, V3, V4 and V-FU visit:

- General appearance
- Skin
- Neck (including thyroid)
- Eyes, ears, nose, throat

- Lungs
- Heart
- Abdomen
- Neurological examination
- Lymph nodes

Physical examination of heart auscultation is also performed at V3bis visit.

5.12.6.2 Analysis

Physical examination data will be listed only.

6 INTERIM ANALYSIS

No interim analyses are planned.

7 CHANGES TO PLANNED PROTOCOL ANALYSIS

Changes to planned protocol analysis are the following:

- Modification of the primary analysis of the primary efficacy endpoint. Modification of the imputation method for missing data due to deaths occurring during the study. The ANCOVA model will be used instead of the MMRM to account for different sources of missing data.

- Deletion of the Bayesian analysis. A Bayesian analysis was planned in the protocol to combine prior data from literature (Promise study) with current study data. However, as the populations differ (all-comers ALS patients in the Promise study vs bulbar-onset ALS patients only in P288ALS study), this Bayesian analysis will be further considered in a *post hoc* manner if appropriate.

8 REFERENCES

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