TITLE PAGE

Title:	**A double-blind, randomized, placebo-controlled, combined single and multiple ascending dose study to investigate the safety, tolerability and pharmacokinetic profile of IFB-088, in healthy volunteers**

Compound Number:

Effective Date:

EudraCT number: 2018-000443-29

SPONSOR SIGNATORY:

_____ _____

Date

SPONSOR/MEDICAL MONITOR INFORMATION PAGE

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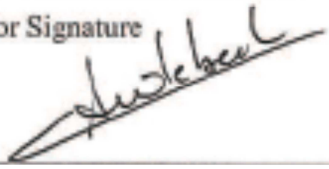
INVESTIGATOR PROTOCOL AGREEMENT PAGE

- I confirm agreement to conduct the study in compliance with the protocol.
- I acknowledge that I am responsible for overall study conduct. I agree to personally conduct or supervise the described clinical study.
- I agree to ensure that all associates, colleagues and employees assisting in the conduct of the study are informed about their obligations. Mechanisms are in place to ensure that site staff receives the appropriate information throughout the study.

SAD

Investigator Name:	Dr Christine Audebert
Investigator Address:	CIC-CPCET Timone hospital Assistance Publique des Hôpitaux de Marseille
Investigator Phone Number:	33 (0)4 91 38 46 41
Investigator Signature 	Date 12 JUIN 2018

MAD

Investigator Name:	Dr Yves Donazzolo
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Investigator Phone Number:	+ 33 438 372 747
Investigator Signature	Date

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SYNOPSIS

Name of Sponsor: InFlectis BioScience	Protocol ID or Number P188	**Name of Investigational product**: IFB-088 Oral capsule	**Name of active ingredients**: IFB-088
Title of Study		A double-blind, randomized, placebo-controlled, combined single and multiple ascending dose study to investigate the safety, tolerability and pharmacokinetic profile of IFB-088, in healthy volunteers	
Principal Investigators		SAD: Dr C. Audebert	Study center CIC-CPCET Hôpital de la Timone- Assistance Publique des Hôpitaux de Marseille
		MAD: Dr Y. Donazzolo	Eurofins Optimed 1 rue des Essarts 38610 Gières
Phase of development		Phase I	
Rationale		This is the first study of single and multiple doses of IFB-088 in human subjects. The current study is designed to assess in the first part, the safety, tolerability, plasma and urine pharmacokinetics (PK) of single oral doses of IFB-088 in healthy subjects (SAD) and in a second part safety, tolerability, plasma and urine pharmacokinetics (PK) of multiple oral doses of IFB-088 in healthy subjects (MAD)	
Objectives		**Primary** To investigate the safety and tolerability of IFB-088 after single and multiple oral doses in healthy volunteers **Secondary** To investigate the pharmacokinetics of IFB-088 after single and multiple oral doses in healthy volunteers	
Study Design		Randomized, double blind, placebo controlled study of single ascending doses (SAD) and multiple ascending doses (MAD) The SAD part consists of 6 cohorts of 8 healthy young male subjects, each receiving a single oral dose of IFB-088 or placebo (6 verum and 2 placebo). In each cohort, 2 subjects (1 verum and 1 placebo) will be dosed first. If the safety and tolerability results are acceptable, the 6 remaining subjects will be dosed by 2 successive groups of 3 subjects, with an adequate period between the 2 groups to detect the occurrence of any reaction or adverse events, namely at least 48H for the first cohort and at least 36H for the following cohorts. Indeed, in the first cohort (2.5 mg IFB-088 base), dosing will be in the morning only. From the second cohort, the planned daily dose will be divided into 2 doses separated by an interval of 12 hours (1 dose in the morning fasting and 1 dose in the evening 2 hours before dinner). The MAD part consists of 3 cohorts of 8 healthy young male subjects, each receiving an oral dose divided into two doses of IFB-088 or placebo (6 verum and 2 placebo) for 14 days. In each cohort, the 2 first subjects will be dosed on Day 1 (one on active treatment and one on placebo). The 6 remaining subjects will be dosed by 2 successive groups of maximum 3 subjects with an adequate period between the groups to observe for any reaction and adverse events. This period will be of at least 36H, corresponding to at least 5-fold the half-life of the drug (based on results obtained during the SAD part) when steady state will be achieved. In each MAD cohort, the total daily dose will be divided into 2 doses separated by an interval of 12 hours.	

<table>
<tr><td>Number of subjects</td><td>Seventy-two (72) healthy young subjects (48 in the SAD part and 24 in the MAD part)</td></tr>
<tr><td>Test product, 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>Capsules</td><td>2.5 to 30 mg</td><td>Oral</td></tr>
<tr><td>Placebo</td><td>Capsules</td><td>NA</td><td>Oral</td></tr>
</table>
<u>SAD</u>

Cohort 1: A single dose of 2.5 mg IFB-088 oral capsule (n=6) or matching placebo (n=2) on Dl

Cohort 2: A single daily dose of 5 mg of IFB-088 oral capsule (n=6) or matching placebo (n=2) divided into 2 doses of 2.5 mg separated by an interval of 12 hours on D1.

Cohort 3: A single daily dose of 10 mg IFB-088 oral capsule (n=6) or matching placebo (n=2) divided into 2 doses of 5 mg separated by an interval of 12 hours on Dl

Cohort 4: A single daily dose of 20 mg IFB-088 oral capsule (n=6) or matching placebo (n=2) divided into 2 doses of 10 mg separated by an interval of 12 hours on Dl

Cohort 5: A single daily dose of 40 mg IFB-088 oral capsule (n=6) or matching placebo (n=2) divided into 2 doses of 20 mg separated by an interval of 12 hours on Dl

Cohort 6: A single daily dose of 60 mg IFB-088 oral capsule (n=6) or matching placebo (n=2) divided into 2 doses of 30 mg separated by an interval of 12 hours on Dl

Escalation to the next higher dose and any dose adjustments of the next dose levels will be based on safety and tolerability results of the previously administered dose and PK data of the previous dose cohorts. There will be a 2-fold increase from the first dose level (2.5 mg) to the second dose level. The dose levels of the following groups will be increased by 2-fold from the previous dose level from cohort 3 to cohort 5 and 1.5-fold from cohort 5 to the last cohort.

<u>MAD</u>

Cohort 1: A daily dose of 15 mg of IFB-088 oral capsule (n=6) or matching placebo (n=2), divided into 2 doses of 7.5 mg, separated by an interval of 12 hours from D1 to D14

Cohort 2: A daily dose of 30 mg of IFB-088 oral capsule (n=6) or matching placebo (n=2), divided into 2 doses of 15 mg, separated by an interval of 12 hours from D1 to D14

Cohort 3: A daily dose of 50 mg of IFB-088 oral capsule (n=6) or matching placebo (n=2), divided into 2 doses of 25 mg, separated by an interval of 12 hours from D1 to D14

Escalation to the next higher dose and any dose adjustments of the next dose levels will be based on safety, tolerability and PK results of the previously administered dose.</td></tr>
<tr><td>Inclusion criteria</td><td>Subjects must satisfy all of the following inclusion criteria:

1. Healthy male 18 to 40 years of age inclusive, Caucasian.
2. Healthy as determined by a responsible and experienced physician, based on a medical evaluation including medical history, physical examination, laboratory tests, vital signs and ECG.
3. AST, ALT, alkaline phosphatase and bilirubin ≤ 1.5xULN (isolated bilirubin >1.5xULN is acceptable if bilirubin is fractionated and direct bilirubin <35%).
4. ECG (12 leads) normal (120<PR<200ms; QRS<120ms; QTcF<450ms) and/or without clinically relevant impairments as judged by investigator.</td></tr>
</table>

	5. Non-smoker or user of not more than tobacco- or nicotine-containing products ≤ 5 cigarettes a day. 6. Negative screen for alcohol and drugs of abuse at screening and admission. 7. No history of psychiatric disorders assessed by a clinical psychological evaluation and the Mini International Neuropsychiatric Interview (MINI). 8. Body mass index (BMI) between 19 and 27 kg/m² inclusive. 9. Subject with female partners of child bearing potential must agree to use one of the contraception methods listed in Section 6.6.1 (Contraception requirements). This criterion must be followed from the time of the first dose of study medication until the follow up visit (for female partners) and with an additional period of 90 days (for subjects themselves). 10. Willing and able to understand and sign an approved Informed Consent Form. 11. Able to understand the protocol and to come to the visits. 12. Who is, in the judgement of the investigator likely to be compliant during the study. 13. Subject registered in the VRB file (volontaires se prêtant à des recherches impliquant la personne humaine). 14. Covered by Health Insurance System and / or in compliance with the recommendations of National Law in force relating to biomedical research.
Exclusion criteria	A subject will not be eligible for inclusion in this study if any of the following criteria apply: 1. History of asthma, anaphylaxis or anaphylactoid reactions, severe allergic responses. 2. History of relevant atopy or drug hypersensitivity. 3. Known allergy to any component of IFB-088 oral capsule or its placebo (HPMC or cellulose microcrystalline). 4. History of major medical, psychiatric illness or surgery which, in the judgment of the investigator, puts them 'at risk' or is likely to modify their handling of the study drug. 5. Acute or chronic systemic disease or disorder (respiratory, gastrointestinal, renal, hepatic, haematological, lymphatic, neurological, cardiovascular, psychiatric, musculoskeletal, genitourinary, immunological, dermatological, endocrine). 6. Impaired renal function defined by a creatinine clearance < 90 mL/min calculated using the Cockcroft-Gault equation (according the FDA Guidance for Industry: Pharmacokinetics in patients with Impaired Renal Function, March 2010). 7. History of nephritic colic and/or renal calculi. 8. History of drug abuse and/or regular use of tobacco- or nicotine-containing products > 5/day within three months of the study. 9. History of alcohol consumption exceeding, (on average 21 drinks/week for men) within 6 months of the first dose of study medication. 10. Drinking excessive amounts of tea, coffee, chocolate and/or beverage containing caffeine (> 4 cups / day). 11. Vital signs with a clinically significant abnormality at screening. 12. ECG with a clinically significant abnormality at screening. 13. Laboratory test values outside the clinically acceptable 'normal range' for healthy volunteers at screening. 14. Positive HIV, Hepatitis B or Hepatitis C at screening. 15. Positive urine drug test or positive breath alcohol test at screening or at admission to the clinical unit. 16. Any medication (including St John's Wort) within 14 days before administration, or within 5 times the elimination half-life of that drug, whichever is the longest (except paracetamol). 17. Treatment with an investigational drug within 30 days or 5 half-lives (whichever is longer) prior to screening. 18. Unable to refrain from consumption of grapefruit or grapefruit juice within 7 days prior to the first dose of study medication.

	19. Unwillingness to abstain from sexual intercourse with pregnant or lactating women or to use a condom and spermicide and another form of contraception (e.g., IUD, birth control pills taken by female partner, diaphragm with spermicide) if engaging in sexual intercourse with a woman who could become pregnant until discharge from the study and during 90 additional days. 20. Subjects unlikely to co-operate in the study, and/or poor compliance anticipated by the investigator. 21. Subject being in the exclusion period of a previous trial. 22. Subject having exceeded the earnings for the last 12 months, including the indemnities for the present study. 23. Subject who could not be contacted in case of emergency. 24. Subject refusing to give written informed consent. 25. Subject who has received blood or plasma derivatives in the year preceding the study. 26. Subject who has given blood within the past 3 months or has planned to give blood or sperm within the 90 days following the study. 27. Subject who has forfeited their freedom by administrative or legal award, or who is under guardianship or under limited judicial protection.
Concomitant medications and study restrictions	Subjects must abstain from taking prescription or non-prescription drugs (including vitamins and dietary or herbal supplements), within 14 days or 5 half-lives (whichever is longer) prior to the first dose of study medication until completion of the follow-up visit. Occasional use of paracetamol at doses up to 2 grams/day will be permitted at the principal investigator's discretion. Subjects will abstain from alcohol-, caffeine- or xanthine-containing beverages (e.g. coffee, tea, cola drinks, chocolate) for 24 hours prior to the start of dosing until collection of the final PK sample during each session. Subjects will abstain from grapefruit or grapefruit-containing products for 7 days prior to the first dosing until follow-up visit and from strenuous exercise for 48 hours prior to each blood collection for clinical laboratory tests.
Procedures	**<u>Screening</u>** Subjects will be screened for eligibility between D-21 and D-2 before the first study drug administration. Written informed consent will be obtained before any study procedure. The screening will consist of: medical history, full physical examination (inc1uding height and body weight), vital signs (supine and standing systolic and diastolic blood pressure, pulse rate and tympanic body temperature), 12-lead ECG, haematology, coagulation, plasma biochemistry and urinalysis tests, viral serology testing (hepatitis B antigen, Hepatitis C antibodies, Anti-HIV1 and Anti HIV2 antibodies), drugs of abuse and alcohol screen, psychological interview and review of the eligibility criteria. The results of screening must be known to the investigator prior to the subject's admission. **<u>Admission</u>** Subjects will be admitted to the clinical unit one day prior to dosing (D-1) to undergo the following assessments: • Medical history and physical examination update, • Vital signs measurement (inc1uding tympanic body temperature), • 12-lead ECG, • Laboratory evaluation (haematology, coagulation, biochemistry, urinalysis), • Urine drug screen, • Alcohol breath test, • AE monitoring,

	• Review of concomitant medications, • Verification of the eligibility criteria, • Randomization to the treatment group after check of all criteria. The results of admission assessments must be known by the investigator prior to the subjects dosing. **Hospitalization period** ♦ **SAD** Subjects will be required to stay in the unit for up to 2 nights for the treatment period from Day-1 to Day 2. Subjects will be dosed on Day 1 and will be discharged after completion of all assessments and with medical authorization at Day 2, 32 hours after first dosing. ♦ **MAD** Subjects will be required to attend the unit on the day (Day -1) prior to the first dose and to stay in the unit for 17 days. Subjects will be dosed from Day 1 to Day 14 and will be discharged after completion of all assessments and with medical authorization at Day 17. **Follow-up (FU)** Subjects will be required to return to the unit for a follow-up visit between 7 days and 14 days after last dosing or early discontinuation, for safety assessments: physical examination; vital signs (supine and standing systolic and diastolic blood pressure, pulse rate and tympanic body temperature), 12-lead ECG, haematology, coagulation, plasma biochemistry and urinalysis tests.
Evaluation criteria	**Safety assessments** ♦ **SAD** • Adverse events (AEs): throughout the study period i.e. from screening to follow-up visit • Full physical examination at screening, discharge and FU and physical examination update at Day-1 and throughout the study on medical indication at the discretion of the investigator • Supine and standing vital signs: on D-1; Dl at pre-dose (in triplicate), and 0.5, 1, 2, 3, 4, 6, 8, 12, 24 and 32h post dose; FU • Tympanic body temperature: on D-1; D1 pre-dose; and 1.5, 12, 24 and 32h post dose; FU • ECG on D-l; Dl at pre-dose (in triplicate) and 1, 2, 4, 8, 12, 24 and 32h post dose; FU • Laboratory evaluation: haematology, coagulation and biochemistry on D-1, D2 24h post dose and FU; urinalysis on D-l; Dl at pre-dose and 12h, 24h and 32h post dose; FU • Vigilance (Bond and Lader VAS) on D1 pre-dose and 1.5, 12 and 32h post dose ♦ **MAD** • Adverse events (AEs): throughout the all study period i.e. from screening to follow-up visit • Full physical examination at screening, discharge and FU and physical examination update at Day-1, D3, D6, D10 and D13 before morning dose and throughout the study on medical indication at the discretion of the investigator; • Supine and standing vital signs: on D-1; Dl at pre-dose (in triplicate), and 0.5, 1, 2, 3, 4, 6, 8, 12 post first dose; D2 24h post first dose; every morning pre dose from D3 to D17; FU

- Tympanic body temperature: on D-1; D1 pre-dose, 1.5 and 12h post first dose; on D2, D4, D6, D8, D10 and D12 every morning before dosing; on D14 at pre dose, and 1.5, 12h post dose; on D17 before discharge; FU
- ECG on D-l; Dl at pre-dose (in triplicate) and 1, 2, 4, 8, 12h post first dose; D3, D6, D10 and D13 in the morning before dosing and D17 at discharge; FU
- Laboratory evaluation: haematology, coagulation and biochemistry on D-1, D3, D6, D10 and D13 in the morning before dosing and D17 at discharge; FU; urinalysis on D-l; Dl at pre-dose, D3, D6, D10 and D13 in the morning before dosing and D17 at discharge; FU
- Vigilance (Bond and Lader VAS) on D1 pre-dose and 1.5, 12h post dose; on D14 pre dose and 1.5, 12h post dose

Pharmacokinetic Assessments in blood

♦ **SAD**

First cohort (dosing in the morning): blood sampling for PK of IFB-088 and its metabolite at pre-dose and 0.16, 0.33, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 5, 8, 12, 24 and 32h post dose

From Cohort 2 (daily dose separated in 2 administrations with a 12-hour interval): blood sampling for PK of IFB-088 and its metabolite at pre-dose and 0.33, 0.66, 1, 2, 3, 4, 5, 12, 12.25, 12.5, 13, 14, 16, 18, 24 and 32h post morning dose

♦ **MAD**

Blood sampling for PK of IFB-088 and its metabolite at D1and D14 pre-dose and 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 13, 14, 17, 19 and 21h post morning dose; D2 24h post first dose just before the morning administration; D7 pre dose, 1h and 2 h post dose, D15 at 24, 30, 36 and 42h post D14 morning dose; D16 at 48h and 60h post D14 morning dose

Pharmacokinetic Assessments in urine

♦ **SAD**

Urine sampling for PK of IFB-088 and its metabolite IFB-139:

Cohort 1: pre-dose and 0-4, 4-8, 8-16, 16-32 h post-dose intervals.

From Cohort 2: pre-dose and 0-4, 4-8, 8-12, 12-24, 24-32h post-dose intervals

♦ **MAD**

Urine sampling for PK of IFB-088 and its metabolite: on D1 and D14: at pre-dose and 0-4, 4-12, 12-24 h post first dose; D6 at pre-dose, 0-4 and 4-12h; D15 at 24-36 and D16 at 36-48h post D14 morning dose

Biomarker assessments (exploratory)

There is currently no validated specific biomarker. So subjects will be asked to donate blood samples that could be used as healthy controls for research purposes in patients. A specific part in the informed consent will be signed.

This assessment will be performed only for SAD part.

♦ **SAD**

Samples will be drawn at specific time points: Pre-dose, 1.5h and 24h post dose

- One sample (3 ml) on heparin lithium tube for PBMC extraction
- One sample (2.5ml) on PAXgene tube for RNA extraction

Samples will be stored in the CRB (Centre de Ressources Biologiques) at -150°C for 5 years after final database freeze and then destroyed.

	♦ **MAD** Sampling for biomarkers will not be performed during the MAD part.
Statistical methods	Data entry and statistical analysis will be performed under the responsibility of the CIC-CPCET Pharmacometrics unit. The statistical software SAS® version 9.4 (SAS Institute Inc. Cary NC USA) will be used in statistical analysis. **Safety parameters** Laboratory results, vital signs measurements (blood pressure, heart rate, tympanic temperature), ECG parameters and VAS scores will be summarized using appropriate descriptive statistics. The incidence of all AEs and treatment-emergent AEs will be described by MedDRA® 21.0 or the latest available version at the study completion date (preferred term and system organ class). **PK parameters** The following parameters for IFB–088 and IFB-139 will be derived, where appropriate: ***Plasma*** • Maximum observed plasma concentration (Cmax), • Time of occurrence of Cmax (Tmax), • Terminal elimination rate constant (Kel), • Terminal half-life (t1/2), • Area under plasma concentration-time curve from hour 0 to last sample with measurable plasma concentrations (AUClast), • Area under plasma concentration-time curve from hour 0 to infinity (AUC0-∞), • Apparent volume of distribution (Vd/F), • Apparent body clearance (CL/F), • Last measurable plasma concentration (Clast), • Time to reach last measurable plasma concentration (Tlast) • Concentration at the end of a dosing interval before the next dose administration (Ctrough), ***Urine*** • Maximum observed urine concentration (CmaxU), • Time of occurrence of Cmax (tmaxU), • Area under urine concentration-time curve from hour 0 to last sample with measurable urine concentrations (AUClast), • Area under urine concentration-time curve from hour 0 to infinity (AUC0-∞), • Renal clearance (CLr), • Total amount excreted in urine (Ae), • Percent of drug recovered in urine (Ae %dose). Pharmacokinetic modelling will be performed using a non-linear mixed effects model with NONMEM software (ICON Development Solutions v7.4). Data will be analysed using a first order conditional estimation method. The R software (www.r-project.org, v3.2.2) will be used for goodness-of-fit diagnostics and graphical displays. Descriptive statistics and graphs will be performed for plasma and urine concentrations and PK parameters.

ABBREVIATIONS

AE	Adverse Event
ALT	Alanine aminotransferase (SGPT)
ANOVA	Analysis of Variance
AST	Aspartate aminotransferase (SGOT)
ATF4	Activating transcription factor 4
AUC	Area under concentration-time curve
AUC(0-∞)	Area under the concentration-time curve from time zero (pre-dose) extrapolated to infinite time
AUC(last)	Area under the concentration-time curve from time zero (pre-dose) to last time of quantifiable concentration within a subject across all treatments
AUC(0-24)	Area under the concentration-time curve from time zero (pre-dose) to 24 hour
BID	Twice a day
BMI	Body mass index
BP	Blood pressure
CI	Confidence Interval
CL/F	Apparent clearance following oral dosing
Cmax	Maximum observed concentration
CMT	Charcot Marie Tooth
Ct	Last observed quantifiable concentration
CPK	Creatine phosphokinase
CRF	Case Report Form
CV	Coefficient of variance
DBP	Diastolic blood pressure
DEC	Dose Escalation Committee
DMP	Data Management Plan
DNA	Desoxyribonucleic acid
ECG	Electrocardiogram
EMA	European Medicine Agency
ER	Endoplasmic Reticulum
FDA	Food and Drug Administration
FIH	First in human
GCP	Good Clinical Practice

GFR	Glomerular Filtration Rate
GGT	Gamma glutamyltransferase
GLP	Good Laboratory Practice
GMP	Good Manufacturing Practice
HBsAg	Hepatitis B surface antigen
HIV	Human Immunodeficiency Virus
h	Hour(s)
HED	Human Equivalent Dose
hERG	Human Ether-à-go-go-Related Gene
HNPP	Hereditary neuropathy with liability to pressure palsies
HR	Heart rate
IB	Investigator's Brochure
ICH	International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use
IC_{50}	Half maximal inhibitory concentration
IgM	Immunoglobulin M
IMP	Investigational Medicine Product
IMPD	Investigational Medicine Product Dossier
INR	International Normalized Ratio
IUD	Intrauterine device
Kg	Kilogram
L	Liter
LDH	Lactate Deshydrogenase
µg	Microgram
µL	Microliter
MAD	Multiple Ascending Dose
MPZ	Myelin Protein Zero
MPZS63del	Transgenic myelin protein zero S63del mouse model
MPZR98C/+	Heterozygous myelin protein zero R98C knock-in mouse model
MRSD	Maximum Recommended Starting Dose
MTD	Maximal Tolerated Dose
NOAEL	No Observed Adverse Effect Level

PK	Pharmacokinetic
PMP22	Peripheral myelin protein 22
PP1	Protein Phosphatase 1
PP/PPP1R15A	Stress-induced holophosphatase complex
PPP1R15A	Protein phosphatase 1 regulatory subunit 15A
PPP1R15B	Protein phosphatase 1 regulatory subunit 15B
QoL	Quality of Life
QTcB	QT duration corrected for heart rate by Bazett's formula
QTcF	QT duration corrected for heart rate by Fridericia's formula
RBC	Red blood cells
RNA	Ribonucleic acid
SAD	Single ascending dose
SAE	Serious adverse event(s)
SAP	Statistical Analysis Plan
SID	Once a day
SPM	Study Procedures Manual
TEAE	Treatment-emergent adverse event
Tlast	Time to reach last measurable plasma concentration
t½	Terminal phase half-life
Tmax	Time of occurrence of Cmax
ULN	Upper limit of normal
UPR	Unfolded Protein Response
VAS	Visual Analogic Scale
WBC	White blood cells
WT	Wild Type

1. INTRODUCTION

1.1. Background

The unfolded protein response (UPR) is a stress response of the endoplasmic reticulum (ER) to a disturbance in protein folding and homeostasis (Hetz et al, 2013), observed in many diseases, including cancer, diabetes, autoimmune conditions, liver disorders, obesity, and neurodegenerative disorders. When this adaptive mechanism is insufficient to handle the unfolded protein load, cells undergo apoptosis. By targeting components of this signaling response, it may become possible to reverse their toxic effects and so to treat a wide range of neurodegenerative disorders (Charcot-Marie-Tooth, Amyotrophic Lateral Sclerosis, Multiple Sclerosis…) (Roussel et al, 2013).

Charcot-Marie -Tooth (CMT), a group of rare inherited peripheral neuropathies, is one of the most common degenerative neurological disorders (mean prevalence: 1/2500). It is characterized by progressive development of symptoms, the main ones being symmetric distal limb weakness, atrophy, foot deformity, musculoskeletal and neuropathic pain, loss of dexterity, fatigue, and sensory loss (Pareyson et al, 2009). Quality of life (QoL) is highly compromised as patients encounter muscle atrophy and thus difficulties in carrying out fine motor skills (El-Abassi et al, 2014). CMT is thus a debilitating disease which, while qualified as rare, still presents a "high" prevalence rate for an orphan condition**.** CMT1A subtype is by far the most common form of CMT (prevalence 1:5000)**.** It is a demyelinating neuropathy associated with an autosomal dominant 1.4 Mb duplication on chromosome 17p11.2 that includes the peripheral myelin protein 22 gene (PMP22) expressed predominantly in the compact myelin of Schwann cells (SC).

Preclinical and clinical studies strongly suggest that the duplication of PMP22 gene is responsible for CMT1A. Correct stoichiometry of PMP22 is required to maintain compact myelin integrity; too much PMP22 (i.e. duplication) causes CMT1A; too little (PMP22 gene deletion) causes hereditary neuropathy with liability to pressure palsies (HNPP). PMP22 protein folds with low efficiency under normal conditions (Sanders et al., 2001) and nearly 80% of the newly synthetized protein is rapidly degraded. In response to PMP22 overexpression in CMT1A, excessive PMP22 polypeptides accumulate in cytosolic aggregates and are targeted to degradation by the ubiquitin proteasome system (Fortun et al 2006). Autophagic and lysosomal components as well as heat shock proteins (HSPs) are recruited to ubiquitin positive PMP22 aggregates, likely reflecting an attempt by the cells to clear them through alternate pathways. Evidences suggest that PMP22 overexpression in SC, leads to the activation of UPR as accredited by the expression of PPP1R15A in the sciatic nerve of CMT1A transgenic rats (N. Callizot, P. Miniou & P. Guédat, Presentation at the 6th International Consortium Meeting of CMT and related neuropathy, September 2016, Italy).

This process likely contributes to SC dysfunction, peripheral nerve demyelination, and secondary axonal damages which are the major cause of weakness in CMT1A (van Paassen et al, 2014). SC which are involved in axonal support, survival and protection of neurons, and the formation of myelin sheath of the peripheral nervous system (PNS), acquire a persistent differentiation defect in CMT1A. As a result, SC lose their differentiated molecular markers and structural features leading to re-expression of more immature molecular markers, such as Neural Cell Adhesion Molecule (NCAM) (Klein et al. 2014) and c-Jun (Hanemann et al. 1996; Hutton et al. 2011; Sociali et al. 2016) and the proliferation of immature non-myelinating SC ensues.

The last decade has seen the first CMT clinical trials, however there is still no cure for CMT and no effective treatment to reverse or slow the underlying disease process (Micallef et al. 2009, Gess, et al. 2015). While several drugs are under examination, there are only few ongoing clinical trials (PXT3003 [combination of baclofen, naltrexone, and sorbitol], and ulipristal).

1.1.1. *Targeting PPP1R15A in CMT*

Myelin-forming cells such as Schwann cells in peripheral nervous system which synthetize a large amount of myelin proteins and lipids are particularly susceptible to endoplasmic reticulum quality control failure. Activation of ER stress/UPR have been involved in several myelin disorders, such as CMT neuropathies (Pennuto et al., 2008, Neuron; Saporta at al., 2012, Brain; Okamoto et al., 2013, Hum Mol Genet, D'Antonio et al., 2009, J. Neurosci. Res.). Indeed, the translation arm of the UPR has been shown to be activated and PPP1R15A expression level increased in sciatic nerve from *MPZ*S63del mice model of CMT1B (Pennuto et al., 2008, Neuron, D'Antonio et al., 2013, JEM). Activation of UPR response has also been described in the *MPZ*R98C mice CMT1B model (Saporta et al., 2012, Brain). In defective nerve tissues of a transgenic rat model over-expressing PMP22 gene that closely mimics autosomal dominant CMT1A (Sereda et al., 1996) PPP1R15A protein expression has been detected in CMT1A sciatic nerve samples from 110 days old rats. In contrast PPP1R15A expression was detected in none of the analysed WT samples. In addition, the expression of the transcription factor ATF-4 which regulates PPP1R15A expression is detected exclusively in the CMT1A samples analysed (Unpublished data - InFlectis Bioscience, Neurosys - IIS2017001/IIS2016001).

In the context of these pathologies, restoring protein homeostasis by prolongating protein translation attenuation by a selective inhibitor of PPP1R15A activity might provide a therapeutic strategy to cure and or limit CMT pathology evolution.

1.1.2. *IFB-088*

The first validated selective inhibitor of PPP1R15A is Guanabenz (2-(2,6-dichlorobenzylidene)-hydrazinecarboximidamide) (Tsaytler et al. Science 2011), an alpha-2 adrenergic receptor agonist that was used as an antihypertensive drug for decades under the trademark Wytensin. The hypotensive activity of Guanabenz limits its potential use in CMT.

A medicinal chemistry research program using Selective Optimization of Side Activity (SOSA) strategy (*Wermuth CG, Drug Discovery Today 2006)* was launched to identify selective inhibitors of PPP1R15A without alpha-2 adrenergic activity.

IFB-088 (2-(2-chlorobenzylidene) hydrazinecarboximidamide acetate), a close chemical derivative of Guanabenz (GBZ), selective inhibitor of PPP1R15A correcting protein misfolding defects, devoid of alpha-2 adrenergic activity, was identified from this program.

InFlectis BioScience's drug candidate, IFB-088, a first-in-class, orally available, would be the first treatment ever targeting the UPR in the frame of neurological diseases (Charcot- Marie-Tooth disease type 1A and type 1B - CMT1A and CMT1B).

IFB-088 mechanism of action in CMT is validated and published (Das et al. 2015; 6th International Consortium Meeting of CMT and related neuropathy September 2016, Italy). The implementation of a series of in vitro and in vivo CMT1 (types A & B) studies demonstrate that IFB-088 is a promising potential therapeutic agent for CMT patients.

An Orphan drug designation was granted to IFB-088 for the treatment of CMT, by the FDA (USA, 10 September 2015) and by the EMA (EU, 14 December 2015).

1.1.2.1. *Non-clinical data*

Full details on nonclinical data are provided in the Investigator's Brochure. Nonclinical studies have been performed in accordance with ICH guidance, and Good Laboratory Practices.

1.1.2.1.1. *Pharmacology*

In vitro pharmacology studies demonstrated that the selective PPP1R15A inhibition by IFB-088 prolongs protein translation attenuation, delays and attenuates the activation of the stress response pathway in stressed cells only, thereby restoring proteostasis and improving stressed cells survival. The absence of effect under non-stressed conditions has also been confirmed.

Therapeutic IFB-088 efficacy has been demonstrated in different CMT1 animal models: decreased PMP22 protein expression in sciatic nerves and improvement of the motor deficits and sensory performances in CMT1A rats, improvement of myelination in ex vivo dorsal root ganglia cultures of CMT1B models, improvement of motor deficits and increased myelin thickness in CMT1B MPZS63del mouse model.

Potential off target effects reported with IFB-088 as well as a moderate hERG channel blocker effect are observed in IC50 ranges which are not expected to be reached in humans. The moderate effect on the hERG channel was identified in vitro whereas the in vivo telemetry conducted in dogs confirmed that IFB-088 has no effect on dog cardiovascular function. Finally, three in vivo safety pharmacology studies conducted in rats and dogs demonstrated that IFB-088 has no effect on CNS, respiratory and cardiovascular functions up to 9 mg/kg/day when administered once or twice daily.

1.1.2.1.2. *Pharmacokinetics and metabolism*

Several pharmacokinetic studies were conducted in mice, rats and dogs. IFB-088 is absorbed after single oral administration in Sprague Dawley rats with an oral bioavailability of 15%. The pharmacokinetic and the tissue distribution profile of IFB-088 in Sprague Dawley rats indicate that IFB-088 reach the CMT target organ (sciatic nerve). In rats, PK was found to be non-linear based on AUC values, and different between males and females. In dogs, no relevant difference was seen in the exposure between the sexes, and the exposures (AUC0-24) increased proportionally within the dose and remained similar on day 28, after repeated dosing, compared to day 1 (single dose). An in vitro study on CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6 and CYP3A4 did not evidence potential for IFB-88 to inhibit CYP450. No major

differences were observed regarding metabolism profile in human hepatocytes and other animal species especially dog and rat hepatocytes models.

1.1.2.1.3. *Toxicology*

The safety of IFB-088 was evaluated in CD1 mice, Sprague Dawley rats and Beagle dogs. Preliminary toxicity studies emphasized renal findings after 10 days of daily administration from 10 mg/kg in rats and for daily administration of 6 mg/kg for 14 days in dogs (minimal to slight tubule vacuolation in the kidneys). In rats, in a 28-day toxicity study, daily administration of 9 mg/kg was also considered as adverse mainly due to renal and hematologic findings. The No Observed Adverse Effect Level (NOAEL) was established at 3 mg/kg/day or 1.5 mg/kg/BID in Sprague Dawley rats. In Beagle dogs, twice daily administration of 4.5 mg/kg (9 mg/kg/day) for 28 days was well tolerated (NOAEL in this species) without any renal findings, suggesting that when administered twice daily IFB-088 is better tolerated than when daily administered.

In vitro genotoxicity studies conducted with IFB-088 showed a weak mutagenic effect in non-mammalian and mammalian cell systems at the maximum. Following equivocal or positive in vitro genotoxicity results, an in vivo genotoxicity study was performed in rats to ward off a genotoxic risk for human; in Sprague Dawley rats after 3 days daily administration up to 20 mg/kg, the in vivo micronucleus test in bone marrow tissue combined to an in vivo Comet assay on liver tissue indicate that IFB-088 had no genotoxic activity in vivo. More information on genotoxicity is available in the expert report provided in appendix of the IMPD.

1.2. **Rationale**

1.2.1. *Study Rationale*

IFB-088 is intended to be developed for the treatment of Charcot-Marie-Tooth disease and also other degenerative pathologies resulting from the accumulation of misfolded proteins. IFB-088 has not been previously administered in human subjects.

The current First-In-Human (FIH) study (Phase 1) aims at examining the safety, tolerability, plasma pharmacokinetics (PK) of single doses and repeated doses of IFB-088 in healthy volunteers. This Phase 1 study is designed to support future clinical developments of IFB-088 in CMTs and other degenerative pathologies, such as Multiple Sclerosis, Amyotrophic Lateral Sclerosis, Oculo-Pharyngeal Muscular Dystrophy and Retinitis Pigmentosa for which preclinical efficacy in animal models has been obtained.

The current study will include 2 sequential parts.

The first part of the current study will examine the safety, tolerability, plasma and urine pharmacokinetics (PK), of single doses of IFB-088 in healthy subjects (SAD).

The second part of this study will examine the safety, tolerability, plasma and urine pharmacokinetics (PK), of multiple doses of IFB-088 in healthy subjects (MAD).

1.2.2. *Dose Rationale for the SAD part*

The dose selection of IFB-088 is based on the No Observed Adverse Effect Level (NOAEL) in animals as per the principles laid down in the Guideline on strategies to identify and mitigate risks for first-in human clinical trials with IMPs (EMEA/CHMP/SWP/28367/07 Rev.1) and the anticipated pharmacokinetic and pharmacodynamic response in humans, using available preclinical biology, toxicology, and pharmacokinetic data from mice, rats, and dogs.

This range of selected doses has the double objective 1) to ensure the safety of subjects participating in the study and 2) to cover the doses at which this New Chemical Entity is expected to exert a beneficial effect in patients affected with CMT as well as in the above-mentioned degenerative diseases.

1.2.2.1. *Calculation of Safety Coverage*

Toxicology studies in rodent (mice, rat) and non-rodent animal species (dog) were conducted to assess IFB-088 safety (see section 4.3.3 & 4.3.4 of Investigator's Brochure):

- Non GLP toxicology studies in CD1 mice, Sprague Dawley rats and Beagle dogs;
- GLP repeated dose toxicity studies in Sprague Dawley rats and Beagle dogs at doses up to 9 mg/kg/day for up to 28 days. Observations and pharmacokinetic parameters are summarized in Table 1.

Table 1: Summary of results from toxicology studies up to 28 days in rats and dogs

Study	Regimen	Main findings	sexe	Plasma Tmax	Cmax (ng/ml)	AUCt (ng/ml*h)	AUC0-24 (ng/ml*h)
44287 TSR 10-day toxicology non-GLP study in Sprague Dawley Rats	Repeated administration 10 days P.O.: 5 mg/kg SID	No clinical signs were reported and pathological changes or renal microscopic examination (tubular basophilia and dilatation) were considered as non-adverse	Female & Male	N.D.	N.D.	N.D.	N.D.
	Repeated administration 10 days P.O.: 10 mg/kg SID	10 mg/kg administered daily for days was considered as adverse due to: -thin appearance, hunched posture, ptyalism and renal impairment in both genders	Female & Male	N.D.	N.D.	N.D.	N.D.
44288 TSC 14-day toxicology non-GLP study in Beagle Dogs	Repeated administration 14 days P.O.: 6 mg/kg SID	Dose was clinically well tolerated in dogs (MTD), but: - increased liver enzymes activity (in the female dog) - increased thrombocytes count were considered as adverse - microscopy, histologically, both dogs had minimal to slight tubule vacuolation in the kidneys. In the female, this adverse renal effect was associated with microscopic renal findings (i.e. tubular degeneration/necrosis and tubule dilation)	Female & Male	N.D.	N.D.	N.D.	N.D.
44289 TSR 28-day toxicology GLP study in Sprague Dawley Rats	Repeated administration 28 days P.O.: 1 mg/kg SID	No adverse effect	Female	1h	0.37	ND	ND
			Male	ND	ND	ND	ND
			Female	1h	1.02	ND	ND
			Male	1h	0,36	ND	ND
	Repeated administration 28 days P.O.: 1.5 mg/kg BID	No adverse effect	Female	T1: 30min T2: 2h	T1: 5.64 T2: 7.98	4.49	
			Male	T1: 30min T2: 2h	T1: 5.64 T2: 7.98	4.48	
			Female	T1: 30min T2: 2h	T1: 5.64 T2: 7.98	9.54	
			Male	T1: 30min T2: 2h	T1: 5.64 T2: 7.98	3.76	
	Repeated administration 28 days P.O.: 3 mg/kg SID	NOAEL	Female	1h	3.76	8.54	12.55
			Male	1h	2.33	4.53	6.46
			Female	1h	4.73	8.71	11.49
			Male	1h	1.85	4.38	6.63
	Repeated administration 28 days P.O.: 9 mg/kg SID	9 mg/kg administered daily for 4 weeks was considered as adverse due to: - Higher leucocyte counts and fibrinogen levels, higher urea, creatinine calcium and inorganic phosphorus concentrations - Adverse microscopic findings in kidneys and non-adverse findings were noted in the liver and bone marrow (males only) - Ongoing but incomplete recovery seen in kidneys and liver in microscopic samples of this group.	Female	1h	20.58	44.17	63.49
			Male	1h	6.30	22.38	38.66
			Female	30min	10.38	38.84	60.99
			Male	3h	6.80	31.27	65.79
44290 TSC 28-day toxicology GLP study in Beagle dogs	Repeated administration 28 days P.O.: 0.5 mg/kg BID (6h between the 2 treatments)	No adverse effect	Female	T1: 30min T2: 2h	T1: 5.64 T2: 7.98		30.9
			Male	T1: 30min T2: 2h	T1: 3.70 T2: 8.77		34.5
			Female	T1: 30min T2: 2h	T1: 5.77 T2: 5.51		37.0
			Male	T1: 30min T2: 2h	T1: 3.98 T2: 3.81		64.3
	Repeated administration 28 days P.O.: 1.5 mg/kg BID (6h between the 2 treatments)	No adverse effect	Female	T1: 30min T2: 2h	T1: 13.57 T2: 11.25		89.4
			Male	T1: 30min T2: 2h	T1: 13.26 T2: 12.97		94.1
			Female	T1: 30min T2: 2h	T1: 16.78 T2: 16.26		119
			Male	T1: 30min T2: 2h	T1: 17.38 T2: 10.78		90.4
	Repeated administration 28 days P.O.: 4.5 mg/kg BID (6h between the 2 oral treatments)	NOAEL Highest tested dose	Female	T1: 3h T2: 2h	T1: 26.82 T2: 39.29		297
			Male	T1: 3h T2: 2h	T1: 39.60 T2: 74.88		472
			Female	T1: 3h T2: 2h	T1: 29.62 T2: 49.11		391
			Male	T1: 30min T2: 2h	T1: 34.70 T2: 48.20		338

A non-GLP toxicity study in rats showed renal toxicity after repeated (10 days) oral administration at ≥10 mg/kg once a day (study 44287 TSR). In the 28-day GLP toxicity study in rats, the administration of 9 mg/kg once daily led to adverse effects, notably renal and haematology abnormal findings. ***Thus, the NOAEL was established at 3 mg/kg/day, administered in one or two doses in Sprague Dawley rats*** (study 44289 TSR)**.**

In the 14-day non-GLP toxicity study in dogs, the 6 mg/kg/day dose administered once daily was well tolerated clinically but females had adverse microscopic tubular degeneration/necrosis in the kidneys. The once daily dose of 6 mg/kg is considered to be the Maximum Tolerated Dose for a 2-week treatment period (study 44288 TSC). In the 28-day GLP toxicity study in dogs (study 44290 TSC), twice daily administration up to 9 mg/kg/day (i.e. 4.5 mg/kg/BID) was well tolerated without any renal toxicity or other findings suggesting that, for a given dose, twice daily administration is better tolerated than once daily administration, due to the minimisation of C_{max}, while AUC remains similar. Therefore, in the present clinical study, it is planned to administer IFB-088 twice daily starting from cohort 2, to reduce the Cmax exposure.

The NOAEL in Beagle dogs was established at 4.5 mg/kg administrated twice a day (9 mg/kg/day).

1.2.2.2. *Determination of the maximum recommended start dose in human volunteers*

The dose of 2.5 mg per day was selected as the maximum recommended start dose for the present clinical trial, based on the following considerations:

1. According to the applicable guidelines on dose selection for first in human trials (paragraph 7.2 of EMEA/CHMP/SWP/28367/07 Rev. 1 and FDA guidance 2005 - Guidance for Industry Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers), the calculation of the maximum recommended start dose (MRSD) is based on the no observed adverse effect level (NOAEL) converted into a human equivalent dose (HED) from which a safety factor is applied to derive the MRSD. As suggested by the above guidelines, the rat species was selected, both in terms of toxicology (most sensitive species) and in terms of pharmacology (species used for some animal models).
2. The NOAEL in this species being 3mg/kg/day, the 6.2 division factor should be applied to convert animal dose to HED (FDA Guidance 2005), it translates into 0.484 mg/kg in humans, that corresponds to a daily dose of 29 mg for a human weighing 60 kg. A safety factor of 10 (FDA Guidance 2005) is then applied to calculate the **MRSD, which is thus estimated at 2.90 mg**.

1.2.2.3. *Determination of the maximal tested dose in human*

Assessment of the target dose based on efficacy data in rodents

IFB-088's biological target PPP1R15A is present only in defective cells. PPP1R15A protein being conformation sensitive and part of a protein complex, there is no validated *in vitro* assays available to evaluate IFB-088 efficacy. Therefore, the efficacy of IFB-088 was evaluated *in vivo* in different validated rodent models (transgenic mouse and rat models). A non-exhaustive list of animal efficacy studies is presented in Table 2.

Table 2: Summary of in vivo pharmacology studies for IFB-088 in several animal models

Therapeutic indication	Animal model (targeted organ)	Tested doses	Efficacy	Remarks
Charcot-Marie-Tooth-1A	PMP22 transgenic rat (Sciatic nerve tissue)	P.O.: 0.76 mg/kg SID	Very weak and partial	First partial effect observed Report CMT1A-REPORT-NC-2017-01
		P.O.: 2.29 mg/kg SID	Good	Report CMT1A-REPORT-NC-2017-01
Charcot-Marie-Tooth-1B	MPZ-S63del transgenic mouse (Sciatic nerve tissue)	P.O.: 1 mg/kg BID (2 mg/kg/day)	Good	Das et al. Science 2015
Amyotrophic Lateral Sclerosis	SOD1-G93A transgenic mouse (Brain tissue)	P.O.: 1 mg/kg BID (2 mg/kg/day)	Partial	Das et al. Science 2015
		P.O.: 5 mg/kg SID	Good	
Multiple Sclerosis	mouse immunized with CFA and MOG35–55 to induce chronic EAE (Brain tissue)	I.P.: 4 mg/kg SID only female	Good, but not maximal	Prosecution of US patent application N°14/773,088 to USPTO Declaration of Dr Brian Popko (University of Chicago) dated January 3, 2017 under 37 C.F.R § 1.131 Exhibit D (IFB-088 is named Sephin1)
		I.P.: 8 mg/kg SID only female	Good	

P.O.: Oral administration
I.P.: intraperitoneal administration
SID: once a day administration
BID: twice a day administration

IFB-088 was administered by the oral route in the CMT1A, CMT1B and ALS animal models but by intra peritoneal (IP) route in the MS mouse model.

The lowest daily dose administered *per os* resulting in a weak and partial efficacy in the CMT1A transgenic rat model was 0.76mg/kg. Applying the allometric conversion mentioned above, this corresponds to **7.35 mg in humans** (assuming a conversion factor of 6.2 and based on a Human body weight of 60 kg).

IFB-088 administered by IP route in female MS mouse model showed optimal efficacy at the dose of 8 mg/kg/day, the highest tested in the study (Table 2). To convert this dose into a *per os* dose, pharmacokinetic data were generated following IP and oral administration of IFB-088 at 4mg/kg and 8mg/kg (studies PSN 11-233 and 17-672 Table 3). The following results were obtained. (Of note, in mice the pharmacokinetics are similar in females and males) (Table 3).

- Oral administration of 10 mg/kg gives an $AUCt_{oral}$ of 215.9 ng/ml.h in male mice
- IP administration of 4mg/kg gives an $AUCt_{IP}$ of 105 ng/ml.h in female mice
- IP administration of 8 mg/kg gives an $AUCt_{IP}$ of 337 ng/ml.h in female mice

Based on the results, a population pharmacokinetics model was developed for the IP route (NONMEM v7.3 Software), allowing the estimation of pharmacokinetic parameters (Clearance (CL), central volume of distribution (V_c)) and inter- and intra-individual variability in mice and the simulation of $AUCt_{IP}$.

Simulations were performed using this population pharmacokinetic model to predict the pharmacokinetic response in a typical mouse, after once daily IP administration of various IFB-088 doses. Parameter estimates (CL_{IP} and $AUCt_{IP}$) and their reported variability were used to simulate time-concentration curves in 1000 mice.

For IP administration of 10 mg/kg, the $AUCt_{IP,predicted}$ was estimated at 443.3 ng/ml.h.

The ratio of $AUCt_{IP,\ predicted}/AUCt_{oral}$ = 443.3/215.9 = 2.05 for the single dose of 10 mg/kg ($AUCt_{oral}$ was the result of pharmacokinetics study (study PSN 11-233), a non-compartmental analysis that does not take account the variability).

The IP administration of 8 mg/kg gives an $AUCt_{IP,predicted}$ of 354.7 ng/ml.h and $CL_{IP,predicted}$ of 0.676 L/h.

Therefore, based on these results we can predict the oral dose to get the corresponding $AUCt_{IP}$ of 8mg/kg:

$$Dose_{oral,predicted} = 2.05\ (AUCt_{IP} \times CL_{IP}) = 0.492 \text{ mg, corresponding to } 16.41 \text{ mg/kg}$$

Taking into account the allometric conversion of animal doses to HED, **the corresponding highest efficient dose in Human is 80.1 mg** (16.41 (highest efficient dose in mice) / 12.3 (allometric factor for mouse) x 60 (body weight in kg for human)).

Based on this extrapolation, the maximal dose daily administered to cover all kind of therapeutic indications listed above is 80.1 mg in human (corresponding AUC of 111.14 ng/ml.h).

Therefore, the range of Pharmacologically Active Dose (PAD) is estimated to be comprised between 7.35 mg and 80.1 mg in humans (Table 2).

To add additional safety, we propose to not exceed 75% of this highest value 80.1 x 75% = 60 mg, **the maximal dose planned to be administered in humans is therefore 60 mg**.

Table 3: Summary of pharmacokinetic studies in different species

Study N°	Species	sexe	Regimen	Study status	sampling time	Plasma Tmax	Cmax (ng/ml)	AUCt (ng/ml*h)
PSN 11-232	Mouse CD1	Male	Single I.V.: 2 mg/mg	Discovery	2min, 15min, 30min, 1h, 3h, 6h, 8h, 24h	2min	143.55	79.23
PSN 11-233			Single P.O.: 10 mg/kg		15min, 30min, 1h, 2h, 3h, 6h, 8h, 24h	15min	132.67	215.9
17-672	Mouse C57BL/J6	Male	Single .I.P: 4mg/kg	Non-GLP	10min, 30min, 1h, 2h, 4h, 6h, 8h, 24h	10min	127.98	128.5
		Female				10min	87.1	105.7
		Male	Single I.P.: 8mg/kg			10min	380.7	363.8
		Female				10min	380.0	337.3
12-640	Sprague Dawley Rat	Male	Single P.O.: 10 mg/kg	Non-GLP	10min, 30min, 1h, 2h, 4h, 6h, 8h, 24h	10min	46.4	34.23
15-035A	Sprague Dawley Rat	Male	Repeated administration 7 days P.O.: 1.15 mg/kg SID	Non-GLP	after last administration: 2h, 4h, 8h, 24h	2h	4.32	ND
			Repeated administration 7 days P.O.: 1.15 mg/kg BID			2h	2.03	ND
			Repeated administration 7 days P.O.: 2.29 mg/kg SID			2h	3.89	19.89
			Repeated administration 7 days P.O.: 7.66 mg/kg SID			2h	9.01	43.02
16-326	Sprague Dawley Rat	Male	Single P.O.: 2.29 mg/kg	Non-GLP	10min, 30min, 1h, 2h, 4h, 6h, 8h, 24h	10min	17.35	12.52

Estimation of the safety of the target dose of 60 mg in Humans

Based on toxicity and pharmacokinetic studies in rats and dogs, population pharmacokinetics models were developed (NONMEM v7.3 software). These population pharmacokinetics models allow to assess pharmacokinetic parameters ((apparent clearance (CL/F) and apparent central volume of distribution (V_c/F)) in rats and dogs.

A first population pharmacokinetics model was developed with rats' data to assess pharmacokinetic parameters in rats and a second population pharmacokinetics model was developed with dogs' data to assess pharmacokinetic parameters in dogs (Results and validation of models were presented in the report IFB-088preclinicmodeling v270717.pdf).

Then, a third population pharmacokinetics model was developed with all the data (rats' and dogs' data). This third model is an interspecies population pharmacokinetics model that was used to assess pharmacokinetic parameters in rats and dogs and to compare these results with the two previous developed models (Results and validation of model were presented in the report IFB-088preclinicmodeling v270717.pdf). This interspecies population pharmacokinetics model was also used to predict pharmacokinetic parameters in humans. Estimated and predicted pharmacokinetics parameters are summarized in Table 4.

Table 4: Estimated PK parameters in rats and dogs and predicted PK parameters in humans, using the NONMEM v7.3 Software

	Rat	Dog	Human*(60kg)
CL/F (L/h/kg)	145.2	19.2	9.7 (9.6 – 9.9)
Vc/F (L/kg)	389.9	44.5	20.9 (20.4 – 21.3)
*Pharmacokinetic parameters derived from bootstrap analysis (95%CI)			

Simulations were performed using this interspecies population pharmacokinetic model to predict the pharmacokinetic response in a standard 60 kg individual, after once and twice daily administrations of various IFB-088 doses over 14 days. Concentration-time profiles were simulated for 1000 typical 60kg humans. C_{max} and $AUC_{0\text{-}24h}$ were simulated for 1000 rats (effective dose and NOAEL dose in rats), 1000 dogs (NOAEL rat dose and NOAEL dog dose) and 1000 typical 60kg humans to determine C_{max} and $AUC_{0\text{-}24h}$ safety coverage.

The maximum clinical dose was determined from dog data as the pharmacokinetic is not linear in rats with differences between sexes. Based on these elements and the Guidance for Industry M3(R2) FDA. The maximum clinical dose should not be higher than half of the AUC at the highest dose tested in dogs not showing toxicity (considered as NOAEL).

The mean AUC at the highest dose not showing toxicity in dogs (4.5mg/kg twice daily) is 376.46 ng/ml.h^{-1} (Table 1). Therefore, half of the AUC is 188.23 ng/ml.h, which corresponds to a dose of 135.79 mg in a human being weighing 60kg.

According to the model, the corresponding AUC for a 60 mg daily dose in humans is 83.15 ng/ml.hr, which is far below 188.23 ng/ml.h (Table 5).

Data on C_{max} are consistent with those on AUC. The half of the C_{max} in dogs receiving 4.5 mg/kg twice daily is 23.77 ng/ml while the C_{max} in humans receiving a total daily dose of 60 mg is 10.57 ng/ml. Thus, the target dose of 60 mg/day (30 mg BID) can be considered as safe in humans.

Table 5: Predicted mean plasma Cmax and AUC(0-inf) in humans following first day dosing of IFB-088 and associated safety coverage

Daily dose (mg)	Daily administered doses (n x mg)	Escalating dose ratio	Expected Human Cmax (ng/ml)	Expected Human AUC (0-inf) (ng/ml.hr)	Cmax safety coverage % of corresponding maximal Cmax in human	AUC safety coverage % of AUC in dog
2.5	1 x 2.5		0.84	3.69	3.5%	0.98%
5	2 x 2.5	2	0.88	6.93	3.7%	1.84%
10	2 x 5	2	1.76	15.32	7.4%	4.07%
20	2 x 10	2	3.52	27.72	14.8%	7.36%
40	2 x 20	2	7.04	55.44	29.6%	14.73%
60	2 x 30	1.5	10.57	83.15	44.47%	22.09%

The escalating dose levels (Table 5) were chosen to not exceed an escalating ratio of 2 except for the last dose level with a smaller ratio of 1.5.

It might be worth noting that the antihypertensive drug Guanabenz, a structural analogue of IFB-088 (with only one atom difference), displays a similar biological activity on PPP1R15A (Tsaytler et al., Science 2011; Das et al., Science 2015) and similar pharmacokinetic profile in Sprague Dawley rats (plasma, brain, retina and sciatic nerve – Report 16-326). Guanabenz is still marketed in certain countries (including Japan) at doses up to 64 mg/day as 32mg/day BID.

Criteria to stop escalating dose:

The dose escalation will be stopped if in a given volunteer:

- The AUC is higher than 111.14 ng/ml.h (simulated AUC for the highest efficient dose in Human and which is lower than the half of AUC in dogs at the NOEL)
- The Cmax reaches 23.77 ng/ml.h (half of the C_{max} in dogs at the NOAEL).

The maximum recommended starting dose (MRSD) for IFB-088 in adult healthy volunteers was evaluated at 2.90 mg/day in Humans.

The maximal daily dose to be administered in Humans to cover the different envisioned therapeutic indications was evaluated at 80 mg/day. Therefore, in order ensure optimal safety, **the dose ranges selected are:**

- **2.5 mg/day for the first therapeutic dose;**
- **60 mg/day given as 2 administrations of 30 mg for the highest therapeutic dose.**

1.2.3. *Dose Rationale for the MAD part*

1.2.3.1. *Update on Safety coverage*

Additional toxicology studies have been conducted in rodent (rat) and non-rodent (dog) animal species to further assess IFB-088 safety (see sections 4.3.1 & 4.3.4 of Investigator's Brochure):

- GLP repeated dose toxicity studies in Sprague Dawley rats and Beagle dogs at doses up to 12 mg/kg/day for 13 weeks. Observations and pharmacokinetic parameters of IFB-088 and its major metabolite (IFB-139) are summarized in Table 6.

Table 6: Summary of results from the GLP 13-week toxicology studies in rats and dogs

Study	Regimen	Main findings	analyte	Day of admin.	sexe	Plasma Tmax	Cmax (ng/ml)	AUCt (ng/ml*h)
46232 TSR 13-week toxicology GLP study in Sprague Dawley Rats	Repeated administration 13 weeks P.O.: 1.5 mg/kg BID (6h between the 2 treatments) 3 mg/kg/day	NOAEL	IFB-088	1	Female	T1: 30min	1.69	8.07
					Male	T1: 30min	0.79	5.86
				90	Female	T1: 30min	1.63	8.86
					Male	T1: 10min	0.50	3.79
			Major metabolite IFB-139	1	Female	T1: 3h	1.20	7.85
					Male	T1: 7h	0.98	7.04
				90	Female	T1: 9h	0.93	9.43
					Male	T1: 7h	1.56	8.98
	Repeated administration 13 week P.O.: 3 mg/kg BID (6h between the 2 treatments) 6 mg/kg/day	At 6 mg/kg/day administered as 3 mg/kg BID for 13 weeks was considered as adverse due to: - 2 out of 10 males presented enlarged kidneys, with irregular colour and/or surface; these changes correlated with the presence of crystal nephropathy at microscopic examination. - One female presented non-adverse, reversible, minimal bile duct hyperplasia. There were no other remarkable, test item-related signs at 6 mg/kg/day	IFB-088	1	Female	T1: 30min	2.95	26.4
					Male	T1: 30min	2.01	13.4
				90	Female	T1: 30min	2.73	29.9
					Male	T1: 10min	2.94	23.6
			Major metabolite IFB-139	1	Female	T1: 7h	1.20	15.1
					Male	T1: 3h	4.67	36.6
				90	Female	T1: 9h	2.96	36.6
					Male	T1: 9h	13.7	99.1
	Repeated administration 13 weeks P.O.: 6 mg/kg BID (6h between the 2 treatments) 12 mg/kg/day	12 mg/kg/day administered as 6 mg/kg BID for 13 weeks was considered as adverse due to: - crystal nephropathy in the kidneys was the main histopathological finding. This observation was adverse and not reversible after the 4-week treatment-free period. - In addition to deaths (males) and effects on body weight, the high-dose level of 12 mg/kg/day produced numerous other treatment-related findings, and it could be concluded that most of them were limited to this dose level. Most of the findings recorded at the high-dose were not reversible after a 4-week recovery period.	IFB-088	1	Female	T1: 30min	12.9	64.5
					Male	T1: 30min	5.50	58.0
				90	Female	T1: 30min	9.42	72.5
					Male	T1: 3h	5.90	113
			Major metabolite IFB-139	1	Female	T1: 30min	5.18	71.2
					Male	T1: 3h	3.19	149
				90	Female	T1: 9h	9.92	123
					Male	T1: 13h	35.3	628
IFB088-13W-DOGS 13-week toxicology GLP study in Beagle Dogs	Repeated administration 13 weeks P.O.: 1.5 mg/kg BID (6h between the 2 treatments) 3 mg/kg/day	No adverse effect	IFB-088	1	Female	T1: 30min	9.3	102.7
					Male	T1: 30min	12.0	105.1
				90	Female	T1: 30min	13.4	78.8
					Male	T1: 30min	17.0	93.4
			Major metabolite IFB-139	1	Female	T1: 8h	1.20	10.0
					Male	T1: 3h	2.2	25.5
				90	Female	T1: 30min	3.7	20.9
					Male	T1: 8h	3.1	28.8
	Repeated administration 13 week P.O.: 3 mg/kg BID (6h between the 2 treatments) 6 mg/kg/day	No adverse effect	IFB-088	1	Female	T1: 3h	16.2	182.9
					Male	T1: 3h	17.4	188.8
				90	Female	T1: 3h	21.4	198.9
					Male	T1: 3h	20.0	197.4
			Major metabolite IFB-139	1	Female	T1: 8h	2.5	37.4
					Male	T1: 8h	4.6	56.5
				90	Female	T1: 6h	7.8	61.2
					Male	T1: 12h	5.1	69.0
	Repeated administration 13 weeks P.O.: 6 mg/kg BID (6h between the 2 treatments) 12 mg/kg/day	NOAEL	IFB-088	1	Female	T1: 3h	41.2	467.6
					Male	T1: 3h	51.2	650.0
				90	Female	T1: 30min	84.8	517.0
					Male	T1: na	78.6	759.8
			Major metabolite IFB-139	1	Female	T1: 8h	7.5	102.7
					Male	T1: 8h	6.7	110.6
				90	Female	T1: 30min	21.8	158.6
					Male	T1: 12h	20.9	225.4

In the 13-week GLP toxicity study in rats, the administration of IFB-088 at dose levels ≥ 6 mg/kg/day (i.e. 3 mg/kg BID) led to adverse effects similar to those observed in the 28-day study, *i.e.* renal and haematology abnormal findings. ***Thus, the NOAEL remained at 3 mg/kg/day, administered in one*** (studies 44289 TSR – 28-day treatment) ***or two doses in Sprague Dawley rats*** (studies 46232 TCR – 13-week treatment)**.**

In the 13-week GLP toxicity study in dogs (study IFB088-13W-DOGS), the administration of IFB-088 up to 12 mg/kg/day (i.e. 6 mg/kg BID) was well tolerated without any renal toxicity or other findings.

The NOAEL in Beagle dogs increased and was established at 12 mg/kg administrated in two doses (6 mg/kg BID).

After 13 weeks of IFB-088 treatment, the maximal exposures (AUC_t) at the NOAEL in Beagle Dogs were 517.0 and 759.8 ng/ml.h with C_{max} of 84.8 and 78.6 ng/ml in females and males respectively. At the NOAEL, IFB-139 the major metabolite has an AUC_t of 158.6 and 225.4 ng/ml.h with C_{max} of 21.8 and 20.9 ng/ml in females and males respectively (IFB-139 is an inactive metabolite).

1.2.3.2. *Determination of the maximal tested dose in the Multiple Ascending Dose part of the study*

This Phase 1 study was initially designed to support future clinical developments of IFB-088 in CMT and other degenerative pathologies, such as Multiple Sclerosis, Amyotrophic Lateral Sclerosis, Oculo-Pharyngeal Muscular Dystrophy and Retinitis Pigmentosa for which preclinical efficacy in animal models has been obtained.

Based on the extrapolation presented in paragraph 1.2.2.3, the maximal daily exposure to cover all targeted therapeutic indications was estimated at 111.14 ng/ml.h (simulated AUC in human for the highest efficient dose in animals). According to the interspecies population pharmacokinetics model, the simulated AUC_{0-24} for a 60 mg daily dose (2x30 mg/day) in humans was 83.15 ng/ml.h. Pharmacokinetic results of the SAD show an AUC_{0-24h} of 51.41 +/- 20.45 ng/ml.h, which is less than half of the maximal daily exposure expected to cover all targeted therapeutic indications (111.14 ng/ml.h). As initial targeted exposures will not be met in this study, the company made the strategic decision to focus only on the treatment of Charcot-Marie-Tooth disease, in which exposures required in animals for efficacy are lower.

Based on the optimal doses to treat CMT in animal models (Table 2, paragraph 1.2.2.3), the targeted exposure for optimal efficacy in CMT patients will be in the range of 19.89 – 43.18 ng/ml.h (targeted AUC_{0-inf}), corresponding to a Cmax for 2 administrations per day between 2.5 and 3.89 ng/ml (Table 7).

Table 7: Estimated exposure for optimal efficacy in CMT patients

Targeted therapeutic indication	Animal model	Optimal doses for efficacy in animals	Extrapolated C_{max} expected in humans	Extrapolated AUC_t expected in humans
Charcot-Marie-Tooth 1A	PMP22 transgenic rats	2.29 mg/kg once a day	3.89 ng/mL	19.89 ng/ml.h(1)
Charcot-Marie-Tooth 1A	MPZS63Del transgenic mice	1 mg/kg twice a day	2.5 ng/ml	43 ng/ml.h(2)

(1) Data from study report 15-035A

(2) Data extrapolated from study report PSN 11-233, based on pharmacokinetics data of CD1 mice administered with single dose of IFB-088 10 mg/kg *per os* (Table 2, paragraph 1.2.2.3). At 10 mg/kg, AUC_t was 215.9 ng/ml.h. Taking into account that IFB-088 AUC_t is dose-proportional at these dose levels, estimated AUC_t of 2x1mg/kg/day is 43 ng/ml.h (= 2 x 215.9/10).

Pharmacokinetics results of the single day of IFB-088 in healthy subjects (SAD) are quite similar to those observed in animal studies and population pharmacokinetics model based on preclinical data (rats and dogs) is considered satisfactory with a 30-40% margin of error to predict SAD results. Indeed, as observed in preclinical studies, IFB-088 pharmacokinetics is characterized by an important volume of distribution, suggesting that IFB-088 rapidly disappears from the blood compartment. Moreover, following two administrations of IFB-088 separated by an interval of 12 hours, there is no accumulation of IFB-088 at T24h after the first daily administration. Concerning pharmacokinetics-dose linearity, increases in IFB-088 C_{max} and $AUC_{0\text{-}24h}$ are dose-proportional between 20 and 60 mg daily. As expected, IFB-139 the inactive major metabolite of IFB-088 is present in a similar range as observed in preclinical studies.

Based on results obtained in different CMT animal models, the exposure ($AUC_{0\text{-}24h}$) for optimal efficacy is estimated between 19.89 and 43.18 ng/ml.h and C_{max} for 2 administrations per day in the range of 2.5 to 3.89 ng/ml (Table 7). According to the pharmacokinetics results from the SAD in healthy volunteers, the three following doses of 15, 30 and 50 mg daily cover to the range of optimal efficacy (exposure between 12.82 and 42.62 ng/ml.h, C_{max} between 1.20 and 3.77 ng/ml). The escalating ratio is 2 between the low and middle doses and 1.66 between the middle and high doses. To conclude, the maximal ascending dose will be 50 mg daily (2 x 25 mg) according to the preclinical population pharmacokinetics model and the preliminary pharmacokinetics results of the single day.

Table 8: Predicted mean plasma Cmax and AUC(0-inf) in humans following 14-day dosing of IFB-088 and associated safety coverage

Daily dose (mg)	Daily administered doses (n x mg)	Escalating dose ratio	Expected Human C_{max} (ng/ml)[(1)]	Expected Human AUC (0-inf) (ng.hr/ml)[(1)]	C_{max} safety coverage % of C_{max} in dogs[(2)]	AUC safety coverage % of AUC in dogs[(2)]
15	2 x 7.5	-	1.20	12.82	1.5%	2.5%
30	2 x15	2	2.26	25.57	2.9%	4.9%
50	2 x 25	1.67	3.77	42.62	4.9%	8.2%
Stopping criteria			**23.77**	**111.14**	**30.2%**	**21.5%**

(1) Data are extrapolated from pharmacokinetics results of the SAD observed at a daily dose of 60mg (30mg BID), taking into account that IFB-088 C_{max} and AUC are dose-proportional at these dose levels.
(2) Safety coverage is calculated based on C_{max} and AUC_t at NOAEL for the sex with the lowest C_{max} and the lowest AUC_t

Criteria for dose modifications or stopping dose escalation:

The criteria to modify planned doses or stop dose escalation will be the same as the ones used for single ascending dose escalation, as NOAEL in Sprague Dawley rats is maintained and NOAEL in Beagle dogs increased following the results of the 13-week GLP toxicity study, *i.e.*

- AUC > 111.14 ng/ml.h, and
- Cmax > 23.77 ng/ml.

Based on the SAD exposure in humans and on the additional toxicity studies performed in animals, the daily doses for the multiple ascending dose to be administered in Humans to cover

the envisioned therapeutic indications (Charcot-Marie-Tooth) and to ensure maximum safety are:

- **15 mg/day given as 2 administrations of 7.5 mg in the first cohort;**
- **30 mg/day given as 2 administrations of 15 mg in the second cohort;**
- **50 mg/day given as 2 administrations of 25 mg in the third cohort.**

1.2.4. *Rationale for Study population*

As is usual in first-in-man studies, the population to be included is healthy young males between 18 and 40 years old (inclusive) at screening. The selection of healthy subjects is justified on the basis that PK, safety, and tolerability, can be investigated accurately in this population.

In this population, the study can be performed under standardized conditions, reducing the influence of confounding factors.

No medical benefit for the subjects can be expected beyond the thorough medical check-up that each subject will receive prior to treatment and at the end of the study. The potential risks associated with IFB-088 are not known, as this product has not yet been tested in humans.

The study protocol is designed to ensure a careful observation and medical management in order to minimize any associated risk in this study.

The participation of healthy subjects in this study is justified with regard to the expected benefit for the target population that will receive this therapy after registration of the compound.

1.3. Summary of Risk Management

1.3.1. *Cardiovascular Monitoring*

Since IFB-088 is devoid of alpha-2 adrenergic activity, a potent reduction in blood pressure is not expected with IFB-088. In addition, even if in vitro, in a HERK study IFB-088 was classified as a potential moderate HERK channel inhibitor, IFB-088 at 1, 3 or 9 mg/kg/day (0.5, 1.5, 4.5mg/kg/administration) had no effect on heart rate, systolic, diastolic and mean arterial blood pressure, ECG parameters or body temperature in conscious male beagle dogs and no treatment-related arrhythmias were observed at any of the dose-levels administered in dog (see Investigator Brochure section 4.1.3.2 and section 4.1.3.5). Nevertheless, as usual in a first in man study a particular attention will be focused on cardiovascular monitoring. Vital signs (systolic and diastolic blood pressure, heart rate) will be closely monitored at regular intervals post dosing, both in supine and standing position and ECG parameters will be controlled at regular intervals post-dosing.

1.3.2. *Drug-induced renal crystals*

Preliminary toxicity studies pointed out renal findings after 10 or 14 days once a day administration from 10 mg/kg in dogs and rats. In particular, intratubular crystals were observed

at the upper dose tested. IFB-088 was localized in the crystals observed in each kidney from each treated animal whereas IFB-139 metabolite was distributed in the entire kidney tissue with the most intense signals localized in the crystals.

In Beagle dogs, twice daily administration for 28 days was well tolerated up to 9mg/kg (NOAEL in this species) without any renal findings suggesting that when administered twice daily IFB-088 is better tolerated than when daily administered.

A twice-daily administration is thus proposed from cohort 2 and an appropriate monitoring plan will be set up with a regular check of renal function parameters (creatinine, urea, estimated Glomerular Filtration Rate and urinary dosage of Beta2 microglobulin, proteins and creatinine), volume of diuresis and urinary parameters by dipstick (pH, hematuria). An increased excretion of beta2microglobulin in the urine is a sensitive indicator of proximal tubular dysfunction (Ostermann M., Joannidis M (2016).

A minimal intake of at least 2L of water post dosing will be mandatory to ensure a sufficient hydration (250 mL with study drug, then 500 mL between dosing and T4H, 1000 mL between T4H and T12H and 500 ml between T12H and T24H).

1.3.3. *Liver monitoring*

Hepatic enzymes are systematically recorded in phase 1 trials but, considering that at the highest dose tested in the 28-day GLP study in rats (9 mg/kg/day SID), an increase of liver enzymes was observed, and that there is a known important hepatic first pass effect for guanabenz, a strategy will be implemented according the FDA guidance (Drug-Induced Liver Injury: Premarketing Clinical Evaluation) to initiate close observation immediately upon detection and confirmation of early signals of possible impairment of liver function (See Section 3.4.3.1 and 8.10).

2. OBJECTIVES AND ENDPOINTS

2.1. **Primary objectives**	End Points
To investigate the safety and tolerability after single and multiple oral doses of IFB-088 in healthy volunteers	- Adverse events - Laboratory parameters (haematology, chemistry, urinalysis) - 12-lead ECG parameters - Vital signs (supine and standing systolic and diastolic blood pressure, pulse rate, tympanic temperature) - Vigilance (Bond and Lader scale)
2.2. **Secondary objectives**	End Points
To investigate the pharmacokinetics after single and multiple oral doses of IFB-088 and its metabolite in healthy volunteers	- Plasma - Peak plasma concentration (Cmax) - Time of peak plasma concentration (tmax) - Area under the plasma concentration-time curve from time-zero to last sample with measurable concentration (AUClast) and from time 0 extrapolated to infinity (AUC0-∞) - Terminal half-life (t½) - Apparent total body clearance (CL/F) - Urine - Maximum observed concentration (Cmax) - Time of occurrence of CmaxU (tmaxU) - Area under concentration-time curve from time-zero to last sample with measurable concentration (AUClast) and from time 0 to infinity (AUC0-∞) - Renal clearance - Total amount excreted in urine (Ae) - Concentration at the end of a dosing interval before the next dose (Ctrough)
2.3. **Exploratory objectives**	End Points
To explore UPR related biomarkers	- Blood samples

3. INVESTIGATIONAL PLAN

Protocol waivers or exemptions are not allowed with the exception of immediate safety concerns. Therefore, adherence to the study design requirements, including those specified in the Time and Events Table, are essential and required for study conduct.

3.1. Study Design

This is a double blind, randomized, placebo-controlled combined SAD and MAD study.

3.1.1. *Overview SAD study design*

This part of the study will be conducted according to a randomized, double blind, placebo controlled, single oral ascending dose design in 6 independent cohorts of 8 healthy male subjects. If a subject is withdrawn from this study part, the subject may be replaced as necessary with another subject assigned to the same treatment with respect to active and placebo doses at the discretion of the sponsor's team in consultation with the investigator.

Each potential subject will undergo:

- A Screening visit prior to initiation of any study-specific procedures:
  - It can take place up to 21 days before the first dosing session,
  - Informed consent will be obtained prior to any procedure with an assessment of inclusion and exclusion criteria.
- A Treatment Phase:
  - Subjects will be required to join the unit on the day prior to dosing (Day -1) and will be required to stay in the unit for up to 2 nights for the treatment period. Subjects will be dosed on Day 1. A continuous monitoring of adverse events, regular check of vital signs, ECG and diuresis will be set up. PK blood samples and urine samples will be taken for up to 32 h post-morning dose.
  - Vigilance will be assessed at regular intervals by Visual Analogic Scales (VAS) from pre-dose to discharge (Bond Lader).
  - Safety parameters particularly vital signs, in the opinion of the investigator, must have returned to baseline values prior to discharge. Duration of both subject stay and PK sampling may be subject to changes, depending on emerging safety and PK results.
- A Follow-up visit:
  - Subjects will be required to return to the unit for a follow-up visit 7 to 14 days after dosing.

Cohort features:

- It is anticipated that 6 cohorts of 8 subjects will be studied.
- In each cohort, 6 subjects will receive active treatment and 2 will receive placebo.

IFB-088 dosing

- The planned doses of IFB-088 are 2.5 mg, 5, 10, 20, 40 and 60 mg administered as oral capsule.

- The maximum daily predefined dose will be 60 mg.
- The planned dosing schedule may be adjusted to allow administration of intermediate doses if dose-limiting toxicity becomes evident at any dose level or to obtain additional safety, tolerability, or PK data.
- In the first group of each cohort, two (2) subjects will be dosed on Day 1 (one on active treatment and one on placebo). The 6 remaining subjects will be dosed by successive groups of 3 subjects, with an adequate period between groups to observe for any reaction and adverse events (at least 36 hours between last assessment of the subjects and dosing of the following).
- In the first cohort (2.5 mg), dosing will be in the morning under fasting state (see Section 6.6.2)
- From the second cohort, the planned daily dose will be divided into 2 doses separated by an interval of 12 hours (1 dose in the morning fasting and 1 dose in the evening 2 hours before dinner) (See paragraph 1.2.2.1).

- A dose escalation committee (DEC) comprised of members from the sponsor's team and the investigator's team, will be convened prior to the start of each subsequent dosing session to interpret the clinical safety, tolerability, laboratory, and PK exposure, and make a decision to proceed to the next higher dose.

- Data gathered from previous cohort will be available prior to dosing the next cohort in order to carefully monitor each dose level.

3.1.2. *Overview MAD study design*

This part of the study will be conducted according to a randomized, double-blind, placebo controlled, multiple oral ascending dose design in 3 independent cohorts of 8 healthy male subjects.

Each potential subject will undergo:

- A Screening visit prior to initiation of any study-specific procedures:
  - This can take place up to 21 days before the first dosing session,
  - Informed consent will be obtained prior to any procedure with an assessment of inclusion and exclusion criteria
- A Treatment Phase:
  - Subjects will be required to join the unit on the day (Day -1) prior to the first dose and will be required to stay in the unit for 17 days for the treatment period. Subjects will be dosed from Day 1 to Day 14. A continuous monitoring of adverse events, regular check of vital signs, ECG and diuresis will be set up and serial PK blood and urine samples will be collected.
  - Vigilance will be assessed at regular intervals by Visual Analogic Scales (VAS) from pre-dose to discharge (Bond Lader).
- A Follow-up visit:

- Subjects will be required to return to the unit for a follow-up visit 7 to 14 days after last dosing.

Cohort features:

- It is anticipated that 3 cohorts of 8 subjects each will be studied.
- In each dosing session, 6 subjects will receive active treatment and 2 will receive placebo.

IFB-088 dosing

- The planned doses are 15 mg per day (2 x 7.5 mg at 12-hour interval) in cohort 1, 30 mg per day (2 x 15 mg at 12-hour interval) in cohort 2 and 50 mg per day (2 x 25 mg at 12-hour interval) in cohort 3.
- The maximum predefined dose will be 60 mg daily (30 mg twice).
- The planned dosing schedule may be adjusted to allow administration of intermediate doses if dose-limiting toxicity becomes evident at any dose level or to obtain additional safety, tolerability, PK data.
- In the first group of each cohort, two (2) subjects will be dosed on Day 1 (one on active treatment and one on placebo). The 6 remaining subjects will be dosed by successive groups of maximum 3 subjects, with an adequate period between dosing to observe for any reaction and adverse events (*i.e.* at least 36 hours between last assessment of the subjects and dosing of the following, corresponding to at least 5 fold the half-life of the drug according to PK results obtained in the SAD part). This period could be modified on the basis of safety and PK results.
- A dose escalation committee (DEC) comprised of members from the sponsor's team and the investigator's team, will be convened prior to the start of each subsequent dosing session to interpret the clinical safety, tolerability, laboratory, and PK exposure, and make a decision to proceed to the next higher dose.

Data will be available prior to dosing the next cohort in order to carefully monitor each dose level.

3.2. Study endpoints

3.2.1. *Safety endpoints*

Safety and tolerability parameters evaluated during this study are:

- Clinical safety: adverse event information, prior and concomitant medications, physical examination (including body weight), vital signs, 12-lead ECGs, vigilance (Bond and Lader VAS)
- Laboratory safety assessments: standard hematology, biochemistry analyses, urinalysis, serology, alcohol breath test, urine drug screen.

3.2.2. *Pharmacokinetic end points*

The following parameters for IFB-088 and IFB-139 will be derived, where appropriate:

Plasma:

- Maximum observed plasma concentration (Cmax),
- Time of occurrence of Cmax (tmax),
- Terminal elimination rate constant (Kel),
- Terminal half-life (t1/2.),
- Area under plasma concentration-time curve from hour 0 to last sample with measurable plasma concentrations (AUClast),
- Area under plasma concentration-time curve from hour 0 to infinity (AUC0-∞),
- Apparent volume of distribution (Vd/F),
- Apparent total body clearance (CL/F),
- Last measurable plasma concentration (Clast),
- Time to reach last measurable plasma concentration (Tlast),
- Concentration at the end of a dosing interval before the next dose administration (Ctrough),

Urine:

- Maximum observed urine concentration (CmaxU),
- Time of occurrence of Cmax (tmaxU),
- Area under urine concentration-time curve from hour 0 to last sample with measurable urine concentrations (AUClast),
- Area under urine concentration-time curve from hour 0 to infinity (AUC0-∞),
- Renal clearance (CLr),
- Total amount excreted in urine (Ae),
- Percent of drug recovered in urine (Ae %dose).

Additional parameters could be calculated if deemed necessary.

3.2.3. *Biomarker assessment (exploratory)*

There is no biological UPR biomarker identified in fluids in healthy subjects.

With the subject's consent, blood samples will be collected during this SAD part of the study and may be used as healthy controls for the purpose of research of UPR biomarkers in patients' populations.

3.3. **Discussion of the Design**

The selected designs for the 2 parts of this study are as follows:

The SAD part consists of 6 cohorts of 8 healthy young male subjects, each receiving a single oral dose of IFB-088 or placebo (6 verum and 2 placebo) administered daily in the morning (cohort 1) or divided into 2 doses, 12h apart (cohort 2 to 6).

In each cohort, 2 subjects (1 verum and 1 placebo) are to be dosed first; if the safety and tolerability results are acceptable after at least 36 hours (48 hours for cohort 1), two groups of 3 subjects will be dosed at least 36 hours apart (48 hours for cohort 1).

The MAD part consists of 3 cohorts of 8 healthy young male subjects, each receiving an oral dose of IFB-088 or placebo (6 verum and 2 placebo) daily for 14 days.

In each cohort, 2 subjects (1 verum and 1 placebo) are to be dosed first and after an adequate period of time to observe for any reaction or adverse event (at least 36h), and if the safety and tolerability results are acceptable, a maximum of 3 subjects will be dosed then a maximum of 3 subjects after at least 24 hours. The 36-hour interval between the administration for the first 2 subjects in a cohort and the remaining subjects have been specified according to the available safety and PK data obtained in the SAD part of the study.

These designs are well-established for first-in-man studies and appropriate to assess the preliminary safety and tolerability of new drug candidate.

For the SAD part, the sample size of 8 subjects per dose level (6 verum and 2 placebo) is deemed adequate for this type of study (Buoen C et al 2003).

For the MAD part, there will be 8 healthy young subjects per dose level (6 verum and 2 placebo). This number is deemed adequate for the objectives of this study part (Buoen C et al 2003).

During this study, safety parameters will be closely followed by regular measurements, to enable a detection of any changes as early as possible and to be able to stop dosing before occurrence of major deleterious effect.

The follow-up observation for the end of study visit will take into account the results of previously available PK parameters (especially for the potential occurrence of delayed AEs) and could be changed if necessary for safety reasons.

The use of placebo as a control is necessary to provide reliable scientific evidence of safety and tolerability and to ensure a reliable evaluation of the tested drug.

A double-blind design is used in conjunction with a placebo control to prevent a potential bias generated by the knowledge of the administered test drug.

The sequential ascending dose design will be used for a safe determination of the maximal exposure in human.

This study design is in line with the current regulatory guidance and appropriate to reach the objectives of the study. (Guideline on strategies to identify and mitigate risks for first-in-human and early clinical trials with investigational medicinal products - EMEA/CHMP/SWP/28367/07 Rev. 1 20 July 2017).

3.4. Dose Adjustment/Stopping Criteria

In the SAD part, a starting dose of 2.5 mg of IFB-088 administered as oral capsule was judged to be safe for a first-in-human administration. Escalation to the next higher dose and any dose

adjustments of the next dose levels will be based on safety and tolerability results and available PK data of the previously administered dose group.

In the tentative dosing scheduled, there will be a 2-fold increase from the first dose level (2.5 mg) to the second dose level. The dose levels of the following groups will be increased by 2-fold from the previous dose level, until the dose level of 40 mg, and then 1.5-fold between the 2 last dose levels. The planned dosing schedule may be adjusted to allow administration of intermediate doses.

In the MAD part, the planned doses are 15 mg per day (2 x 7.5 mg at 12-hour interval) in cohort 1, 30 mg per day (2 x 15 mg at 12-hour interval) in cohort 2 and 50 mg per day (2 x 25 mg at 12-hour interval) in cohort 3.

The decision to proceed to the next dose level of IFB-088, will be made by the Dose Escalation Committee based on safety, tolerability and preliminary PK data obtained at the prior dose level.

Dose escalation will stop at doses, which are predicted on the basis of ongoing PK projections, to correspond to concentrations greater than the PK stopping limits (Section 3.4.2).

3.4.1. *Data Package Requirements for Dose Escalation Committee Meetings*

Dataset from a dosing session including IFB-088 plasma concentrations from the previous dose(s)/cohort(s) and safety data including AEs, vital signs, ECG (12-lead) and laboratory parameters up to at least 32 hours post last dose will be available prior to dosing the next group.

The DEC, comprised of the Investigator, the Pharmacologist of the clinical center, the statistician, a representative of the Pharmacovigilance platform, and a Sponsor's representative, will be convened prior to the start of each subsequent dosing session to interpret the clinical safety, tolerability, laboratory data and PK exposure, and make a decision to proceed to the next higher dose. Additional advisors could be invited as required.

A DEC charter will be provided to the members before the start of the study.

The DEC may unblind the treatment assignment for any subject with an SAE or for any treatment group after reasonable justification (e.g. subjects experiencing grade 3 events or findings) (Sibille et al, 2010).

The DEC will make a recommendation to proceed to the next higher dose as per protocol, or to modify the next dose or to stop the trial.

At the end of each meeting, the DEC will document in writing the decision that has been taken.

3.4.2. *Dose Adjustment/Stopping Pharmacokinetic Criteria*

As pharmacokinetic is not linear in rats with significant differences between males and females, and is linear without sex differences in dogs, dog rather than rat data have been used to modelize human pharmacokinetics.

Dosing will be stopped if the observed AUC(0-inf), at a given dose level in **any** subject, is > 111.14 ng/ml.h^{-1} (simulated AUC for the highest efficient dose in Human, lower than the half AUC in dogs at the NOAEL) or if the observed Cmax is > 23.77 ng/ml which corresponds to the half of the Cmax at the NOAEL in dog.

If the predicted upper limit of 95% CI of AUC(0-inf) at the next dose level is > 111.14 ng/ml.h^{-1} or of Cmax is > 23.77 ng/ml, the next dose level may be decreased, or a decision could be made not to dose further.

3.4.3. *Stopping Safety Criteria*

The dose should not be escalated further if one of the circumstances listed below occurs in subjects within the same cohort, unless it is obvious that the occurrence is not related to the administration of the treatment:

- Occurrence of a Serious Adverse Event considered at least possibly related to the IMP administration in one subject (See Section 8.3). In that case, study has to be immediately interrupted.
- Possibly drug related severe AE in 2 subjects in the same cohort, independent of within or not within the same system-organ-class (EMA Guidelines on strategies to identify and mitigate risks for first-in-human and early clinical trials with investigational medicinal products)
- Clinically significant drug-related laboratory abnormalities of the same character in 2 or more subjects e.g.
  - Alanine aminotransferase (ALT) or aspartate aminotransferase (AST)> 5 times the upper limit of normal (5 ULN) (Grade 3),
  - Creatinine > 120 µmol/L (1.2 ULN) (Grade 1)

  (FDA's Guidance on Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials; Sibille et al 2010).

Moreover, the occurrence of moderate adverse reactions within the same system-organ-class in more than one subject, and/or clinically significant changes from baseline measurements will be considered as a safety signal and will have to lead to increase caution and a closer assessment of safety parameters in the other subjects. In such a case, data from all subjects would be to be reviewed.

3.4.3.1. *Liver Chemistry Stopping Criteria*

Liver chemistry threshold stopping criteria have been designed to ensure subject safety and to evaluate liver event etiology (in alignment with the FDA premarketing clinical liver safety guidance).

Study treatment will be stopped **for a subject** if the following liver chemistry stopping criteria is met:

- ALT or AST ≥ 3xULN

Refer to Section 8.10, Liver Chemistry Follow-up Procedures, for details of the required assessments if a subject meets the above criteria.

3.4.3.2. *Triglycerides Stopping Criteria*

Several subjects experienced hypertriglyceridemia during the SAD part of the study. All cases were below the threshold of 5.6 mmol/L (i.e. 5 g/L), above which hypertriglyceridemia is considered clinically significant (European Atherosclerosis Society, European Society of Cardiology, French Society of Endocrinology) and no correlation or possible dose relationship was established. Moreover, some subjects with hypertriglyceridemia were in the placebo groups.

Nevertheless, a stopping criterion for triglycerides has been added in the MAD part of the study:

Study treatment will be stopped for a subject if the following criterion is met:

- Serum triglycerides ≥ 5.6 mmol/L and the subject is receiving IFB-088

Study treatment will be stopped for the whole cohort and the dose will not be escalated, if the following criterion is met:

- Serum triglycerides ≥ 5.6 mmol/L in 2 subjects receiving IFB-088.

Next cohort could be administered a lower dose intermediate between the previous cohort and the present cohort.

3.4.3.3. *Renal disorders*

The dose should not be escalated further if one of the circumstances listed below occurs **in 2 or more subjects** within the same cohort:

- Creatinine > 120 µmol/L (1.2 ULN) (Grade 1)
- Or a change from baseline ≥30µmol/L within 24 h
- Or a change from baseline ≥ 50% within one week

(KDIGO definition Ostermann and Joannidis, 2016)

- Hematuria > 2+, verified by cytologic exam, on 2 samples and considered at least possibly related to the IMP
- Urinary ratio Beta 2 microglobulin/ creatinine ≥ 2N

One subject that will meet one of these criteria above will be withdrawn from the study.

3.4.3.4. *QTc Withdrawal Criteria*

A subject that meets the criteria below will be withdrawn from the study. The QT correction formula used to determine discontinuation should be the same throughout the study.

- QTcB and QTcF > 500 msec, OR
- Change from baseline: QTc >60 msec

Withdrawal decisions are to be based on an average QTc value of triplicate ECGs. If an ECG demonstrates a prolonged QT interval, obtain 2 more ECGs over a brief period, and then use the averaged QTc values of the 3 ECGs to determine whether the subject should be discontinued from the study.

The dose should not be escalated further if 2 or more subjects present a prolonged QT interval as defined above.

3.4.3.5. *Vital Sign Withdrawal Criteria*

The dose should not be escalated further if 2 or more subjects, within a dose-level, receiving IFB-088 experience a grade 3 vital signs (heart rate and/or blood pressure) adverse event that are deemed possible or probably related to study drug by the investigator.

The grading for adverse events is based on FDA's Guidance on Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials:

Vital Signs*	**Grade 3**
Tachycardia (beats per minute)	> 130
Bradycardia - beats per minute**	< 45
Hypertension (systolic) - mm Hg	> 155
Hypertension (diastolic) – mm Hg	> 100
Hypotension (systolic) – mm Hg	< 80

*Assuming supine position, at least 5 min at rest conditions, not sleeping subjects, measurements on the same arm and several concordant results
**When resting heart rate is between 60-100 beats per minutes. Use clinical judgment when characterizing bradycardia among some healthy subject populations, for example, conditioned athletes.

Stopping rules for an individual subject, for the dose escalation and within a cohort are summarized in Appendix 2.

3.5. Flow-Chart: Time and events

3.5.1. *SAD part Cohort 1*

Procedures	Screening (D-21 to Day -2)	Day -1	Study days: Day 1 Pre-dose	0 h	0.16h	0.33h	0.5 h	0,75h	1 h	1,5h	2 h	2,5h	3h	4h	5h	6h	8h	12h	Day 2 24h	Day 2 32h	Follow-up **Day 7 to Day 14** (7 to 14 days post-last dose)
Informed Consent	X																				
Demographics	X																				
Medical / drug and alcohol history	X																				
Psychological interview	X																				
Full Physical Exam	X																			X	X
HIV, Hepatitis B & C serologies	X																				
Vital signs (SBP, DBP, HR)[1]	X	X	X[2]				X		X		X		X	X		X	X	X	X	X	X
Tympanic temperature	X	X	X							X								X	X	X	X
Urine Drug abuse & Breath Alcohol	X	X																			
Hematology/Coagulation/Biochemistry	X	X																	X		X
Urinalysis	X	X	X															X	X	X	X
Eligibility criteria	X	X																			
Admission to Unit		X																			
Brief Physical Exam		X																			
12-lead ECG	X	X	X[3]						X		X			X			X	X	X	X	X
Randomization		X																			
Study treatment dosing[4]				X																	
Vigilance assessment			X							X								X		X	
Plasma PK Sampling			X		X	X	X	X	X	X	X	X	X	X	X		X	X	X	X	
Blood sampling for exploratory biomarkers			X							X									X		
Urine PK Sampling[5]			X	←														→			
Adverse Event Review		X	←															→			X
Concomitant Medication Review	X	X	←															→			
Discharge																				X	

1 SBP, DBP and HR in supine and standing position
2 SBP, DBP and HR in triplicate with an interval of 10-30 minutes between each measurement
3 ECG in triplicate with an interval of 10-30 minutes between each measurement
4 In the first cohort one single dose fasting in the morning
5 Urine sampling for PK of IFB-088 and metabolites: pre-dose and 0-4, 4-8, 8-16, 16-32

3.5.2. *SAD part: from Cohort 2*

Procedures	Screening (D-21 to Day -2)	Day -1	Study days																					Follow-up
			Day 1																			Day 2		**Day 7 to Day 14**
			Pre-dose	0 h	0,33h	0,5h	0.66h	1 h	1,5h	2 h	3h	4h	5h	6h	8h	12h	12,25h	12,5 h	13h	14h	16h	18h	24h	32h	(7 to 14 days post-last dose)
Informed Consent	X																							
Demographics	X																							
Medical / drug and alcohol history	X																							
Psychological interview	X																							
Full Physical Exam	X																						X	X
HIV, Hepatitis B & C serologies	X																							
Vital signs (SBP, DBP, HR)[1]	X	X	X[2]			X		X		X	X	X		X	X	X		X	X	X			X	X	X
Tympanic temperature	X	X	X						X							X							X	X	X
Urine Drug abuse & Breath Alcohol	X	X																							
Hematology/Coagulation/Biochemistry	X	X																					X		X
Urinalysis	X	X	X													X				X			X	X	X
Eligibility criteria	X	X																							
Admission to Unit		X																							
Brief Physical Exam		X																							
12-lead ECG	X	X	X[3]					X		X		X			X	X							X	X	X
Randomization		X																							
Study treatment dosing[4]				X												X[6]									
Vigilance assessment			X						X							X								X	
Plasma PK Sampling			X		X		X	X		X	X	X	X			X	X	X	X	X	X	X	X	X	
Blood sampling for exploratory biomarkers			X						X														X		
Urine PK Sampling[5]			X	←	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	→	
Adverse Event Review		X	←	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	→	X
Concomitant Medication Review	X	X	←	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	→	
Discharge																								X	

1 SBP, DBP and HR in supine and standing position
2 SBP, DBP and HR in triplicate with an interval of 10-30 minutes between each measurement
3 ECG in triplicate with an interval of 10-30 minutes between each measurement
4 From the cohort 2 the total dose is separaeted in two intakes with an interval of 12 hours
5 Urine sampling for PK of IFB-088 and metabolites: pre-dose and 0-4, 4-8, 8-12, 12-24 and 24-32
6 Evening dosing after PK sampling

3.5.3. *MAD part*

Procedures	Screening (up to 21 days prior to Day 1)	Day -1	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14	Day 15	Day 16	Day 17	Follow up 7 to 14 days post last dose
Informed Consent	X																			
Demographics	X																			
Medical/drug &Alcohol history	X																			
Clinical psychological evaluation and MINI	X																			
Full Physical Exam	X				X			X				X			X				X	X
HIV, Hepatitis B, Hepatitis C serologies	X																			
Vital signs (SBP, DBP, HR)[1]	X	X	X[2]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Tympanic temperature[3]	X	X	X	X		X		X		X		X		X		X			X	X
Urine Drug abuse&Breath Alcohol	X	X																		
Hematology/Coagulation/ Biochemistry	X	X			X			X				X			X				X	X
Urinalysis	X	X	X		X			X				X			X				X	X
Eligibility criteria	X	X																		
Admission to Unit		X																		
Brief Physical Exam		X			X			X				X			X					
12-lead ECG	X	X	X[4]		X			X				X			X				X	X
Randomization		X																		
Study treatment dosing[5]			X	X	X	X	X	X	X	X	X	X	X	X	X	X				
Vigilance (VAS)[10]			X													X				
Plasma PK Sampling			X[6]	X[7]					X[8]							X[6]	X[7]	X[7]		
Urine PK Sampling			X[9]	X				X[9]								X[9]	X[9]	X[9]		
Adverse Event Review	X	←																	→	X
Concomitant Medication Review	X	←																	→	X
Discharge																			X	

1 SBP, DBP and HR in supine and standing position, Day 2 : 24h post first dose, from Day 3 to Day 14 : every morning pre dose , from Day 15 to Day 17 : in the morning

2 Pre dose : SBP, DBP and HR in triplicate with an interval of 10-30 minutes between each measurement and then 0.5h, 1h, 2h, 3h, 4h, 6h, 8h, 12h post first dose

3 On Day -1; on Day 1 and Day 14: pre dose, T1.5h and T12h post first dose; on Day 2, Day 4, Day 6, Day 8, Day 10 and Day 12 every morning pre dose; on Day 17 before discharge; FU

4 Pre dose: ECG in triplicate with an interval of 10-30 minutes between each measurement then 1h, 2h, 4h 8h and 12h after first dose

5 Dosing twice daily, two intakes being seperated by a 12-hour interval

6 Plasma sampling: Day 1 and Day 14: pre dose, then 0.5h, 1h, 2h, 3h, 4h, 6h, 8h, 10h, 12h (just before evening dose), 13h and 14h post morning dose. **Reference time is the time of the morning dose**

7 Plasma sampling: Day 2: 17h, 19h, 21h and 24h post Day 1 first dose, and just before the morning administration; Day 15 : 17h, 19h, 21h, 24h, 30h, 36h and 42h; Day 16: 48h and 60h post Day 14 morning dose

8 Plasma sampling: Day 7 pre dose, then 1h and 2h post morning dose

9 Urine sampling for PK of IFB-088 and metabolites: Day 1 and Day 14: pre-dose and 0-4h, 4-12h, 12-24h post morning dose; Day 6: pre dose, 0-4h, 4-12h; Day15: 24-36h, Day 16: 36-48h post D14 morning dose

10 Assessment of vigilance: Day 1 and Day 14: pre dose, 1.5h and 12h post morning dose

4. STUDY POPULATION

4.1. Number of Subjects

A sufficient number of subjects will be enrolled so that 8 subjects will complete dosing and critical assessments for each of the cohorts. Six cohorts are planned in the SAD part (48 subjects) and 3 cohorts in the MAD part (24 subjects).

If a subject is withdrawn from the study, the subject may be replaced as necessary with another subject assigned to the same treatment with respect to active and placebo doses at the discretion of the sponsor's team in consultation with the investigator.

Subjects will be recruited from the clinical unit's database and via advertisements through mailing lists or announcement. The advertisement will be submitted to the Ethics Committee.

Inclusion of the same subjects in the 2 successive parts of the study (SAD then MAD) is possible, provided that the subjects have not met any discontinuation criteria during the first part, that they are not in the exclusion period (i.e. one month for SAD) and that they do not exceed the earnings for the last 12 months (*EMA Guidelines on strategies to identify and mitigate risks for first-in-human and early clinical trials with investigational medicinal products Paragraph 8.2.5*).

4.2. Eligibility Criteria

4.2.1. *Inclusion Criteria*

A subject will be eligible for inclusion in this study only if all of the following criteria apply:

1. Healthy male 18 to 40 years of age, inclusive, Caucasian.
2. Healthy as determined by a responsible and experienced physician, based on a medical evaluation including medical history, physical examination, laboratory tests, vital signs and ECG. A subject with a clinically significant abnormality or laboratory parameters significantly outside the reference range for the population being studied may be included only if according to the investigator's opinion, the finding is unlikely to introduce additional risk factors and will not interfere with the study procedures.
3. AST, ALT, alkaline phosphatase and bilirubin ≤ 1.5xULN (isolated bilirubin >1.5xULN is acceptable if bilirubin is fractionated and direct bilirubin <35%).
4. ECG (12 leads) normal (120<PR<200 ms; QRS<120ms; QTcF<450ms) and/or without clinically relevant impairments as judged by investigator.
5. Non-smoker, or user of tobacco- or nicotine-containing products ≤ 5/day.
6. Negative screen for alcohol and drugs of abuse at screening and admission.
7. No history of psychiatric disorders assessed by a clinical psychological evaluation and the Mini International Neuropsychiatric Interview (MINI).

8. Body mass index (BMI) between 19 and 27 kg/m2 inclusive where:

$$BMI = \frac{\text{weight in kg}}{(\text{height in meters})^2}$$

9. Subject with female partners of child-bearing potential must agree to use one of the contraception methods listed in Section 6.6.1 (Contraception requirements). This criterion must be followed from the time of the first dose of study medication until the follow up visit (for female partners) and with an additional period of 90 days (for subjects themselves).
10. Willing and able to understand and sign an approved Informed Consent Form.
11. Able to understand the protocol and to come to the visits.
12. Who is, in the judgement of the investigator likely to be compliant during the study.
13. Subject registered in the VRB file (volontaires se prêtant à des recherches impliquant la personne humaine).
14. Covered by Health Insurance System and / or in compliance with the recommendations of National Law in force relating to biomedical research.

4.2.2. *Non-selection and non-inclusion Criteria*

A subject will not be eligible for inclusion in this study if any of the following criteria apply:

1. History of asthma, anaphylaxis or anaphylactoid reactions, severe allergic responses.
2. History of relevant atopy or drug hypersensitivity.
3. Known allergy to any component of IFB-088 oral capsule or its placebo (HPMC or cellulose microcrystalline).
4. History of major medical, psychiatric illness or surgery which, in the judgment of the investigator, puts them ‘at risk’ or is likely to modify their handling of the study drug.
5. Acute or chronic systemic disease or disorder (respiratory, gastrointestinal, renal, hepatic, hematological, lymphatic, neurological, cardiovascular, psychiatric, musculoskeletal, genitourinary, immunological, dermatological, endocrine).
6. Impaired renal function defined by a creatinine clearance < 90 mL/min calculated using the Cockcroft-Gault equation (FDA Guidance for Industry: Pharmacokinetics in patients with Impaired Renal Function, March 2010).
7. History of nephritic colic and/or renal calculi.
8. History of drug abuse and/or regular use of tobacco- or nicotine-containing products > 5/day within three months of the study.
9. History of alcohol consumption exceeding, (on average 21 drinks/week for men) within 6 months of the first dose of study medication.

10. Drinking excessive amounts of tea, coffee, chocolate and/or beverage containing caffeine (> 4 cups / day).
11. Vital signs with a clinically significant abnormality at screening.
12. ECG with a clinically significant abnormality at screening.
13. Laboratory test values outside the clinically acceptable 'normal range' for healthy volunteers at screening.
14. Positive HIV, Hepatitis B or Hepatitis C at screening.
15. Positive urine drug test or positive breath alcohol test at screening or at admission to the clinical unit.
16. Any medication (including St John's Wort) within 14 days before administration, or within 5 times the elimination half-life of that drug, whichever is the longest (except paracetamol).
17. Treatment with an investigational drug within 30 days or 5 half-lives (whichever is longer) prior to dosing.
18. Unable to refrain from consumption of grapefruit and grapefruit juice within 7 days prior to the first dose of study medication.
19. Unwillingness to abstain from sexual intercourse with pregnant or lactating women or to use a condom and spermicide and another form of contraception (e.g., IUD, birth control pills taken by female partner, diaphragm with spermicide) if engaging in sexual intercourse with a woman who could become pregnant until discharge from the study and during 90 additional days.
20. Subjects unlikely to co-operate in the study, and/or poor compliance anticipated by the investigator.
21. Subject being in the exclusion period of a previous trial.
22. Subject having exceeded the earnings for the last 12 months, including the indemnities for the present study.
23. Subject who could not be contacted in case of emergency.
24. Subject refusing to give written informed consent.
25. Subject who has received blood or plasma derivatives in the year preceding the study.
26. Subject who has given blood within the past 3 months or have planned to give blood or sperm within the 90 days following the study.
27. Subject who has forfeited their freedom by administrative or legal award, or who is under guardianship or under limited judicial protection.

4.3. Screen and Baseline Failures

Data for screen and baseline failures will be collected in source documentation at the site. These data will be filled in the eCRF but will not be fully exported in the clinical database.

Only the following data will be exported and transmitted to the sponsor: reason of withdrawal, acknowledgement of consent obtainment and inclusion / non-selection / non-inclusion criteria.

5. STUDY TREATMENT

5.1. Blinding and emergency code-break procedure

This will be a double-blind study.

One copy of the randomization list will be provided to the unblinded pharmacist for preparation of the treatment.

One set of the sealed code break envelopes will be provided to the investigational site, one for the DEC if needed, one set to the Pharmacy and one to Stragen Services, the CRO in charge of SAE processing.

The DEC will be authorized to un-blind for subjects experiencing grade 3 events or findings to help the dose escalation process (Sibille et al 2010).

The investigator or treating physician may unblind a subject's treatment assignment **only in the case of an emergency**, when knowledge of the study treatment is essential for the appropriate clinical management or welfare of the subject.

Whenever possible, the investigator will first discuss options with the sponsor **before** unblinding the subject's treatment assignment. If this is impractical, the investigator must notify the sponsor as soon as possible.

The documentation must include the name of the individual breaking the blind, the date on which the blind was broken, and a description of the event that led to the unblinding. The investigator must also indicate in source documents and in the CRF that the blind was broken and provide the date, time, and reason for breaking the blind.

5.2. Investigational Medicinal products description (active and placebo product)

The active investigational product IFB-088 is formulated as an oral capsule dosage form. During the clinical study, the Investigational Medicinal Product (IMP) will be manufactured as unit doses ranging from a low strength of 2.5 mg dose to a high strength 30 mg dose.

The Placebo for IFB-088 Capsule will be manufactured as unit doses ranging from 2.5 mg fill weight to 30 mg fill weight with a placebo excipient i.e. Cellulose microcrystalline. The capsule used for the placebo formulation will thus be identical to that used for the active formulation.

The composition of the 2.5 mg to 30 mg anticipated capsule strength and matching placebo are presented in Table 9.

Table 9: Composition of the 2.5 mg to 30 mg IFB-088 capsule strength and matching placebo

Product name	Pharmaceutical Form	Unit dose strength	Mode of administration	Dosing instructions
IFB-088 oral capsule	Size 5 white opaque capsules	2.5 mg to 30 mg	Oral	Study medication will be administered by the study personnel during each dosing day with 250 ml of water at room temperature
Placebo oral capsule	Size 5 white opaque capsules	2.5 mg to 30 mg	Oral	

5.3. Manufacturing, Packaging and Labeling

5.3.1. *Manufacturing of Study Drug at the Site*

The IMPs (IFB-088 oral capsules and matching placebo capsules) will be manufactured by the Pôle Pharmacie, Assistance Publique-Hôpitaux de Marseille, which has the authorization for experimental drug preparation.

5.3.2. *Packaging and Labeling*

The IMPs will be packed and labeled according to GMP for medicinal products and all applicable regulatory requirements at the Pôle Pharmacie, Assistance Publique-Hôpitaux de Marseille. The IMPs will be packed into 50 mL white high-density polyethylene (HDPE) bottle with HDPE screw caps. Bottle filling depend on the cohort of the study. The number of capsules per bottle may vary depending on the dose and the phase of the clinical study.

The trial medication will be labelled according to the regulations set in Annex 13 to the Current Edition of the GMP Guideline.

Packaging of all the investigational products will allow blinded administration.

5.4. Randomization, storage, dispensation and accountability

5.4.1. *Randomization*

Randomization lists will be generated by *Amatsi* prior to the start of the study, using the website randomization.com, and validated by the QC team. A randomisation list will be generated for each part of the study (SAD, MAD), based on a 2-block size design by cohort. The first blocks will contain two units with a ratio of 1:1, the second block will contain six units, 1 placebo and 5 verum.

5.4.2. *Dispensing*

The study drugs will be dispensed to the investigating site by the pharmacist of the clinical site after regular prescription by the investigator or delegated medical physician and administered under investigator/medical supervision. Only subjects enrolled in the study may receive study treatment.

5.4.3. *Storage*

Study drug will be stored in a locked area, accessible only to appropriate study personnel in the pharmacy in accordance with the storage conditions defined on the IMP labels. Storage temperature will be followed by an automated reporting system.

The pharmacist on study site will be responsible for the correct storage and handling of the IMPs.

5.4.4. *Study Drug Accountability*

An accurate and current accounting of the dispensing and return of study drug for each subject will be maintained on an on-going basis by the Pharmacy.

The responsible person(s) in the Pharmacy will document the amount of study treatment produced and the amount dispensed to subjects.

All unused IMPs must be returned in the original containers. Non-used, returned study drug and empty IMP containers may be destroyed after the Pharmacist and the monitor have performed accountability and the Sponsor has confirmed in writing that destruction can be done.

Each time a dose is dispensed to a subject, the following information should be recorded: the protocol and cohort, the subject's study number, the daily dose prescribed, the number of capsules dispensed, the batch number, and the signature of the pharmacist dispensing the drugs.

As the monitor of the study must remain blinded of study treatment, a final drug accountability review and reconciliation will be completed at the completion or termination of the study, and any discrepancies must be investigated and their resolution documented.

5.4.5. *Assessment of Compliance*

All IMP doses will be administered in the clinical unit under the direct supervision of the Investigator or his designee.

After administration of IMP, a hand check and a mouth check will be performed to verify that the subject has swallowed the dose.

Drug administration information will be recorded in the source documents and in the drug accountability form.

5.5. Treatment of Study Treatment Overdose

An overdose is defined as any dose of IFB-088 greater than that specified in the randomization code for that subject.

In such a case, the investigator will use symptomatic treatment to treat any overdose, depending on the signs and symptoms.

5.6. Concomitant Medications and Non-Drug Therapies

5.6.1. *Permitted Medications*

Occasional use of paracetamol at doses of ≤ 2 grams/day will be permitted at the principal investigator's discretion. Other concomitant medication may be considered on a case-by-case basis by the investigator and the sponsor, and use should be restricted to 4 hours after dosing if possible.

All concomitant medications taken during the study will be recorded in the CRF with drug name, dates of administration, the dosage and the reason of the prescription.

5.6.2. *Prohibited Medications and Non-Drug Therapies*

Subjects must abstain from taking prescription or non-prescription drugs (including vitamins and dietary or herbal supplements), within 14 days or 5 half-lives (whichever is longer) prior to the first dose of study medication until completion of the follow-up visit, unless in the opinion of the Investigator and sponsor the medication will not interfere with the study.

6. STUDY ASSESSMENTS AND PROCEDURES

This section lists the parameters of each planned study assessment. The exact timing of each assessment is listed in the Time and Events Tables (Section 3.5). Detailed procedures for obtaining each assessment are provided below.

Whenever vital signs, 12-lead ECGs and blood draws are scheduled for the same nominal time, the assessments should occur in the following order: 12-lead ECG, vital signs, blood draws. The timing of the assessments should allow the blood draw to occur at the exact nominal time.

The timing and number of planned study assessments and procedures may be altered during the course of the study based on newly available data (e.g. to obtain data closer to the time of peak plasma concentrations) to ensure appropriate monitoring. Additional safety tests (such as vital signs, physical exams, and clinical laboratory tests (up to a maximum of approximately 500 mL of blood from the subject within a 30-day period)) may also be obtained during the course of the study based on newly available data, to ensure appropriate safety monitoring.

The Ethics Committee will be informed of any safety issues that require alteration of the safety monitoring scheme.

The actual date and time should be recorded for all procedures and the Investigator should make every effort to perform procedures at the scheduled nominal dates and times.

6.1. Demographic/Medical History Assessments

Demographic data will include date of birth, height in centimeters, and body weight in kilograms.

Medical/medication/alcohol and drug abuse histories will be assessed as related to the eligibility criteria listed in Section 4.2 as well as life habits (tobacco, tea, coffee, chocolate and/or beverage containing caffeine).

6.2. Safety

Planned timepoints for all safety assessments are listed in the Time and Events Table (Section 3.5). Additional time points for safety tests (such as vital signs, physical exams and laboratory safety tests) may be added during the course of the study based on newly available data to ensure appropriate safety monitoring.

Physical Exam

- A complete physical examination will include assessments of the head, eyes, ears, nose, throat, skin, thyroid, neurological, lungs, cardiovascular, abdomen (liver and spleen), lymph nodes and extremities. Height and weight will also be measured and recorded.
- A brief physical examination will include assessments of the skin, lungs, cardiovascular system, and abdomen (liver and spleen).
- Physical examination can also be performed throughout the study on medical indication at the discretion of the investigator.
- A clinical psychological evaluation will be conducted at screening

Examinations will be performed by the Investigator or his designee.

Vigilance

Vigilance will be assessed at regular intervals by a Visual Analogic Scale, namely the Bond-Lader VAS of Mood and Alertness: this questionnaire of 16 analogue scales derives 3 factors that assess change in Self-rated Alertness, Self-rated Calmness and Self-rated Contentment.

Vital Signs

- Vital sign measurements will include tympanic temperature, systolic (SBP) and diastolic blood pressure (DBP), pulse rate (HR) in supine and standing positions. The subject should be allowed to relax at least 5 minutes before taking the first BP in supine position. Standing BP and pulse rate will be measured at both 1 and 3 min of standing.
- At baseline in Day 1 pre-dose, vital sign measurements (except tympanic temperature) will be conducted in **triplicate** (with an interval of approximately 10 - 30 minutes between each record). The average value of each parameter will be considered as baseline value.
- Tympanic temperature, SBP, DBP and HR will be checked post dose at specified time points (see Flow-Chart Time and Events Section 3.5.).
- If possible, BP measurements will be taken from the same arm (opposite to the arm that is used for blood sampling) by an automated BP monitor using the oscillometric method (e.g. Dinamap). Normal ranges for vital signs parameters will be according to the CPCET's standard operating procedures (SOP).
- In the case of an out-of-range value, measurements will be repeated immediately to confirm the change and the investigator or designee will be informed:

SBP	> 150 mm Hg or < 100 mm Hg
DBP	> 90 mm Hg or < 45 mm Hg
HR	> 100 bpm or < 45 bpm

Electrocardiogram (ECG)

- 12-lead ECGs will be obtained at each time point during the study using an ECG machine that automatically calculates the heart rate and measures PR, QRS, QT, and QTc intervals. Subjects must rest in the supine position for at least 5 minutes before recording. The ECG may be recorded during the period of rest required before the measurements of supine BP and pulse. A qualified physician will review the ECGs promptly and any clinically important finding will be recorded on the appropriate CRF.
- At baseline in Day 1 pre-dose, ECG recordings will be conducted in **triplicate** (with an interval of approximately 10 - 30 minutes). The average value of each parameter will be considered as baseline value.
- ECG recordings will be performed post dose at specified time points (see Flow-Chart Time and Events).
- Parameters:
  - Measured parameters: HR, PR, QRS duration, QRS axis, QT;
  - Derived parameters: two corrections of the QT interval will be investigated: Fridericia's correction (QTcF) and Bazett's correction (QTcB);
  - Observations and comments on the quality of trace, on normality or abnormality.
- Refer to Section 3.4.3.3 for QTc withdrawal criteria and additional QTc readings that may be necessary.

Clinical Laboratory Assessments

The following parameters will be determined:

- Hematology:

Red blood cell (RBC) count, hemoglobin, hematocrit, white blood cell (WBC) count with differential (neutrophils, lymphocytes, monocytes, eosinophils, basophils), platelets, reticulocyte count.

- Coagulation:

Activated partial thromboplastin time (APTT), international normalized ratio (INR).

- Biochemistry:

Sodium, potassium, chloride, calcium, total bilirubin, alanine aminotransferase (ASAT), aspartate aminotransferase (ALAT), gamma-glutamyl transferase (GGT), alkaline phosphatases, total protein, albumin, urea, uric acid, bicarbonate, creatine phosphokinase (CPK), creatinine, glycaemia, lactate dehydrogenase (LDH), total cholesterol, HDL and LDL cholesterol, triglycerides.

- Urinalysis:

Specific gravity, pH, glucose, protein, blood, nitrites, leucocytes and ketones by dipstick, cytobacteriological exam (if abnormal results on dipstick); beta 2 microglobulin (B2M), proteinuria and creatinuria on urinary sample

- Other screening tests:

Hepatitis B antigen (HBs Ag), Hepatitis C antibodies (Anti-HCV Ab), Anti-HIV1 and Anti HIV2 antibodies (only at screening)

- Alcohol breath test.
- Drug screen (amphetamines, cocaine, opiates, cannabinoids and benzodiazepines).

All clinically significant abnormal laboratory test values identified after IMP administration will be reported as an adverse event and had to be controlled until the return to normal or baseline. If laboratory values do not return to normal or baseline within a reasonable period, the etiology should be identified

Laboratory tests will be performed in local labs of the hospital for the SAD part, and by the Groupe Oriade (Mr Bernard CADOUX, 83 Avenue Gabriel Péri, 38400 St Martin d'Hères) for the MAD part.

6.3. Pharmacokinetics

6.3.1. *Blood Sample Collection*

Venous blood samples (8 mL) for the determination of plasma concentrations of IFB-088 and its metabolite IFB139 will be drawn by direct venipuncture or via an intravenous

catheter into heparin - lithium Vacutainer polyethylene tubes during the SAD and MAD parts of the study at the time points indicated in Section 3.5, Time and Events Table.

The actual date and time of each blood sample collection will be recorded.

Details of PK blood sample processing, storage and shipping procedures will be provided in a Laboratory Procedures Manual (LPM) provided by the sponsor.

6.3.2. *Urine Sample Collection*

Urine samples for pharmacokinetic analysis of IFB-088 and its metabolite will be collected at the time points listed in Section 3.5, Time and Events Table.

Urine will be collected in containers (adapted to the collected urine volume) at the designated time points in the SAD and MAD parts of the study. The total volume collected by period will be recorded in the CRF. After homogenization, 3 aliquots of 5 ml of the resulting urine will be transferred into polypropylene tubes and stored at -80°C until required for analysis.

Sufficient hydration (at least 2L/day) will be provided and the diuresis will be carefully monitored.

Details of PK urine sample collection, processing, storage and shipping procedures are provided in a LPM provided by the sponsor.

6.3.3. *Sample Analysis*

Plasma and urine samples analysis will be performed under the management of ADME Bioanalyses (Eurofins) after completion of each cohort.

Concentrations of IFB-088 and its metabolite will be determined in plasma and urine samples using the currently approved analytical methodology.

The bioanalytical and pharmacokinetic parts of this study will be described in details in a EUROFINS ADME BIOANALYSES protocol.

IFB-088 and its metabolite will be analyzed in plasma and urine samples using the PKH/MOA/982, PKH/MOA/983 and PKH/MOA/1137 bioanalytical methods which will be previously validated at EUROFINS ADME BIOANALYSES according to Bioanalytical Method Validation EMA July 2011 and Bioanalytical Method Validation FDA draft September 2013.

6.4. **Exploratory Marker(s)**

There is currently no validated specific biomarker.

So, in the SAD part of the study, healthy subjects will be asked to donate blood samples that could be used for research purposes as healthy controls in future studies conducted in patients. A specific part in the informed consent will be signed.

Two samples will be drawn:

- One sample (3 ml) on heparin lithium tube for PBMC extraction
- One sample (2.5 mL) on PAXgene tube for RNA extraction

Times of sampling are summarized on the Flow-Charts of SAD in Section 3.5.

Samples will be stored at -150°C for 5 years after final database freeze and then destroyed.

Blood samples for the analysis of biomarkers will be stored in the CRB (Centre de Ressources biologiques).

No blood sampling for exploratory biomarkers will be performed during the MAD part of the study.

6.5. Investigational schedule

See Section 3.5, Time and Events Table

6.5.1. *Screening visit*

The screening visit will include the following:

- Signature and date of an ICF before any study specific screening procedures,
- Verification of the eligibility criteria,
- Medical history,
- Weight (kg) and height (cm), BMI,
- Physical examination and clinical psychological evaluation and MINI,
- Vital signs measurement (Systolic and diastolic blood pressure, heart rate in supine and standing position, tympanic body temperature),
- 12-lead ECG,
- Laboratory evaluation (hematology, coagulation, biochemistry and urinalysis),
- Viral serology testing [hepatitis B antigen (HBs Ag), Hepatitis C antibodies (Anti-HCV Ab), Anti-HIV1 and Anti HIV2 antibodies],
- Urine drug of abuse screening,
- Alcohol breath test.

6.5.2. *Admission Visit (D-1, one day prior to dosing)*

Subjects will be admitted to the unit one day prior to dosing and will undergo the following investigations:

- Medical history update,
- Physical examination update,
- Vital signs measurement (inc1uding tympanic body temperature),
- 12-lead ECG,

- Laboratory evaluation (hematology, coagulation, biochemistry, urinalysis),
- Urine drug screen,
- Alcohol breath test,
- AE monitoring,
- Review of concomitant medications,
- Verification of the eligibility criteria,

6.5.3. *SAD*

6.5.3.1. *Dosing Day (D1)*

The study drug will be administered after an overnight fast of at least 8 hours.

Subjects will have the following procedures performed at **pre-dose**:

- Verification of the eligibility criteria,
- Randomization to the group treatment (verum or placebo) after check of all criteria,
- Vital signs in triplicate except tympanic temperature,
- 12-lead ECG in triplicate,
- PK blood sampling,
- PK urine sampling,
- Blood sampling for exploratory assessment of biomarkers,
- AE monitoring,
- Vigilance assessment.

In the first cohort, the planned dose (2.5 mg) will be administered in the morning in a single intake.

From the second cohort, the total dose planned in the cohort will be administered in 2 intakes separated by a 12-hour interval, the first intake in the morning at around 8:00 am and the second one at around 8:00 pm in the evening.

The intake hour (H0) on Dl (morning dose) will be the reference time for implementing the following procedures at several time-points (see Flow chart Section 3.5.1 and 3.5.2)

- Vital signs (inc1uding tympanic body temperature),
- 12-lead ECG,
- Diuresis monitoring,
- PK blood sampling,
- PK urine sampling,
- Blood sampling for exploratory assessment of biomarkers,

- AE monitoring,
- Vigilance assessment.

In cohort 1, meals will be served at H4 and H14 post-dose.

From the cohort 2, meals will be served at H4 (around 12:00 a.m) and at H14 post first dose, 2 hours after the second administration in the evening (around 10:00 p.m).

6.5.3.2. *Day 2 (D2)*

The following procedures will be performed at H24 and H32 post first dose (see Flow chart Section 3.5.1 and 3.5.2):

- Vital signs (inc1uding tympanic body temperature) (H24 post-dose and H32 post dose),
- Physical examination update at H32 post dose,
- 12-lead ECG,
- PK blood sampling (H24 post-dose and H32 post dose),
- PK urine sampling,
- Blood sampling for exploratory assessment of biomarkers,
- Laboratory evaluation (hematology, biochemistry, urinalysis) at H24 post dose
- Urinalysis only at H32 post dose,
- Vigilance assessment at H32 post dose,
- AE monitoring.

A breakfast will be offered after the H24 post-dose sampling has been drawn.

After medical authorization, the subject will be discharged after assessment of evaluations planned at H32 post first dose.

The subject will be asked to return for a follow-up visit, scheduled 7 to 14 days post dose.

6.5.3.3. *Follow-up / End of study visit (7 to 14 days post dose)*

The following procedures will be performed

- Vital signs measurement (inc1uding tympanic body temperature),
- Physical examination update,
- 12-lead ECG,
- Laboratory evaluation (hematology, coagulation, biochemistry and urinalysis),
- AE monitoring,
- Review of concomitant medications.

6.5.4. *MAD*

See Flow-Chart Section 3.5.3

6.5.4.1. *Dosing Day (D1)*

The first administration of the study drug will be done after an overnight fast of at least 8 hours.

Subjects will have the following procedures performed at pre-dose:

- Verification of the eligibility criteria,
- Vital signs in triplicate except tympanic temperature,
- 12-lead ECG in triplicate,
- PK blood sampling,
- PK urine sampling (baseline),
- AE monitoring,
- Vigilance assessment.

The daily dose planned in each cohort will be administered in 2 intakes separated by a 12-hour interval, the first intake in the morning at 8:00 am and the second one at 8:00 pm in the evening.

The intake hour (H0) on Day 1 morning will be the reference time for implementing the following procedures at several time-points (see Flow chart Section 3.5.3):

- Vital signs (inc1uding tympanic body temperature),
- 12-lead ECG,
- Diuresis monitoring,
- PK blood sampling at several time points,
- PK urine sampling,
- AE monitoring,
- Vigilance assessment at pre-dose, 1.5h post first dose and 12h post first dose before evening dose.

All assessments planned at 12h post first dose will be performed before the evening dose.

6.5.4.2. *Day 2*

The following procedures will be performed at several time points:

- Study treatment dosing in 2 daily administrations separated by an interval of 12 hours,

- Vital signs (inc1uding tympanic body temperature) before morning dose,
- Diuresis monitoring,
- PK blood sampling (17h, 19h, 21h and 24h post first dose of Day 1, just before the Day 2 morning dose),
- PK urine sampling (end of collection interval T12-T24h of Day 1),
- AE monitoring.

6.5.4.3. *Day 3 to Day 13*

The following procedures will be performed at several time points:

- Study treatment dosing in 2 daily administrations separated by an interval of 12 hours,
- Vital signs every morning before dosing,
- Tympanic body temperature in the morning before dosing on Day 4, Day 6, Day 8, Day 10 and Day12,
- 12-lead ECG on Day 3, Day 6, Day 10 and Day 13,
- Diuresis monitoring on Day 6,
- Safety laboratory tests (hematology, coagulation, biochemistry) in fasting state, at Day 3, Day 6, Day 10 and Day 13,
- Urinalysis on Day 3, Day 6, Day 10 and Day 13,
- PK blood sampling in the morning at Day 7 pre-dose, then 1h and 2h post morning dose,
- PK urine sampling on Day 6: pre dose, 0-4h and 4-12h,
- AE monitoring.

6.5.4.4. *Day 14*

The following procedures will be performed at several time points (see Flow chart Section 3.5.3):

- Study treatment dosing in 2 daily administrations separated by an interval of 12 hours,
- Vital signs (inc1uding tympanic body temperature) in the morning pre-dose,
- Diuresis monitoring,
- PK blood sampling,
- PK urine sampling,
- AE monitoring,

- Vigilance assessment at pre-dose, 1.5h post first dose and 12h post morning dose.

6.5.4.5. *Day 15 to Day 17*

The following procedures will be performed:

- Full physical exam at Day 17 before discharge,
- Vital signs every morning,
- Tympanic body temperature at Day 17,
- Safety laboratory tests (hematology, coagulation, biochemistry) in fasting state at Day 17,
- Urinalysis on Day 17,
- 12-lead ECG on Day 17,
- Diuresis monitoring on Day 15 and Day 16,
- PK blood sampling on Day 15 (17h, 19h, 21h, 24h, 30h, 36h and 42h post Day 14 morning dose) and on Day 16 (48h and 60h post Day 14 morning dose),
- PK urine sampling on Day 15 (end of collection of T12-24h and T24-36h post Day 14 morning dose) and on Day 16 (T36-48h post Day 14 morning dose),
- AE monitoring,
- Discharge after assessment of scheduled evaluations and medical authorization.

The subject will be asked to return for a follow-up visit, scheduled 7 to 14 days post last dose.

6.5.4.6. *Follow-up / End of study visit (7 to 14 days post last dose)*

The following procedures will be performed

- Vital signs measurement (inc1uding tympanic body temperature)
- Full physical examination,
- 12-lead ECG,
- Laboratory evaluation (hematology, coagulation, biochemistry and urinalysis),
- AE monitoring,
- Review of concomitant medications.

6.6. Lifestyle And/or Dietary Restrictions

6.6.1. *Contraception Requirements*

Subjects with female partners of child-bearing potential must use condom while the female partner must use a highly effective contraceptive, such as IUD, birth control pills or diaphragm with spermicide, after the first dose of study treatment and until the end of study visit (for female partners) and during an additional 90 days period (for subjects themselves).

6.6.2. *Meals and Dietary Restrictions*

The first dose of the study drug in the morning will be done in the fasting state. Subjects will be required to fast from all food and drink with the exception of water for 8 hours prior to study drug administration. Water is permitted and may be consumed ad libitum post-dosing with a minimal intake of 2L/day to assure a sufficient diuresis, distributed as follows: 500 mL between dosing and T4H, 1000 mL between T4H and T12H and 500 mL between T12H and T24H.

Moreover, subjects will be asked on Day-1:

- To ensure sufficient hydration (around 2L over the 24-hour period) with a normal salt intake,
- To refrain from any fruit juice and sparkling water,
- These recommendations will be maintained during the hospitalization period.

In the SAD part, a standard lunch will be provided approximately 4 hours post-dosing. Dinner will be served 14 hours post morning dose (2 hours after the evening administration and after completion of assessments scheduled at this time from Cohort 2).

In the MAD part, a breakfast will be provided 2 hours post-morning dose, standard lunch 5 hours post-morning dose, a snack 9 hours post-morning dose and a dinner will be served 2 hours post-evening dose. Therfore, breakfast will be served at 10 AM, lunch at 1 PM, snack at 5 PM and dinner at 10 PM

Subjects will be requested to abstain from consumption of grapefruit or grapefruit-containing products) for 7 days prior to first dosing and until the follow up visit.

6.6.3. *Caffeine, Alcohol*

During each dosing session, subjects will abstain from alcohol and xanthine-containing beverages (e.g. coffee, tea, cola drinks, chocolate) for 24 hours prior to the start of dosing until collection of the final pharmacokinetic sample during each session.

6.6.4. *Activity*

Subjects will abstain from strenuous exercise for 48 hours prior to each blood collection for clinical laboratory tests. Subjects may participate in light recreational activities during studies (e.g., watch television, read*).*

6.7. Total volume of blood collected

Blood samples will be obtained according to the following schedule:

6.7.1. *SAD part: Collection schedule and Blood volume*

- Screening Visit (D-21 to D-2)

Biochemistry, Hematology, Coagulation, Serologies (5+2+4+8.5 mL)
Total =19.5 mL

- Admission Visits D-l

Biochemistry, Hematology, Coagulation (5+2+4 mL)
Total = 11 mL

- Dosing Visit Dl

PK samples: 14 samples (14 x 8 mL)
Exploratory biomarkers samples: 2 samples ([3+2.5 mL] x 2)
Total = 123 mL

- D2

PK samples: 2 samples (Cohort 1) (2 x 8 mL), 3 samples (Cohorts 2 to 6) (3 x 8 mL)
Exploratory biomarkers samples 1 sample (3+2.5 mL)
Biochemistry, Hematology, Coagulation (5+2+4 mL)
Total = 32.5 mL (Cohort 1), 40.5 mL (From Cohort 2 to 6)

- Follow up visit

Biochemistry, Hematology, Coagulation (5+2+4 mL)
Total = 11 mL

Total on the overall duration of the study: 197 mL (Cohort 1) and 205 mL (Cohorts 2 to 6)

6.7.2. *MAD part: Collection schedule and Blood Volume*

- Screening Visit (D-21 to D-1)

Biochemistry, Hematology, Coagulation, Serologies (9+2+2.7+5 mL)
Total =18.7 mL

- Admission Visits D-l

Biochemistry, Hematology, Coagulation (5.5+2+2.7 mL)
Total = 10.2 mL

- Dosing Visit Dl

PK samples: 12 samples (12 x 8 mL)
Total = 96 mL

- Dosing Visit D2

PK samples: 4 samples (4 x 8 mL)
Total = 32 mL

- Dosing Visit D3

Biochemistry, Hematology, Coagulation (5.5+2+2.7 mL)
Total = 10.2 mL

- Dosing Visit D6

Biochemistry, Hematology, Coagulation (5.5+2+2.7 mL)
Total = 10.2 mL

- Dosing Visit D7

PK samples: 3 samples (3 x 8 mL)
Total = 24 mL

- Dosing Visit D10

Biochemistry, Hematology, Coagulation (5.5+2+2.7 mL)
Total = 10.2 mL

- Dosing Visit D13

Biochemistry, Hematology, Coagulation (5.5+2+2.7 mL)
Total = 10.2 mL

- Dosing Visit Dl4

PK samples: 12 samples (12 x 8 mL)
Total = 96 mL

- Dosing Visit D15

PK samples: 7 samples (7 x 8 mL)
Total = 56 mL

- Dosing Visit D16

PK samples: 2 sample (2 x 8 mL)
Total = 16 mL

- Dosing Visit D17

Biochemistry, Hematology, Coagulation (5.5+2+2.7 mL)
Total = 10.2 mL

- Follow up visit

Biochemistry, Hematology, Coagulation (5.5+2+2.7 mL)
Total = 10.2 mL

Total on the overall duration of the study: 410.1 mL

7. COMPLETION OR EARLY WITHDRAWAL OF SUBJECTS

7.1. Subject Completion

A completed subject is one who has completed all phases of the study including the follow-up visit.

The end of the study is defined as the last subject's last visit.

7.2. Subject Withdrawal Criteria

Refer to Section 3.4 for dose adjustment/stopping criteria based on safety and PK criteria.

A subject may withdraw from study treatment at any time at his own request, or may be withdrawn at any time at the discretion of the investigator for safety, behavioral or administrative reasons.

7.3. Subject Withdrawal Procedures

In the event a randomized subject is withdrawn from the study for any reason, the Investigator should make every effort to perform a final study visit 7 to 14 days after last administration of investigational product and complete the assessments and procedures outlined in the Time & Events Table for 'Follow Up'.

Subjects who withdraw due to an AE(s) must have the AE followed as indicated in Follow-Up of AEs/SAEs.

Subjects who withdraw may be replaced at the discretion of the sponsor's team in consultation with the investigator.

8. ADVERSE EVENTS (AE) AND SERIOUS ADVERSE EVENTS (SAE)

The investigator or site staff is responsible for detecting, documenting and reporting events that meet the definition of an AE or SAE.

AEs will be collected from the signature of Informed Consent and until the follow-up visit.

This period will be extended on all on-going AEs after the last visit until all AEs are finally resolved.

SAEs will be collected over the same time period as stated above for AEs.

There is no time limit on the collection of SAEs that are considered related to study drug. Nevertheless and by default, the collection of SAE is primarily set as 1 month after the end of study participation. If the Investigator detects an SAE in a study subject after the end of the period of observation, and considers the event possibly related to prior study treatment

or procedures, he must contact the sponsor to determine how the SAE should be documented and reported.

8.1. Definitions of Adverse Events and New Facts

❖ Definition of an Adverse Event

An AE is **any untoward medical occurrence** in a patient or clinical investigation subject, temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the use of a medicinal product.

Events meeting the definition of an AE **include**:

- Any abnormal laboratory test results (hematology, coagulation, clinical chemistry, or urinalysis) or other safety assessments (e.g., ECGs, vital signs measurements), including those that worsen from baseline, and felt to be clinically significant in the medical and scientific judgment of the investigator.
- Exacerbation of a chronic or intermittent pre-existing condition including either an increase in frequency and/or intensity of the condition.
- New conditions detected or diagnosed after study treatment administration even though it may have been present prior to the start of the study.
- Signs, symptoms, or the clinical sequelae of a suspected interaction.
- Signs, symptoms, or the clinical sequelae of a suspected overdose of either study treatment or a concomitant medication (overdose per se will not be reported as an AE/SAE unless this is an intentional overdose taken with possible suicidal/self-harming intent; this should be reported regardless of sequelae).

❖ Treatment Emergent Adverse Event and Repetition of Adverse Event

- A treatment-emergent AE is any AE that occurs after dosing, or that was present prior to dosing but is exacerbated after dosing.
- AE occurring after subject enrolment (ICF signature) but prior to first study drug administration will be recorded as a non-treatment emergent adverse event (NTEAE).
- A clinically relevant worsening of an AE (e.g., relevant change in severity, seriousness) must result in a new entry. The original entry remains unresolved and is given an end date reflecting the date of the worsening and a comment must be entered stating that the AE is continuing with a changed severity/seriousness (e.g., "continues as event name with onset date and new severity/seriousness"). The onset date of the new entry is also the date of worsening.

❖ Events **that do not meet** the definition of an AE include:

- Medical or surgical procedure (e.g., endoscopy, appendectomy); the condition that leads to the procedure is an AE.
- Situations where an untoward medical occurrence did not occur (social and/or convenience admission to a hospital).
- Anticipated day-to-day fluctuations of pre-existing disease(s) or condition(s) present or detected at the start of the study that do not worsen.

❖ **Definition of New Facts**

A New Fact is defined as an incident, experience, or outcome that generally will warrant consideration of changes to the protocol or consent in order to protect the safety, welfare, or rights of participants. A New Fact may warrant corrective actions. Examples of corrective actions or changes that might need to be considered include:

- Modification of inclusion or exclusion criteria to mitigate the newly identified risks
- Implementation of additional safety monitoring procedures
- Suspension of enrollment of new participants or halting of study procedures for enrolled participants
- Modification of informed consent documents to include a description of newly recognized risks
- Provision of additional information about newly recognized risks to previously enrolled participants.

The sponsor informs immediately the competent authorities of those new events and the measures taken and ensure that the Ethics Committee is notified at the same time (France: ANSM, CPP, ARS).

8.2. Definition of Serious Adverse Events

❖ General definitions

A SAE or Serious Adverse Drug Reaction (ADR) is any untoward medical occurrence that at any dose:

- Results in death
- Is life-threatening (refers to immediate risk of death as the event occurred)
- Requires inpatient hospitalization or prolongation of an existing hospitalization
- Results in a persistent or significant disability or incapacity (defined as a substantial disruption in a person's ability to conduct normal life functions)
- Is a congenital anomaly or birth defect

Additionally, **important medical events** may be considered SAEs when, based on appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of

such events inc1ude allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in hospitalization, or development of drug dependency or drug abuse.

NOTE: In general, hospitalization signifies that the subject has been detained (usually involving at least an overnight stay) at the hospital or emergency ward for observation and/or treatment that would not have been appropriate in the physician's office or out-patient setting. Complications that occur during hospitalization are AEs. If a complication prolongs hospitalization or fulfills any other serious criteria, the event is serious.

Hospitalization for elective treatment of a pre-existing condition that did not worsen from baseline is not considered an AE.

8.3. Expected and unexpected adverse events

As this is a first-in-man (FIM) study, no AEs are defined as expected in humans.

Potential adverse reactions were identified from findings in the non-clinical toxicity studies and are mentioned in the Section 1.3 "Summary of Risk Management".

According to the French legislation and to the directives of the Health Authority (ANSM: "Obligations de déclarations immediates du promoteur" Mai 2017), in a FIM study, a serious adverse drug reaction is considered as a New Fact and **New Facts identified by the Investigator are to be notified without delay to the sponsor**.

In case of New Facts, the administrations of the IMP will be withheld for all subjects, emergency measures will be implemented. The Authorities (ANSM, CPP and ARS) will be notified without delay by the sponsor's representative.

In the event of a suspension of the trial, before restarting, the subjects will have to be informed and, when needed, to sign a new ICF approved after amendment of the study.

8.4. Method of Detecting AEs and SAEs

Care will be taken not to introduce bias when detecting AEs and/or SAEs. Open-ended and non-leading verbal questioning of the subject is the preferred method to inquire about AE occurrence. Appropriate questions include:

- "How are you feeling?"
- "Have you had any (other) medical problems since your last visit/contact?"
- "Have you taken any new medicines, other than those provided in this study, since your last visit/contact?"

Subjects will be provided with a study card containing important information on the study particularly contact of the study staff (investigator) and an emergency phone number to be contacted 24/24 with a physician of the clinical center on duty.

8.5. Recording of AEs and SAEs

When an AE/SAE occurs, it is the responsibility of the investigator to review all documentation (e.g., hospital progress notes, laboratory, and diagnostics reports) relative to the event. The investigator will then record all relevant information regarding an AE/SAE in the appropriate pages in the CRF.

The investigator will attempt to establish a diagnosis of the event based on signs, symptoms, and/or other clinical information. In such cases, the diagnosis will be documented as the AE/SAE and not the individual signs/symptoms.

The following will also be specified:

- Other actions (none, medication required, tests required, hospitalization required or prolonged, treatment unblinded, other-specify)
- Outcome and date of outcome according to the following definitions:
  - Recovered/resolved
  - Recovering/resolving
  - Not recovered/not resolved
  - Recovered with sequelae/resolved with sequelae
  - Fatal
  - Unknown
- Seriousness: yes or no (criteria for SAE see above)

8.6. Evaluating AEs and SAEs

8.6.1. *Assessment of Intensity*

The investigator will make an assessment of intensity for each AE and SAE reported during the study and will assign it to one of the following categories:

- ❖ Mild: An event that is easily tolerated by the subject, causing minimal discomfort and not interfering with everyday activities (Grade 1).
- ❖ Moderate: An event that is sufficiently discomforting to interfere with normal everyday activities, no treatment except paracetamol (Grade 2).
- ❖ Severe: An event that prevents normal everyday activities or requires treatment (Grade 3).

An AE that is assessed as severe will not be confused with an SAE. Severity is a category utilized for rating the intensity of an event; and both AEs and SAEs can be assessed as severe.

8.6.2. *Assessment of Causality*

The investigator has to assess the relationship between study treatment and the occurrence of each AE/SAE.

The relationship of an AE to the IMP is based on all available information at the time of the completion of the CRF and is graded as follows:

- **Not related:** a reaction for which sufficient information exists to indicate that the etiology 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 etiology 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).

8.7. Follow-up of AEs and SAEs

After the initial AE/SAE report, the investigator is required to proactively follow each subject at subsequent visits/contacts. All AEs and SAEs will be followed until resolution.

8.8. Prompt Reporting of SAEs to the sponsor

Once the investigator determines that an event meets the protocol definition of an SAE, the SAE will be reported to the sponsor **without delay, within 24h**.

Any follow-up information on a previously reported SAE will also be reported to the sponsor within 24 hours.

The SAE form must be completed and faxed [Fax number +33 478425571] or emailed [email: InFlectisPV@stragen.fr] to Stragen Services. Stragen Services 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 Stragen Services by any means for ensuring the receipt of SAE information at the earliest opportunity.

The initial report must be as complete as possible, including details of the Serious Adverse Event, and most importantly an assessment of the causal relationship between the SAE and the study medication. Information not available at the time of the initial report (e.g., end date of the Serious Adverse Event or laboratory values received after the report) must be documented on a follow-up "SAE report form".

The SAE report form and the instructions on completion are provided in the investigator's study file.

8.9. Pregnancy

Because the effect of the study drug on sperm is unknown, pregnancies in partners of male subjects included in the study will be reported by the study-site personnel within 24 hours of their knowledge of the event using the SAE form.

Follow-up information regarding the outcome of the pregnancy and any postnatal sequelae in the infant will be required. For this purpose, follow-up requests will be initiated by Stragen Services, at the end of each trimester of the pregnancy and at least until delivery, using a specific Pregnancy Form.

Abnormal pregnancy outcomes (e.g., spontaneous abortion, fetal death, stillbirth, congenital anomalies, and ectopic pregnancy) are considered SAEs and must be reported using the Serious Adverse Event Form.

8.10. Liver chemistry Follow-up procedures

Refer to the diagram in Appendix 1 for a visual presentation of the procedures listed below.

The procedures listed below are to be followed if a subject meets the liver chemistry stopping criteria defined in Section 3.4.3.1:

- Immediately withdraw the subject from study treatment.
- Notify the medical monitor of the sponsor within 24 hours of learning of the abnormality to confirm the subject's study treatment cessation and follow-up.
- Complete the "Safety Follow-Up Procedures" listed below.
- If the event also meets the criteria of an SAE (see Section 8.2), all remaining subjects of the cohort will be informed immediately, and the IMP administration immediately stopped. The sponsor will be notified without any delay. The SAE form will be completed with the relevant details.

Safety Follow-Up Procedures for subjects with ALT ≥ 3xULN:

- Monitor subjects weekly until liver chemistries (ALT, AST, alkaline phosphatase, bilirubin) resolve, stabilize or return to within baseline values.

Safety Follow-Up Procedures for subjects with ALT ≥3xULN and total bilirubin ≥2xULN (>35% direct bilirubin); or ALT ≥ 3xULN and INR > 1.5:

- This event is considered an SAE (see Section 8.2). Serum bilirubin fractionation should be performed if testing is available. If fractionation is unavailable, urinary bilirubin is to be measured via dipstick (a measurement of direct bilirubin, which would suggest liver injury).
- All remaining subjects of the cohort will be informed immediately and the IMP administration immediately stopped. The sponsor will be notified without any delay.
- Make every reasonable attempt to have subjects return to the clinic within 24 hours for repeat liver chemistries, additional testing, and close monitoring (with specialist or hepatology consultation recommended).
- Monitor subjects twice weekly until liver chemistries (ALT, AST, alkaline phosphatase, bilirubin) resolve, stabilize or return to within baseline values.

In addition, for all subjects with ALT ≥ 3xULN, every attempt must be made to also obtain the following:

- Viral hepatitis serology including:
  - Hepatitis A IgM antibody.
  - Hepatitis B surface antigen and Hepatitis B Core Antibody (IgM).
  - Hepatitis C RNA.
  - Cytomegalovirus IgM antibody.
  - Epstein-Barr viral capsid antigen IgM antibody (or if unavailable, obtain heterophile antibody or monospot testing).
  - Hepatitis E IgM antibody.
- Blood sample for pharmacokinetic (PK) analysis. Record the date/time of the PK blood sample draw and the date/time of the dose of study treatment prior to blood sample draw on the source documents and the CRF.
- Serum creatine phosphokinase (CPK) and lactate dehydrogenase (LDH).
- Fractionate bilirubin, if total bilirubin ≥ 2xULN.
- Assess eosinophilia.
- Record the appearance or worsening of clinical symptoms of hepatitis (fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash or eosinophilia) on the AE CRF.
- Record use of concomitant medications, on the appropriate pages of the CRF.

The following are required for subjects with ALT ≥ 3xULN **and** bilirubin ≥ 2xULN (>35% direct) but are optional for other abnormal liver chemistries:

- Anti-nuclear antibody, anti-smooth muscle antibody, and Type 1 anti-liver kidney microsomal antibodies.
- Liver imaging (ultrasound, magnetic resonance, or computerized tomography) to evaluate liver disease.

9. DATA MANAGEMENT AND STATISTICAL CONSIDERATIONS

9.1. Data management

CIC-CPCET Pharmacometry unit will be responsible for activities associated with the data management of this study. This will include setting up a relevant database and data transfer mechanisms, along with appropriate validation of data and resolution of queries. Data generated within this clinical study will be handled according to the relevant SOPs of the data management and biostatistics unit.

A detailed Data Management Plan (DMP) will be established before the study initiation.

9.1.1. *Data collection*

All the results will be recorded in an electronic CRF for each subject. Designated study site staff will enter data required by the protocol into CRFs.

All data obtained in this study should be source-documented. The appropriate study site staff will record all data on source documents immediately. If any form of printout is available, each copy of such printouts should be dated, signed by the investigator and attached to the subject's file.

It is the responsibility of the investigator to ensure that CRFs are kept up to date so that they always contain the latest observations on the subjects enrolled. Corrections to CRFs will be made directly into the CRF by the investigator or an authorised staff member. For any correction made, user's identification, date and reason(s) for the change will be reported in the audit trail.

All supportive documentation in addition to the CRF, such as laboratory results, ECG traces must be clearly identified with the study code, the subject's number and initials. Any personal information, including the subject's name, must be removed or rendered illegible to preserve individual confidentiality.

9.1.2. *External Data*

External data will concern PK data (Excel files provided by laboratory and by PK modeling). A data transfer protocol will be validated, and the data will be directly imported into the study database.

9.1.3. *Data validation*

Consistency checks will be implemented according to the DMP. If inconsistencies are detected, queries will be generated using eCRF queries and all queries will be documented in an audit file.

9.1.4. *Data coding*

Adverse events (AEs) and medical histories will be coded according to the Medical Dictionary for Regulatory Activities (MedDRA 21.0 or the latest available version at the study completion date). Concomitant medications will be coded according to the ATC codes.

9.1.5. *Data Review meeting*

A Data Review meeting will be organized before locking the database at the end of each study part (*i.e.* end of SAD and end of MAD). Reconciliation of pharmacovigilance database and study database will be done before each meeting.

The study database will be locked in SAS export format. Formatted database will be transferred for statistical analysis.

A data management report will document all steps at the end of the study.

9.2. Sample Size Considerations

No formal sample size calculation has been performed (no formal hypothesis will be tested). The sample size is based on empirical considerations and on the literature.

Nevertheless, a sample size of 6 subjects receiving an active drug provides a chance of 26% to observe at least one adverse event, with a true adverse event rate estimated to 5%. This level of predictability is deemed adequate for this FTIH study.

No sample size re-estimation will be planned.

9.3. Data Analysis Considerations

Statistical analysis will be performed by the Pharmacometrics unit of the Clinical Pharmacology department.

Complete details of the planned statistical analyses will be provided in the Statistical Analysis Plan (SAP).

Patients' characteristics and safety data will be analyzed using SAS® software version 9.4 (SAS Institute Inc., Cary, NC, USA).

Pharmacokinetic data will be analyzed using NONMEM ICON Development Solutions v7.4) and R software (www.r-project.org, v3.2.2).

9.3.1. *Description of data sets*

Several populations will be considered:

The Included set will be defined as all included subjects.

The Randomized set will be defined as all randomized subjects.

The Safety set will be defined as all included subjects having taken at least one dose of IMP.

The PK set will be defined as all randomized subjects having taken at least one dose of IMP without protocol deviation affecting PK evaluation and with available PK data.

The analysis sets will be validated during the final data review meeting.

A summary of protocol deviations by treatment group and the corresponding listing by subject will be generated.

9.3.2. *Hypothesis and statistics*

No formal statistical hypothesis will be tested. Safety data including but not limited to AEs, vital signs, ECGs, and laboratory safety tests will be descriptively summarized by doses.

Estimation approach will be adopted to estimate both the between- and within-subject PK variability and to assess the dose proportionality following single dosing of IFB-088.

Descriptive statistics will be supplied according to the nature of the criteria:

- Quantitative variables: sample size, arithmetic mean, standard deviation (SD), median, minimum and maximum (with geometric mean and coefficient of variation (CV) for PK);

- Qualitative variables: sample size, absolute and relative frequencies per class.

9.3.3. *Interim Analysis and/or schedule of analyses*

No formal interim analysis is planned. During dose escalation phases, PK and safety data will be reviewed on an in-stream basis to inform the dose escalation decision.

Statistical analyses will be performed after achievement of each cohort in order to provide ongoing safety, PK data so that the DEC can have access to all information they need to decide the dose escalation. A statistical report will be generated for each DEC meeting, the content will be detailed in the DEC charter.

A final analysis will be done after SAD part and after MAD part, statistical results will be presented in the clinical study report.

9.3.4. *Handling of dropouts and missing data*

Dropout subjects will not be analyzed for pharmacokinetics.

No repositioning of visits will be done.

If not otherwise stated:

- Missing data other than baseline values will not be replaced.
- Baseline for safety parameters will be defined as the last available parameter value before and closest to the first dosing.

9.3.5. *Demographic and baseline characteristics*

The following analysis will be performed on the Randomized set.

The subjects' demographic and baseline characteristics will be summarized by treatment group and listed by subject.

Tables by treatment group with the number of subjects having at least one medical or surgical history and the corresponding listing by subject will be edited; by system organ class and preferred term, if relevant (MedDRA). Tables by treatment group with the number of subjects having at least one previous treatment and concomitant treatment and the corresponding listing by subject will be prepared.

Baseline safety parameters:

Individual safety data (clinical laboratory, vital signs, ECG and physical examination) measured before the first drug administration will be checked for validity of entrance criteria, and abnormalities will be documented. Abnormal physical findings at baseline will be listed. Individual abnormalities before dosing will be flagged in data listings and presented along with post-dose measurements in the statistical appendices.

9.3.6. *Study drug and concomitant therapy*

Drug dispensing information and details of drug dosing (actual treatment received, actual dose received, date and time of drug intake) for each subject will be listed by treatment group.

Subjects who received concomitant treatments along with the study drug will be listed by treatment group and subject. If relevant, concomitant medications will also be summarized by anatomic class and therapeutic class (ATC drug dictionary) for each treatment group and overall subjects, presenting the frequency of subjects (n) taking a given medication and the number of occurrence of each medication.

9.3.7. *Safety Analyses*

Safety data will be presented in tabular and/or graphical format and summarized descriptively according to standard international guidelines.

9.3.7.1. *Criteria*

- Adverse events
- Vital signs: supine and standing systolic and diastolic blood pressure, pulse rate, tympanic temperature
- Standard 12-lead ECG
- Laboratory parameters: hematology, coagulation, biochemistry, urinalysis
- Physical examination
- Vigilance (Bond and Lader VAS)

9.3.7.2. *Methodology*

Laboratory data (hematology, biochemistry, coagulation and urinalysis), vital signs, vigilance VAS and ECG parameters, (raw data and changes from baseline) will be described.

In addition, vital signs, ECG and laboratory data could be compared to potentially clinically significant range (PCSA) to be specified in the SAP.

All these parameters will be listed by subject and assessment time. Data that is out of normal ranges will be flagged as well clinical significance.

Adverse events (AEs) will be coded according to the Medical Dictionary for Regulatory Activities (MedDRA version 21.0). They will be classified into pre-defined standard categories according to chronological criteria:

- Pre-treatment AEs are defined as AEs that occurred or worsened (or become serious) prior to the first study drug administration.
- The treatment-emergent AEs (TEAEs) are defined as AEs that occurred or worsened (or become serious) during an exposure of study drug.

The number of subjects with at least one adverse event and number of AEs for each treatment group will be summarized by categories, and if relevant detailed by system organ class and preferred term.

All safety analyses will be based on the TEAEs using the safety set. Pre-treatment AEs will be listed by subject in statistical appendices.

TEAEs will be summarized by treatment group:

- The number and percentage of subjects with at least one TEAE overall, any severe TEAE, any serious TEAE, and any TEAE leading to treatment discontinuation.
- The number and percentage of subjects with at least one TEAE by system-organ class and preferred term.
- The number of occurrences of TEAE by system-organ class and preferred term.

Subjects presenting TEAEs will be listed, sorted by, treatment, system organ class, and preferred term.

Serious adverse events will be listed, sorted by treatment and subject.

9.3.8. *Pharmacokinetic Analyses*

A non-compartmental analysis to calculate pharmacokinetic parameters of IFB-088 and IFB-139 in plasma and urine (described in §3.2.2) will be performed by the Pharmacometrics Unit.

Plasma concentrations of IFB-088 will be used to estimate pharmacokinetics parameters in human and compare to estimated pharmacokinetics parameters from inter-species model. Pharmacokinetic modeling will be performed using a non-linear mixed effects model with

NONMEM software (ICON Development Solutions v7.4). Data will be analysed using a first order conditional estimation method. The R software (www.r-project.org, v3.2.2) will be used for goodness-of-fit diagnostics and graphical displays.

Pharmacokinetics data of SAD will be analyzed using a population pharmacokinetic modeling approach to develop a pharmacokinetic model in human. This new pharmacokinetic model will be used to estimate pharmacokinetic parameters and variability in human and simulate new dosing schemes (with AUC and Cmax information) to help choosing the doses for MAD.

Pharmacokinetic modeling will be performed by the Pharmacometrics Unit.

Pharmacokinetic data will be listed and presented in graphical and/or tabular form as appropriate to the data, and will be summarized descriptively.

10. STUDY CONDUCT CONSIDERATIONS

10.1. Regulatory and Ethical Considerations, Including the Informed Consent Process

10.1.1. *Ethics Committee opinion*

It is the responsibility of the Sponsor to seek and obtain the favorable opinion of the CPP (Independent Ethics Committee).

The present trial will not be initiated until this favorable opinion is obtained.

10.1.2. *ANSM authorisation*

It is the responsibility of the Sponsor to seek and obtain the authorization from ANSM (Agence Nationale de Sécurité du Médicament) for conducting the trial.

The present trial will not be initiated until the ANSM authorization is received.

10.1.3. *Protocol Amendments*

Any significant change (substantial modification) in the study requires a protocol amendment sent to ANSM and/or CPP. The investigator must not make any changes to the study without regulatory authorities and sponsor approval except when necessary to eliminate apparent immediate hazards to the subjects.

10.1.4. *Regulatory requirements and ethical considerations*

In compliance with French law, according the articles R1121-10 to R1121-15 of the Public Health Code (Code de Santé Publique), an approval as a site for Phase 1 biomedical research was granted to the clinical unit by the regional Agency of Health (ARS) (Renewal numbers N° 2017-02 for CIC-CPCET, N°2017-8358 for Eurofins|Optimed)

The study will be conducted in accordance with all applicable regulatory requirements and in accordance with ICH Good Clinical Practice (GCP E6 R2), all applicable subject privacy requirements, and the guiding principles of the Declaration of Helsinki (last amendment 2013).

Written informed consent must be obtained from each subject prior to participation in the study. Two Inform Consent forms will be prepared, one for the SAD part and one for the MAD part. The form for the MAD, updated before implementation of this part, with the safety and PK results collected during the SAD part and with the MAD planned dose levels, will be submitted to the CPP.

Subjects will be informed by an investigator of all pertinent aspects of the trial: the purpose of the study, the study drug, the trial procedures to be followed, including all invasive procedures, its possible risks and restrictions, the duration of subject's participation, and the fee that they will receive. Each subject must be informed that participation in the study is voluntary and that he may withdraw from the study at any time, that records identifying the subjects will be kept in confidential manner, except for the trial monitor and the regulatory authorities which will be granted direct access to medical records for verification of data and study procedures, and that data collected on CRFs during the study will be documented in an pseudonymous fashion. An information sheet will be given to each subject. The language used in the oral and written information about the trial and written informed consent form, should be easily understandable to the subject.

Before informed consent may be obtained, the investigator should provide the subject ample time and opportunity to inquire about details of the trial and to decide whether or not to participate in the trial. The subject should carefully read before signing and dating the informed consent form. The informed consent form must be signed and personally dated by both the subject and the investigator. A copy of the signed document should be given to the subject. A copy will be kept for 15 years by the investigator (the second copy given to the subject).

Whenever important new information becomes available that may be relevant to the subject's consent, the written subject information sheet and any other written information provided to subjects will be revised by the sponsor and be submitted again to the Ethics committee for review and approval. The approved, revised information will be provided to each subject in the trial for signing and dating. The Investigator will explain the changes to the previous version.

10.1.5. *Subject confidentiality*

All personal data collected and processed for the purposes of this study should be managed by the Investigator and his/her staff with adequate precautions to ensure confidentiality of those data, and in accordance with the Health Insurance Portability and Accountability Act applicable to French national laws and regulations on personal data protection and the General Data Protection Regulation n° 2016/679.

Monitors, auditors, and other authorized agents of the sponsor and/or its designee, the ethics committee approving this research, and ANSM, as well as those of any other applicable agency, will be granted direct access to the study subjects' original medical records for verification of clinical study procedures and/or data, without violating the

confidentiality of the subjects to the extent permitted by the law and regulations. In any presentations of the results of this study or in publications, the subjects' identity will remain confidential.

10.1.6. *Urgent Safety Measures*

If an event occurs that is related to the conduct of the study or the development of the study treatment, and this new event is likely to affect the safety of subjects, the sponsor and the investigator will take appropriate urgent safety measures to protect subjects against any immediate hazard. See paragraph 8.3.

10.2. **Quality Assurance and Quality Control**

10.2.1. *Monitoring*

The study will be monitored by the CRO Qualissima.

In accordance with GCP, the monitor, representative of the sponsor will monitor the study and site activities to verify that the:

- Data are authentic, accurate, and complete.
- Safety and rights of subjects are being protected.
- Study is conducted in accordance with the currently approved protocol and any other study agreements, GCP, and all applicable regulatory requirements.

The monitor will check that the CRFs have been correctly completed, with comparison of source documents and check that AEs have been documented.

Source data must be available at the study site to document the existence of the research subjects and to substantiate the integrity of study data collected. Source data must include the original documents relating to the study, as well as the medical treatment and medical history of the subject.

The investigator agrees to allow the monitor direct access to all relevant documents.

This study will be monitored at regular intervals, by mutual agreement of the investigator and the monitor.

10.2.2. *Audit/Inspection*

The Sponsor or its representative may conduct audits at the investigative site, before, during or after the study. The Regulatory Authorities may also carry out an inspection at any time. In this case, the Investigator must inform the Sponsor as soon as he receives the notification of inspection.

The investigator must allow the auditor(s) and inspector(s) to:

- Inspect the site, facilities and material used for the study,
- Meet the members of the team involved in the study,

- Have direct access to study data and source documents,
- Consult all relevant documents

10.2.3. *Study and Site Closure*

Upon completion or premature discontinuation of the study, the monitor will conduct site closure activities with the investigator or site staff, as appropriate, in accordance with applicable regulations and GCP.

In addition, the sponsor reserves the right to temporarily suspend or prematurely discontinue this study at any time for reasons including, but not limited to, safety or ethical issues or severe non-compliance.

10.2.4. *Records Retention*

Following closure of the study, the investigator must maintain all site study records and investigator file, in a safe and secure location. The records must be maintained to allow easy and timely retrieval, when needed (e.g., audit or inspection), and, whenever feasible, to allow any subsequent review of data in conjunction with assessment of the facility, supporting systems, and staff.

To comply with all applicable regulatory requirements, the minimum retention time will be not less than 15 years.

10.3. **Report and Publication**

10.3.1. *Report*

The results of the study will be reported in a Clinical Study Report. This report will be prepared by the investigational site according to the guidance ICH E3 “Structure and Content of Clinical Study Report”.

10.3.2. *Publications*

The sponsor must review and approve any results of the study or abstracts for professional meetings prepared by the Investigator(s). Published data must not compromise the objectives of the study.

11. REFERENCES

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7. U.S. Food and Drug Administration. (2007) Guidance on Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials

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9. U.S. Food and Drug Administration (2009) Drug-Induced Liver Injury: Premarketing Clinical Evaluation

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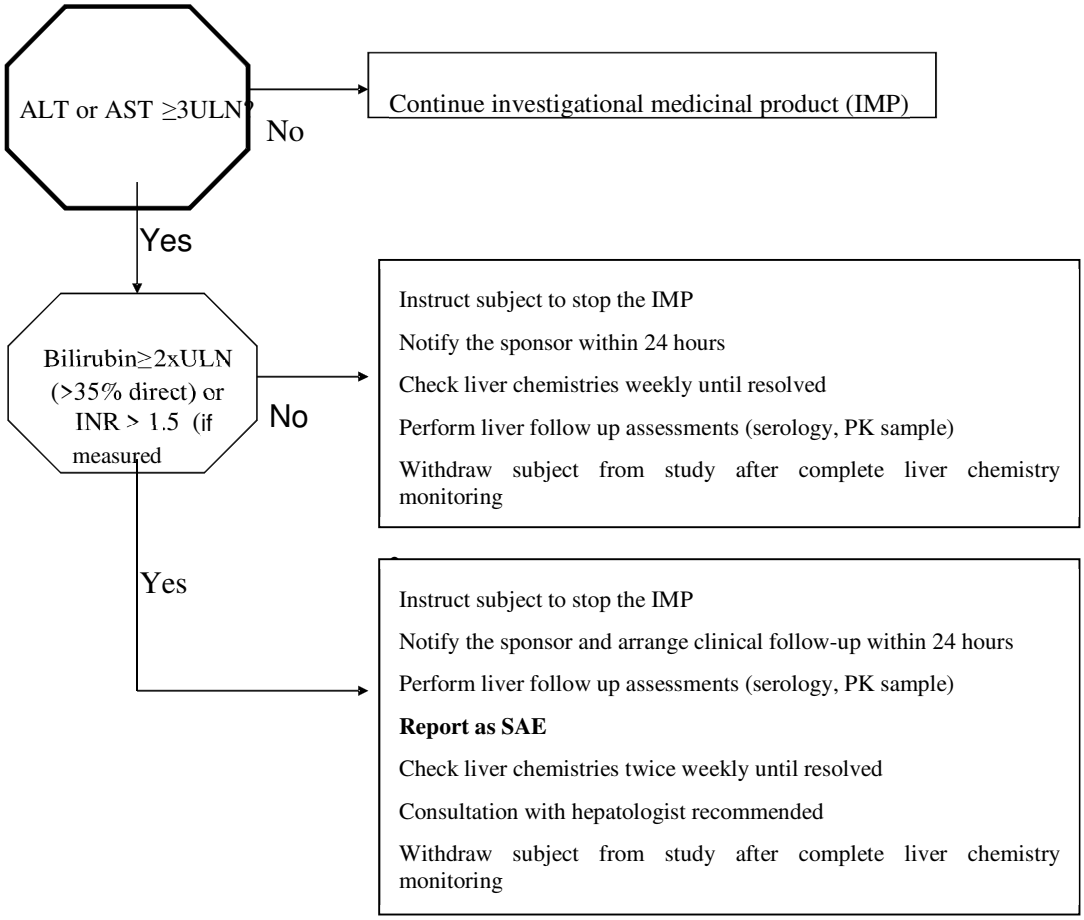
12. APPENDICES

12.1. Appendix 1: Liver Safety Algorithms



12.2. Appendix 2: Stopping rules

Stopping rules	Individual subject	Within a cohort/dose escalation
ALT or AST ≥ 3 x ULN	1 subject	
ALT or AST > 5 x ULN		2 subjects
Triglycerides ≥ 5.6 mmol/L	1 subject	2 subjects
Creat > 1,2 x ULN or Increase in serum creat by ≥ 30 µmol/L within 24h or Increase in serum creat to ≥ 1,5 x baseline within 7 days	1 subject	2 subjects
β2 microglobuline ≥ 2 x ULN	1 subject	2 subjects
Hematuria > 2+ on dipstick verified by cytologic exam, on 2 samples and considered at least possibly related to the IMP	1 subject	2 subjects
QTcB and QTcF > 500 msec or Change from baseline: QTc > 60 msec	1 subject	2 subjects
HR < 45 or HR > 130 bpm*	1 subject	2 subjects
SBP < 80 or SBP > 155 mmHg	1 subject	2 subjects
DBP > 100 mmHg	1 subject	2 subjects
Occurrence of a Serious Adverse Event considered at least possibly related to the IMP administration	1 subject	1 subject
Possibly drug related severe AEs in the same cohort, independent of within or not within the same system-organ		2 subjects
Clinically significant drug-related laboratory abnormalities of the same character		2 subjects
$AUC_{(0\text{-}inf)}$ > 111.14 ng/ml.h^{-1} or Cmax > 23.77 ng/ml		1 subject (only for dose escalation)

* When resting heart rate is between 60-100 beats per minutes. Use clinical judgment when characterizing bradycardia among some healthy subject populations, for example, conditioned athletes.