STUDY PROTOCOL

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

Treatment combining **R**iluzole and **I**FB-088 in bulbar **A**myotrophic **L**ateral **S**clerosis: the **TRIALS** study

Protocol number	P288ALS
EUDRACT number	2021-003875-32
Protocol date	08 April 2024
Protocol version	Version 4.0 (Final)
Investigational product name	IFB-088
Indication	Bulbar-onset amyotrophic lateral sclerosis
Sponsor	InFlectis BioScience 21 Rue La Noue Bras de Fer, 44200 Nantes, France Sponsor signatory: Anne Visbecq, MD, Chief Medical Officer. Tel: +33 6 30 63 20 20 Sponsor medical expert: Anne Visbecq, MD, Chief Medical Officer. Tel: +33 6 30 63 20 20
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PROTOCOL SPONSOR SIGNATURE PAGE

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

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PROTOCOL INVESTIGATOR SIGNATURE PAGE

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Protocol number	P288ALS
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I certify that I will conduct the study in compliance with the protocol, any amendments, GCP and all applicable regulatory requirements.

Investigator	Pr Shahram Attarian
Institution	Head of Department of neuromuscular disorders and ALS. *Assistance Publique Hôpitaux de Marseille*, Marseille, France.

Date 12/4/2024

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PROTOCOL INVESTIGATOR SIGNATURE PAGE

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 of Amyotrophic Lateral Sclerosis

Treatment combining **R**iluzole and **I**FB-088 in bulbar **A**myotrophic **L**ateral **S**clerosis: the **TRIALS** study

Protocol number	P288ALS
EUDRACT number	2021-003875-32
Protocol date	08 April 2024

I certify that I will conduct the study in compliance with the protocol, any amendments, GCP and all applicable regulatory requirements.

Investigator	Pr Giuseppe Lauria
Institution	Department of clinical neurosciences. IRCCS Carlo Besta Institute of Milan, Milan, Italy.

Date 15/4/2024

Signature DocuSigned by: Pr Giuseppe LAURIA C1C9B563418B443...

SYNOPSIS

Protocol number: P288ALS	**EUDRACT number:** 2021-003875-32
Indication: Amyotrophic Lateral Sclerosis (ALS)	**Phase**: Phase II
Investigational product: IFB-088, tablets	**Active ingredient**: IFB-088
Protocol date: 08 April 2024	
Sponsor: InFlectis BioScience	

Study 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.
Rationale for the study:	Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease of motor neurons in the brain and spinal cord, resulting in progressive paralysis, with death typically occurring within 2 to 4 years of symptom onset. The prognosis has not been very much improved by the only approved medication in Europe, riluzole. ALS can be divided into 2 onsets, namely spinal and bulbar, the latter being characterised by difficulties in swallowing, chewing and speaking, a more rapid evolution and a poorer prognosis. 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 (the drug has been commercialised as an anti-hypertensive drug in several countries). 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.
Objectives and endpoints:	**Primary objective** To assess the safety of IFB-088 50 mg/day in patients with bulbar-onset ALS. **Primary endpoints** Safety endpoints include: • Incidence, grade and relationship to IFB-088 for treatment emergent AEs, SAEs, and AESIs, • AEs leading to dose interruption or premature discontinuation.

	Secondary objectives • 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). **Secondary and exploratory endpoints** Efficacy: • 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 3 and at 6 months compared to the baseline, • Change in King's College score from baseline to month 3 and 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 PK parameters: • Plasma concentration of IFB-088 and IFB-139, • AUC of IFB-088 and IFB-139, • Maximum observed plasma concentration (C_{max}), with associated T_{max}, • Terminal or apparent terminal half-life ($t_{1/2}$), • Apparent systemic clearance, apparent volume of distribution. Biomarkers: • Change in TDP-43 plasmatic concentration from baseline to 6 months, compared to placebo, • Change in neurofilament (NfL) light chain and NfL heavy chain plasmatic concentration from baseline to 6 months, compared to placebo, • Change in plasmatic (neuro)inflammatory biomarkers from baseline to 3 months, and from baseline to 6 months, compared to placebo, • Change in plasmatic oxidative stress biomarkers from baseline to 3 months and from baseline to 6 months, compared to placebo. QoL: • Change in ALS assessment questionnaire (ALSAQ-40) from baseline to 6 months.
Study Population, Countries and sites:	Adult patients with bulbar-onset ALS. Patients will be recruited in France and in Italy.

<table>
<tr><td>Study design:</td><td>Prospective, international, randomised, double-blind, placebo controlled, multicentre, parallel group study.
Patients will be randomised in a 2:1 allocation ratio to receive either IFB-088 + riluzole 100 mg or placebo + riluzole 100 mg.
Patients will be treated over a 6-month period. After a screening/consent visit, patients will undergo clinic visits at randomisation (V0), at 2 weeks (V1), and at months 1 (V2), 3 (V3), 4.5 (V3bis) and 6 (V4). In addition to these hospital visits, patient will undergo urine analysis (dipstick) and blood sampling for measurement of creatinine one week after V0, as well as blood sampling for measurement of creatinine and calculation of eGFR at months 2, 4 and 5. At the V2 visit, in addition to other assessments, patients will undergo blood sampling for PK measurements and urine sampling for crystalluria examination. Laboratory parameters, as well as physical examination and vital signs assessment to assess safety will be performed at each visit for safety purpose and crystalluria examination will be repeated at the follow-up visit, performed one month ± one week after V4, or in case of premature discontinuation.
Safety will be monitored over the course of the study by a data and safety monitoring board (DSMB).
If needed, compliance with coronavirus disease 2019 (COVID-19)-related rules will be followed and adjustments might be necessary accordingly.
Unless they already obtained treatment research initiative to cure ALS (TRICALS) certificates, study examiners will undergo online training to ensure uniformity of study procedures across sites, and inter-rater variability will be documented. For investigators who cannot undergo the TRICALS training, certification from the Barrow Neurological Institute is acceptable.
Management of frail functions will be standardised as much as possible.</td></tr>
<tr><td>Planned number of patients:</td><td>The minimum required sample size for this study has been estimated at 42 evaluable patients, 28 receiving IFB-088 and 14 receiving placebo. Assuming a 15% drop-out rate, 50 patients will be randomised.</td></tr>
<tr><td>Investigational products, dose and mode of administration:</td><td>
<table>
<tr><th>NAME</th><th>PHARMACEUTICAL FORM</th><th>UNIT DOSE STRENGTH</th><th>MODE OF ADMINISTRATION</th></tr>
<tr><td>IFB-088</td><td>Tablets</td><td>25 mg</td><td>Oral</td></tr>
<tr><td>PLACEBO</td><td>Tablets</td><td>NA</td><td>Oral</td></tr>
</table>
IFB-088 and placebo will be provided as white, oblong, immediate release tablets. IFB-088 and placebo tablets contain the following excipients: microcrystalline cellulose, colloidal silica, crospovidone and magnesium stearate.
The drug product and placebo will be manufactured and formulated under current good manufacturing practice (GMP) conditions. Tablets should be kept in the containers provided to patients and stored at room temperature.
IFB-088 or placebo will be administered orally twice a day (morning and evening uptakes), as an add-on therapy to riluzole 100 mg. Intervals for dosing should ideally be about 12 hours (± one hour). Tablets will be swallowed with a glass of water 30 minutes before the meal, in fasting condition. For patients who have swallowing difficulties, tablets will be administered in a spoon filled with jellified water to prevent swallowing disorders.</td></tr>
<tr><td>Inclusion criteria:</td><td>Patients must satisfy all the following inclusion criteria:</td></tr>
</table>

	1. Diagnosis of probable or definite ALS according to the revised El Escorial criteria, with bulbar onset of disease, familial or sporadic form, 2. Onset of symptoms ≤ 18 months prior to screening, as reported by the patient, 3. Adult males or females, aged at least 18 years old, 4. SVC > 60% of predicted value for age and sex, 5. ALSFRS-R score ≥ 36, 6. Treatment with riluzole 100 mg/day, at stable dose since at least one month and well tolerated, 7. Male or female patient of childbearing potential[1] who agrees to use highly effective mechanical contraception methods (sexual abstinence, intrauterine device, bilateral tubal occlusion, vasectomised partner) throughout the study, and for 3 months after the end of the treatment, 8. Patient who read, understood and signed the informed consent form (ICF), 9. Patient who is willing to adhere to the study visit schedule and is capable to understand and comply with protocol requirements.
Exclusion criteria:	A patient will not be eligible for inclusion in this study if at least one of the following criteria applies: 1. Known other significant neurological disease(s), 2. Serious illness(es) or medical condition(s) (e.g. unstable cardiac disease, cancer, hematologic disease, hepatitis or liver failure, renal failure) that is not stabilised or that could require hospitalisation and may jeopardise the participation in the study, 3. Abnormal renal function at screening defined as estimated glomerular filtration rate (eGFR) < 60 mL/min/1.73m^2, 4. Abnormal liver function at screening defined as total bilirubin levels >1.5 ULN, and/or AST and/or ALT >3 ULN, 5. Neutropenia (ANC <1.5 x 10^9/L) at screening, 6. Other causes of neuromuscular weakness, 7. Non progressive or very rapidly progressing ALS (ALSFRS-R decline from disease onset to randomisation ≤ 0.1 / month or ≥ 1.2 / month)[2], 8. Non-invasive ventilation, 9. Tracheotomy,

[1] According to the CTFG guideline V2.1 September 2020, a woman is considered of childbearing potential (WOCBP), i.e., fertile, following menarche and until becoming post-menopausal unless permanently sterile. Permanent sterilisation methods include hysterectomy, bilateral salpingectomy and bilateral oophorectomy. A postmenopausal state is defined as no menses for 12 months without an alternative medical cause. A high follicle stimulating hormone (FSH) level in the postmenopausal range may be used to confirm a post-menopausal state in women not using hormonal contraception or hormonal replacement therapy. However, in the absence of 12 months of amenorrhea, a single FSH measurement is insufficient. A man is considered fertile after puberty unless permanently sterile by bilateral orchidectomy.

[2] Decline will be calculated as follows: 48 minus ALSFRS-R score at the time of inclusion divided by number of months since occurrence of first symptoms of disease, as reported by the patient.

	10. Dementia or other severe active psychiatric illness, including suicidal ideation assessed using the Columbia-Suicide Severity Rating Scale (C-SSRS), 11. Patient with a significant pulmonary disorder not attributed to ALS or who require treatments that might complicate the evaluation of the effect of ALS on respiratory function, 12. Patient treated by edaravone for ALS, 13. Patient using unauthorised concomitant treatments, namely moderate or strong inhibitors or inducers of cytochrome P450 family 1 subfamily A member 2 (CYP1A2), strong inhibitors or inducers of CYP2D6 or 2C19 and strong inhibitors of OCT2. Combined oral contraceptives containing ethinylestradiol are forbidden concomitant medications, 14. Smoker of > 10 cigarettes per day (e-cigarettes and nicotine patches are permitted), 15. Known hypersensitivity to any of the ingredients or excipients of the investigational medicinal products (IMPs), 16. Pregnant, lactating women, 17. Patient who participated in another trial of investigational drug(s) within 30 days prior to randomisation, or 5 half-lives of the previous investigational product, whichever is longer, 18. Patient who has forfeited their freedom by administrative or legal award, or who is under guardianship or under limited judicial protection.
Evaluation criteria:	**Safety Assessments** Safety assessments will include the surveillance and recording of adverse events (AEs) including serious adverse events (SAEs), treatment emergent AEs (TEAEs), AEs of special interest (AESIs). Identified AEs will be characterised in relation to seriousness, incidence, severity, and relationship to the study product. The percentage of patients requiring dosing interruption or definitive treatment discontinuation due to toxicity will be recorded. Safety evaluation will be performed at all visits. **Efficacy Assessments** Clinical scores: changes between baseline and 3 months, and between baseline and 6 months will be calculated for: • ALSFRS-R score, • ALS- ALS-MITOS score, • King's College score, • Respiratory function (SVC, sniff test, ABG). • Bioelectrical Impedance Analysis **PK assessments** Blood samples will be collected at V2 (5 samples/patient: pre-IMP dose, and at one, 2, 4 and 6 hours post dose). After processing, plasma samples will be stored at -80°C pending analyses within the same week. IFB-088 and its main metabolite IFB-139 concentrations will be measured using a validated high-performance liquid chromatography with tandem mass spectrometry (HPLC/MS-MS) method. **Biomarker assessments** Blood potential biomarkers (neurofilament [NfL] light chain, NfL heavy chain, and TAR DNA-binding protein 43 [TDP-43]) will be measured at baseline and 6 months

	while biomarkers of neuro-inflammation and oxidative stress will be measured in blood at baseline, 3 and 6 months. Blood, cerebrospinal fluid (CSF)[3] and urine will be stored frozen in a biobank for further biomarker analyses[4].
Statistical methods:	**Statistical Methods** Statistical methods will be further detailed in the statistical analysis plan (SAP). **General considerations:** Statistical analysis will be performed by the sponsor or under the supervision of the sponsor. Complete details of the statistical analyses will be provided in the SAP, which will be finalised prior to the locking and unblinding of the database. In general, summary tabulations will be presented by treatment arm and will display the number of observations, mean, standard deviation (SD), median, Q1, Q3 quartiles minimum, and maximum for continuous variables, and the number and percent per category for categorical data. Nominal alpha level of significance: as it is an exploratory study, statistical tests will be performed at the alpha one-sided level of 0.10. 80% and 95% 2-sided confidence intervals (CIs) will be provided. No adjustment for multiplicity issues will be considered. Missing data handling: as a general rule, missing data will not be replaced. Sensitivity analyses based on multiple imputation will be proposed and further detailed in the SAP. **Baseline characteristics description:** Baseline characteristics will be described by treatment arm according to general considerations. **Analysis sets:** The following analysis sets will be considered. Additional details of analysis sets of patients may be defined in the SAP. • Safety set (SS): randomised patients having received at least one dose of the IMPs and analysed according to the treatment actually received. Efficacy sets: • Randomized Set (RS): all patients “as randomised” (i.e. according to the treatment group to which they were randomised, regardless of the treatment actually taken), • Full analysis set (FAS): as randomized patients having received at least one dose of the IMPs and having a non-missing baseline value, • Per protocol set (PPS): FAS patients fulfilling the following criteria. Criteria definition will be further detailed during a blinded data review meeting and specified in the SAP: o Major inclusion/exclusion criteria satisfied, o Absence of relevant protocol violations likely to affect treatment efficacy,

[3] In patients willing to undergo lumbar puncture.

[4] Patients will be asked in the ICF if they want to consent to the storage of their biological samples in the biobank.

	○ Adequate study medication compliance, defined as intake of at least 80% of the planned total dose. **Primary Outcome:** Usual descriptive statistics will be used and detailed in the SAP to analyse the safety parameters. **Secondary Outcomes:** Efficacy: Analyses will be further detailed in the SAP. The analysis of efficacy endpoints will be primarily performed in the FAS. A secondary analysis will be proposed in the PPS. • Change in ALSFRS-R score from baseline to 6 months: the change from baseline to 6 months will be analysed using a mixed model for repeated measures (MMRM) assuming missing at random (MAR) and an unstructured covariance matrix and including fixed factors for treatment, visit (categorical variable), treatment-by-visit interaction, random patient factor and baseline ALSFRS-R as a continuous covariate. The degrees of freedom will be estimated using the Kenward-Roger's approximation. Least square means (LSMs) of change from baseline and LSM differences in change from baseline between active doses and placebo will be presented along with 80% and 95% CIs and the comparison p-value. A one-sided nominal significance level of 0.10 will be used for treatment comparison. Model assumptions will be checked (plot of studentized residuals vs predicted values, etc.). If the model assumptions do not hold, a rank semi-parametric analysis of covariance (ANCOVA) model (or a rank non-parametric Quade's ANCOVA model if the assumptions of this latter do still not hold) will be proposed instead. As the MAR assumption is untestable and may not hold for subjects withdrawing at their own will, a sensitivity analysis will be based on a pattern mixture model that uses a multiple imputation technique analysed with an ANCOVA with pre-specified fixed factors and covariates. The imputation model will be further clarified in the SAP. In addition, a sensitivity Bayesian analysis using the historical placebo data from E. Dalla Bella's ProMISe study will be also performed and further detailed in the SAP. An intrapatient analysis of ALSFRS-R changes will be performed. • Worsening, i.e. progression to a higher stage at 6 months compared to the baseline stage defined by ALS-MITOS score: the proportion of progressors will be compared between both treatment groups. The difference in binomial proportions will be estimated along with an exact 95% CI. A sensitivity Bayesian analysis using the historical placebo data from Dalla Bella's et al. ProMISe study of 2021 will be also performed and further detailed in the SAP. Progressors will also be analysed in a logistic regression model adjusting for treatment, randomisation stage and additional relevant covariates (specified in the SAP). The treatment effect will be estimated in terms of an odds ratio associated with the 95% CI and p-value and also in terms of a difference in progression rates with its 95% CI using the method proposed by Ge and al. in 2011. • Change in King's College score: the endpoint will be analysed with an approach similar to the analysis of ALS-MITOS score. Analyses of the changes in efficacy endpoints from baseline to month 3 (as inferential analysis) and from month 3 to month 6 (as descriptive analysis) will be also proposed and further detailed in the SAP.

	• Respiratory function: changes from baseline to 3 months and from baseline to 6 months will be analysed. Further details will be provided in the SAP. • Bioelectrical impedance analysis: changes from baseline to 3 months and from baseline to 6 months will be analysed for selected variables. Further details will be provided in the SAP. PK: Individual plasma concentrations of IFB-088 and IFB-139 (i.e. IFB-088 metabolite) with corresponding nominal sampling times, will be tabulated for each subject and scheduled sampling time, together with descriptive statistics. Mean plasma IFB-088 concentrations profiles by cohort will be presented over time graphically, using blood sampling time (x axis) and concentrations (y axis) on both linear and semi-log scales. Derived and observed PK parameters will be evaluated by noncompartmental analysis. Plasma concentrations of IFB-088 will also be analysed using a population PK modelling approach to develop a population PK model in patients with ALS. PK and pharmacodynamic (PD) data will be listed and presented in graphical and/or tabular form as appropriate to the data, and will be summarized descriptively. A PK/PD model could be developed according to the available data. Biomarkers: Details of analysis will be provided in the SAP. Changes in plasma concentration of TDP-43, NfL heavy chain and NfL light chain will be measured from baseline to 6 months. Changes in plasma concentration of inflammatory and oxidative stress biomarkers will be measured from baseline to 3 months, and from baseline to 6 months. All changes will be compared between both arms. Correlation of biomarkers with clinical endpoints will be assessed. QoL: Change in ALS-AQ40 QoL scores from baseline will be assessed at 6 months and compared between both arms. **Sample size determination:** 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 will be randomised.
Study timelines:	First patient in: first quarter 2022 Last patient out: third quarter 2024

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

µg	Microgram
µL	Microliter
µM	Micromolar
ABG	Arterial blood gases
AE	Adverse event
AESI	Adverse event of special interest
ALS	Amyotrophic lateral sclerosis
ALSAQ-40	ALS assessment questionnaire
ALSFRS-R	Revised ALS functional rating scale
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
ANC	Absolute Neutrophil Count
ANCOVA	Analysis of covariance
AST	Aspartate aminotransferase
AUC	Area under the plasma concentration-time curve
AUC_{inf}	Area under the plasma concentration-time curve until infinity
AUC_t	Area under the plasma concentration-time curve until the last observation time
BDNF	Brain-derived neurotrophic factor
b.i.d.	Twice a day (*bis in die*)
BMI	Body mass index
C9ORF72	Chromosome 9 open reading frame 72
CA	Competent authorities
CBEU	Cytobacteriological examination of urine
CDISC	Clinical data interchange standards consortium
CI	Confidence interval
C_{max}	Maximum plasma concentration
COVID-19	Coronavirus disease 2019
CRA	Clinical research associate
CRO	Clinical research organisation
CSF	Cerebrospinal fluid

CSR	Clinical study report
CYP	Cytochrome P450
CYP1A2	Cytochrome P450 family 1 subfamily A member 2
CYP2C19	Cytochrome P450 family 2 subfamily C member 19
CYP2D6	Cytochrome P450 family 2 subfamily D member 6
dL	Decilitre
DNA	Deoxyribonucleic acid
DRM	Data review meeting
DSMB	Data and safety monitoring board
ECG	Electrocardiogram
eCRF	Electronic case report form
EDTA	Ethylenediaminetetraacetic acid
eIF2α	Eukaryotic initiation factor 2α
ER	Endoplasmic reticulum
FAS	Full analysis set
FTD	Frontotemporal dementia
FU	Follow-up
FUS	Fused in sarcoma
FVC	Forced vital capacity
g	Gram
GCP	Good clinical practice
GGT	Gamma-glutamyl transferase
GMP	Good manufacturing practice
h	Hour
HCG	Human chorionic gonadotropin
HDL	High-density lipoprotein
hERG	Human ether-à-go-go-related gene
HPLC/MS-MS	High-performance liquid chromatography with tandem mass spectrometry
HTG	Hypertriglyceridaemia
ICF	Informed consent form
ICH	International Conference on Harmonisation
ICMJE	International committee of medical journal editors

IEC	Independent ethics committee
IL	Interleukin
IMP	Investigational medicinal product
INR	International Normalized Ratio
IFNγ	Interferon γ
IWRS	Interactive web response system
kg	Kilogram
KLS	Keyrus Life Science
L	Litre
LDH	Lactate dehydrogenase
LDL	Low-density lipoprotein
LLOQ	Lower limit of quantification
LP	Lumbar Puncture
LPM	Laboratory procedures manual
LSM	Least square means
MAD	Multiple ascending dose
MAE	Mean absolute error
MAR	Missing at random
MCP-1	Monocyte chemoattractant protein-1
MDAPE	Median absolute prediction error
MDPE	Median prediction error
MedDRA	Medical dictionary for regulatory activities
miRNA	microRNA
MITOS	Milano-Torino staging system
mIU	Milli-international units
mg	Milligram
mL	Millilitre
mmol	Millimole
MMRM	Mixed model for repeated measures
MPE	Mean prediction error
mRNA	Messenger RNA
NfL	Neurofilament
ng	Nanogram

NGF	Nerve growth factor
nM	Nanomolar
NOAEL	No observed adverse effect level
NTEAE	Non-treatment emergent adverse event
PD	Pharmacodynamics
PE	Prediction error
pH	Potential of hydrogen
PK	Pharmacokinetics
popPK	Population pharmacokinetics
PPP1R15A/PP1c	Protein phosphatase 1 regulatory subunit 15A/protein phosphatase 1c
PPS	Per protocol set
QA	Quality assurance
QoL	Quality of life
RNA	Ribonucleic acid
SAD	Single ascending dose
SAP	Statistical analysis plan
SD	Standard deviation
SO	Safety officer
SmPC	Summary of Product Characteristics
SOD1	Superoxide dismutase 1
SS	Safety set
SUSAR	Suspected unexpected serious adverse reaction
SVC	Slow vital capacity
$T_{1/2}$	Half-life
TARDBP	TAR DNA binding protein
TDP-43	TAR DNA-binding protein 43
TEAE	Treatment emergent adverse event
T_{max}	Time corresponding to C_{max}
TMF	Trial master file
TNFα	Tumour necrosis factor α
TRICALS	Treatment research initiative to cure ALS
ULN	Upper Limit of Normal

UPR	Unfolded protein response
V	Visit
VEGF	Vascular endothelial growth factor
WHO	World health organisation
WOCBP	Women of childbearing potential

1. INTRODUCTION

1.1 BACKGROUND

1.1.1 Amyotrophic lateral sclerosis

Amyotrophic lateral sclerosis (ALS) is an adult-onset progressive, fatal, neurodegenerative disorder that results in the gradual degeneration of the motor neurons in the motor cortex and spinal cord. ALS is the most common type of motor neuron disease with an incidence rate of 2.8 per 100,000 persons/year and a mean prevalence rate of 5.4 per 100,000 people in Europe [1]. This disease predominantly affects males, with a male/female incidence rate ratio of 1.4, and people in their late adulthood, with median age at diagnosis of 65.2 years for men and 67.0 years for women [2]. Clinical diagnosis of ALS is often a prolonged process due to heterogeneity in patient presentation and prognosis as well as a lack of robust diagnostic biomarker [3]. ALS is characterised by rapidly progressive paralysis and death typically occurs from respiratory failure within 2 to 4 years of symptom onset, although about 10% of ALS patients manifest a slow form of the disease with a survival of 10 years or longer [4]. Although the primary symptoms of ALS are associated with motor dysfunction, up to 50% of patients develop cognitive and/or behavioural impairment during the course of disease, and 13% of them may present with concomitant behavioural variant frontotemporal dementia (FTD) [5]. Cognitive and/or behavioural symptoms in patients with ALS significantly relate to the involvement of bulbar regions affecting speech and swallowing [6].
There is no curative treatment for ALS. Treatment and therapy options aim at slowing down the progression of symptoms, preventing complications and improving quality of life (QoL). Riluzole, the only approved medication in Europe, slows the rate of progression and prolongs median survival in patients with ALS by 2 to 3 months and despite its marginal benefits it represents the current standard of care [7]. In Asia, North America and Switzerland an antioxidant which slows the loss of physical function, edaravone, is also approved for the treatment of ALS [8].

Depending on the anatomic location firstly affected by the neuropathology, ALS can be divided into 2 main phenotypes:

- Limb onset, which begins with weakness in the limbs represents about two-thirds of all cases. The median survival from symptoms onset is 39 months for lower limb onset and 27 for upper limb onset [9],
- Bulbar-onset, in which the muscles of speech, mastication and swallowing are first affected, accounts for about a third of ALS cases [4]. The bulbar-onset form has generally a more rapid evolution and a worse prognosis than the limb-onset one, and is often associated with emotional lability. The median survival from symptom onset is 25 months [9].

About 10% of ALS is classified as familial, whereas the remaining 90% of cases are considered sporadic. The genetic aetiology is known only for two thirds of familial cases and about 11% of sporadic ALS cases. Among the genes identified to be involved in ALS, there are genes that encode proteins involved in pathways of protein production, trafficking and degradation. The 4 genes most often found mutated are chromosome 9 open reading frame 72 (*C9ORF72*), superoxide dismutase 1 (*SOD1*), TAR DNA binding protein

(*TARDBP*), and fused in sarcoma (*FUS*) [10]. A specific pathological hallmark of ALS is the presence of 2 representative cytoplasmic inclusions, Bunina bodies and TAR DNA-binding protein 43 (TDP-43)-positive cytoplasmic inclusions in degenerating motor neurons and surrounding glial cells [11]. However, neuronal susceptibility to ALS is not only confined to motor neurons [12]. It has been suggested that pathological processes underlying ALS begin in motor neurons and propagate to other regions of the brain in a fashion that is predictable based on proximity and connection by a corticofugal axonal spread, consistently with the inclusion and dissemination of TDP-43 pathology [13, 14]. Growing evidence suggests that an impairment of proteome homeostasis is responsible for the cytoplasmic presence of ubiquitylated protein inclusions and for ALS pathogenesis [15, 16]. One evidence of the proteome homeostasis impairment is the endoplasmic reticulum (ER) stress and the activation of unfolded protein response (UPR) signalling pathways demonstrated in motor neurons of *in vitro* and *in vivo* models of ALS [17, 18] as well as in human post-mortem tissues from patients with a familial or sporadic form of ALS [19-21]. Targeting components of the UPR pathway and consequently modulating the proteostatic capacity of motor neurons is considered a powerful tool to delay the symptomatic phase of ALS [16]. The phosphatase complex protein phosphatase 1 regulatory subunit 15A/protein phosphatase 1c (PPP1R15A/PP1c) has been one of these targets. The prolongation of protein translation attenuation by PPP1R15A/PP1c phosphatase complex inhibition could be beneficial to decrease the build-up of misfolded proteins in the ER and favour the cells survival. This activity was first shown with guanabenz, an α2 adrenergic receptor agonist [22]. Several preclinical studies have shown that guanabenz improves misfolded protein clearance, reducing ER stress and neuronal death and prolonging the survival in *in vitro* and *in vivo* models of ALS [23-25]. These positive data have been confirmed in the ProMISe phase II clinical study [26] where 201 patients were randomised to receive guanabenz 64 mg, 32 mg, 16 mg, or placebo as an add-on therapy to riluzole for 6 months. Guanabenz at 32 mg or 64 mg administered together with riluzole reduced the proportion of patients progressing to higher stages of disease in 6 months as measured by the ALS Milano-Torino staging score (ALS-MITOS) and decreased the decline rate in the revised ALS functional rating scale (ALSFRS-R) total score. The benefits were more pronounced in patients with bulbar-onset ALS. However, because of its α2 adrenergic activity, guanabenz also has a hypotensive effect. Due to this effect, the proportion of patients who experienced at least one adverse event (AE) was higher in any guanabenz arm than in the placebo arm leading to a higher drop-out rate, which discouraged further development in ALS. A selective inhibitor of PPP1R15A/PP1c phosphatase complex devoid of α2 adrenergic activity and consequent side-effect profile represents a promising therapeutic approach for ALS patients.

1.1.2 IFB-088

The small synthetic molecule IFB-088, also referred to as icerguastat or Sephin1, is a selective inhibitor of PPP1R15A/PP1c phosphatase complex developed by InFlectis BioScience. A medicinal chemistry program based on selective optimisation of side activity was developed to design guanabenz analogues that would be devoid of agonist α2 adrenergic activity but would retain its cytoprotective effect; this has allowed the identification of IFB-088 [27]. Similarly to guanabenz, IFB-088 is intended to prolong the UPR as a way to protect the cell against toxic effects of cellular stress. In stress conditions, the inhibition of PPP1R15A/PP1c phosphatase complex by IFB-088 increases the

phosphorylation of eukaryotic initiation factor 2α (eIF2α), which in turn leads to the prolongation of protein translation attenuation. This decrease in protein synthesis attenuates the ER stress and therefore causes a reduction in the activation of phosphorylated eIF2α downstream pathway, helping with ER stress resolution. Ultimately, the reduction of ER stress by IFB-088 allows the survival of the cell against ER stress and reduces consequent cell death (IFB-report: IFB_R_009_v2).

1.1.2.1 *Preclinical studies*

The efficacy of IFB-088 was evaluated *in vitro* and *in vivo* in models relevant for ALS. In TDP-43-overexpressing human bone osteosarcoma epithelial cells, IFB-088 at 50 µM reduced the number of TDP-43-positive cytoplasmic inclusions without impacting TDP-43 localisation in the nucleus. In combination with riluzole 5 µM, IFB-088 from 5.5 µM to 50 µM reduced the number of TDP-43 stress granules in the cytoplasm (Report Inflectis 21-S30710; Report 29102021). In rat primary spinal motor neurons injured with glutamate, an *in vitro* excitotoxicity assay, IFB-088 at 100 nM and 500 nM reduced the cytoplasmic TDP-43 mislocalisation similarly to what was observed for riluzole 5 µM. The coadministration of IFB-088 with riluzole did not lower the protective effect of riluzole (Report IIS21.01).

In a mouse model of ALS using mice overexpressing the superoxide dismutase 1 containing the mutation G93A (SOD1 G93A), IFB-088 orally administrated at 1 mg/kg twice a day or at 5 mg/kg once a day, was shown to respectively partially and almost completely prevent the motor deficit associated with motor neurons loss in these mice. Moreover, IFB-088 prevented the accumulation of insoluble SOD1 mutant and decreased ER stress markers in SOD1 G93A spinal cords [27].

Based on in vitro and in vivo data in rats and dogs, the primary metabolic pathway of IFB-088 was oxidation by cytochrome P450 (CYP) family 1 subfamily A member 2 (CYP1A2) and subsequent glucuronidation. The main metabolite of the oxidation process was named IFB-139, which was demonstrated to be pharmacologically inactive. CYP family 2 subfamily D member 6 (CYP2D6) and CYP family 2 subfamily C member 19 (CYP2C19) could also contribute to the metabolism of IFB-088, albeit to a much lower extent.

To support the safety of repeated-dose oral administration in humans, IFB-088 was assessed in a series of nonclinical studies that included safety pharmacology, general toxicology, genetic toxicology and embryofetal developmental toxicity studies.

Safety pharmacology studies

No causes of concerns were reported in the safety pharmacology program (in vitro selectivity tests against 44 potential off-targets, in vitro study for effects on the human ether-à-go-go-related gene [hERG] channel and in vivo studies for effects on cardiovascular function in Beagle dogs and on the central nervous system and respiratory function in Sprague-Dawley rats).

General toxicology studies

The toxicity profile of repeated oral administration of IFB-088, in a twice daily regimen, was fully characterised in one rodent (Sprague Dawley rat) and one non-rodent (Beagle dog) species up to 26 weeks and 39 weeks, respectively. The primary and dose-limiting effects in repeated-dose toxicity studies in rats were crystal nephropathy and associated secondary changes at doses above 3 mg/kg/day. Therefore, the no observed adverse effect

level (NOAEL) in this species was set at 1.5 mg/kg/twice a day [b.i.d.], i.e. 3 mg/kg/day. No signs of crystal nephropathy were however reported in dogs and rabbits (embryofoetal development study) at dose levels of up to 16 mg/kg/day (8 mg/kg/b.i.d.) and 24 mg/kg/day (12 mg/kg b.i.d), respectively. Based on the toxicity data gathered in the 4-week toxicity study in rats, it was hypothesised that the toxicity was more related to the maximal concentration (C_{max}) than to the area under the curve (AUC); therefore, IFB-088 schedule of administration was changed from once daily administration to twice daily administration for all subsequent studies, in order to minimize peak plasma concentrations and keep targeted plasma exposures. The twice daily schedule of administration was used during phase I P188 Study and is intended to be used for all the upcoming clinical trials.

Genotoxicity studies

The genotoxicity of IFB-088 was evaluated in in vitro and in vivo studies. In the in vitro Ames test a weak and equivocal increase in mutant frequency was observed in the presence of metabolic activation on one out of the 5 tested strains. No genotoxic effect was observed in the in vitro study to assess the potential to induce chromosome aberrations in cultured human peripheral blood lymphocytes. The in vivo study was performed on Sprague-Dawley rats combining a micronucleus test in the bone marrow tissue and a Comet assay in the liver. Animals were treated orally once a day for 3 consecutive days, 24 hours apart, at dose levels of 0, 5, 10 and 20 mg/kg/day. Both endpoints showed no evidence of genotoxicity. Based on these results, IFB-088 is anticipated to have no genotoxic hazard to humans.

Reproductive toxicity studies

Reproductive toxicity potential of IFB-088 was assessed in one study for effects on fertility and general reproductive performance in Sprague Dawley rats, and 2 studies for effects on embryofoetal development in Sprague Dawley rats and New Zealand white rabbits.

In the fertility and general reproductive performance study in male and female Sprague Dawley rats (at IFB-088 doses of 1.5, 3 or 6 mg/kg b.i.d., i.e. 3, 6 or 12 mg/kg/day), an impact on reproductive parameters (prolonged oestrus cycle, decreased number of corpora lutea and implantation sites), associated with evidence of parental toxicity comparable to that observed in the repeated-dose toxicology studies (clinical signs, decreased food consumption and body weight and evidence of kidney damage), were reported at the dose of 12 mg/kg/day. No treatment-related effects on parental and reproductive parameters were observed at the doses of 3 and 6 mg/kg/day (NOAEL was thus established at 3 mg/kg/b.i.d).

In the embryofoetal development study in female Sprague Dawley rats (IFB-088 doses of 1.5, 3 or 6 mg/kg b.i.d., i.e. 3, 6 or 12 mg/kg/day), skeletal malformations, associated with evidence of maternal toxicity (clinical signs, decreased appetite and body weight, signs of renal toxicity), were observed in some foetuses at doses of 6 and 12 mg/kg/day. The NOAELs for maternal parameters and for embryofoetal development were considered to be 1.5 mg/kg b.i.d.

In the embryofetal development study in female New Zealand White rabbits, there was no evidence of test item related effects on maternal parameters or on hysterectomy data up the highest administered dose of 24 mg/kg/day. Therefore, 12 mg/kg b.i.d. was concluded to be the NOAELs for maternal parameters and for embryofetal development.

At this stage of IFB-088 development, the pre- and post-natal developmental toxicity study in laboratory animals is not required. Therefore, women who are pregnant or lactating will be excluded from this trial. According to the preclinical tests results, the risk assessment for IFB-088 resulted in possible human teratogenicity/foetotoxicity in early pregnancy. Women with childbearing potential will be asked to take contraceptive measures according to this evaluation.

1.1.2.2 *Clinical studies*

Phase I study P188 (NCT03610334, EudraCT 2018-000443-29) was a randomised, double-blind, placebo-controlled, single and multiple ascending dose study to evaluate the safety, tolerability and pharmacokinetics (PK) of IFB-088 when administered to healthy adult subjects. The study consisted of a single ascending dose (SAD) part, and a multiple ascending dose (MAD) part. The study is complete and a total of 72 subjects received IFB-088 or placebo. In the SAD part, 36 subjects received single daily doses of IFB-088 ranging from 2.5 to 60 mg; in the MAD part, 18 subjects received multiple doses of IFB-088 ranging from 15 mg to 50 mg over 14 days. Drug absorption was rapid and maximum concentrations were observed at approximately 1-2 hours (median time at which the C_{max} is observed [T_{max}]) following single and multiple oral dose administration of IFB-088. Dose proportionality was observed for IFB-088 C_{max} and AUC following single daily doses of 10 to 60 mg and multiple daily doses of 15 to 50 mg for 14 days. IFB-088 half-life was 4.6 to 6.2 hours following single doses and 5.7 to 8.4 hours following multiple dosing. The terminal elimination half-lives were similar between Days 1 and 14, suggesting the stationarity (time-independence) of the IFB-088 PK over 14 days. Following repeated bi-daily administration, the drug accumulation ratio ranged between 1.4 and 1.6. Very low concentrations of IFB-088 were evident in urine; the percentage of the administered dose excreted unchanged in urine was < 1%. The primary metabolic pathway proposed in nonclinical studies included the oxidation of IFB-088 to IFB-139 (phase I metabolite) followed by glucuronidation (phase II metabolite), and was confirmed in the P188 study. Based on the population PK model, renal clearance likely marginally contributes to IFB-088 total clearance, while the formation of metabolite IFB-139 by hydroxylation has a major contribution to IFB-088 elimination.

In terms of safety, IFB-088 was well tolerated in healthy subjects at all doses evaluated, including single daily doses up to 60 mg and repeated doses up to 50 mg daily for 14 days. No deaths, serious AEs (SAEs) or severe drug-related treatment emergent AEs occurred during the study. No AEs led to study discontinuation, discontinuation of dosing, or discontinuation of dose escalation. There was no relationship between IFB-088 or placebo treatment and the frequency or nature of treatment emergent AEs (TEAEs). There was also no relationship between increasing dose and the frequency or type of TEAEs.

1.2 RATIONALE FOR THE STUDY

The pathological hallmark of ALS is the presence of ubiquitin-positive inclusions consisting of misfolded protein aggregates in the affected motor neurons and glial cells of the spinal cord and motor cortex, suggesting that the proteome homeostasis is impaired in ALS [11, 15, 16]. One evidence of the proteome homeostasis impairment is the ER stress and the activation of UPR signalling pathways in human post-mortem tissues from patients with familial or sporadic form of ALS. In addition, biochemical and morphological

findings correlate the development of ALS with the markers of ER stress and the activation of UPR pathway [19, 21].
Targeting the UPR pathway, in particular the phosphatase complex PPP1R15A/PP1c could be beneficial to ALS patients. Encouraging results were obtained with guanabenz in an exploratory phase II study in ALS patients, especially in patients with the bulbar onset of ALS. IFB-088 has demonstrated activity in the so far best studied animal model of ALS, i.e. transgenic rodents overexpressing the gene encoding SOD-1 [27]. Moreover, IFB-088 is devoid of α2 adrenergic activity, which is responsible for the hypotensive effect of guanabenz, despite maintaining its effect on UPR. The clinical development program of IFB-088 to date has shown no particular safety concerns at all doses evaluated, including single daily doses up to 60 mg/day and repeated doses up to 50 mg daily for 14 days. Since a similar efficiency to guanabenz is expected from IFB-088 without hypotensive effects, an exploratory phase II study has been designed to assess the safety and the efficacy of IFB-088 as an add-on to the standard of care riluzole in patients with bulbar onset of ALS. Bulbar-onset ALS population was selected to be the target of this study as the benefit of guanabenz was higher and primarily observed in bulbar-onset ALS patients compared to limb-onset ALS patients [26].
Based on the results of preclinical toxicity studies, IFB-088 will be administered twice a day in order to minimize peak plasma concentrations and keep targeted plasma exposures. The dosage of 50 mg/day has been selected based on the results obtained with guanabenz in the ProMISe phase II clinical trial [26] (see Section 3.3). Patients will be treated during 6 months. This duration is considered acceptable in view of the exploratory aim of the study.

1.3 BENEFIT-RISK ASSESSMENT

To date, therapeutic options for ALS are still very limited and no curative treatment exists. Riluzole, the only approved medication in Europe, improves survival in ALS patients by 2 or 3 months only. Although relatively well tolerated, it can cause hepatotoxicity [28]. Apart from riluzole, treatment of ALS is mainly palliative. Considering the seriousness of the disease and the limited options for treatment, there is still a high unmet medical need for patients suffering from ALS.

In the ProMISe phase II clinical study [26], guanabenz at 64 mg and 32 mg significantly reduced the proportion of patients progressing to higher stages of disease compared to what was expected under the hypothesis of non-futility, as measured by the ALS-MITOS. Moreover, a slowing down in the decline rate of daily living activities was recorded as measured by ALSFRS-R total score. The benefits were more pronounced in patients with bulbar-onset ALS.

Thus, the ProMISe study confirmed that controlling the UPR pathway may be of benefit in slowing the progression of ALS. However, the hypotensive effect of guanabenz linked to its α2 adrenergic receptor activity represented a limit to its practical application, and further clinical development was interrupted.

IFB-088, developed by InFlectis Bioscience is a close analogue of guanabenz devoid of agonist α2 adrenergic activity. Therefore, it is expected to have similar efficacy compared to guanabenz, without hypotensive adverse effects. In the phase I first-in-human study P188 (NCT03610334), IFB-088 was well tolerated at all doses evaluated, including single daily doses up to 60 mg and repeated doses up to 50 mg daily for 14 days.

The safety results from the phase I study are reassuring and no serious adverse reactions are considered expected by the sponsor for the purpose of expedited reporting of suspected unexpected serious adverse reactions (SUSARs). However, human safety data with IFB-088 are limited to the results of this first-in-human study on healthy volunteers. Since IFB-088 has never yet been tested in ALS patients, who may present with potential additional risk factors linked to their condition, a set of measures will be implemented in this study in order to minimise those potential risks, including i) Restriction of eligibility criteria to patients with no other serious illness(es) or medical condition(s) such as cancer, cardiac, liver, renal or haematological diseases, ii) Regular monitoring of safety laboratory and clinical AEs in all patients enrolled in the present clinical trial, since as with any new drug, there is a risk that unidentified or unexpected side effects may occur, iii) Definition, monitoring and specific reporting procedure for AEs of special interest (AESIs), iv) Implementation of a data safety monitoring board (DSMB) for periodic benefit risk assessment.

In preclinical studies, kidneys appeared to be the only affected organs in Sprague Dawley rats at IFB-088 doses above the NOAEL (1.5 mg/kg/b.i.d., i.e. 3 mg/kg/day). Adverse, dose-related crystal nephropathy was observed in both sexes, but at slightly lower doses in males compared to females and was the first cause of moribundity and death. No signs of crystal nephropathy were reported in dogs and rabbits at dose levels of up to 16 mg/kg/day (i.e. 8 mg/kg b.i.d.) and 24 mg/kg/day (i.e. 12 mg/kg b.i.d), respectively. In the phase I P188 study no changes in the renal-associated biochemistry parameters and no IFB-088 related AEs from the renal system organ class were reported. Although the renal findings in the Sprague Dawley rat strain are not completely understood yet, the current data suggest that it likely represents a species-specific toxicity not representative of the risk in humans. Nonetheless renal toxicities will be reported in this study as AESIs and renal function will be closely monitored, along with microscopic examination of urine to detect crystals.

Definitive pre- and post-natal developmental toxicity studies with IFB-088 have not been conducted. For this reason, the use of IFB-088 is contraindicated in women who are pregnant or lactating and precautions will be used in women of childbearing potential.

Based on the preclinical and phase I safety results, the risk associated to the administration of IFB-088 is considered to be acceptable for ALS patients, particularly in view of a possible beneficial effect in slowing down the progression of the disease and in improving the daily living functionality, as demonstrated for guanabenz. An efficacy comparable to that of guanabenz at corresponding doses is expected. This clinical trial is an exploratory study, designed to confirm the results of guanabenz in ALS patients with the aim of assessing the safety and providing proof of efficacy of IFB-088.

Overall, considering the poor prognosis of ALS patients, the urgent need for developing more efficacious ALS therapeutic alternatives, the promising efficacy results from guanabenz phase II study and the reassuring safety results of the IFB-088 phase I study, the potential benefits expected for IFB-088 outweigh the known and potential risks of IFB-088 administration in patients with bulbar-onset ALS during a phase II exploratory study.

2. STUDY OBJECTIVES AND ENDPOINTS

Objectives	Endpoints
Primary	
To assess the safety of the test product, IFB-088 50 mg/day, in patients with bulbar-onset ALS.	• Incidence, grade and relationship to IFB-088 for treatment emergent AEs, SAEs, and AESIs, • AEs leading to dose interruption or premature discontinuation.
Secondary and exploratory	
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.	• Change in ALSFRS-R score from baseline to month 3 and to month 6, • Worsening according to ALS-MITOS score, i.e. progression to a higher stage at 3 and at 6 months compared to the baseline, • Change in King's College score from baseline to month 3 and to month 6. Exploratory endpoints: • Assessment of respiratory function (slow vital capacity [SVC], sniff test [optional], arterial blood gases [ABG]). • Evaluation of nutritional status and body composition by bioelectrical impedance.
To determine the PK parameters of IFB-088.	Measurements of PK parameters: • Plasma concentration of IFB-088 and IFB-139, • AUC of IFB-088 and IFB-139, • Maximum observed plasma concentration (C_{max}), with associated T_{max}, • Terminal or apparent terminal half-life ($t_{1/2}$), • Apparent systemic clearance, apparent volume of distribution.

To investigate the effects of IFB-088 on potential biomarkers.	• Change in TDP-43 plasmatic concentration from baseline to 6 months, compared to placebo, • Change in neurofilament (NfL) light chain and NfL heavy chain plasmatic concentration from baseline to 6 months, compared to placebo, • Change in plasmatic (neuro)inflammatory biomarkers from baseline to 3 months, and from baseline to 6 months, compared to placebo, • Change in plasmatic oxidative stress biomarkers from baseline to 3 months and from baseline to 6 months, compared to placebo.
To investigate the QoL of ALS patients.	Change in ALS assessment questionnaire (ALSAQ-40) from baseline to 6 months.

3. STUDY DESIGN

3.1 STUDY DESIGN OVERVIEW

This is a prospective, international, randomised, double-blind, placebo controlled, and multicentre, parallel group phase II study. Patients with bulbar-onset ALS will be recruited in University hospitals in France and Italy. Patients will be randomised in a 2:1 allocation ratio to receive either IFB-088 50 mg/day + riluzole 100 mg/day or placebo + riluzole 100 mg/day.

Patients will be treated for a period of 6 months (26 weeks). Only patients who have consented and meet the inclusion and exclusion criteria will be eligible for participation in the study.

Patients will undergo 8 visits, as illustrated in Figure 1: screening visit (within 2 weeks before V0), baseline visit (V0), visit 1 (V1, 2 weeks ± 3 days after V0), visit 2 (V2, 4 weeks ± 4 days after V0), visit 3 (V3, 9 weeks ± 4 days after V2), visit 3bis (V3bis, 6.5 weeks ± 4 days after V3), visit 4 (V4, 13 weeks ± 4 days after V3), and a follow-up (FU) visit (V-FU, 4 weeks ± 1 week after V4). In addition to these hospital visits, patients will undergo urine analysis (dipstick) and blood sampling for measurement of creatinine one week after V0, as well as blood sampling for measurement of creatinine and calculation of eGFR at months 2, 4 and 5; these exams can be done in any laboratory, depending on the patient's choice. In case of premature study discontinuation, a final visit (premature discontinuation visit) will take place as soon as possible and no later than 4 weeks after the last investigational medicinal product (IMP) intake. A detailed list of procedures performed at each visit is presented in Sections 3.7 and 4.

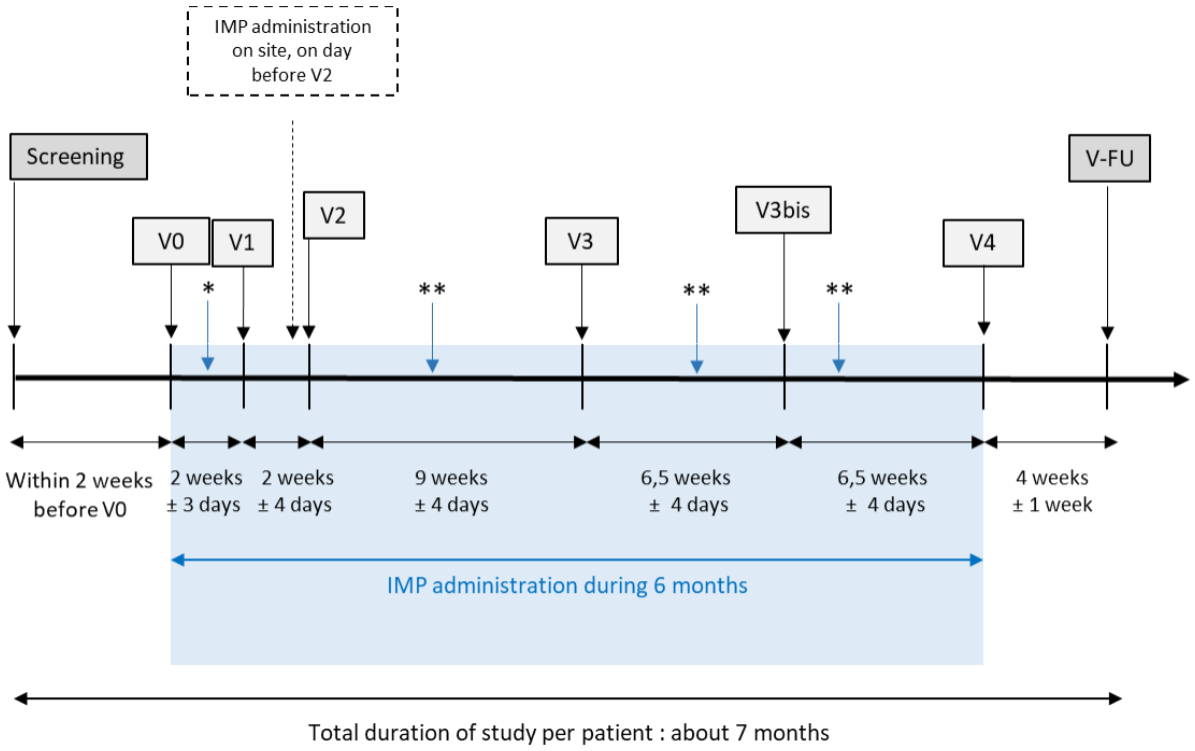
During the screening visit, patient eligibility will be assessed. Patients will be randomised at the baseline (V0) visit after confirmation of eligibility. Safety will be monitored over all the course of the study by a DSMB.

The study will be submitted for approval to the independent ethics committees (IECs) and signed informed consent will be obtained from all patients before any study procedures are conducted.

Compliance with coronavirus disease 2019 (COVID-19)-related rules will be followed and adjustments might be necessary accordingly. In particular, if hospital visits must be limited due to governmental or local hospitals' decisions, or for practical reasons due to insufficient resources in healthcare professionals, the V1 visit (2 weeks after V0), could be replaced by laboratory assays performed in a local lab (for which the patient would receive a written prescription), and a phone call to investigate for any symptom evocative of adverse event.

Unless they already obtained treatment research initiative to cure ALS (TRICALS) certificates, study examiners will undergo online training to ensure uniformity of study procedures across sites, and inter-rater variability will be documented. For investigators who cannot undergo the TRICALS training, certification from the Barrow Neurological Institute is acceptable. Management of frailty will be performed according to standard of care, under the responsibility of each site's investigator.

Figure 1- Study scheme



* Urine analysis and blood sampling will be additionally performed one week after V0.
** Blood sampling for measurement of creatinine and calculation of eGFR will be additionally performed at 2, 4 and 5 months.
IMP: investigational medicinal product, V: visit, FU: follow-up.

3.2 RATIONALE FOR THE STUDY DESIGN

To assess the effects of medicinal products for treatment of ALS, parallel, double-blind, randomised placebo-controlled trials are optimal. Riluzole is the only available drug in Europe for the treatment of ALS and despite its limited effect in reducing the disease progression, it is the current standard of care. Therefore, for ethical reasons, as IFB-088 has not yet demonstrated efficacy in this indication, it will be administered as an add-on treatment in combination with riluzole. Patients will be randomised to receive either the new drug or the placebo; the trial objectives will be to assess the safety of IFB-088 in patients with bulbar-onset of ALS and to show a signal of efficacy.

3.3 DOSE JUSTIFICATION

The 50 mg/day dose of IFB-088 selected for this study is based on the results obtained with guanabenz combined to riluzole 100 mg/day in the ProMISe trial [26]. In the ProMISe study, the intermediate dose (16 mg twice a day; 32 mg/day) and high dose (32 mg twice a day; 64 mg/day) of guanabenz showed efficacy in patients with bulbar-onset ALS as reflected by a statistically significant decrease in the percentage of patients who progressed to a higher ALS-MITOS score after 6 months (primary endpoint). The rate of ALSFRS-R decline was also significantly lower in the guanabenz high and intermediate doses arms compared to placebo and guanabenz low dose arms.

Guanabenz and IFB-088, both targeting the PPP1R15A/PP1c phosphatase complex, have similar pharmacological activity in vitro (results from preclinical studies on cytoprotective effect on ER stress: reports IFB_R_009_V2, and on TDP-43 cytoplasmic stress granule reduction: Inflectis 21-S30710 and 29102021). Furthermore, these compounds, which differ only by one chlorine atom, have similar metabolic pathways and the exposure to IFB-088 in tissues of interest (central and peripheral nervous system) is at least as high as that of guanabenz in rats (report 16-326, see Table 1).

Table 1 – Tissues distribution of IFB-088 and guanabenz in rats

	C_{max} (ng/mL for plasma and ng/g for tissues)	AUC_t (ng/mL*h for plasma and ng/g*h for tissues)	AUC_{inf} (ng/mL*h for plasma and ng/g*h for tissues)	$T_{1/2}$ (h)
IFB-088 (3 mg/kg)				
Plasma	17.4	12.5	22.8	2.98
Brain	31.2	192.6	NC	
Sciatic nerve	751.3	5674.9	5717	
Guanabenz (3 mg/kg)				
Plasma	47.2	19.74	21.0	2.29
Brain	18.8	70.2	NC	
Sciatic nerve	1108	2874.6	NC	

C_{max}: maximum plasma concentration, AUC_t: area under the plasma concentration-time curve until the last observation time, AUC_{inf}: area under the plasma concentration-time curve until infinity, ng: nanogram, mg: milligram, mL: milliliter, g: gram, kg: kilogram, h: hour, $T_{1/2}$: half-life, NC: not calculated.

The molecular weights of free bases of guanabenz and IFB-088 are 231.08 g/mol and 196.64 g/mol respectively, leading to a guanabenz: IFB-088 molecular weight ratio of 1.18. Hence, IFB-088 doses corresponding to the intermediate and high doses of guanabenz in the ProMISe trial are:

Intermediate dose: 2 x 16 mg of guanabenz corresponding to 2 x 13.6 mg of IFB-088

High dose: 2 x 32 mg of guanabenz corresponding to 2 x 27.1 mg of IFB-088

IFB-088 is therefore anticipated to be efficient in the range of 2 x 13.6 mg/day (27.2 mg/day) to 2 x 27.1 mg/day (54.2 mg/day).

This clinical trial is an exploratory study, designed to confirm the results of guanabenz in ALS patients, therefore, to show a signal of efficacy of IFB-088. It will not investigate any dose effect relationship: only one dose of IFB-088 will be tested. In order to maximise the probability of showing efficacy, it has been decided to select the dose corresponding to the higher end of the targeted range. As 50 mg/day (25 mg twice a day), the highest dose tested in the MAD part of the phase I study, was safe and well tolerated, this dose will be used in the phase II study.

3.4 STUDY DURATION

The overall study duration will be approximately 2 years and 6 months. The IMPs will be administered for 6 months (26 weeks) and each patient will undergo 8 visits over a period of about 7 months (32 weeks maximum). The V-FU will take place 4 weeks ± one week after V4.

3.5 STUDY COMPLETION AND FOLLOW-UP

A patient is considered to have completed the study if he/she has completed all study visits and attended the V-FU, which must take place 4 weeks (time window ± one week) after the V4 visit.

The end of the study is defined as the date of the V-FU for the last subject in the study.

All patients who are prematurely withdrawn from the study will undergo a final assessment as soon as possible and no later than 4 weeks after the last study drug intake.

3.6 PREMATURE TERMINATION OF THE STUDY

The sponsor reserves the right to temporarily suspend or prematurely discontinue this study at any time if, in the opinion of the sponsor, the DSMB or the investigator, there is reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party. The IECs and pertinent regulatory authorities will be informed according to the national regulations of the suspension or termination of the study and the reason(s) for this action. Circumstances that may warrant termination include:

- Failure to enrol patients,
- Major protocol violations,
- Inaccurate or incomplete data,
- Unsafe or unethical practices,
- Questionable safety of the study product,
- Plans to modify, suspend, or discontinue the development of the IMP.

3.7 SCHEDULE OF ACTIVITIES

The scheduling of study procedures is outlined in Table 2 and detailed in Section 4.1.

All data obtained from the assessments listed in the schedule of activities and described in detail in Section 4.2 must be supported in the patient's source documentation (e.g. medical charts or patient notes).

Visit dates should be adhered to as closely as possible. If one visit is postponed or brought forward, it should not result in the next visit being postponed or brought forward. The next visit, if at all possible, should adhere to the original time schedule.

Table 2 – Schedule of activities

Visits	**Screening**	**Baseline V0**	**V1**	**V2**	**V3**	**V3bis**	**V4**	**Premature discontinuation**	**V-FU**
Study Weeks	**Within 2 weeks before V0**	**0**	**2 ± 3 days**	**4 ± 4 days**	**13 ± 4 days**	**19.5 ± 4 days**	**26 ± 4 days**	**-**	**30 ± 1 week**
Informed consent (1)	X								
Inclusion/exclusion criteria	X	X							
Demographic characteristics	X								
Medical history	X								
Physical examination and vital signs	X	X	X	X	X	X[x]	X	X	X
Weight / BMI	X	X	X	X	X		X	X	X
ECG	X	X*		X	X	X	X	X	X
Serum/urine chemistry	X	X*†	X	X††	X†††	X[y]	X	X	X
Serum pregnancy test	X								X
Urine pregnancy test[f]		X		X	X		X	X	
Neuropsychological assessment	X								
Concomitant medications and AEs	X	X	X	X	X	X	X	X	X
Crystalluria examination				X				X	X
Randomisation		X							
Study drug delivery		X[a]		X	X				
ALSFRS-R	X	X**			X		X	X	
ALS-MITOS		X			X		X	X	
King's College		X			X		X	X	
C-SSRS evaluation (4)	X				X		X	X	
Bioelectrical Impedance Analysis		X			X		X	X	
ALSAQ-40		X					X	X	
SVC	X				X		X	X	
Sniff test (2)	X				X		X	X	
ABG	X				X		X	X	
Plasma biomarkers		X			X		X	X	

Whole blood biomarkers		X					X	X	
Urine biomarkers		X					X	X	
International Normalized Ratio[ƒƒ]		X				X[z]	X	X	
CSF sampling[ƒƒƒ]		X					X	X	
Blood samples for PK				X					
Administration of study drug on site				X[(3)]					

*Will be performed only if deemed necessary by the investigator.
[ƒ] In addition to tests mentioned in the table, pregnancy tests will be performed at monthly intervals during treatment and until the end of relevant systemic exposure.
** Will be performed only if V0 is performed > 15 days after the screening visit.
[†] A **prescription** will be given to patients at V0 to perform urine analysis and blood sampling in a local laboratory at V0 + one week.
[††] A **prescription** will be given to patients at V2 to perform blood sampling for creatininemia and eGFR in a local laboratory at V2 + 1 month (i.e., V0 + 2 months).
[†††] A **prescription** will be given to patients at V3 to perform blood sampling for creatininemia and eGFR in a local laboratory at V3 + 1 month and V3 + 2 months (i.e., V0 + 4 months and 5 months respectively).
[ƒƒ] Prior to CSF sampling, only in case CSF sampling is planned.
[ƒƒƒ] Only in patients who accepted to undergo lumbar puncture and do not have contraindications (see section 4.2.2.3).
a: first intake of IMP on site at baseline V0 visit (see section 4.1.2).
x: cardiac auscultation, heart rate, systolic and diastolic blood pressure.
y: selected blood markers, detailed in section 4.1.6.
z: independent of CSF sampling.
(1) Patient will have to sign informed consent form for the study before any study procedure.
(2) Sniff test is optional. It will be performed only in sites that have the equipment and resources to perform this test.
(3) The day before V2 the patient will go on site to be administered the IMP at 20:00.
(4) C-SSRS "Lifetime" version at screening visit. C-SSRS "Since Last Visit" version at V3, V4 and premature discontinuation visits.
BMI: body mass index, ECG: electrocardiogram, AEs: adverse events, ALSFRS-R: revised ALS functional rating scale, ALS-MITOS: ALS Milano-Torino staging, ALSAQ-40: ALS assessment questionnaire, SVC: slow vital capacity, ABG: arterial blood gases, PK: pharmacokinetics, CSF: cerebrospinal fluid, V: visit, FU: follow-up, mIU/ml: milli-international units per millilitre.

3.8 IDENTIFICATION OF PATIENTS

All patients who have signed an informed consent form (ICF) and meet the study inclusion/exclusion criteria will enter the study by means of an electronic case report form (eCRF). After eligibility is confirmed, patients will be assigned a number. Patients' numbers will be allocated in ascending order, the order in which patients are enrolled. The investigator or a staff member will enter the patient's number in the eCRF, the confidential patient identification list and the drug dispensing log. The drug dispensing log will be filled in with the treatment number without knowing what the treatment is. The confidential patient identification list must contain sufficient information so that it would be possible to contact the study patient in the event of an emergency or if any further FU is required.

3.9 RANDOMISATION AND BLINDING

All participants will be centrally randomised in a 2:1 allocation ratio to receive either IFB-088 + riluzole or placebo + riluzole, respectively. No stratification factor will be taken into account. The randomisation will be 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 will be provided to each site. Investigators will be supplied with user guides for the IWRS. Once a set of medical characteristics is entered, the IWRS will immediately assign a patient to one of the 2 arms. After the randomisation visit, the investigator will automatically receive 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 will be aware of treatment assignments prior to the database lock at the conclusion of the study.

3.9.1 Unblinding process

Unblinding before the conclusion of the study can be necessary in the cases detailed in this Section.

Blind break for reasons other than those mentioned in this Section should be first discussed with the sponsor's medical monitor.

3.9.1.1 *Unblinding for emergency*

In case of an emergency, the investigator has the sole responsibility for determining if unblinding of a participants' intervention assignment is necessary for the effective treatment of the patient. Participant safety must always be the first consideration in making such a determination. However, the investigator should make all attempts to contact the sponsor's medical monitor before proceeding with the unblinding.
The code breaks will be available 24 hours a day and 7 days a week using the IWRS implemented via the eCRF. The IWRS will require access credentials only available to staff members named on the delegation log for the trial recorded in the trial master file (TMF) of each site. The investigators and delegated members with unblinding responsibilities are responsible for testing their credentials prior to the treatment of patients to ensure unblinding is possible, and for ensuring appropriately trained staff members are available to action code breaks when required for medical emergencies.

If a participant's intervention assignment is unblinded the sponsor must be notified immediately after breaking the blind even if consultation occurred in advance. Details of any emergency unblinding must be recorded in the source documentation and eCRF.
This includes, but may not be limited to:

- Date,
- Subject details,
- Reason for unblinding,
- The results,
- Name and role of the individual requesting the unblinding,
- Name and role of the individual carrying out the unblinding.

Following unblinding and appropriate patient's care if needed, the administration of the IMP should be discontinued and should not be resumed. Subsequent monitoring of patients is however encouraged, i.e. maintenance of visits as per schedule of activities.

3.9.1.2 *Unblinding for DSMB*

If requested, information will be provided to the DSMB unblinded. To that effect, a delegated person not involved with the final data analysis or with the study, shall receive the relevant codes. A record shall be kept in the investigator TMF of the name of the statistician, the date they were supplied the relevant code breaks and the location of the results. The unblinded data and the results supplied to the DSMB shall not be accessible by the investigators or trial staff.

3.9.1.3 *Unblinding at the end of trial*

The statistical analysis plan (SAP) shall be finalised prior to the release of the randomisation codes. Changes to the SAP should be version-controlled. A record shall be kept in the investigator TMF to confirm when the randomisation code was requested and when provided.

3.9.1.4 *Unblinding by the pharmacovigilance and safety institution*

In case of SUSARs associated with an IMP, the blind needs to be broken by the pharmacovigilance and safety institution (Stragen) for the purpose of SUSARs processing and reporting. Unblinding IWRS credentials will be provided to Stragen project manager and his/her backup for this purpose.

4. STUDY VISITS AND PROCEDURES/ASSESSMENTS

Study procedures and their timing are summarised in the schedule of activities (Section 3.7).

COVID-19 special measures
Specific measures will be put in place according to local regulations as appropriate.

4.1 STUDY VISITS

4.1.1 Screening visit

Prior to any study activities, the patient will be asked to read and sign an ICF that has been approved by an IEC and which complies with regulatory requirements. Patients will be given adequate time to consider any information concerning the study given to them by the investigator. As part of the informed consent procedure, patients will be given the opportunity to ask the investigator any question regarding potential risks and benefits of a participation in the study.

Investigators must keep a record (screening log) of patients who were screened. The screening log must be dated and signed by the investigator and filled into the investigator site file.

The following assessments and investigations will be conducted during this visit:

- Explanations about the study and signature of ICF. The patient will be able to consent separately to i) the participation in the study, ii) the storage of biological samples in a biobank and iii) the collection of cerebrospinal fluid (CSF),
- Collection of the patient demographic characteristics (date of birth, age, sex, ethnicity),
- Physical examination and vital signs assessment, according to Sections 4.2.1.2 and 4.2.1.5,
- Height and weight measurement, and calculation of BMI,
- 12-lead electrocardiogram (ECG) (Section 4.2.1.6),
- Blood and urine samples collection for haematology, blood chemistry, and urinary analysis according to Section 4.2.1.8,
- Evaluation of patient eligibility to the study (according to inclusion/exclusion criteria, see Section 5.1 and 5.2) and documentation in patient's file:
    - Recording of ALS history and diagnosis:
        - ALS diagnosis according to the revised El Escorial criteria [29],
        - Date of first ALS symptom/sign,
        - ALS disease duration from diagnosis to the screening visit,
    - SVC assessment,
    - ALSFRS-R score measurement and calculation of the ALSFRS-R score progression rate (point/month) from symptom onset to screening visit,
    - Recording of medication history including but not limited to:
        - Riluzole treatment (100 mg/day) and treatment start date,
        - Concomitant medications and/or non-drug therapies, including the reason for administration,
        - Knowledge of hypersensitivity to IMPs ingredients,
    - Documentation of smoking habits,
    - Documentation of the relevant past medical history, any planned surgery and current medical conditions not related to the diagnosis of ALS,
    - Blood pregnancy test in females of childbearing potential,
    - Check of contraception methods used by patient (and his/her partner when applicable),
    - Neuropsychological assessment (Section 4.2.1.3),
    - C-SSRS evaluation,
    - Calculation of patient's weight variation between symptoms onset and screening visit,

  - Check of patient participation in a clinical trial prior to screening and eventual date of trial termination,
  - Check that the patient understands and is able to follow the protocol procedures and is willing to adhere to the study visit schedule,
- Sniff test[5],
- ABG,
- Appointment for the next visit,
- eCRF recording of data collected.

Screen Failure

A patient who has given his/her consent is considered as a screen failure if:

- He/she does not meet all the inclusion criteria or meets a non-inclusion criterion,
- He/she was withdrawn from the study prior to randomisation and study drug intake.

For patients considered as screen failure the following eCRF pages must be completed:

- Screening visit applicable pages (all performed assessments),
- Baseline visit pages if applicable (all performed assessments and randomisation/inclusion decision).

4.1.2 Baseline – V0 visit

The baseline visit (V0) will occur 2 days to 2 weeks after the screening visit.

The following assessments will be conducted before the randomisation:

- Confirmation of patient eligibility to the study (as in screening visit, see Section 4.1.1),
- Delivery of patient card,
- Physical examination and vital signs assessment, according to Sections 4.2.1.2 and 4.2.1.5,
- Weight and calculation of BMI,
- Query for changes in concomitant medications,
- Ensure that the patient is still willing to follow the protocol procedures and the visits schedule,
- Urine pregnancy test in females of childbearing potential (WOCBP)[6]. In addition to tests performed at hospital visits, pregnancy tests will be performed at monthly intervals during

[5] The sniff test is optional. It will be performed only in sites that have the equipment and resources to perform this test.
[6] High sensitivity urine pregnancy test must be used (sensitivity 25 mIU/ml or more).

treatment and until the end of relevant systemic exposure. If a positive result is detected with urine pregnancy test, a serum pregnancy test will be performed to confirm the results.

- 12-lead ECG (Section 4.2.1.6), blood and urine samples collection for haematology, blood chemistry, and urinary analysis according to Section 4.2.1.8, will not be repeated unless any intercurrent event might have modified eligibility criteria,

Once patient eligibility is confirmed:

- Recording of AEs (if any),
- Randomisation and treatment allocation,
- Dispensation of study product to the patient,
- Patients will be given a prescription for urine analysis (dipstick for quantification of blood, leukocytes, pH, glucose, and proteinuria) and blood sampling (creatininemia) to be performed at V0 + one week in a local laboratory. Analyses will be performed according to Section 4.2.1.8. Results of these analyses will be sent to the site in real time.
- Instructions to the patient:
  - Study product must be taken at approximately the same time each morning and each evening, about 12 hours apart, with a glass of water and 30 minutes before the meal, in fasting condition. For patients who have swallowing difficulties, tablets can be taken with a spoon filled with jellified water. The patient will take the first tablet at the V0 visit under a healthcare professional supervision. If this first intake is in the morning, the patient will take his next dose in the evening at home. If the first intake is in the afternoon, the patient will take the next dose at home the next morning. From the second intake, intervals for dosing should ideally be about 12 hours (± 1 hour).
  - Study drug must be kept in the original container, at room temperature,
  - Daily dose of riluzole must be taken at approximately the same time each morning and each evening, together with the study product,
  - Both used and unused study drug bottles must be returned at the next visit for treatment accountability,
  - The patient will be encouraged to stay well hydrated throughout the duration of the study.
- ALSFRS-R measurement if V0 is performed > 15 days after screening visit,
- ALS-MITOS measurement,
- King's College score measurement,
- Bioelectrical Impedance Analysis
- Patient's completion of ALSAQ-40,
- International Normalized Ratio (INR) if CSF sampling is planned,
- Collection of blood, urine and CSF samples for biomarkers analysis according to Section 4.2.2.3,

- Appointment for the next visit,
- eCRF recording of data collected.

4.1.3 V1 visit

The V1 visit will occur 2 weeks after V0 (± 3 days). The following assessments will be conducted at V1:

- Physical examination and vital signs assessment, according to Sections 4.2.1.2 and 4.2.1.5,
- Weight and calculation of BMI,
- Blood and urine sample collection for haematology, blood chemistry, urinary analysis according to Section 4.2.1.8,
- Query for changes in concomitant medications,
- Recording of AEs (if any),
- Appointment for the next visit,
- eCRF recording of data collected.

4.1.4 V2 visit

The V2 visit will occur 4 weeks after V0 (± 4 days).

The day before V2 the patient will go on site to be administered the IMP at 20:00. The day of V2 the patient must be present on site around 7:30 as a blood sample for PK will be collected in the morning of V2 before the IMP administration that will take place at 08:00. Patients can, but are not forced to, spend the night at the site between these 2 drug administrations, upon agreement between both parties. Patients who leave far away from the investigational site and cannot be hospitalized during the night before V2 for logistical reasons are allowed to take the evening study treatment at home on the day before V2. In this case, the time of dosing must be set by the study team, depending on the time of planed first PK blood sampling at V2, allowing a 12-hour interval between last drug intake and blood sapmpling. If the patient cannot take the drug at the exact time requested by the hospital, he must note the actual time of drug intake. Patients who do not spend the night in the hospital must be provided with a sterile container to collect morning urine. It will be given to the patient at V1 for those who will not come to the hospital for drug administration on the day before V2.

The following assessments will be conducted at V2:

- Physical examination and vital signs assessment, according to Sections 4.2.1.2 and 4.2.1.5,
- Weight and calculation of BMI,
- 12-lead ECG (Section 4.2.1.6),
- Blood and urine sample collection for haematology, blood chemistry, urinary analysis according to Section 4.2.1.8,
- Crystalluria analyses according to Section 4.2.1.9,
- PK blood samples collection according to Section 4.2.2.2,

- Query for changes in concomitant medications,
- Recording of AEs (if any),
- Pregnancy test in WOCBP. In addition to tests performed at hospital visits, pregnancy tests will be performed at monthly intervals during treatment and until the end of relevant systemic exposure. If a positive result is detected with urine pregnancy test, a serum pregnancy test will be performed to confirm the results.
- Dispensation of study product to the patient,
- Appointment for the next visit,
- Patients will be given a prescription for blood sampling (creatininemia and eGFR) to be performed at V2 + 1 month in a local laboratory. Results of these analyses will be sent to the site in real time,
- eCRF recording of data collected.

4.1.5 V3 visit

The V3 visit will occur 9 weeks after the V2 (± 4 days).

The following assessments will be conducted:

- Physical examination and vital signs assessment, according to Sections 4.2.1.2 and 4.2.1.5,
- Weight and calculation of BMI,
- 12-lead ECG (Section 4.2.1.6),
- Blood and urine sample collection for haematology, blood chemistry, and urinary analysis according to Section 4.2.1.8,
- Query for changes in concomitant medications,
- Recording of AEs (if any),
- ALSFRS-R measurement,
- ALS-MITOS measurement,
- King's College score measurement,
- Bioelectrical Impedance Analysis
- C-SSRS evaluation,
- SVC assessment,
- Sniff test[7],
- ABG,
- Collection of blood samples for biomarkers analysis according to Section 4.2.2.3,

[7] The sniff test is optional. It will be performed only in sites that have the equipment and resources to perform this test.

- Pregnancy test in WOCBP. In addition to tests performed at hospital visits, pregnancy tests will be performed at monthly intervals during treatment and until the end of relevant systemic exposure. If a positive result is detected with urine pregnancy test, a serum pregnancy test will be performed to confirm the results.
- Dispensation of study product to the patient,
- Appointment for the next visit,
- Patients will be given a prescription for blood sampling (creatininemia and eGFR) to be performed at V3 + 1 month and V3 + 2 months in a local laboratory. Results of these analyses will be sent to the site in real time,
- eCRF recording of data collected.

4.1.6 V3bis visit

The V3bis visit will occur 6.5 weeks after the V3 visit (± 4 days).

The following assessments will be conducted:

- Physical examination: cardiac auscultation, heart rate, systolic and diastolic blood pressure),
- 12-lead ECG,
- Blood sample collection for measurement of electrolytes (Na, K, Ca, Cl, Mg, Ph, HCO3), BNP, troponin, D-Dimers, INR, and prothrombin time.

For patients who have already completed the V3 visit and are very close (≤2 weeks) to the V4 visit when the decision is made to add the V3bis visit, a phone call should be made to investigate the patient's condition. If any sign or symptom is mentioned, the patient should come to the hospital as soon as possible for further investigations, otherwise the V3bis visit will not be performed and the patient will undergo the V4 visit as planned.

For patients who have completed the V3 visit and have the V4 visit expected in more than 2 weeks but less than 6.5 weeks when the decision is made to add the V3bis, the V3bis visit should be made as early as possible, depending on the site's and patient's possibilities. If an onsite visit is impossible, a phone call must be made to investigate the patient's condition.

4.1.7 V4 visit

The V4 visit will occur 6.5 weeks after the V3bis (± 4 days).

The following assessments will be conducted:

- Physical examination and vital signs assessment, according to Sections 4.2.1.2 and 4.2.1.5,
- Weight and calculation of BMI,
- 12-lead ECG (Section 4.2.1.6),
- Blood and urine sample collection for haematology, blood chemistry, and urinary analysis according to Section 4.2.1.8,
- Query for changes in concomitant medications,

- Recording of AEs (if any),
- ALSFRS-R measurement,
- ALS-MITOS measurement,
- King's College score measurement,
- Bioelectrical Impedance Analysis,
- C-SSRS evaluation,
- Patient's completion of ALSAQ-40,
- SVC assessment,
- Sniff test[8],
- ABG,
- International Normalized Ratio (INR) if CSF sampling is planned,
- Collection of blood, urine and CSF samples for biomarkers analysis according to Section 4.2.2.3,
- Pregnancy test in WOCBP. In addition to tests performed at hospital visits, pregnancy tests will be performed at monthly intervals during treatment and until the end of relevant systemic exposure. If a positive result is detected with urine pregnancy test, a serum pregnancy test will be performed to confirm the results.
- Appointment for the next visit,
- eCRF recording of data collected.

4.1.8 FU visit – V-FU

The V-FU will occur 4 weeks (± 1 week) after V4.

The following assessments will be conducted:

- Physical examination and vital signs assessment, according to Sections 4.2.1.2 and 4.2.1.5,
- Weight and calculation of BMI,
- 12-lead ECG (Section 4.2.1.6),
- Crystalluria analyses according to Section 4.2.1.9,
- Blood and urine samples collection for haematology, blood chemistry, and urinary analysis according to Section 4.2.1.8,
- Serum pregnancy test,

[8] The sniff test is optional. It will be performed only in sites that have the equipment and resources to perform this test.

- Query for changes in concomitant medications,
- Recording of AEs (if any),
- eCRF recording of data collected.

4.1.9 Premature discontinuation visit

Patients who prematurely withdraw from the study for any reason (Section 5.3), should be scheduled for a final visit as soon as possible and no later than 4 weeks after the last IMP intake.

The following assessments will be conducted:

- Physical examination and vital signs assessment, according to Sections 4.2.1.2 and 4.2.1.5,
- Weight and calculation of BMI,
- 12-lead ECG (Section 4.2.1.6),
- Blood and urine sample collection for haematology, blood chemistry, and urinary analysis according to Section 4.2.1.8,
- Crystalluria analyses according to Section 4.2.1.9,
- Query for changes in concomitant medications,
- Recording of AEs (if any),
- ALSFRS-R measurement,
- ALS-MITOS measurement,
- King's College score measurement,
- Bioelectrical Impedance Analysis,
- C-SSRS evaluation,
- Patient's completion of ALSAQ-40,
- SVC assessment,
- Urine pregnancy test in WOCBP,
- Sniff test[9],
- ABG,
- International Normalized Ratio (INR) if CSF sampling is planned,
- Collection of blood, urine and CSF samples for biomarkers analysis according to Section 4.2.2.3,
- eCRF recording of data collected.

[9] The sniff test is optional. It will be performed only in sites that have the equipment and resources to perform this test.

4.2 SPECIFIC ASSESSMENTS

4.2.1 Measurements of the primary variable

4.2.1.1 *Safety assessment*

The safety will be assessed on the occurrence of AEs (including SAEs, TEAEs, AESIs (see Section 7).

Identified AEs will be characterised in relation to type, seriousness, incidence, severity, and relationship to study treatment. Safety will be monitored over the course of the study by a DSMB. The percentage of patients requiring dosing interruption or definitive treatment discontinuation due to toxicity will be recorded.

Abnormal laboratory values, vital signs, ECG and other objective measurements

Laboratory values (Section 4.2.1.8), crystalluria (Section 4.2.1.9), physical examination (Section 4.2.1.2), vital signs (Section 4.2.1.5), ECGs (Section 4.2.1.6) and other objective measurements will be collected in related pages of eCRF. In addition, any of the abovementioned should be reported as AE if assessed as clinically significant by the investigator and as SAE or AESI if it meets the corresponding criteria (see Section 7.3).

In the relevant page of the eCRF, a clinical diagnosis should be recorded rather than the abnormal value itself, if this is available.

4.2.1.2 *Physical examination*

A physical examination will be performed at each visit. It will include the examination of general appearance, skin, neck (including thyroid), eyes, ears, nose, throat, lungs, heart, abdomen, lymph nodes, and neurological examination. Height in centimetres (cm) will be measured at screening. Body weight (to the nearest 0.1 kilogram [kg]) will be measured in patients' underwear.

If indicated based on medical history and/or symptoms, additional exams will be performed. Information about all physical examinations must be present in the source documentation at the study site.

4.2.1.3 *Neuropsychological assessment*

In order to assess the presence of active psychiatric illnesses, including dementia, patients will undergo a neuropsychological evaluation during the screening visit. The investigator will conduct the visit and perform a basic neuropsychiatric evaluation. No formal tests will be required.

4.2.1.4 *C-SSRS evaluation*

Considering the stage of development of IFB-088 (phase 2 with limited safety data in healthy volunteers), suicidal risk will be evaluated at screening and throughout the study using the Columbia-Suicide Severity Rating Scale.

4.2.1.5 *Vital signs*

This will include temperature, systolic and diastolic arterial pressure and heart rate. Information about the vital signs must be present in the source documentation at the study site. Blood pressure and heart rate will be measured after the patient has rested for 5 minutes, in both supine position and standing.

4.2.1.6 *Electrocardiogram*

A standard 12-lead ECG will be performed in triplicates in a resting position. Prior to the recording the patient should be at rest for at least 5 minutes. The ECG printout will be reviewed by the investigator.

4.2.1.7 *Laboratory assessments*

Accredited local and central laboratories will be used for analysis of specimens collected at each visit, according to Table 3. Analyses will include:

- Complete laboratory evaluations (hematology, biochemistry, electrolytes, liver function, renal function, urinalysis) for safety assessment,
- Biomarker assessment (Section 4.2.2.3),
- Pregnancy test,
- Crystalluria (Section 4.2.1.9),
- PK.

Laboratory values that exceed the thresholds of normal laboratory values must be commented by the investigator on source documents and respective patient's laboratory eCRF page.

For each visit, the drawn amount of blood, urine and CSF required is detailed in Table 3.

Table 3 – Schedule and location of the blood, urinary and cerebrospinal fluid tests

Sample	Assessment	Screening	Baseline (V0)	V1	V2	V3	V3bis	V4	V-FU	Premature discontinuation	Bio bank	Quantity per visit	Laboratory
Whole blood (EDTA/lithium heparin tube)	Safety (haematology)	X	X*	X	X	X		X	X	X		20 ml	Local
Serum (serum-separating tube)	Safety (electrolytes, biochemistry, liver function, renal function)	X	X*[a]	X	X[aa]	X[aaa]	X	X	X	X		20 ml	Local
Plasma (EDTA tube)	Biomarkers		X			X		X		X	X	20 ml	Central
Whole blood (PAXgene blood RNA tube)	Biomarkers		X					X		X	X	5 ml	Central
Plasma (lithium heparin tube)	PK				X							10 ml	Central
Plasma (lithium heparin tube)	Efficacy (arterial blood gases)	X				X		X		X		2 ml	Local
Urine	Safety	X	X*†	X	X	X		X	X	X		50 ml	Local
Urine	Crystalluria				X				X	X		50 ml	Central
Urine	Biomarkers		X					X		X	X	100 ml	Central
Blood/urine	Pregnancy test	X**	X***		X***	X***		X***	X**	X***		2 ml/1 ml	Local
CSF	Biomarkers		X					X		X	X	1.8 ml	Central

CSF: cerebrospinal fluid, EDTA: ethylenediaminetetraacetic acid, V: visit, FU: follow-up.
* If needed, based on investigator's judgement.
† Additional urine analysis (dipstick) at V0 + one week.
[a] Additional blood sampling for creatinine measurement at V0 + one week.
[aa] Additional blood sampling for creatinine measurement and calculation of eGFR at V2 + 1 month.
[aaa] Additional blood sampling for creatinine measurement and calculation of eGFR at V3 + 1 month and V3 + 2 months.
** Blood pregnancy test at screening and FU visit (V-FU).
*** Urine pregnancy test on a monthly basis until end of exposure to study treatment.

4.2.1.8 *Blood and urine safety analysis*

Blood and urine safety analyses will be performed at each visit after screening, except at baseline (V0) unless required by occurrence of intercurrent event since screening. Blood will be drawn via a hypodermic needle inserted into a vein in the arm and collected in the appropriate tube(s), according to Table 3. The patient must be fasting overnight from 22:00. Urine specimens will be obtained by providing participants with a collection cup and instructions for the collection.

Further details on blood and urine sample processing, storage and shipping procedures will be provided in the laboratory procedures manual (LPM) provided by the sponsor.

The following parameters will be assessed for safety assessment:

Haematology

Haematology will include count of red blood cells, haemoglobin, haematocrit, total white blood cells count, platelet count, and a differential count including neutrophils, lymphocytes, monocytes, eosinophils, basophils.

Biochemistry

Biochemistry will include glucose, total cholesterol, high-density lipoprotein (HDL) cholesterol and calculated low-density lipoprotein (LDL) cholesterol, triglycerides, C-reactive protein, troponin T, creatine kinase, and uric acid.

Electrolytes

Electrolytes will include sodium, potassium, chloride, bicarbonate, calcium, magnesium, phosphorus.

Renal function

Renal function panel will include creatinine, blood urea nitrogen, estimated glomerular filtration rate, total protein.

Liver function

Liver function panel will include total protein, albumin, total bilirubin, direct bilirubin, alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), lactate dehydrogenase (LDH).

According to section 4.4 of riluzole SmPC, ALT should be measured every month during the first 3 months of treatment, every 3 months during the remainder of the first year, and periodically thereafter. This monitoring should be performed more frequently in patients with ALT increase under treatment. Depending on the date when riluzole has been initiated and the kinetics of ALT, additional measurements of ALT might therefore be necessary.

Urinary analysis

It includes blood, leukocytes, pH, glucose, and proteinuria assessed by means of commercial urine dipsticks. In case of proteinuria ≥ 30 mg/dL on the dipstick, 24-hour protein is also performed.

Urinary analysis will also include measurement of beta 2 microglobuline or albumin-to-creatinine ratio in mg/g or mg/mmol, microalbumine, creatinine, and proteins, and cytobacteriological examination of urine (CBEU). All these analyses will be performed in a spot urine sample.

4.2.1.9 *Crystalluria*

Microscopic analysis of urine will be performed to investigate drug-related crystalluria. Sample of first-morning micturition will be collected at the V2 visit. The sample will be collected by the hospital staff if the patient has been hospitalised the day before (see Section 4.1.4), or by the patients themselves if they have spent the night outside the hospital. In this case, patients will receive a sterile dedicated container either on the day before the V2 visit when they go on site for the IMP administration for PK assessment, or at the end of the V1 visit for those who will take the evening treatment at home before V2. A sample of 50 ml will be frozen at -20°C and sent to a central laboratory that will perform microscopic analysis within 7 days after urine collection. Urine freezing should be done within 3h of collection.

Crystalluria will also be investigated at the V-FU visit or at the premature discontinuation visit if any. Urine will be collected when the patient arrives in the hospital for his visit.

4.2.1.10 *Pregnancy testing*

All female patients of childbearing potential will have to perform a blood beta-human chorionic gonadotropin (beta-HCG) testing at screening. For WOCBP, high sensitivity urine pregnancy test will be performed at baseline and repeated on a monthly basis during treatment and until the end of relevant systemic exposure, as well as in case of premature discontinuation and at any time if pregnancy is suspected (sensitivity 25 mIU/ml or more).

In case of pregnancy, collection of information is needed as described in Section 7.8.

4.2.2 Measurements of secondary and exploratory variables

4.2.2.1 *Efficacy assessment*

Efficacy assessment will be performed at baseline (V0), 3 months (V3) and 6 months (V4). It will consist of:

- ALSFRS-R score,
- ALS-MITOS score,
- King's College score,
- Assessment of respiratory function: SVC and ABG. Sniff test will be optional, performed in sites that have the equipment and resources to perform it.
- Bioelectrical Impedance analysis

The ALSFRS-R, which compared to ALSFRS includes also respiratory function, is the most widely used and validated rating instrument for monitoring the progression of disability in patients with ALS. It consists of a disease-specific questionnaire with 12 questions aimed at assessing functionality [30-32]. Functional decline averages about one point per month in untreated patients [33].

The ALS-MITOS score is a more recent staging system composed by 4 key domains included in the ALSFRS-R (walking/self-care, swallowing, communicating and breathing). It is a validated tool for predicting long-term survival of ALS patients [34, 35].

The King's College score is based on the number of neurological regions affected and requirement for gastrostomy or non-invasive ventilation [36, 37]. While ALS-MITOS staging has greater

resolution for late disease stage corresponding to functional impairment, King's College score is able to differentiate early to mid-disease better, making the two systems complementary [38].

The monitoring of respiratory function is achieved by the measurement of SVC, assessed by spirometry: the patient is asked to take a breath as deep as possible, make a tight seal around the device mouthpiece, and then exhale. In SVC assessment, exhalation is performed slowly. SVC is simple, easy to perform and convenient for serial measurements over a long time to evaluate disease progression [39]. Recently, SVC has been chosen more and more frequently over forced vital capacity (FVC) test in ALS clinical trials as it is easier to perform for the patient while providing equivalent information [40]. However, it can be difficult for bulbar-onset ALS patients who experience facial weakness. The sniff test is a simple, portable, inexpensive test that expects patients to inhale through their noses with one nostril occluded and a probe connected to a pressure transducer. This test is easy to perform also for patients with facial weaknesses [39].

ABG test measures the pH and the levels of oxygen and carbon dioxide in the blood from an artery. ABG is a sensitive and inexpensive tool for monitoring respiratory function in ALS patients, and turned out to correlate with FVC. ABG does not require patient collaboration and is not influenced by bulbar involvement [41]. Arterial blood samples will be drawn through radial arterial puncture into heparin - lithium Vacutainer polyethylene tubes and immediately analysed using blood gas analyser, routinely calibrated, as appropriate.

Bioelectrical Impedance analysis will be performed using the non-invasive device BIODY XPERT^{ZM}, commercialized by Aminogram. The device is maintained by the patient on his ankle, in seated position, during approximately one minute, during which various parameters are recorded and proceeded by a software. All sites will be trained by Aminogram prior to utilization of the device. Once trained, members of the study team will be allowed to train additional members.

4.2.2.2 *Pharmacokinetic assessment*

Blood sampling for PK assessment will be performed at the V2 visit. The day before V2 the patient will go on site to be administered the IMP at 20:00. If the patient cannot come to the hospital on the day before V2, he will take study treatment at home at the time requested by the site (see Section 4.1.4). The day of V2 the patient will be administered again the IMP at 08:00. The blood samples for PK will be collected in the morning of V2. Patients can, but are not forced to, spend the night at the site between these 2 drug administrations, upon agreement between both parties.

Venous blood samples (10 mL) for the determination of plasma concentrations of IFB-088 and its hydroxylated metabolite IFB-139 will be drawn by direct venipuncture or via an intravenous catheter into heparin - lithium Vacutainer polyethylene tubes (5x2mL). Samples will be collected at the following time points in the morning: pre-IMP dose, 1, 2, 4 and 6 hours post dose. The actual date and time of each blood sample collection will be recorded.

IFB-088 and IFB-139 concentrations will be measured within one week, using a validated high-performance liquid chromatography with tandem mass spectrometry (HPLC/MS-MS) method. After collection, plasma samples will be stored at -80°C pending analyses. Details of PK blood sample processing, storage and shipping procedures will be provided in the LPM provided by the sponsor.

4.2.2.3 *Biomarkers assessment*

Potential biomarkers known to be associated with the evolution of the disease and potentially modulated by IFB-088 treatment will be assessed before and during study treatment. Blood, urine

and CSF samples will be collected for this assessment (see Table 3). Further details on blood, urine and CSF sample processing, storage and shipping procedures will be provided in the LPM provided by the sponsor. Frozen samples will be kept in a biobank for further analyses (see Section 4.2.2.5).

Blood biomarkers

Blood will be collected in 2 different types of tubes to measure potential biomarkers in both plasma and whole blood (messenger RNA [mRNA], microRNA [miRNA]). Blood should be first drawn in Ethylenediaminetetraacetic acid (EDTA) tubes and then in PAXgene Blood RNA Tube, as described below.

Plasma

Blood for plasma biomarkers assessment will be collected at V0, V3 and V4 according to schedule of activities (Sections 3.7 and 4.1) in 2 tubes of 10 ml containing EDTA properly labelled with patient identification. After processing, plasma will be aliquoted in and stored at -80°C pending analyses. Samples will be stored at a facility designated by the sponsor. The following parameters will be analysed at a laboratory approved by the sponsor:

- NfL light chain: at baseline and 6 months,
- NfL heavy chain: at baseline and 6 months,
- TDP-43: at baseline and 6 months,
- Inflammation biomarkers (interleukin [IL]-6, tumour necrosis factor-α [TNFα], interferon γ [IFNγ], IL-1β, IL-8, IL-10, monocyte chemoattractant protein-1 [MCP-1], nerve growth factor [NGF], brain-derived neurotrophic factor [BDNF], vascular endothelial growth factor [VEGF]), transforming growth factor beta [TGFβ]: at baseline, 3 and 6 months,
- Oxidative stress biomarkers (reactive oxygen species): at baseline, 3 and 6 months.

Unused plasma aliquots will be kept in the biobank (Section 4.2.2.5) for potential further analyses.

Whole blood

For whole blood biomarkers assessment, blood will be drawn at V0 and V4 in PAXgene Blood RNA tubes. Tubes must be at room temperature prior to use and properly labelled with patient identification. The tube should be gently inverted 8 to 10 times and store at -80°C pending analyses. Samples will be stored at a facility designated by the sponsor and will be kept in a biobank (Section 4.2.2.5) for potential further analyses.

Urine biomarkers

Urine will be collected at V0 and V4.

Urine samples of 100 ml will be collected and stored in accordance with the Urine & Kidney Proteome Projects standards [42]. Sampling for urine biomarkers and routine safety/crystalluria must be collected at different time points. After processing the urine will be aliquoted, properly labelled with patient identification and stored at -80°C pending analyses. Urine samples will be stored at a facility designated by the sponsor and will be kept in a biobank (Section 4.2.2.5) for potential further analyses.

Cerebrospinal fluid biomarkers

CSF will be collected at V0 and V4 in patients willing to undergo lumbar puncture unless they present any contraindication to CSF sampling. These contraindications include the presence of risk for increased or uncontrolled bleeding and/or risk of bleeding that if not managed optimally could place a participant at an increased risk for intraoperative or postoperative bleeding. These could include, but are not limited to, anatomical factors at or near the LP site (e.g., vascular abnormalities, neoplasms, or other abnormalities) and underlying disorders of the coagulation cascade, platelet function, or platelet count (e.g., hemophilia, Von Willebrand's disease, liver disease). They also include anticipated need, in the opinion of the Investigator, for administration of any antiplatelet or anticoagulant medication (e.g., clopidogrel) that cannot be safely continued or held for an LP procedure, if necessary, according to local or institutional guidelines and/or Investigator determination.
INR should be measured prior to CSF sampling.
Patients with contraindications to lumbar puncture can be included in the study but will not undergo CSF sampling.
Lumbar puncture will be performed by a clinician according to the site's usual practice. The CSF will be collected and then aliquoted and properly labelled with patient identification. The tubes will be snap frozen in liquid nitrogen and immediately stored at -80°C, pending analyses. CSF samples will be stored at a facility designated by the sponsor and will be kept in a biobank (Section 4.2.2.5) for potential further analyses.

4.2.2.4 *Quality of life assessment*

QoL will be assessed using the validated ALSAQ-40 questionnaire, specifically used to measure the subjective well-being of patients with ALS [43]. ALSAQ-40 has psychometric robustness, and it provides an interpretable picture of the impact of the condition on the subjective functioning and well-being of patients on areas that are of concern to them. It provides scores for 5 areas of health state: physical mobility (10 items), activities of daily living and independence (10 items), eating and drinking (3 items), communication (10 items), and emotional reactions (10 items). The questionnaire must be completed by the patient. Patients need to tick the box which best describes his/her own experience or feelings. Patients should try to answer every question even though some may seem rather similar to others, or may not seem relevant to them. QoL will be assessed as the change in absolute value and percentage of ALSAQ- 40 between baseline and 6 months.

4.2.2.5 *Biobank*

Blood, urine and CSF samples will be stored at -80°C at a facility designated by the sponsor to measure biomarker candidates as needed. These samples will be kept for 5 years after final database lock for further analysis and then destroyed if unused.

5. STUDY POPULATION

The study population will consist of patients with bulbar-onset ALS. A total of 50 patients from France and Italy will be enrolled in the study, primarily through University hospitals setting. A voluntary signed ICF will be obtained from the patients prior to participation in the study.

The recruitment of patients is competitive and will be terminated once the required sample size has been reached.

Patients who meet all the following inclusion criteria and none of the exclusion criteria will be eligible to participate in the study.

5.1 INCLUSION CRITERIA

Patients must satisfy all the following inclusion criteria to be enrolled in the study:

1. Diagnosis of probable or definite ALS according to the revised El Escorial criteria [29], with bulbar onset of disease, familial or sporadic form,
2. Onset of symptoms ≤ 18 months prior to screening, as reported by the patient,
3. Adult males or females, aged at least 18 years old,
4. SVC > 60% of predicted value for age and sex,
5. ALSFRS-R score ≥ 36,
6. Treatment with riluzole 100 mg/day, at stable dose since at least one month and well tolerated,
7. Male or female patient of childbearing potential[10] who agrees to use highly effective mechanical contraception methods (sexual abstinence, intrauterine device, bilateral tubal occlusion, vasectomised partner) throughout the study, and for 3 months after the end of the treatment,
8. Patient who read, understood and signed the ICF,
9. Patient who is willing to adhere to the study visit schedule and is capable to understand and comply with protocol requirements.

5.2 EXCLUSION CRITERIA

A patient will not be eligible for inclusion in this study if at least one of the following criteria applies:

1. Known other significant neurological disease(s),
2. Serious illness(es) or medical condition(s) (e.g. unstable cardiac disease, cancer, hematologic disease, hepatitis or liver failure, renal failure) that is not stabilised or that could require hospitalisation and may jeopardise the participation in the study,
3. Abnormal renal function at screening defined as estimated glomerular filtration rate (eGFR) < 60 mL/min/1.73m^2,

[10] According to the CTFG guideline V2.1 September 2020, a woman is considered of childbearing potential (WOCBP), i.e., fertile, following menarche and until becoming post-menopausal unless permanently sterile. Permanent sterilisation methods include hysterectomy, bilateral salpingectomy and bilateral oophorectomy. A postmenopausal state is defined as no menses for 12 months without an alternative medical cause. A high follicle stimulating hormone (FSH) level in the postmenopausal range may be used to confirm a post-menopausal state in women not using hormonal contraception or hormonal replacement therapy. However, in the absence of 12 months of amenorrhea, a single FSH measurement is insufficient. A man is considered fertile after puberty unless permanently sterile by bilateral orchidectomy.

4. Abnormal liver function at screening defined as total bilirubin levels >1.5 ULN, and/or AST and/or ALT >3 ULN,
5. Neutropenia (ANC <1.5 x 10^9/L) at screening,
6. Other causes of neuromuscular weakness,
7. Non progressive or very rapidly progressing ALS (ALSFRS-R decline from disease onset to randomisation ≤ 0.1 / month or ≥ 1.2 / month)[11],
8. Non-invasive ventilation,
9. Tracheotomy,
10. Dementia or other severe active psychiatric illness, including suicidal ideation assessed using the Columbia-Suicide Severity Rating Scale (C-SSRS),
11. Patient with a significant pulmonary disorder not attributed to ALS or who require treatments that might complicate the evaluation of the effect of ALS on respiratory function,
12. Patient treated by edaravone for ALS,
13. Patient using unauthorised concomitant treatments, namely moderate or strong inhibitors or inducers of CYP1A2, strong inhibitors or inducers of CYP2D6 or 2C19 and strong inhibitors of OCT2, as listed in Section 6.2. Combined oral contraceptives containing ethinylestradiol are forbidden concomitant medications,
14. Smoker of > 10 cigarettes per day (e-cigarettes and nicotine patches are permitted),
15. Known hypersensitivity to any of the ingredients or excipients of the IMPs,
16. Pregnant, lactating women,
17. Patient who participated in another trial of investigational drug(s) within 30 days prior to randomisation, or 5 half-lives of the previous investigational product, whichever is longer,
18. Patient who has forfeited their freedom by administrative or legal award, or who is under guardianship or under limited judicial protection.

5.3 WITHDRAWAL OF THE PATIENT

Patients may withdraw from the study at any time without having to provide justification and without penalty to their continuing medical care.

The investigators may withdraw patients from the study for the following reasons:

- Patient withdrawing the consent,
- If, in the investigator's opinion, continuation in the study would be detrimental to the patient’s well-being, in particular in case of an AE,
- If, in the investigator’s opinion, the patient is unable to maintain the schedule or is non-compliant,

[11] Decline will be calculated as follows: 48 minus ALSFRS-R score at the time of inclusion divided by number of months since occurrence of first symptoms of disease, as reported by the patient.

- If the patient becomes pregnant during the study,
- If the patient meets one of the following criteria for permanent drug discontinuation:
    - Increased ALT or AST levels ≥ 5 x ULN.
    - ALT or AST > 3 ULN and total bilirubin levels > 2 ULN.
    - Neutropenia.
    - Symptoms evocative of interstitial lung disease: dry cough and/or dyspnoea, bilateral diffuse lung opacities.
- Lost to FU. A participant will be considered lost to FU if he/she repeatedly fails to return for scheduled visits and is unable to be contacted by the study site. Site personnel are expected to make diligent attempts to contact participants who fail to return for a scheduled visit or are otherwise unable to be followed up by the site.

The date of withdrawal and the principal reason for withdrawal must be recorded in the eCRF and in the patient's records. No additional data will be collected for withdrawn patients. Every effort must be made to contact patients who did not come back for a planned visit/contact.

Every effort will be made to ensure that patients who do not complete all study visits return to the site for the premature discontinuation visit (Section 4.1.9), as soon as possible and no later than 4 weeks after the last IMP intake. Patients who withdraw due to an AE or SAE will be given appropriate care under medical supervision until the symptoms resolve or the patient's condition becomes stable.

Patients who prematurely withdrew from the study or are lost to FU will not be replaced by new patients.

Any temporary discontinuation of 10 days or less, related to any medical condition that prevents treatment administration based on investigator's judgment, should be allowed and recorded in the eCRF. Any associated AE shall be reported. In case of treatment discontinuation above 10 days, the patient should be withdrawn from the study.

If the participant withdraws consent for disclosure of future information, the sponsor may retain and continue to use any data collected before such a withdrawal of consent. If a participant withdraws from the study, they may request destruction of any samples taken and not tested, and the investigator must document this in the site study records.

6. STUDY PRODUCTS

The study product is defined as "a pharmaceutical form of an active substance or placebo being tested or used as a reference in a clinical study, including products already with a marketing authorisation but used or assembled (formulated or packaged) in a way different from the authorised form, or when used for an unauthorised indication, or when used to gain further information about the authorised form". In this trial, the study products are IFB-088 and its matching placebo.
IFB-088 and placebo will be provided as white, oblong, immediate release 200 mg tablets, with the same composition except for the active ingredient, i.e. 25mg of IFB-088 per tablet for the IFB-088 tablets.
IFB-088 and placebo tablets excipients are listed in Table 4.

Table 4 - Composition of IFB-088 and placebo tablets

Compound	Function
IFB-088 acetate salt	Active pharmaceutical ingredient
Microcrystalline cellulose	Filler
Silica, colloidal hydrated	Glidant
Crospovidone	Disintegrant
Magnesium stearate	Lubricant

6.1 INVESTIGATIONAL MEDICINAL PRODUCTS

6.1.1 Dosage and dosage schedule of the investigational medicinal products

The test product, IFB-088, will be administered in 50 mg/day dosage consisting of two uptakes of 25 mg each, as an add-on therapy to riluzole 100 mg. The placebo will be administered in two uptakes, as an add-on therapy to riluzole 100 mg.

Administration of riluzole 100 mg, tablet or suspension, will be at the patient's and/or investigator's choice, as per summary of product characteristics [44]. The daily dose of 100 mg will be taken in two 50 mg doses every 12 hours, at the same time than the IMPs.

6.1.2 Administration of the investigational medicinal products

Only patients enrolled in the study will receive the study products and only trained and authorised site staff will handle and supply them. Patients will receive the IMPs kit directly from the investigator or the medicine will be sent directly to the patient from the site pharmacy.

The patients will receive the IMP at baseline visit (V0: 2 bottles), V2 (2 bottles) and V3 (2 bottles) visits. They will be instructed to take the IMP orally twice a day (morning and evening uptakes). Intervals for dosing should ideally be about 12 hours (± 1 hour). Tablets shall be swallowed with a glass of water 30 minutes before the meal, in fasting condition. For patients who have swallowing difficulties, tablets can be taken in a spoon filled with jellified water. IFB-088 and riluzole will be taken at the same time. Dosage modifications are not permitted. However, in case of unplanned dosage modification by the patient, it shall be documented in the eCRF. Missed doses will not be made up for. In case of vomiting shortly after ingestion of the study product, the patient will leave out the potential missed dose and will take the next dose as normal. Study product doses withheld due to toxicities and AEs will not be made up later. Should the patient or the investigator wish to discontinue treatment for any reason, they may do so at any time. The date of the last uptake will be documented in the eCRF.

6.1.3 Supply and storage conditions of the investigational medicinal products

IFB-088 and placebo supplied by the sponsor to be used in this study will be manufactured, tested, handled and stored according to current Good Manufacturing Practice (GMP) requirements for clinical trials.

The sponsor will provide the study centre with the test product and the placebo. The investigator or pharmacist (if applicable) will receive numbered products. The investigator/pharmacist is responsible for a safe and proper handling and storage of the study drug at the investigational site. The study drug must be stored in a locked facility with restricted access to the investigator/pharmacist and authorised personnel. The study products have to be stored at room

temperature. The investigator or designee must confirm appropriate temperature conditions have been maintained during transit for each study product received.

The investigator and/or a pharmacist or another appropriately trained individual is obliged to document the receipt, dispensation, and return of all study products received during this study.

Records on receipt, use, return, loss, or other disposition of study drugs must be maintained. The investigator and/or a pharmacist or another appropriately trained individual must sign the receipt forms. Records on study drug delivery to the site, the inventory at the site, the use by each patient, and the return to the sponsor must be maintained by the investigator and/or a pharmacist or another appropriately trained individual at the investigational site. Patients will be asked to return all unused study drug and packaging at each visit, and at the time of study drug discontinuation. The following information will be recorded in the patient's file: dates, quantities, batch numbers, and the unique code numbers assigned to the study products and the patients. The investigators must maintain records documenting that the patients were provided with their respective doses specified in the protocol. Furthermore, they should reconcile all study drugs received from the sponsor. It is the responsibility of the investigator to give reasons for any discrepancies in IMPs accountability

All remaining IMPs, used and unused, shall be collected and returned to the sponsor for destruction at the end of the study.

6.1.4 Packaging and labelling of the investigational medicinal products

The study products will be packaged and labelled according to current GMP guidelines, good clinical practice (GCP) guidelines, and national legal requirements and instruction will be given in local language. KLS will be responsible for label compliance. Eurofins Gent will be responsible for the packaging, and Eurofins St Gely will be responsible for the labelling and shipping of the IMP and placebo to the investigating sites.

IFB-088 and placebo tablets are packaged in 40 mL high-density polyethylene bottles with polypropylene screw cap lids that contain desiccant. Each bottle contains 65 tablets covering the needs for a bi-daily administration over one month.

6.2 CONTRAINDICATIONS

Definitive pre- and post-natal developmental toxicity studies with IFB-088 have not been conducted. For this reason, the use of IFB-088 is contraindicated in women who are pregnant or lactating.

Edaravone, that is not commercially available in Europe but can be administered via compassionate use programs, is contraindicated during the whole study period, until completion of the follow-up visit.

IFB-088 is a substrate of CYP1A2. The concomitant use of drugs that are strong or moderate inhibitors or inducers of CYP1A2 is not permitted for inclusion in the study or during treatment (Table 5). The concomitant use of weak inhibitors or inducers of CYP1A2 will be allowed. IFB-088 is also metabolized, to a lesser extent, by CYP2D6 and CYP2C19. Therefore, concomitant use of strong inhibitors or inducers of these 2 isoenzymes will be prohibited for inclusion and during treatment. Finally, IFB-088 is certainly a substrate of OCT2, a renal transporter expressed at the basal membrane of tubular epithelial cells that might thus be involved in the renal tubular secretion

of IFB-088. For this reason, strong inhibitors of OCT2 will be prohibited for inclusion and during treatment. The list of prohibited drugs is displayed in Table 5.

If treatment by one of the abovementioned drugs is required during the study because there is no therapeutic alternative, treatment with IFB-088 will be interrupted during the administration of the prohibited therapy and during a period of 5 elimination half-lives of the administered treatment. If IFB-088 discontinuation lasts more than 10 days, the patient will be withdrawn from the study.

Tobacco consumption exceeding 10 cigarettes per day is prohibited during the study. E-cigarettes and nicotine patches are allowed.

Table 5 - Forbidden concomitant medications

	CYP1A2	CYP2D6	CYP2C19	OCT2
Strong inhibitors	Ciprofloxacin Enoxacin Fluvoxamine Norfloxacin	Bupropion, Fluoxetine, Paroxetine, Quinidine, Terbinafine	Fluconazole, Fluoxetine, Fluvoxamine, Ticlopidine	Cimetidine Doxepin Pantoprazole
Moderate inhibitors	Amiodarone Atazanavir Cannabidiol Ethinylestradiol Fluoxetine Methoxsalem Mexiletine Moclobemide Oral contraceptives Paroxetine Propafenone Quetiapine Sertraline Tipranavir, Verapamil			
Strong inducers			Rifampicin	
Moderate inducers	Carbamazepine Lansoprazole Modafinil Omeprazole Phenytoin Rifampicin Ritonavir Teriflunomide			

CYP: Cytochrome P450 family.

As *in vitro* data suggest that CYP1A2 is also the main isoenzyme involved in the metabolism of riluzole, and according to section 4.5 of riluzole SmPC, it should be noted that inhibitors of CYP1A2 (e.g. caffeine, diclofenac, diazepam, nicergoline, clomipramine, imipramine, fluvoxamine, phenacetin, theophylline, amitriptyline and quinolones) could potentially decrease the rate of riluzole elimination, while inducers of CYP1A2 (e.g. cigarette smoke, charcoal-broiled food, rifampicin and omeprazole) could increase the rate of riluzole elimination.

6.3 USE OF CONCOMITANT MEDICATIONS

Any medication, investigational agent, or vaccine, including over the counter or prescription medicines, vitamins, and/or herbal supplements, or other specific categories of interest that the participant is receiving at the time of enrolment or receives during the study must be recorded along with:

- Reason of use,
- Dates of administration including start and end dates,

- Dosage information including dose and frequency for concomitant therapy of special interest.

Other concomitant medication may be considered on a case-by-case basis by the investigator in consultation with the sponsor if required. The sponsor should be contacted if there are any questions regarding concomitant or prior therapy. Patients must be informed by the investigators about the fact that they may not take any drug, food supplement, vitamins, etc. on their own without the agreement of their referent investigator.

6.4 COMPLIANCE

Patient compliance with study medication will be assessed at each visit and at the time of study products discontinuation by the investigator by direct questioning and by recollection of IMP packaging. Patients will be asked to return all unused study products.

If the time for one visit exceeds the time window, this will be recorded as a deviation from the protocol. Any deviation from the prescribed dosage regimen is to be recorded in the eCRF.

7. ADVERSE EVENTS

7.1 DEFINITION OF AN ADVERSE EVENT

According to international conference on harmonisation (ICH), an AE is any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product. A clinically relevant worsening of a pre-existing condition during the study must also be recorded as an AE.

7.2 DEFINITION OF A TREATMENT EMERGENT ADVERSE EVENT

A TEAE is defined as any AE not present prior to the initiation of the treatments (that is before baseline visit: V0) or any event already present that worsens in either intensity or frequency following exposure to the treatments. AEs occurring after subject enrolment (ICF signature) but prior to first study drug administration will be recorded as a non-treatment emergent adverse event (NTEAE).

7.3 DEFINITION OF A SERIOUS ADVERSE EVENT

A SAE is defined as any untoward medical occurrence that:

- Results in death,
- Is life-threatening (the term ‘life-threatening’ refers to an event in which the patient was at risk of death at the time of the event; it does not refer to an event that hypothetically might have caused death if it was more severe),
- Requires in-patient hospitalisation or prolongation of existing hospitalisation,
- Results in persistent or significant disability or incapacity,

- Is a congenital anomaly or birth defect.

Medical and scientific judgement should be exercised in deciding whether expedited reporting is appropriate in other situations, such as important medical events that might not be immediately life threatening or result in death or hospitalisation but might jeopardise the patient or might require intervention to prevent one of the other outcomes listed above.

7.4 DEFINITION OF AN ADVERSE EVENT OF SPECIAL INTEREST

An AESI (serious or non-serious) is an AE of scientific and medical concern specific to the sponsor's product or program, for which ongoing monitoring and rapid communication by the investigator to the sponsor can be appropriate.

The following will be considered as AESIs:

- 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 (HTG). 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.

7.5 DEFINITION OF A NEW FACT

New information which might alter the current benefit-risk assessment of the IMP or the trial; modify the use of the IMP, the conduct of the study, or documents related to the trial; or suspend, modify or terminate the clinical trial.

7.6 DEFINITION OF SUSPECTED UNEXPECTED SERIOUS ADVERSE REACTION

SUSAR is the term used to refer to an adverse event that occurs in a clinical trial subject, which is assessed by the sponsor and or study investigator as being unexpected, serious and as having a reasonable possibility of a causal relationship with the study drug.

7.7 RECORDING OF AN ADVERSE EVENT

The investigator must record all AEs presented or reported by the patient in the AE section of the eCRF. The start date, the actions taken (corrective treatments, dose decrease, temporary or definitive discontinuation of study product, premature withdrawal from the study, none, other) and the outcome (ongoing, recovered with/without sequelae, death) must all be recorded, and the investigator must assess the event in terms of seriousness, severity and relationship to the study products.

When possible, signs and symptoms should be reported as a diagnosis i.e. the investigator should avoid reporting only the signs and symptoms. If there is no medical diagnosis and the signs and symptoms have to be reported, the investigator should record a separate AE for each sign and symptom.

The severity of the AEs will be judged by the investigator(s) and recorded as follows:

- Mild: discomfort noticed but no disruption of normal daily activity,

- Moderate: discomfort sufficient to reduce or affect daily activity,
- Severe: inability to work or perform normal daily activity.

The relationship of the AE to the study product will be judged by the investigator(s) and recorded as follows:

- Not related: a reaction for which sufficient information exists to indicate that the aetiology is unrelated to the study drug; the subject did not receive the study medication or the temporal sequence of the AE onset relative to administration of the study medication is not reasonable or the event is c1early related to other factors (subject' s clinical state, therapeutic intervention or concomitant therapy),
- Unlikely: Does not follow a reasonable temporal sequence from administration of the IMP, or is most likely related to another aetiology than the trial drug such as the patient's clinical state, environmental factors or other therapies,
- Possibly: a clinical event, including laboratory test abnormality, with a reasonable time sequence to administration of the drug but which could also be explained by concurrent disease or other drugs or chemicals,
- Probably: a clinical event including laboratory test abnormality, with a reasonable time sequence to administration of the drug, unlikely to be attributed to concurrent disease or other drugs and which follows a clinically reasonable response on withdrawal (de-challenge),
- Definitely: a reaction that follows a reasonable temporal sequence from administration of the drug, or in which the drug level has been established in body fluids or tissues, that follows a known or expected response pattern to the suspected drug, and that is confirmed by improvement on stopping or reducing the dosage of the drug, and reappearance of the reaction on repeated exposure (re-challenge if applicable).

For safety reporting purpose by the sponsor:

- Reasonable possibility: possibly, probably, definitely related AEs,
- No reasonable possibility: not related, unlikely related AEs.

All AEs and SAEs will be collected from the time of signing of the ICF until participation in study has ended. There is no time limit for investigators for reporting to the sponsor related SAEs they are made aware of.

Adverse events that begin before the start of study intervention but after signing of the ICF will be recorded on the AE eCRF (NTEAEs).

7.8 REPORTING OF SERIOUS ADVERSE EVENTS AND NEW FACTS

The investigator will immediately, i.e. within 24 hours from first knowledge, report, by fax or e-mail, any SAE occurring during the study to the safety officer (SO) acting on behalf of the sponsor of the study:

STRAGEN SERVICES S.A.S, 19 rue Jacqueline Auriol, 69008 Lyon, France

Fax number +33 478425571

Email: InFlectisPV@stragen.fr

SAEs must be reported using the supplied SAE/AESI/pregnancy form. The investigator will complete in English and sign an SAE report form and transmit it to the SO by email or telefax not later than 24 hours after the first knowledge of the SAE. The SO acknowledges the receipt of the SAE information by email to the investigational site within one working day. In the absence of email acknowledging the receipt or in case of issue in sending the fax or email, the investigator shall contact the SO by any means for ensuring the receipt of SAE information at the earliest opportunity.

Although all AEs after signing the ICF are recorded in the eCRF, SAEs reporting to sponsor begins after the participant has signed the ICF and has received study intervention. However, if an SAE occurs after signing the ICF, but prior to receiving study intervention, it needs to be reported within the SAE reporting timeframe if it is considered reasonably possibly related to study procedures.

Details of all pregnancies in female participants will be collected for 90 days after end of treatment (V4). If a pregnancy is reported, the investigator should inform the sponsor within 24 hours of learning of the pregnancy, using the SAE/AESI/pregnancy form. The sponsor (via Stragen Services) will then follow up on such pregnancy with the investigator, using a dedicated pregnancy form, at least until pregnancy outcome. Abnormal pregnancy outcomes, for example, spontaneous abortion, foetal death, stillbirth, congenital anomalies, and ectopic pregnancy, are considered SAEs.

New facts which may impact the risk-benefit for enrolled patients and/or impact on the study conduct may not necessarily fulfil the definition and/or criteria for constituting an SAE. However, such information shall be provided to the sponsor in accordance with the process described for SAEs, i.e. within 24h from knowledge and using the SAE/AESI/pregnancy form.

Any FU information will be reported to the SO and to the sponsor as soon as it becomes known, with the same process and timelines as described here above for initial reports. The sponsor will ensure that all relevant information about SAE and SUSAR is recorded and reported to the national competent authorities (CAs) in all member states concerned and to IECs within the applicable regulation and local specificities.

SAEs occurring after the last study visit will only be reported if the investigator believes that the event may have been caused by the IMP or a protocol procedure.

Hospitalisation for a diagnosis or therapeutic procedure planned before study enrolment but performed after the enrolment should not be considered for SAE and should be reported in eCRF in concomitant procedures page.

7.9 REPORTING OF ADVERSE EVENTS OF SPECIAL INTEREST

AESIs should be reported via the same process established for SAEs, regardless of seriousness. AESIs, both serious and non-serious, must be reported within 24h from knowledge using the SAE/AESI form. The form will be named SAE/AESI/pregnancy form, as it is also used for the initial notification of pregnancy.

7.10 CLINICAL EVENTS RELATED TO ALS

Clinical events related to ALS or consisting in known ALS complications will be recorded in the AE section of the eCRF. When serious, these clinical events are exempt from SAE reporting, unless the investigator deems the event to be related to the administration of the study drug, or unless the event results in patient's death: in these 2 cases, the event should be reported as SAE. The following events will be considered clinical events related to ALS or ALS complications:

- Onset, changes or aggravation of speech and/or voice disorders,
- Onset, changes or aggravation of swallowing disorders including drooling, aspiration/choking,
- Onset, changes or aggravation of emotional disorders, including exaggerated and/or involuntary emotional responses, alteration and/or lack of control in emotive expression, fronto-temporal executive dysfunction,
- Onset, changes or aggravation of breathing difficulties,
- Onset, changes or aggravation of muscle and/or joint disorders and their consequences, including muscle weakness, muscle atrophy/wasting, muscle spasticity, muscle cramps, fasciculations, stiffness, joint pain and/or contractures, reduced dexterity, wrist drop, tripping, stumbling, awkwardness, foot drop, spasms, uncontrolled movements,
- Onset, changes or aggravation of upper and/or lower motor neuron signs and symptoms, including reflexes disorders (abnormal reflexes, hypo/hyperreflexia), abnormal motor nerve conduction studies.

7.11 TREATMENT OF OVERDOSE

In the event of an overt overdose, with or without adverse event, the investigator should:

- Contact the sponsor immediately via Stragen Services using the SAE/AESI/pregnancy/overdose form,
- Closely monitor the participant for any AE/SAE and laboratory abnormalities as appropriate and provide supportive care as necessary,
- Document the quantity of the excess dose in the eCRF.

Decisions regarding treatment interruptions or modifications will be made by the investigator, in consultation with the sponsor, based on the clinical evaluation of the patient.

8. STATISTICS

8.1 STATISTICAL AND ANALYTICAL METHODS

8.1.1 General statistical considerations

Statistical analyses will be performed by the sponsor or under the supervision of the sponsor and further detailed in the SAP, which will be finalised prior to the unblinding of data and database lock.

In general, summary tabulations will be presented by treatment arm and will display the number of observations, mean, standard deviation (SD), median, Q1, Q3 quartiles, minimum, and maximum for continuous variables, and the number and percent per category for categorical data.

Patient disposition

An accounting of study patients by disposition will be tabulated and the number of patients in each analysis set will be summarised. Patients who discontinue study treatment and patients who withdraw from the study, with associated reason(s), will be summarised. A summary of protocol deviations by treatment arm and the corresponding listing by patient will be generated.

Nominal alpha level of significance

Unless otherwise noted, as the trial is a safety trial and an estimation efficacy study and not powered for efficacy endpoints, all tests performed on efficacy assessments will be conducted at a 1-sided nominal alpha level of 0.10 and confidence intervals (CIs) will be calculated at 80%, 2-sided 95% confidence intervals will also be provided.

Missing data handling

As a general rule, missing data will not be replaced. Sensitivity analyses based on multiple imputation will be proposed and further detailed in the SAP.

Multiplicity

As it is mainly an estimation trial, there is no adjustment for multiplicity.

8.1.2 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 will be randomised.

8.1.3 Analysis Sets

The following analysis sets will be considered. Additional details of analysis sets of patients may be defined in the SAP.

- Safety set (SS): randomised patients having received at least one dose of the IMPs and analysed according to the treatment actually received,

- Efficacy sets:
    - Randomised set (RS): all patients "as randomised" (i.e. according to the treatment group to which they were randomised, regardless of the treatment actually taken),
    - Full analysis set (FAS): as randomised patients having received at least one dose of the IMPs and having a non-missing baseline value,
    - Per protocol set (PPS):

      FAS patients fulfilling the following criteria. Criteria definition will be further detailed during a blinded data review meeting and specified in the SAP:

      - Major inclusion/exclusion criteria satisfied,
      - Absence of relevant protocol violations likely to affect treatment efficacy,
      - Adequate study medication compliance, defined as intake of at least 80% of the planned total dose.

8.1.4 Population and baseline characteristics description

Demographics, concomitant medications, and other baseline characteristics will be described by treatment arm according to general considerations. Details will be provided in the SAP.

8.1.5 Efficacy / safety analysis

8.1.5.1 *Primary outcome*

Usual descriptive statistics will be used and detailed in the SAP to analyse the safety parameters.

AEs will be classified by system organ class and preferred term using the Medical Dictionary for Regulatory Activities (MedDRA). AEs will be listed and summarised by, MedDRA preferred term, severity, and relationship to study drug. In the event of multiple occurrences of the same AE with the same preferred term in one patient, the AE will be counted once as the occurrence.

8.1.5.2 *Secondary outcomes*

<u>Efficacy endpoints</u>
Analyses will be further detailed in the SAP.

The analysis of efficacy endpoints will be primarily performed in the FAS. A secondary analysis will be proposed in the PPS.

- Change in ALSFRS-R score: from baseline to 6 months will be analysed using a mixed model for repeated measures (MMRM) assuming missing at random (MAR) and unstructured covariance matrix and including fixed factors for treatment, visit (categorical variable), treatment-by-visit interaction, random patient factor and baseline ALSFRS-R as a continuous covariate. The degrees of freedom will be estimated using the Kenward-Roger's approximation. Least square means (LSMs) of change from baseline and LSM differences in change from baseline between active doses and placebo will be presented along with 80% and 95% CIs and the comparison p-value. A one-sided nominal significance level of 0.10 will be used for treatment comparison. Model assumptions will be checked (plot of studentized residuals vs predicted values, etc.). If the model assumptions do not hold, a rank

semi-parametric analysis of covariance (ANCOVA) model (or a rank non-parametric Quade's ANCOVA model if the assumptions of this latter do still not hold) will be proposed instead. As the MAR assumption is untestable and may not hold for subjects withdrawing at their own will, a sensitivity analysis will be based on a pattern mixture model that uses a multiple imputation technique analysed with an ANCOVA with pre specified fixed factors and covariates. The imputation model will be further clarified in the SAP. Another sensitivity Bayesian analysis using the historical placebo data from the ProMISe study [26] will be also performed and further detailed in the SAP. An intrapatient analysis of ALSFRS changes will be performed.

- Worsening (i.e. progression to a higher stage at 6 months compared to the baseline stage defined by ALS-MITOS score): the proportion of progressors will be compared between both treatment groups. The difference in binomial proportions will be estimated along with an exact 95% CI. A sensitivity Bayesian analysis using the historical placebo data from the ProMISe study [26] will be also performed and further detailed in the SAP. Progressors will also be analysed in a logistic regression model adjusting for treatment and additional relevant covariates (specified in the SAP). The treatment effect will be estimated in terms of an odds ratio associated with the 95% CI and p-value and also in terms of a difference in progression rates with its 95% CI using the method proposed by Ge and al. [45].
- Change in King's Çollege score: the endpoint will be analysed with an approach similar to the analysis of ALS-MITOS score.

Analyses of the changes in efficacy endpoints from baseline to month 3 (as inferential analysis) and from month 3 to month 6 (as descriptive analysis) will be also proposed and further detailed in the SAP.

- Respiratory function: changes from baseline to 3 months and from baseline to 6 months will be analysed. Analyses will be further detailed in the SAP.
- Bioelectrical analysis: changes from baseline to 3 months and from baseline to 6 months will be analysed for the planned following parameters: phase angle, Z200/Z5 impedance ratio, skeletal muscle mass, dry mass, and dry mass without fat. Analyses will be further detailed in the SAP.

PK Analysis

Individual plasma concentrations of IFB-088 and IFB-139 (i.e. IFB-088 metabolite) with corresponding nominal sampling times, will be tabulated for each subject and scheduled sampling time, together with descriptive statistics. Mean plasma IFB-088 concentrations profiles by cohort will be presented over time graphically, using blood sampling time (x axis) and concentrations (y axis) on both linear and semi-log scales.

Derived and observed PK parameters will be evaluated by noncompartmental analysis. The following parameters will be listed and summarised by cohort, as appropriate:

- AUC, ng.h/mL,
- C_{max}, ng/mL, with associated T_{max}, h,

- Terminal or apparent terminal $t_{1/2,}$ h,
- Apparent systemic clearance (Cl/F, L/h) and apparent volume of distribution (Vd/F, L) at steady state, where F is the overall oral bioavailability of IFB-088.

For the calculation of derived PK parameters, concentrations below the lower limit of quantification (LLOQ) will be assigned a value of zero.

Plasma concentrations of IFB-088 will also be analysed using a population PK modelling approach to develop a population PK model in patients with ALS. Results of this model will be used to estimate PK parameters and variability in patients and simulate new dosing schemes (with AUC and C_{max} information) to help choosing the doses for subsequent clinical studies.

Based on our previous PK analyses (Phase I), the developed population pharmacokinetics (popPK) model with plasma concentrations and urine data of IFB-088 and IFB-139 in patients (P188_MAD/PK/2019-001) will be evaluated. To evaluate the predictive performance of the model, goodness-of-fit plots for the predicted and the observed concentration will be first inspected. Then, prediction error (PE) will be calculated to assess the predictive performance of the model. Model bias will be determined with the mean prediction error (MPE) and the median prediction error (MDPE). Model imprecision will be determined with the mean absolute error (MAE) and the median absolute prediction error (MDAPE). This evaluation will be performed using a non-linear mixed effects model with NONMEM software (ICON Development Solutions v7.5). The R software v4.4.1 will be used for goodness-of-fit diagnostics and graphical displays. Then, this model will be optimised and used to estimate PK parameters and variability in patients (CL/F, V/F, $T_{1/2}$ and AUC), and simulated optimal dosing schemes.

A PK/PD (pharmacokinetic/pharmacodynamic) model could be developed according to the available data. The population PD analysis will be performed with a sequential method using the final PK model. In a first step, the PK/PD of each PD data will be fitted separately to define the model more easily then the run simultaneously estimated PD parameters of each PD data.

PK and PD data will be listed and presented in graphical and/or tabular form as appropriate to the data, and will be summarised descriptively.

Analysis of changes in potential biomarkers

Details of analysis will be provided in the SAP.

Changes in plasma concentration of TDP-43, NfL heavy chain and NfL light chain will be measured from baseline to 6 months.

Changes in plasma concentration of inflammatory and oxidative stress biomarkers will be measured from baseline to 3 months, and from baseline to 6 months. All changes will be compared between both arms.

Correlation of biomarkers with clinical endpoints will be assessed.

QoL endpoint

Change in ALS-AQ40 QoL scores from baseline will be assessed at 6 months and compared between both arms.

9. ETHICS

9.1 STATEMENT

This study will be conducted in accordance with all applicable regulatory requirements including the principles of the Declaration of Helsinki and in compliance with this protocol, the requirements of ICH GCP Harmonised Tripartite Guideline Topic E6, Directives 2001/20/EC , 2005/28/ EC of the European Parliament.

The sponsor is obliged to obtain evidence of the investigator's qualification to perform the clinical study. Therefore, the investigator has to provide a dated and signed copy of his professional curriculum vitae (no older than two years and preferably one page-length in English) prior to the start of the clinical study, including information about his experience and training in conducting clinical studies according to the guidelines for GCP.

Written approval must be obtained from the IECs and the CAs in each country before the study can start.

Any modification of the protocol will be made only with the agreement of the sponsor and must be submitted to the IECs and CAs. No changes in the clinical study protocol will be implemented until the amendment and revised ICF (if applicable) have received approval from the IECs and the CAs.

9.2 INDEPENDENT ETHICS COMMITTEE

Prior to the start of the study, the sponsor or investigator will submit the study protocol, patient information, ICF, and other study-related documents, as required by local regulations, to the respective regulatory authorities and the responsible IEC for their written approval. The sponsor or investigator will inform the IEC and regulatory authorities, according to local regulations, about protocol amendments including any new information that require an ethical reconsideration of the study protocol.

When required by the IEC or by local regulation, the sponsor or the investigator will submit to the IEC:

- Information on AEs that are serious and unexpected and associated with the IMP from the investigator’s site, as soon as possible,
- Expedited safety reports from the sponsor, as soon as possible,
- Periodic summaries on the status of the study, in accordance with the local requirements and procedures established by the IEC.

9.3 INDEPENDENT DATA MONITORING COMMITTEE

A DSMB without direct involvement in the conduct of the study will be set up to ensure the safety of the participating patients, the relevance of the study and data integrity. DSMB will be composed of at least 3 ALS experts. Each DSMB member will be respectively independent from the sponsor and from the study. DSMB members will monitor safety data and assess patient safety throughout the duration of the study. All safety data occurring during the trial will be forwarded to the DSMB. Roles, responsibilities as well as safety monitoring rules and conventions will be reviewed and

approved by the DSMB at the organisational meeting and included in the DSMB charter. The roles and responsibilities of the DSMB, their operational procedures, and methods of communication with the investigators are objects of the separate DSMB charter.

9.4 INFORMED CONSENT

Prior to a patient's participation in the study, written informed consent must be obtained after the investigator has fully informed the patient or the patient's legally accepted representative of all pertinent aspects of the study. The ICF must be signed and personally dated by the patient or the patient's legally accepted representative and by the investigator according to ICH GCP. The patient or the patient's legally accepted representative must receive a copy of the signed and dated ICF and any other written information provided to the patients.

Any change to the information sheet and the consent form constitutes an amendment and must be submitted for approval to the IEC and the CA.

With the exception of an amendment needed to eliminate hazards to the patients in the study, such amendments may only be put into practice once written approval from the IEC and the CA has been obtained.

The new ICF must be signed and personally dated by the patient or the patient's legally accepted representative and by the investigator and the patient or the patient's legally accepted representative must receive a copy of this signed and dated ICF.

10. CONTRACT RESEARCH ORGANISATION AND SUBCONTRACTORS

The following activities have been outsourced from the sponsor:

Keyrus Life Science (KLS) 155 rue Anatole France, 92300 Levallois Perret, France Phone: +33 1 41 34 10 00	Project Management Regulatory Affairs Quality assurance Randomisation Clinical operations Data management Data review meeting (DRM) DSMB Clinical data interchange standards consortium (CDISC) Statistics Medical writing Clinical trial supply

STRAGEN Services SAS 19 rue Jacqueline Auriol, 69008 Lyon, France Phone: +33 4 78 42 95 26	Pharmacovigilance and safety
Central laboratories	PK, crystalluria, biomarkers and biobank

11. SOURCE DOCUMENTS AND ACCESS TO SOURCE DATA / DOCUMENTS

Source data and documents are all information, original records of clinical findings, observations or other activities in a clinical study necessary for the reconstruction and evaluation of the study.

Examples of these original documents and data records include concomitant medication forms, laboratory reports, and investigational product accountability records.

According to GCP guidelines, upon request of the CRA, auditor, IEC or regulatory authority, the investigator must provide direct access to all requested source data / documents.

12. QUALITY CONTROL AND QUALITY ASSURANCE

12.1 STUDY MONITORING

In accordance with applicable regulations, GCP and CRO standard operating procedures, an initiation visit will be performed at the investigator centre before any patients are included in the study. The aim of this visit is to review the protocol and data collection procedures with the study personnel as well as to provide a reminder of the investigator responsibilities according to ICH GCP.

During the study, the CRA will regularly contact the centre and will perform on-site monitoring visits. The extent, nature and frequency of on-site visits will be based on the patient inclusion rate and will be discussed with the investigator. The aim of these contacts and visits is to check the progress of the study, review collected study data, conduct source document verification and identify any issues and address their resolution. This will be done in order to verify that the data are authentic, accurate and complete, the safety and rights of patients are being protected and the study is being conducted in accordance with the approved protocol (and any amendments), GCP, and all applicable regulatory requirements.

The investigator agrees to allow the CRA direct access to all relevant documents and to allocate his time and the time of the study personnel to discuss any issues.

Upon completion of the study the CRA, with the collaboration of the investigator, will ensure that:

- All data queries have been finalised,
- The centres' study records are complete.

12.2 AUDIT

To ensure compliance with GCP and all applicable regulatory requirements, the sponsor or a CRO designated by the sponsor may conduct a quality assurance (QA) audit of the investigator centre. Regulatory agencies may also conduct regulatory inspections. Such audits or inspections can occur at any time during or after completion of the study. If an audit or inspection occurs, the investigator centre agrees to allow the auditor / inspector direct access to all relevant documents and to allocate his time and the time of the study personnel to the auditor / inspector to discuss findings and any relevant issues.

13. CONFIDENTIALITY, DATA HANDLING AND RECORD KEEPING

13.1 CONFIDENTIALITY

Information regarding patients' identity obtained as a result of this study is considered confidential and disclosure to unauthorised individuals is prohibited. Patients will not be identified by their names or dates of birth on the eCRF or other study documentation submitted to the sponsor; instead patients will be given a unique identification number as soon as they have signed their ICF. For safety reasons, the investigator will maintain a patient identification log with the name and contact details of each patient. This log and the signed consent forms will be kept in strict confidence by the investigator.

13.2 DATA HANDLING

All clinical data will be reported by the investigator or authorised designee on the eCRF. The eCRF is specifically designed for this study and developed by KLS data management department. A unique number will identify each patient on the eCRF (see Section 3.8). The investigator will ensure that all data are entered after the evaluation has occurred, in accordance with source documents.

In case of missing values, out of range values or data inconsistencies, queries will be generated and submitted to the investigator site for resolution. Coding of AEs will be performed using the MedDRA and coding of previous and concomitant treatments will be performed using the world health organisation (WHO) drug dictionary enhanced.

13.3 RECORD KEEPING

13.3.1 Investigator

Following closure of the study, the investigators must archive, in a safe and secure location, all study records including patient medical files, original ICFs, source documents, copies of the eCRFs, copies of the study product accountability forms, copies of the regulatory authorities' approval and all correspondence. Investigators will retain all study documentation for the maximum period of time required by local requirements.

The records must be maintained in a way to allow easy and timely retrieval when needed e.g. for an audit, inspection or any subsequent review of data in conjunction with assessment of the facility,

supporting systems and staff. Where permitted by local laws and regulations, some or all of these records can be maintained in a format other than hard copy e.g. microfiche, scanned, electronic, however, caution needs to be exercised before such action is taken. The investigator must assure that all reproductions are legible, are a true and accurate copy of the original and meet accessibility and retrieval standards (a hard copy must be regenerated if required). Furthermore, the investigator must ensure that there is an acceptable back-up of these reproductions and that an acceptable quality control process exists for making these reproductions.

The investigator agrees to provide direct access to source documents during monitoring visits.

The investigator must notify the sponsor of any changes in the archiving arrangements, including, but not limited to: archival at an off-site facility and transfer of ownership of the records in the event the investigator leaves the site.

13.3.2 Sponsor

The following documents are to be archived by the sponsor in accordance with GCP and applicable regulatory requirements:

- Final version of the protocol,
- Any protocol amendments,
- Original eCRFs and queries,
- IEC and CA approval forms,
- All correspondence related to the study,
- The investigator's and any co-investigators' curriculum vitae,
- All other documents generated during the study.

All of the above documents must be archived in a room specifically designated for this purpose, access to which is controlled by the person responsible for archiving.

14. INSURANCE AND FINANCES

14.1 INSURANCE

The sponsor, InFlectis BioScience, certifies that a medical insurance policy has been taken out, which covers the liability of the investigator in case of damage or injury resulting from this study, in accordance with Articles 3.2 and 6.3 of Directive 2001 / 20 / EC of the European Parliament.

This insurance does not relieve the investigators of any obligation to maintain their own liability insurance policy as required by applicable law.

14.2 FINANCES

Contracts will be written up between the sponsor / CRO and the sites. A site-specific agreement will be signed by the investigators and the sponsor.

14.3 PATIENT PAYMENTS AND EXPENSES

Patients will be reimbursed for all expenses in relation to their participation to the study. Patients will not receive a compensation for their participation.

15. REPORT AND PUBLICATION POLICY

15.1 REPORT

After completion of the study, the results will be reported in a CSR according to the ICH-E3 note for guidance on structure and content of CSRs. The sponsor will send a summary of this CSR to the regulatory authorities within one year after the end of the trial.

15.2 DISCLOSURE OF DATA

No information provided by InFlectis BioScience to the investigators for the purposes of performing the study, will be published, or passed on to a third party, without prior written approval by InFlectis BioScience.

The investigators will have full access to all study data and will take complete responsibility for the integrity of the data and the relevance of the data analysis and reporting.

After regulatory clearance, the study will be registered by the sponsor or an appointed CRO in the ClinTrials.gov database. The principal investigator or anyone else working on the study will submit all proposed publications, papers, abstracts, other written materials or an outline of any proposed oral presentation related to the study to InFlectis BioScience at least one month prior to (i) submission of such written materials for publication, or (ii) any proposed oral disclosure to a third party. InFlectis BioScience shall have the right to comment on such written material/outline and to take any necessary action to protect its intellectual property; the principal investigator, in determining the final form of disclosure, shall consider such comments in good faith. Notwithstanding any of the above, the principal investigator or anyone else working on the study may not include any confidential information unrelated to the study in any such publication or disclosure.

The investigator will provide InFlectis BioScience with complete test results and all data derived from the study in accordance with the protocol.

Only InFlectis BioScience and its authorised contractor may make information obtained during the study available to regulatory agencies, except as required by regulation.

15.3 PUBLICATION

The sponsor commits to communicating, and otherwise making available for public disclosure, the results of the clinical study, including publication of a paper in a scientific journal, abstract submission with a poster or oral presentation at a scientific meeting, or disclosure by other means. However, the sponsor reserves the right to defer the release of data until specified milestones are reached, for example when the CSR is available.

Study results will be published as manuscripts in peer-reviewed scientific journals, in an objective, accurate, balanced and complete manner.

Authorship Policy

Authorship of any public disclosure must be in accordance with the "Recommendations for the conduct, reporting, editing, and publication of scholarly work in medical journals" of the international committee of medical journal editors (ICMJE, [46]), i.e. fulfilling the following 4 criteria:

- Substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data for the work; and,
- Drafting the work or revising it critically for important intellectual content; and,
- Final approval of the version to be published; and,
- Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Authors will be determined at the end of the study according to their substantial contributions. Those who do not meet all 4 criteria will be acknowledged as contributors.

As far as they fulfil their responsibilities up to the end of the study and meet the ICMJE criteria, the principal investigator and co-principal investigator will have the first and last authorship position respectively.

16. REFERENCE LIST

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17. APPENDICES

17.1 SCALES AND QUESTIONNAIRES

17.1.1 ALSFRS-R

ALS Functional Rating Scale Revised (ALS-FRS-R)

Date:……..Name patient:……Date of Birth:……

Patient's number……..Right-/left-handed

Item 1: SPEECH

4	Normal speech process
3 2	Detectable speech disturbance
1 0	Intelligible with repeating
	Speech combined with non-vocal communication
	Loss of useful speech

Item 2: SALIVATION

4	Normal
3	Slight but definite excess of saliva in mouth; may have nighttime drooling
2	Moderately excessive saliva; may have minimal drooling (during the day)
1	Marked excess of saliva with some drooling
0	Marked drooling; requires constant tissue or handkerchief

Item 3: SWALLOWING

4	Normal eating habits
3	Early eating problems – occasional choking
2	Dietary consistency changes
1	Needs supplement tube feeding
0	NPO (exclusively parenteral or enteral feeding)

Item 4: HANDWRITING

4	Normal
3	Slow or sloppy: all words are legible
2	Not all words are legible
1	Able to grip pen, but unable to write
0	Unable to grip pen

Item 5a: CUTTING FOOD AND HANDLING UTENSILS
Patients <u>without</u> gastrostomy ⇗ Use 5b if >50% is through g-tube

4	Normal
3	Somewhat slow and clumsy, but no help needed
2	Can cut most foods (>50%), although slow and clumsy; some help needed
1	Food must be cut by someone, but can still feed slowly
0	Needs to be fed

Item 5b: CUTTING FOOD AND HANDLING UTENSILS
Patients <u>with</u> gastrostomy ⇗ 5b option is used if the patient has a gastrostomy and only if it is the primary method (more than 50%) of eating .

4	Normal
3	Clumsy, but able to perform all manipulations independently
2	Some help needed with closures and fasteners
1	Provides minimal assistance to caregiver
0	Unable to perform any aspect of task

Item 6: DRESSING AND HYGIENE

4 Normal function
3 Independent and complete self-care with effort or decreased efficiency 2
Intermittent assistance or substitute methods
1 Needs attendant for self-care 0
Total dependence

Item 7: TURNING IN BED AND ADJUSTING BED CLOTHES

4 Normal function
3 Somewhat slow and clumsy, but no help needed
2 Can turn alone, or adjust sheets, but with great difficulty 1
Can initiate, but not turn or adjust sheets alone
0 Helpless

Item 8: WALKING

4 Normal
3 Early ambulation difficulties 2
Walks with assistance
1 Non-ambulatory functional movement 0
No purposeful leg movement

Item 9: CLIMBING STAIRS

4 Normal 3
Slow
2 Mild unsteadiness or fatigue 1
Needs assistance
0 Cannot do

Item 10: DYSPNEA

4 None
3 Occurs when walking
2 Occurs with one or more of the following: eating, bathing, dressing (ADL) 1
Occurs at rest: difficulty breathing when either sitting or lying
0 Significant difficulty: considering using mechanical respiratory support

Item 11: ORTHOPNEA

4 None
3 Some difficulty sleeping at night due to shortness of breath, does not routinely use more than two pillows
2 Needs extra pillows in order to sleep (more than two) 1
Can only sleep sitting up
0 Unable to sleep without mechanical assistance

Item 12: RESPIRATORY INSUFFICIENCY

4 None
3 Intermittent use of BiPAP
2 Continuous use of BiPAP during the night
1 Continuous use of BiPAP during day & night
0 Invasive mechanical ventilation by intubation or tracheostomy

Interviewer's name.....

17.1.2 ALS-MITOS

ALS Milano-Torino Staging (ALS-MITOS)

Date:……Name patient:……

Date of Birth:……

Patient's number……..

ALSFRS domain	Item	Score	Functional score*
Movement (walking/self-care)†	8	4 Normal	0
	Walking	3 Early ambulation difficulties	
		2 Walks with assistance	
		1 Non-ambulatory functional movement only	1
	OR	0 No purposeful leg movement	
	6	4 Normal function	0
	Dressing and hygiene	3 Independent and complete self-care with effort or decreased efficiency	
		2 Intermittent assistance or substitute methods	
		1 Needs attendant for self-care	1
		0 Total dependence	
Swallowing	3	4 Normal eating habits	0
	Swallowing	3 Early eating problems; occasional choking	
		2 Dietary consistency changes	
		1 Needs supplemental tube feeding	1
		0 NPO (exclusively parenteral or enteral feeding)	
Communicating†	1	4 Normal speech processes	0
	Speech	3 Detectable speech with disturbances	
		2 Intelligible with repeating	
		1 Speech combined with non-vocal communication	1
	AND	0 Loss of useful speech	

	4	4 Normal	0
	Handwriting	3 Slow or sloppy; all words are legible	
		2 Not all words are legible	
		1 Able to grip pen but unable to write	1
		0 Unable to grip pen	
Breathing†	10	4 None	0
	Dyspnea	3 Occurs when walking	
		2 Occurs with one or more of: eating, bathing, dressing	
		1 Occurs at rest, difficulty breathing when either sitting or lying	1
	OR	0 Significant difficulty, considering using mechanical respiratory support	
	12	4 None	0
	Respiratory insufficiency	3 Intermittent use of NIPPV	
		2 Continuous use of NIPPV during the night	1
		1 Continuous use of NIPPV during the night and day	
		0 Invasive mechanical ventilation by intubation or tracheostomy	
ALS-MITOS	Stage	Functional domains lost	
	0	None	
	1	1 domain	
	2	2 domains	
	3	3 domains	
	4	4 domains	
	5	Death	

*Staging determined by the sum of functional score of 1 for each domain.
†Where two items were used, scoring was based on either or both item scores as indicated.
ALFSRS, Amyotrophic Lateral Sclerosis Functional Rating Scale; ALS-MITOS, Amyotrophic Lateral Sclerosis Milano-Torino Staging; NIPPV, nasal intermittent positive pressure ventilation; NPO, nothing to mouth.

Chiò A, et al. J Neurol Neurosurg Psychiatry 2013;0:1–7. doi:10.1136/jnnp-2013-306589

17.1.3 King's College scale

Affix Hospital Sticker Here

Or Enter Patient Details

KING'S ALS STAGING FORM

Date: _____

Tester's name: _____

Bulbar region:

- Unaffected ☐
- Function affected (e.g. slurred speech, slowing difficulty, or hypophonia) ☐
- Affected on examination only (accepted signs: tongue atrophy fasciculation, slowness of movement) ☐
- Affected on reflex examination only (accepted sign: pathologically brisk jaw jerks only) ☐

Upper limbs:

- Unaffected ☐
- Function affected (e.g. difficulty with keys, doorknobs, zips, bags) ☐
- Affected on examination only (accepted sign: wasting of the first dorsal interossei) ☐
- Affected on reflex examination only (accepted signs: the presence of pectoral reflexes or Hoffman's sign) ☐

Lower limbs:

- Unaffected ☐
- Function affected e.g. difficulty walking, falls, cramps etc. ☐
- Affected on examination only (accepted signs: gait stiffness or foot drop) ☐
- Affected on reflex examination only (accepted signs: crossed adductor reflexes, pathologically brisk patellar reflexes or ankle clonus) ☐

Weight (in Kg) *an estimate is acceptable if actual weight is not known or measurable*

Current ☐ Baseline Weight ☐

RIG needed/ in-situ: **Yes** ☐ ☐ **No**

Respiratory symptoms (exertional dyspnea, orthopnoea or excessive daytime sleepiness):

Yes ☐ No ☐

Respiratory function *Please complete what is available*

SNIP (cm H2O) ☐ Recent SNIP in last 3 months: ☐

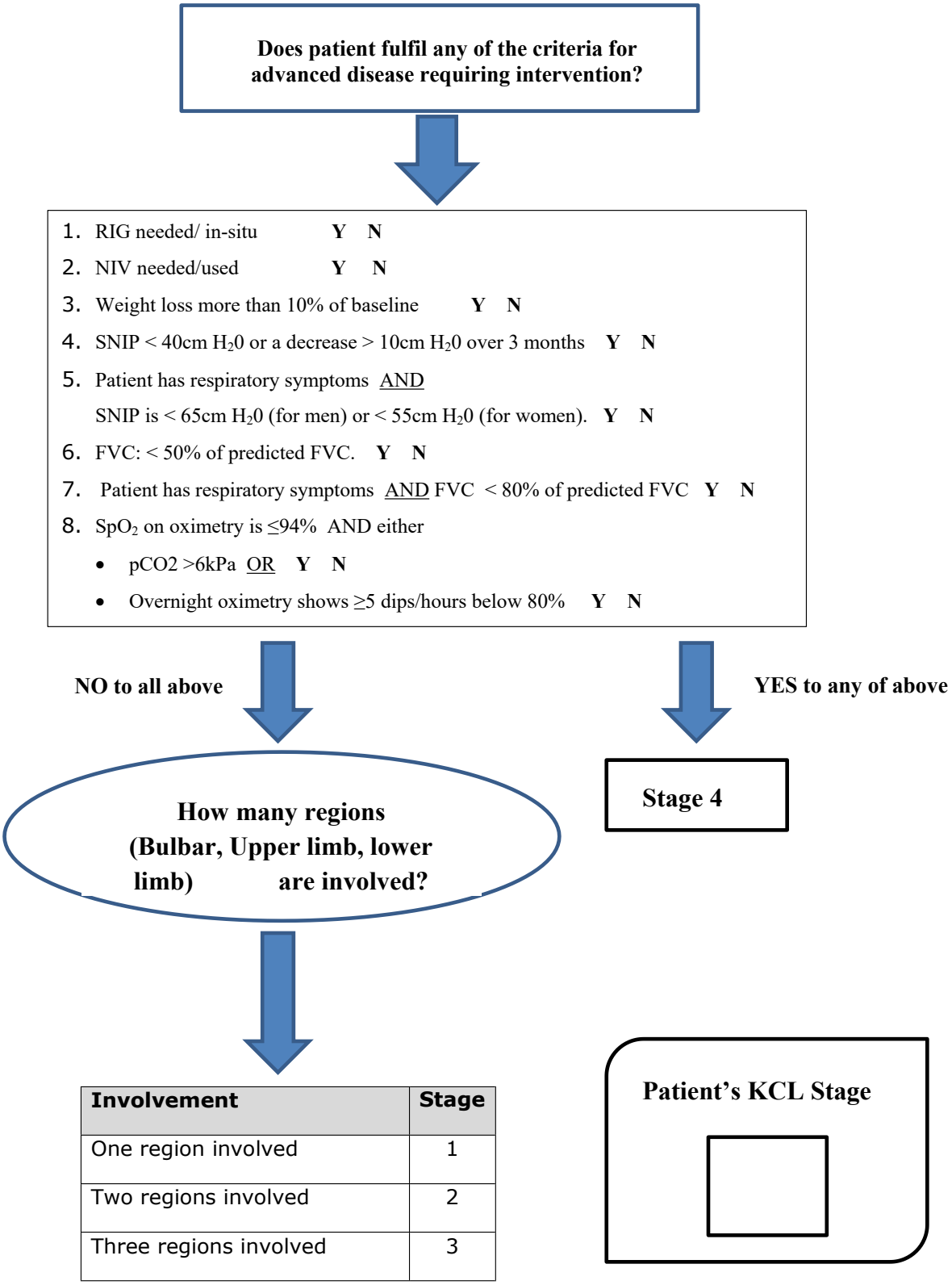
FVC ☐

Pulse Oximetry SpO_2 ☐ pCO_2 ☐

NIV needed/used: **Yes** ☐ ☐ **No**

NOTE: *If pulse oximetry is the only measure used to test respiratory function AND SpO_2 is ≤94% AND pCO_2 <6kPa then arrange for the patient to have overnight oximetry*

Version 3, Feb 2016



Version 3, Feb 2016

17.1.4 ALSAQ-40

ALSAQ-40

Please complete this questionnaire as soon as possible. If you have any difficulties filling in the questionnaire by yourself, please get someone else to help you with it. However it is **your** responses that we are interested in.

The questionnaire consists of a number of statements about difficulties that you may have experienced **during the last 2 weeks.** There are no right or wrong answers: your first response is likely to be the most accurate for you. **Please tick the box which best describes your own experience or feelings.**

Please try to answer every question even though some may seem rather similar to others, or may not seem relevant to you.

All the information you give will be treated in the **strictest confidence**.

The following statements all refer to difficulties that you may have had **during the last 2 weeks**.
Please indicate, by ticking the appropriate box, how often the following statements have been true for you.

The following statements all refer to certain difficulties that you may have had during the last 2 weeks. Please indicate, by ticking the appropriate box, how often the following statements have been true for you.

If you cannot walk at all
please tick **Always/cannot walk at all**.

How often during the last 2 weeks have the following been true?

Please tick **one box** for each question

	Never	Rarely	Some-times	Often	Always or cannot walk at all
1. I have found it difficult to walk short distances, e.g. around the house.	☐	☐	☐	☐	☐
2. I have fallen over whilst walking.	☐	☐	☐	☐	☐
3. I have stumbled or tripped whilst walking.	☐	☐	☐	☐	☐
4. I have lost my balance whilst walking.	☐	☐	☐	☐	☐
5. I have had to concentrate whilst walking.	☐	☐	☐	☐	☐

Please make sure that you have ticked **one box for each question** before going on to the next page.

The following statements all refer to certain difficulties that you may have had during the last 2 weeks. Please indicate, by ticking the appropriate box, how often the following statements have been true for you.

If you cannot do the activity at all
please tick **Always/cannot do at all**.

How often during the last 2 weeks have the following been true?

Please tick **one box** for each question

	Never	Rarely	Some-times	Often	Always or cannot do at all
6. Walking has tired me out.	☐	☐	☐	☐	☐
7. I have had pains in my legs whilst walking.	☐	☐	☐	☐	☐
8. I have found it difficult to go up and down the stairs.	☐	☐	☐	☐	☐
9. I have found it difficult to stand up.	☐	☐	☐	☐	☐
10. I have found it difficult to get myself up out of chairs.	☐	☐	☐	☐	☐

Please make sure that you have ticked **one box for each question** before going on to the next page.

The following statements all refer to certain difficulties that you may have had during the last 2 weeks. Please indicate, by ticking the appropriate box, how often the following statements have been true for you.

If you cannot do the activity at all
please tick **Always/cannot do at all**.

How often during the last 2 weeks have the following been true?

Please tick **one box** for each question

	Never	Rarely	Some-times	Often	Always or cannot do at all
11. I have had difficulty using my arms and hands.	☐	☐	☐	☐	☐
12. I have found turning and moving in bed difficult.	☐	☐	☐	☐	☐
13. I have found picking things up difficult.	☐	☐	☐	☐	☐
14. I have found holding books or newspapers, or turning pages, difficult.	☐	☐	☐	☐	☐
15. I have had difficulty writing clearly.	☐	☐	☐	☐	☐

Please make sure that you have ticked **one box for each question** before going on to the next page.

The following statements all refer to certain difficulties that you may have had during the last 2 weeks. Please indicate, by ticking the appropriate box, how often the following statements have been true for you.

If you cannot do the activity at all
please tick **Always/cannot do at all**.

How often during the last 2 weeks have the following been true?

Please tick **one box** for each question

	Never	Rarely	Some-times	Often	Always or cannot do at all
16. I have found it difficult to do jobs around the house.	☐	☐	☐	☐	☐
17. I have found it difficult to feed myself.	☐	☐	☐	☐	☐
18. I have had difficulty combing my hair or cleaning my teeth.	☐	☐	☐	☐	☐
19. I have had difficulty getting dressed.	☐	☐	☐	☐	☐
20. I have had difficulty washing at the hand basin.	☐	☐	☐	☐	☐

Please make sure that you have ticked **one box for each question** before going on to the next page.

The following statements all refer to certain difficulties that you may have had during the last 2 weeks. Please indicate, by ticking the appropriate box, how often the following statements have been true for you.

If you cannot do the activity at all
please tick **Always/cannot do at all**.

How often during the last 2 weeks have the following been true?

Please tick **one box** for each question

	Never	Rarely	Some-times	Often	Always or cannot do at all
21. I have had difficulty swallowing.	☐	☐	☐	☐	☐
22. I have had difficulty eating solid food.	☐	☐	☐	☐	☐
23. I have found it difficult to drink liquids.	☐	☐	☐	☐	☐
24. I have found it difficult to participate in conversations.	☐	☐	☐	☐	☐
25. I have felt that my speech has not been easy to understand.	☐	☐	☐	☐	☐

Please make sure that you have ticked **one box for each question** before going on to the next page.

The following statements all refer to certain difficulties that you may have had during the last 2 weeks. Please indicate, by ticking the appropriate box, how often the following statements have been true for you.

If you cannot do the activity at all
please tick **Always/cannot do at all**.

How often during the last 2 weeks have the following been true?

Please tick **one box** for each question

	Never	Rarely	Some-times	Often	Always or cannot do at all
26. I have slurred or stuttered whilst speaking.	☐	☐	☐	☐	☐
27. I have had to talk very slowly.	☐	☐	☐	☐	☐
28. I have talked less than I used to do.	☐	☐	☐	☐	☐
29. I have been frustrated by my speech.	☐	☐	☐	☐	☐
30. I have felt self-conscious about my speech.	☐	☐	☐	☐	☐

Please make sure that you have ticked **one box for each question** before going on to the next page.

The following statements all refer to certain difficulties that you may have had during the last 2 weeks. Please indicate, by ticking the appropriate box, how often the following statements have been true for you.

How often during the last 2 weeks have the following been true?

Please tick **one box** for each question

	Never	Rarely	Some-times	Often	Always
31. I have felt lonely.	☐	☐	☐	☐	☐
32. I have been bored.	☐	☐	☐	☐	☐
33. I have felt embarrassed in social situations.	☐	☐	☐	☐	☐
34. I have felt hopeless about the future.	☐	☐	☐	☐	☐
35. I have worried that I am a burden to other people.	☐	☐	☐	☐	☐

Please make sure that you have ticked **one box for each question** before going on to the next page.

The following statements all refer to certain difficulties that you may have had during the last 2 weeks. Please indicate, by ticking the appropriate box, how often the following statements have been true for you.

How often during the last 2 weeks have the following been true?

Please tick **one box** for each question

	Never	Rarely	Some-times	Often	Always
36. I have wondered why I keep going.	☐	☐	☐	☐	☐
37. I have felt angry because of the disease.	☐	☐	☐	☐	☐
38. I have felt depressed.	☐	☐	☐	☐	☐
39. I have worried about how the disease will affect me in the future.	☐	☐	☐	☐	☐
40. I have felt as if I have no freedom.	☐	☐	☐	☐	☐

Please make sure that you have ticked **one box for each question.**

Thank you for completing this questionnaire.

17.1.5 C-SSRS

- **C-SSRS Lifetime**
- **C-SSRS Since Last Visit**

COLUMBIA-SUICIDE SEVERITY RATING SCALE (C-SSRS)

Lifetime Recent

Version 1/14/09 m9/12/17

Posner, K.; Brent, D.; Lucas, C.; Gould, M.; Stanley, B.; Brown, G.; Fisher, P.; Zelazny, J.; Burke, A.; Oquendo, M.; Mann, J.

Disclaimer:

This scale is intended to be used by individuals who have received training in its administration. The questions contained in the Columbia-Suicide Severity Rating Scale are suggested probes. Ultimately, the determination of the presence of suicidal ideation or behavior depends on the judgment of the individual administering the scale.

Definitions of behavioral suicidal events in this scale are based on those used in ***The Columbia Suicide History Form****, developed by John Mann, MD and Maria Oquendo, MD, Conte Center for the Neuroscience of Mental Disorders (CCNMD), New York State Psychiatric Institute, 1051 Riverside Drive, New York, NY, 10032. (Oquendo M. A., Halberstam B. & Mann J. J., Risk factors for suicidal behavior: utility and limitations of research instruments. In M.B. First [Ed.]* Standardized Evaluation in Clinical Practice*, pp. 103 -130, 2003.)*

For reprints of the C-SSRS contact Kelly Posner, Ph.D., New York State Psychiatric Institute, 1051 Riverside Drive, New York, New York, 10032; inquiries and training requirements contact posnerk@nyspi.columbia.edu

SUICIDAL IDEATION		
Ask questions 1 and 2. If both are negative, proceed to "Suicidal Behavior" section. If the answer to question 2 is "yes", ask questions 3, 4 and 5. If the answer to question 1 and/or 2 is "yes", complete "Intensity of Ideation" section below.	**Lifetime: Time He/She Felt Most Suicidal**	**Past 1 month**
1. Wish to be Dead Subject endorses thoughts about a wish to be dead or not alive anymore, or wish to fall asleep and not wake up. ***Have you wished you were dead or wished you could go to sleep and not wake up?*** If yes, describe:	**Yes** ☐ **No** ☐	**Yes** ☐ **No** ☐
2. Non-Specific Active Suicidal Thoughts General non-specific thoughts of wanting to end one's life/die by suicide (e.g., *"I've thought about killing myself"*) without thoughts of ways to kill oneself/associated methods, intent, or plan during the assessment period. ***Have you actually had any thoughts of killing yourself?*** If yes, describe:	**Yes** ☐ **No** ☐	**Yes** ☐ **No** ☐
3. Active Suicidal Ideation with Any Methods (Not Plan) without Intent to Act Subject endorses thoughts of suicide and has thought of at least one method during the assessment period. This is different than a specific plan with time, place or method details worked out (e.g., thought of method to kill self but not a specific plan). Includes person who would say, *"I thought about taking an overdose but I never made a specific plan as to when, where or how I would actually do it…and I would never go through with it."* ***Have you been thinking about how you might do this?*** If yes, describe:	**Yes** ☐ **No** ☐	**Yes** ☐ **No** ☐
4. Active Suicidal Ideation with Some Intent to Act, without Specific Plan Active suicidal thoughts of killing oneself and subject reports having <u>some intent to act on such thoughts</u>, as opposed to *"I have the thoughts but I definitely will not do anything about them."* ***Have you had these thoughts and had some intention of acting on them?*** If yes, describe:	**Yes** ☐ **No** ☐	**Yes** ☐ **No** ☐
5. Active Suicidal Ideation with Specific Plan and Intent Thoughts of killing oneself with details of plan fully or partially worked out and subject has some intent to carry it out. ***Have you started to work out or worked out the details of how to kill yourself? Do you intend to carry out this plan?*** If yes, describe:	**Yes** ☐ **No** ☐	**Yes** ☐ **No** ☐
INTENSITY OF IDEATION		
The following features should be rated with respect to the most severe type of ideation (i.e., 1-5 from above, with 1 being the least severe and 5 being the most severe). Ask about time he/she was feeling the most suicidal. <u>Lifetime</u> - ***Most Severe Ideation:*** _____ ***Type # (1-5)*** _____ ***Description of Ideation*** <u>Recent</u> - ***Most Severe Ideation:*** _____ ***Type # (1-5)*** _____ ***Description of Ideation***	Most Severe	Most Severe
Frequency ***How many times have you had these thoughts?*** (1) Less than once a week (2) Once a week (3) 2-5 times in week (4) Daily or almost daily (5) Many times each day	____	____
Duration ***When you have the thoughts how long do they last?*** (1) Fleeting - few seconds or minutes (2) Less than 1 hour/some of the time (3) 1-4 hours/a lot of time (4) 4-8 hours/most of day (5) More than 8 hours/persistent or continuous	____	____
Controllability ***Could/can you stop thinking about killing yourself or wanting to die if you want to?*** (1) Easily able to control thoughts (2) Can control thoughts with little difficulty (3) Can control thoughts with some difficulty (4) Can control thoughts with a lot of difficulty (5) Unable to control thoughts (0) Does not attempt to control thoughts	____	____
Deterrents ***Are there things - anyone or anything (e.g., family, religion, pain of death) - that stopped you from wanting to die or acting on thoughts of suicide?*** (1) Deterrents definitely stopped you from attempting suicide (2) Deterrents probably stopped you (3) Uncertain that deterrents stopped you (4) Deterrents most likely did not stop you (5) Deterrents definitely did not stop you (0) Does not apply	____	____
Reasons for Ideation ***What sort of reasons did you have for thinking about wanting to die or killing yourself? Was it to end the pain or stop the way you were feeling (in other words you couldn't go on living with this pain or how you were feeling) or was it to get attention, revenge or a reaction from others? Or both?*** (1) Completely to get attention, revenge or a reaction from others (2) Mostly to get attention, revenge or a reaction from others (3) Equally to get attention, revenge or a reaction from others and to end/stop the pain (4) Mostly to end or stop the pain (you couldn't go on living with the pain or how you were feeling) (5) Completely to end or stop the pain (you couldn't go on living with the pain or how you were feeling) (0) Does not apply	____	____

SUICIDAL BEHAVIOR *(Check all that apply, so long as these are separate events; must ask about all types)*	**Lifetime**	**Past 3 months**
Actual Attempt: A potentially self-injurious act committed with at least some wish to die, *as a result of act.* Behavior was in part thought of as method to kill oneself. Intent does not have to be 100%. If there is ***any*** intent/desire to die associated with the act, then it can be considered an actual suicide attempt. ***There does not have to be any injury or harm***, just the potential for injury or harm. If person pulls trigger while gun is in mouth but gun is broken so no injury results, this is considered an attempt. Inferring Intent: Even if an individual denies intent/wish to die, it may be inferred clinically from the behavior or circumstances. For example, a highly lethal act that is clearly not an accident so no other intent but suicide can be inferred (e.g., gunshot to head, jumping from window of a high floor/story). Also, if someone denies intent to die, but they thought that what they did could be lethal, intent may be inferred. ***Have you made a suicide attempt?*** ***Have you done anything to harm yourself?*** ***Have you done anything dangerous where you could have died?*** ***What did you do?*** ***Did you_____ as a way to end your life?*** ***Did you want to die (even a little) when you_____?*** ***Were you trying to end your life when you _____?*** ***Or Did you think it was possible you could have died from_____?*** ***Or did you do it purely for other reasons / without ANY intention of killing yourself (like to relieve stress, feel better, get sympathy, or get something else to happen)?*** *(*Self-Injurious Behavior without suicidal intent) If yes, describe:	**Yes** ☐ **No** ☐ Total # of Attempts _____	**Yes** ☐ **No** ☐ Total # of Attempts _____
Has subject engaged in Non-Suicidal Self-Injurious Behavior?	**Yes** ☐ **No** ☐	**Yes** ☐ **No** ☐
Interrupted Attempt: When the person is interrupted (by an outside circumstance) from starting the potentially self-injurious act *(if not for that, actual attempt would have occurred).* Overdose: Person has pills in hand but is stopped from ingesting. Once they ingest any pills, this becomes an attempt rather than an interrupted attempt. Shooting: Person has gun pointed toward self, gun is taken away by someone else, or is somehow prevented from pulling trigger. Once they pull the trigger, even if the gun fails to fire, it is an attempt. Jumping: Person is poised to jump, is grabbed and taken down from ledge. Hanging: Person has noose around neck but has not yet started to hang - is stopped from doing so. ***Has there been a time when you started to do something to end your life but someone or something stopped you before you actually did anything?*** If yes, describe:	**Yes** ☐ **No** ☐ Total # of interrupted _____	**Yes** ☐ **No** ☐ Total # of interrupted _____
Aborted or Self-Interrupted Attempt: When person begins to take steps toward making a suicide attempt, but stops themselves before they actually have engaged in any self-destructive behavior. Examples are similar to interrupted attempts, except that the individual stops him/herself, instead of being stopped by something else. ***Has there been a time when you started to do something to try to end your life but you stopped yourself before you actually did anything?*** If yes, describe:	**Yes** ☐ **No** ☐ Total # of aborted or self-interrupted _____	**Yes** ☐ **No** ☐ Total # of aborted or self-interrupted _____
Preparatory Acts or Behavior: Acts or preparation towards imminently making a suicide attempt. This can include anything beyond a verbalization or thought, such as assembling a specific method (e.g., buying pills, purchasing a gun) or preparing for one's death by suicide (e.g., giving things away, writing a suicide note). ***Have you taken any steps towards making a suicide attempt or preparing to kill yourself (such as collecting pills, getting a gun, giving valuables away or writing a suicide note)?*** If yes, describe:	**Yes** ☐ **No** ☐ Total # of preparatory acts _____	**Yes** ☐ **No** ☐ Total # of preparatory acts _____

	Most Recent Attempt Date:	Most Lethal Attempt Date:	Initial/First Attempt Date:
Actual Lethality/Medical Damage: 0. No physical damage or very minor physical damage (e.g., surface scratches). 1. Minor physical damage (e.g., lethargic speech; first-degree burns; mild bleeding; sprains). 2. Moderate physical damage; medical attention needed (e.g., conscious but sleepy, somewhat responsive; second-degree burns; bleeding of major vessel). 3. Moderately severe physical damage; *medical* hospitalization and likely intensive care required (e.g., comatose with reflexes intact; third-degree burns less than 20% of body; extensive blood loss but can recover; major fractures). 4. Severe physical damage; *medical* hospitalization with intensive care required (e.g., comatose without reflexes; third-degree burns over 20% of body; extensive blood loss with unstable vital signs; major damage to a vital area). 5. Death	*Enter Code* _____	*Enter Code* _____	*Enter Code* _____
Potential Lethality: Only Answer if Actual Lethality=0 Likely lethality of actual attempt if no medical damage (the following examples, while having no actual medical damage, had potential for very serious lethality: put gun in mouth and pulled the trigger but gun fails to fire so no medical damage; laying on train tracks with oncoming train but pulled away before run over). 0 = Behavior not likely to result in injury 1 = Behavior likely to result in injury but not likely to cause death 2 = Behavior likely to result in death despite available medical care	*Enter Code* _____	*Enter Code* _____	*Enter Code* _____

COLUMBIA-SUICIDE SEVERITY RATING SCALE (C-SSRS)

Since Last Visit

Version 1/14/09

Posner, K.; Brent, D.; Lucas, C.; Gould, M.; Stanley, B.; Brown, G.; Fisher, P.; Zelazny, J.; Burke, A.; Oquendo, M.; Mann, J.

Disclaimer:

This scale is intended to be used by individuals who have received training in its administration. The questions contained in the Columbia-Suicide Severity Rating Scale are suggested probes. Ultimately, the determination of the presence of suicidal ideation or behavior depends on the judgment of the individual administering the scale.

Definitions of behavioral suicidal events in this scale are based on those used in ***The Columbia Suicide History Form****, developed by John Mann, MD and Maria Oquendo, MD, Conte Center for the Neuroscience of Mental Disorders (CCNMD), New York State Psychiatric Institute, 1051 Riverside Drive, New York, NY, 10032. (Oquendo M. A., Halberstam B. & Mann J. J., Risk factors for suicidal behavior: utility and limitations of research instruments. In M.B. First [Ed.]* Standardized Evaluation in Clinical Practice*, pp. 103 -130, 2003.)*

For reprints of the C-SSRS contact Kelly Posner, Ph.D., New York State Psychiatric Institute, 1051 Riverside Drive, New York, New York, 10032; inquiries and training requirements contact posnerk@childpsych.columbia.edu

SUICIDAL IDEATION

Ask questions 1 and 2. If both are negative, proceed to "Suicidal Behavior" section. If the answer to question 2 is "yes", ask questions 3, 4 and 5. If the answer to question 1 and/or 2 is "yes", complete "Intensity of Ideation" section below.	Since Last Visit
1. Wish to be Dead Subject endorses thoughts about a wish to be dead or not alive anymore, or wish to fall asleep and not wake up. ***Have you wished you were dead or wished you could go to sleep and not wake up?*** If yes, describe:	**Yes** ☐ **No** ☐
2. Non-Specific Active Suicidal Thoughts General, non-specific thoughts of wanting to end one's life/commit suicide (e.g., *"I've thought about killing myself"*) without thoughts of ways to kill oneself/associated methods, intent, or plan during the assessment period. ***Have you actually had any thoughts of killing yourself?*** If yes, describe:	**Yes** ☐ **No** ☐
3. Active Suicidal Ideation with Any Methods (Not Plan) without Intent to Act Subject endorses thoughts of suicide and has thought of at least one method during the assessment period. This is different than a specific plan with time, place or method details worked out (e.g., thought of method to kill self but not a specific plan). Includes person who would say, *"I thought about taking an overdose but I never made a specific plan as to when, where or how I would actually do it…and I would never go through with it."* ***Have you been thinking about how you might do this?*** If yes, describe:	**Yes** ☐ **No** ☐
4. Active Suicidal Ideation with Some Intent to Act, without Specific Plan Active suicidal thoughts of killing oneself and subject reports having some intent to act on such thoughts, as opposed to *"I have the thoughts but I definitely will not do anything about them."* ***Have you had these thoughts and had some intention of acting on them?*** If yes, describe:	**Yes** ☐ **No** ☐
5. Active Suicidal Ideation with Specific Plan and Intent Thoughts of killing oneself with details of plan fully or partially worked out and subject has some intent to carry it out. ***Have you started to work out or worked out the details of how to kill yourself? Do you intend to carry out this plan?*** If yes, describe:	**Yes** ☐ **No** ☐

INTENSITY OF IDEATION

The following features should be rated with respect to the most severe type of ideation (i.e., 1-5 from above, with 1 being the least severe and 5 being the most severe). ***Most Severe Ideation:*** _____ _____ ***Type # (1-5)*** ***Description of Ideation***	Most Severe
Frequency ***How many times have you had these thoughts?*** (1) Less than once a week (2) Once a week (3) 2-5 times in week (4) Daily or almost daily (5) Many times each day	____
Duration ***When you have the thoughts, how long do they last?*** (1) Fleeting - few seconds or minutes (2) Less than 1 hour/some of the time (3) 1-4 hours/a lot of time (4) 4-8 hours/most of day (5) More than 8 hours/persistent or continuous	____
Controllability ***Could/can you stop thinking about killing yourself or wanting to die if you want to?*** (1) Easily able to control thoughts (2) Can control thoughts with little difficulty (3) Can control thoughts with some difficulty (4) Can control thoughts with a lot of difficulty (5) Unable to control thoughts (0) Does not attempt to control thoughts	____
Deterrents ***Are there things - anyone or anything (e.g., family, religion, pain of death) - that stopped you from wanting to die or acting on thoughts of committing suicide?*** (1) Deterrents definitely stopped you from attempting suicide (2) Deterrents probably stopped you (3) Uncertain that deterrents stopped you (4) Deterrents most likely did not stop you (5) Deterrents definitely did not stop you (0) Does not apply	____
Reasons for Ideation ***What sort of reasons did you have for thinking about wanting to die or killing yourself? Was it to end the pain or stop the way you were feeling (in other words you couldn't go on living with this pain or how you were feeling) or was it to get attention, revenge or a reaction from others? Or both?*** (1) Completely to get attention, revenge or a reaction from others (2) Mostly to get attention, revenge or a reaction from others (3) Equally to get attention, revenge or a reaction from others and to end/stop the pain (4) Mostly to end or stop the pain (you couldn't go on living with the pain or how you were feeling) (5) Completely to end or stop the pain (you couldn't go on living with the pain or how you were feeling) (0) Does not apply	____

Version 1/14/09

SUICIDAL BEHAVIOR *(Check all that apply, so long as these are separate events; must ask about all types)*	Since Last Visit
Actual Attempt: A potentially self-injurious act committed with at least some wish to die, *as a result of act.* Behavior was in part thought of as method to kill oneself. Intent does not have to be 100%. If there is ***any*** intent/desire to die associated with the act, then it can be considered an actual suicide attempt. ***There does not have to be any injury or harm***, just the potential for injury or harm. If person pulls trigger while gun is in mouth but gun is broken so no injury results, this is considered an attempt. Inferring Intent: Even if an individual denies intent/wish to die, it may be inferred clinically from the behavior or circumstances. For example, a highly lethal act that is clearly not an accident so no other intent but suicide can be inferred (e.g., gunshot to head, jumping from window of a high floor/story). Also, if someone denies intent to die, but they thought that what they did could be lethal, intent may be inferred. ***Have you made a suicide attempt?*** ***Have you done anything to harm yourself?*** ***Have you done anything dangerous where you could have died?*** ***What did you do?*** ***Did you_____ as a way to end your life?*** ***Did you want to die (even a little) when you_____?*** ***Were you trying to end your life when you _____?*** ***Or did you think it was possible you could have died from_____?*** ***Or did you do it purely for other reasons / without ANY intention of killing yourself (like to relieve stress, feel better, get sympathy, or get something else to happen)?*** *(*Self-Injurious Behavior without suicidal intent) If yes, describe: **Has subject engaged in Non-Suicidal Self-Injurious Behavior?**	**Yes No** ☐ ☐ Total # of Attempts _____ **Yes No** ☐ ☐
Interrupted Attempt: When the person is interrupted (by an outside circumstance) from starting the potentially self-injurious act *(if not for that, actual attempt would have occurred).* Overdose: Person has pills in hand but is stopped from ingesting. Once they ingest any pills, this becomes an attempt rather than an interrupted attempt. Shooting: Person has gun pointed toward self, gun is taken away by someone else, or is somehow prevented from pulling trigger. Once they pull the trigger, even if the gun fails to fire, it is an attempt. Jumping: Person is poised to jump, is grabbed and taken down from ledge. Hanging: Person has noose around neck but has not yet started to hang - is stopped from doing so. ***Has there been a time when you started to do something to end your life but someone or something stopped you before you actually did anything?*** If yes, describe:	**Yes No** ☐ ☐ Total # of interrupted _____
Aborted Attempt: When person begins to take steps toward making a suicide attempt, but stops themselves before they actually have engaged in any self-destructive behavior. Examples are similar to interrupted attempts, except that the individual stops him/herself, instead of being stopped by something else. ***Has there been a time when you started to do something to try to end your life but you stopped yourself before you actually did anything?*** If yes, describe:	**Yes No** ☐ ☐ Total # of aborted _____
Preparatory Acts or Behavior: Acts or preparation towards imminently making a suicide attempt. This can include anything beyond a verbalization or thought, such as assembling a specific method (e.g., buying pills, purchasing a gun) or preparing for one's death by suicide (e.g., giving things away, writing a suicide note). ***Have you taken any steps towards making a suicide attempt or preparing to kill yourself (such as collecting pills, getting a gun, giving valuables away or writing a suicide note)?*** If yes, describe:	**Yes No** ☐ ☐
Suicidal Behavior: Suicidal behavior was present during the assessment period?	**Yes No** ☐ ☐
Completed Suicide:	**Yes No** ☐ ☐
Answer for Actual Attempts Only	Most Lethal Attempt Date:
Actual Lethality/Medical Damage: 0. No physical damage or very minor physical damage (e.g., surface scratches). 1. Minor physical damage (e.g., lethargic speech; first-degree burns; mild bleeding; sprains). 2. Moderate physical damage; medical attention needed (e.g., conscious but sleepy, somewhat responsive; second-degree burns; bleeding of major vessel). 3. Moderately severe physical damage; *medical* hospitalization and likely intensive care required (e.g., comatose with reflexes intact; third-degree burns less than 20% of body; extensive blood loss but can recover; major fractures). 4. Severe physical damage; *medical* hospitalization with intensive care required (e.g., comatose without reflexes; third-degree burns over 20% of body; extensive blood loss with unstable vital signs; major damage to a vital area). 5. Death	*Enter Code* _____
Potential Lethality: Only Answer if Actual Lethality=0 Likely lethality of actual attempt if no medical damage (the following examples, while having no actual medical damage, had potential for very serious lethality: put gun in mouth and pulled the trigger but gun fails to fire so no medical damage; laying on train tracks with oncoming train but pulled away before run over). 0 = Behavior not likely to result in injury 1 = Behavior likely to result in injury but not likely to cause death 2 = Behavior likely to result in death despite available medical care	*Enter Code* _____