PPD

CLINICAL STUDY PROTOCOL

STUDY TITLE:

An Open-label, Single-center Study in Healthy Subjects to Investigate the Effect of Oral Givinostat on the Single Dose Pharmacokinetics (PK) of Intravenous or Oral Midazolam and Oral Dabigatran Etexilate

Protocol code:	ITF/2357/55
Type:	Interventional, Pharmacokinetic and Drug-Drug Interaction Trial
EudraCT No.:	2021-005756-11
Version:	1.0
Date:	29NOV2021
Supersedes version:	None
Date:	Not applicable
Sponsor:	ITALFARMACO S.p.A Via dei Lavoratori, 54 20092 Cinisello Balsamo (MI), Italy with its registered office at Viale Fulvio Testi, 330, Milan (MI), Italy www.italfarmaco.com Phone: + 39 02 64431 Fax: + 39 02 644346
Clinical Pharmacology Unit:	PPD

CONFIDENTIALITY DISCLAIMER

1 STUDY SUMMARY

Study Title:	**An Open-label, Single-center, Three-part Study in Healthy Subjects to Investigate the Effect of Givinostat on the Pharmacokinetics of Midazolam and Dabigatran, the Effect of Clarithromycin on the Pharmacokinetics of Givinostat and the Pharmacokinetics of Single and Multiple Doses of Givinostat.**
Study Type:	Interventional, Pharmacokinetic and Drug-Drug Interaction Trial.
Study Code:	ITF/2357/55
EudraCT/IND No.	2021-005756-11/126598
Phase:	Phase I.
Sponsor:	Italfarmaco S.p.A.
Clinical Pharmacology Unit:	PPD
Principal Investigator:	Marlene Fonseca, MD mfonseca@blueclinical.pt
Investigational Products:	ITF2357/Givinostat* 10 mg/mL oral suspension (140 mL/bottle). *Givinostat is used to indicate the whole study drug name givinostat hydrochloride monohydrate. The dosages/concentrations of the study drug are expressed as givinostat hydrochloride monohydrate. Midazolam Aurobindo 1 mg/ml solution for injection/infusion or rectal administration. Midazolam 2.5 mg oromucosal solution (Buccolam®). Dabigatran etexilate 75 mg hard capsules (Pradaxa®).
Background information and trial rationale:	*In vitro* data showed that givinostat (ITF2357) may have a potential for drug-drug interaction (DDI) at level of CYP3A-mediated metabolism and P-glycoprotein (P-gp) transport. In the current study, the perpetrator DDI potential of givinostat as inhibitor and inducer of CYP3A and P-gp activity will be evaluated in Part 1, by determining the impact of givinostat on the pharmacokinetics of midazolam (sensitive index CYP3A substrate) and dabigatran etexilate (P-gp substrate). The perpetrator DDI potential of givinostat at the level of CYP3A activity will be evaluated following oral (effect at both intestinal and hepatic CYP3A) and intravenous (mainly hepatic CYP3A) midazolam administration.
Primary Objectives:	1. To assess the potential inhibitory and inducing effect of oral givinostat on the single dose pharmacokinetics of intravenous and oral midazolam (Part 1). 2. To assess the potential inhibitory and inducing effect of oral givinostat on the single dose pharmacokinetics of oral dabigatran etexilate (Part 1).
Secondary Objectives:	1. To assess the safety and tolerability of concomitant administration of givinostat plus midazolam and dabigatran etexilate (Part 1).

Primary Endpoint	1. Midazolam and dabigatran plasma concentrations and thereof derived pharmacokinetic parameters alone and in combination with Givinostat (Part 1).
Secondary Endpoints	1. Incidence and severity of adverse events (AEs); changes in vital signs, physical examination, electrocardiogram (ECG) and clinical laboratory tests following administration of midazolam and dabigatran etexilate alone and in combination with givinostat (Part 1).
Study Design:	This is an open-label, 3-part, fixed-sequence, non-randomized study in healthy male and female subjects. A summary of procedures and an overview of study design are presented in Section 2 (Flow-Chart and Design Diagram). Study parts may be conducted concomitantly.
Sample Size:	To ensure enough subjects with evaluable data complete each Study part, a total of 54 healthy male and female subjects are planned to be enrolled as follows: – Part 1: 26 subjects – Part 2: 20 subjects – Part 3: 8 subjects Each subject will participate in one study part only.

Inclusion Criteria:	For all subjects: 1. Subject's written informed consent obtained prior to any study-related procedure. 2. Male or female subject, ≥18 and ≤55 years of age, at the time of signing the informed consent. 3. Body mass index (BMI) of 18.5 to 32.0 kg/m^2 inclusive, and body weight ≥55 kg and ≤100 kg for females and body weight ≥60 kg and ≤110 kg for males. 4. Non-smoker or ex-smoker (i.e. someone who abstained from using tobacco- or nicotine-containing products for at least 3 months prior to Screening). 5. No clinically relevant diseases. 6. No major surgery within 4 weeks prior to dosing. 7. No clinically relevant abnormalities on physical examination. 8. No clinically relevant abnormalities on 12-lead ECG. 9. No clinically relevant abnormalities on clinical laboratory tests. 10. Negative test results for anti-Human Immunodeficiency virus 1 and 2 antibodies (anti-HIV-1Ab and anti-HIV-2Ab), Hepatitis B surface antigen (HBsAg) and anti-Hepatitis C virus antibodies (anti-HCVAb). 11. Female subjects are eligible if they are of non-childbearing potential or agree to use a non-hormonal highly effective contraceptive method from 28 days prior to Screening until at least 90 days after the last study drug administration. Nonchildbearing potential female is defined as: a) Menopausal, i.e. no menses for ≥ 12 months without an alternative medical cause other than menopause, and a high follicle-stimulating hormone (FSH) level. b) Pre-menopausal female with hysterectomy, bilateral salpingectomy and/or bilateral oophorectomy. A non-hormonal highly effective contraceptive method is defined as: a) Intrauterine device. b) Bilateral tubal occlusion. c) Total abstinence of heterosexual intercourse, in accordance with the lifestyle of the subject. d) Vasectomized partner, who has received medical assessment of the surgical success, or clinically diagnosed infertile partner. 12. Male subjects who are sexually active with a female partner of childbearing potential (pregnant or non-pregnant) must use contraception (condom) from investigational product administration up to at least 90 days following the last study drug administration. 13. Male subjects must ensure that his non-pregnant female partner of childbearing potential agrees to consistently and correctly use for the same period a highly effective method of contraception (see Section 8.3.3). 14. Male subjects must be willing not to donate sperm until 90 days following the last study drug administration. 15. Willingness and capability to comply with the requirements of the study and ability to understand the study procedures and the risks involved.
Exclusion Criteria:	Subjects to whom any of the following criteria apply will be excluded from the study:

	At Screening 1. Previous use of givinostat. 2. History of anaphylaxis reaction or clinically significant drug hypersensitivity reaction (e.g., angioedema, Stevens-Johnson syndrome, acute generalized exanthematous pustulosis, drug-induced hypersensitivity syndrome, drug-induced neutropenia). 3. Known history of hypersensitivity and/or allergic reactions to givinostat, histone deacetylases (HDAC) inhibitors or to any excipient in the formulation. 4. History of sorbitol intolerance, sorbitol malabsorption or fructose intolerance. 5. Any medical condition (e.g. gastrointestinal, renal or hepatic, including peptic ulcer, inflammatory bowel disease or pancreatitis) or surgical condition (e.g. cholecystectomy, gastrectomy) that may affect drug pharmacokinetics (absorption, distribution, metabolism or excretion) or subject safety. 6. Systolic blood pressure lower than 90 or over 140 mmHg, diastolic blood pressure lower than 60 or over 90 mmHg, or pulse rate lower than 50 or over 100 bpm. 7. QTcF >450 msec. 8. Subjects with history of cardiac arrhythmias (documented), family history of sudden cardiac death or history of additional risk factors for torsades-de-pointes (e.g. heart failure, hypokalemia, long QT syndrome). 9. Having an estimated glomerular filtration (eGFR) < 80 mL/min, based on creatinine clearance calculation by the Cockcroft-Gault formula and normalized to an average surface area of 1.73 m^2. 10. Any of the following abnormal laboratory test values: a) Platelet count below the lower limit of the normal range (LLN) b) Total white blood cells count below the LLN c) Hemoglobin below the LLN d) Triglycerides above the upper limit of normal range (ULN) e) Potassium or magnesium below the LLN 11. Positive urine alcohol, drugs-of-abuse or cotinine screen tests. 12. Positive serum pregnancy test. 13. If woman, she is breast-feeding. 14. History of alcohol abuse within 1 year prior to screening or regular use of alcohol within 6 months prior to the screening visit (i.e. more than 14 units of alcohol per week for males or more than 7 units for females). 15. History of drug abuse within 1 year prior to screening or use of soft drugs (such as marijuana) within 3 months prior to the screening visit or hard drugs [such as cocaine, phencyclidine (PCP), crack, opioid derivatives including heroin, and amphetamine derivatives] within 1 year prior to screening. 16. Participation in any clinical trial within the previous 2 months. 17. Participation in more than 2 clinical trials within the previous 12 months. 18. Blood donation or significant blood loss (≥ 450 mL) due to any reason or had plasmapheresis within the previous 2 months. 19. Veins unsuitable for intravenous puncture on either arm. 20. Difficulty in swallowing capsules, tablets or suspensions.

	21. Any reason which, in the opinion of the Investigator, would prevent the subject from participating in the study. *At Admission to Treatment Period* 22. Any clinically relevant abnormalities on clinical laboratory tests. 23. Positive urine alcohol, drugs-of-abuse or cotinine screen tests. 24. Positive urine pregnancy test. 25. Positive or inconclusive SARS-CoV-2 test prior to admission. 26. Use of prescription or non-prescription medicinal products within 28 days or within 5-half-lives of the medicinal product, whichever is longer. 27. Any reason which, in the opinion of the Investigator, would prevent the subject from participating in the study. **For Part 1 subjects**: *At Screening* 28. Known history of hypersensitivity and/or allergic reactions to midazolam, other benzodiazepines, dabigatran etexilate or to any excipient of the formulations. 29. Clinically relevant history of impaired respiratory function, obstructive sleep apnea, myasthenia gravis, respiratory arrest and/or cardiac arrest. 30. History of glaucoma. 31. Presence of respiratory failure. 32. Presence of active clinically significant bleeding. 33. Lesion or condition considered to pose a significant risk factor for major bleeding including, but not limited to: current or recent gastrointestinal ulceration, malignant neoplasms, recent brain or spinal injury, recent brain, spinal or ophthalmic surgery, recent intracranial hemorrhage, known or suspected esophageal varices, arteriovenous malformations, vascular aneurysms or major intraspinal or intracerebral vascular abnormalities. 34. Presence of a medical condition requiring anticoagulant treatment. *At Admission to Treatment Period* 35. Treatment with anticoagulants within 28 days or 5 drug half-lives, whichever is longer, before admission.
Screening Procedures:	The healthy status will be determined by the following pre-study assessments: – Collection of demographic data, medical history, and medication history. – Complete physical examination, body measurements and vital signs [blood pressure (BP), pulse rate (PR), respiratory rate (RR), and body temperature]. – ECG. – Fasting safety laboratory assessments: ▪ Hematology [complete blood count: red blood count (RBC), white blood cell (WBC) (including differential count), hemoglobin, mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), mean

	corpuscular volume (MCV), red cell distribution width, hematocrit, platelets and mean platelet volume]. ▪ Biochemistry [total bilirubin, direct and indirect bilirubin, alkaline phosphatase (ALP), amylase, alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), cystatin C, C-reactive protein (CRP), gamma-glutamyltransferase (GGT), creatine kinase (CK), total protein, albumin, uric acid, triglycerides, total cholesterol, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), sodium, potassium, chloride, calcium, magnesium, glucose, creatinine, urea and thyroid-stimulating hormone (TSH)]. ▪ Coagulation [prothrombin time, prothrombin rate, international normalized ratio (INR) and activated partial thromboplastin time (aPTT)]. ▪ Urinalysis. – Viral serology (HIV, hepatitis B and C tests). – Serum pregnancy test. – Urine cotinine, alcohol and drug screen test. – FSH test.
Treatments:	Part 1: On Days 1, 6 and 17, single doses of midazolam 1 mg i.v and dabigatran etexilate 75 mg, will be administered 1 hour after the planned (as per Day 4) morning time of givinostat administration. On Days 2, 7 and 18, a single oral dose of midazolam 2.5 mg oral solution will be administered 1 hour after the planned (as per Day 4) morning time of givinostat administration. From Day 4 to Day 18, givinostat 50 mg as oral suspension will be administered twice a day, in the morning and in the evening. On Day 19, only the morning dose will be administered. Midazolam and dabigatran etexilate will be administered following an overnight fasting of at least 8 hours and subjects will remain fasted until at least 3 hours post-dose. Oral midazolam and dabigatran etexilate will be administered with 150 mL of water. Except for water given with the investigational products, no fluids will be allowed from 1 hour before midazolam and dabigatran dosing until 2 hours post dose. Water will be provided ad libitum at all other times. Midazolam and dabigatran etexilate will be administered with the subjects in a semi-recumbent position. Subjects will remain semi-recumbent until at least 3hours post-dose.
Confinement:	Subjects will be confined at the clinical unit from Day -1 of each Part to the morning of Day 20 in Part 1.
Concomitant Treatments and Study Restrictions:	The use of any medications including Over-the-Counter (OTC) products (including herbal medicines such as St John's Wort, homeopathic preparations, vitamins, and minerals) is forbidden from 28 days or within 5 half-lives of the medicinal product, whichever is longer, prior to admission up to last sample for pharmacokinetics assessment, except for medications for the treatment of adverse events (AEs). Use or the consumption of any methylxanthines-containing products (e.g., coffee, tea, chocolate, or cola) is prohibited from 48 hours prior to admission until the end-of-study, i.e. until the last sample collection

	for pharmacokinetic assessment. Use or the consumption of alcoholic beverages is prohibited from 72 hours prior to admission and until the end-of-study. Subjects will also be advised to abstain from strenuous physical activity within 48 hours prior to blood collection for clinical laboratory tests. Subjects will be requested to abstain from consuming pineapple, Seville orange, pomelo, pomegranate, starfruit or grapefruit products (fresh, canned, or frozen) from 7 days prior to admission until the end-of-study. While confined, participants will receive a standardized diet at scheduled times that do not conflict with other study-related activities.
Blood Sampling for Pharmacokinetics Assessment:	Part 1: A total of 117 blood samples will be collected as follows: – 13 blood samples of 3 mL each will be collected in K_2-EDTA collection tubes at pre-dose and at 2 minutes, 0.25, 0.5, 0.75, 1, 2, 3, 4, 6, 9, 12 and 24 hours after the administration of intravenous midazolam, on Days 1, 6 and 17, for the determination of midazolam and 1-hydroxymidazolam plasma concentrations. – 11 blood samples of 3 mL each will be collected in K_2-EDTA collection tubes at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12 and 24 hours after the administration of oral midazolam, on Days 2, 7 and 18, for the determination of midazolam and 1-hydroxymidazolam plasma concentrations. The 24-hour blood collection following intravenous midazolam will be assumed as the pre-dose sampling for oral midazolam. – 15 blood samples of 4 mL each will be collected in K_2EDTA collection tubes at pre-dose and at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 24, 36, 48 and 72 hours after the administration of dabigatran etexilate, on Days 1, 6 and 17, for the determination of total and free dabigatran plasma concentrations.
Pharmacokinetic Assessments:	The following parameters will be estimated for midazolam, 1-hydroxymidazolam, total dabigatran, free dabigatran, following single doses of the parent drug: – Maximum observed plasma concentration (C_{max}); – Time of occurrence of C_{max} (t_{max}); – Area under the plasma concentration versus time curve (AUC) from pre-dose (time zero) to the last sampling time with quantifiable concentrations (AUC_{0-t}); – AUC from time zero to infinity ($AUC_{0-\infty}$); – Residual area or percentage of extrapolated part of $AUC_{0-\infty}$ ($\%AUC_{extrap}$); – Apparent terminal elimination rate constant (λ_z); and apparent terminal elimination half-life ($t_{1/2}$). Other PK parameters will be calculated, if considered appropriate and justified at the time of the PK analysis.
Safety Assessments:	Clinically significant abnormalities in laboratory safety tests, vital signs, 12-lead ECG, physical examination and any other relevant safety variables will be reported as AEs. The following safety endpoints will be considered: – Treatment-emergent AEs (TEAEs) after IMP administration.

	– Treatment-emergent Serious AEs (SAEs). – TEAEs leading to premature study discontinuation.
Statistical Methods:	*Part 1:* Appropriate descriptive statistics will be used to summarize plasma concentrations and PK parameters. Formal statistical analysis of PK data will be performed to characterize the PK interactions: – *Analysis of the effect of givinostat on midazolam PK:* C_{max} and AUC_{0-24} of midazolam and hydroxy-midazolam when it is administered alone and co-administered with givinostat will be the primary pharmacokinetic parameters for the assessment of the effect of givinostat on midazolam PK. – *Analysis of the effect of givinostat on dabigatran PK:* C_{max} and AUC_{0-72} of total and free dabigatran when dabigatran etexilate is administered alone and co-administered with givinostat will be the primary pharmacokinetic parameters for the assessment of the effect of givinostat on dabigatran. An analysis of variance (ANOVA) will be performed on the *ln*-transformed primary pharmacokinetic parameters. A linear mixed effects model will be applied, using Treatment as fixed effects, assessed at a two one-sided 5% significance level ($\alpha = 0.05$). Subject will be included as random effect. Geometric means ratios (GMR) and corresponding 90% confidence intervals (CI) will be calculated for the *ln*-transformed primary pharmacokinetic parameters, using substrates alone as the reference. Wilcoxon signed rank test will be used to test for the difference in t_{max}.
Planned Trial Timelines:	Expected duration of enrolment: 1 month Expected FSI: March 2022 Expected LSLV: April 2022
Trial Procedures:	See Flowchart
Study Exit Procedures/End of Trial Visit/Early Termination Visit	At the end-of-study (i.e., at the scheduled discharge or in case of earlier discontinuation), subjects will undergo: physical examination, safety laboratory assessments including hematology, biochemistry, coagulation and urinalysis, vital signs (BP, PR, RR and body temperature), 12-lead ECG, weight and, if female, serum pregnancy test. AEs will be monitored.
Follow-up Visit (Day 12 after EoS±2 days)	For each study Part, a follow-up visit will be made to all subjects who received at least one dose of the investigational product for safety assessments [safety laboratory assessments including hematology, biochemistry and coagulation (only in Part 1), vital signs (BP, PR, RR and body temperature), 12-lead ECG and, if female, urine pregnancy test] and to monitor ongoing AEs or to check for possible AEs on Day 12±2 days after the EoS.

2 FLOW-CHART AND STUDY DESIGN DIAGRAM

2.1 Study Flow-Chart – Part 1

	Screening	Admission	Treatment Period																			Safety Follow-up
DAYS	–21 to –3	-1	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20 (EoS[1])	10-14 Days after EoS
Informed consent	X																						
Demographic data[2]	X																						
Medical history	X																						
Selection criteria verification	X	X																					
Physical examination	X																					X	
Physical examination update		X																					
Body height, weight and BMI[3]	X	X																				X	
Vital signs[4]	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
12-lead safety ECG[5]	X	X			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Hematology	X	X				X		X			X			X			X			X		X	X
Biochemistry[6]	X	X				X					X											X	X
Urinalysis	X																					X	
Coagulation	X	X				X					X											X	X
Viral serology (HIV and Hepatitis B and C)	X																						
Urine drug, cotinine and alcohol screen	X	X																					
Pregnancy test[7]	X	X																				X	X
FSH test (post-menopausal only)	X																						
SARS-CoV-2 test[8]		X																					
Givinostat administration[9]						X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		
Midazolam i.v. administration			X					X											X				
Midazolam oral administration				X					X											X			
Dabigatran etexilate Administration			X					X											X				
Blood sampling for midazolam PK[10]			X	X	X			X	X	X									X	X	X		
Blood sampling for dabigatran etexilate PK[11]			X	X	X	X		X	X	X	X								X	X	X	X	
Time in clinic	X	←	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	→		X
AE monitoring	←	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	→

	Screening	Admission	Treatment Period																			Safety Follow-up
DAYS	–21 to –3	-1	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20 (EoS[1])	10-14 Days after EoS
Prior and concomitant medication	←																						→

1 End-of-study is defined as the last study procedure on Day 20 or at early discontinuation of subjects administered at least one dose of investigational product. Early termination procedures will be performed as soon as possible after subject withdrawal, within 10-14 days after last participation of the subject in the study, whenever possible. If clinically significant abnormal results are found on the laboratory safety tests performed during end-of-study safety assessments, the subject will be invited to repeat significant abnormal laboratory results.

2 Demographic data including age, sex and race.

3 Only body weight will be determined at admission and end-of-study.

4 Blood pressure, pulse rate, respiratory rate and body temperature will be assessed at screening, admission, end-of-study and follow-up. On Days 1, 2, 6, 7, 17 and 18 blood pressure, pulse rate and respiratory rate will be assessed before midazolam administration and 0.5, 1, 2, 4, 8 and 12 hours post-dose. On Days 4, 5, 8-16 and 19, blood pressure and pulse rate will be assessed prior to each givinostat morning dose. Body temperature will also be assessed once daily, in the morning, during the confinement period as part of the COVID-19 contingency plan.

5 From Day 4 to Day 19, an ECG will be obtained approximately at 5 hours after the administration of givinostat morning dose. Baseline ECG will be obtained on Day 3 at approximately the same time. ECG will also be obtained at screening, admission, end-of-study and follow-up.

6 The following biochemistry parameters will be assessed at all timepoints: total bilirubin, direct and indirect bilirubin, ALP, ALT, AST, LDH, GGT, creatine kinase, sodium, potassium, chloride, calcium, magnesium, glucose, creatinine and urea. Total cholesterol, LDL-C, HDL-C, triglycerides, amylase, c-reactive protein, cystatin C, total protein, albumin, uric acid, TSH will be assessed at screening and end-of-study.

7 The pregnancy test will be performed in serum at screening and end of study, and in urine at admission and follow-up.

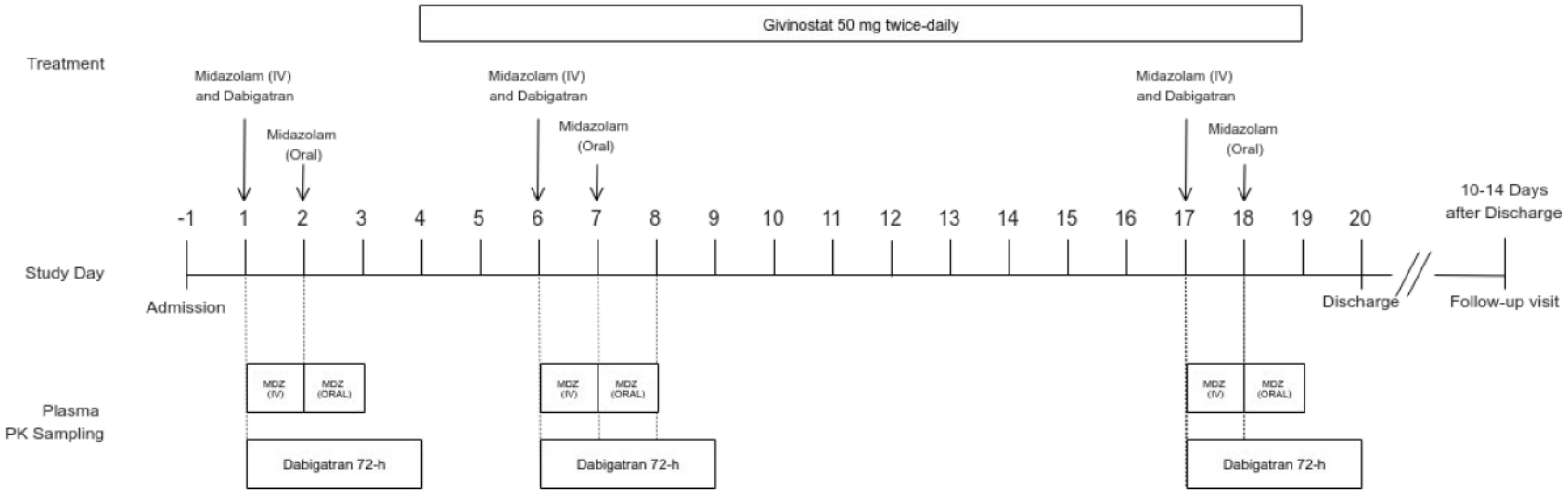
8 SARS-CoV-2 test to be performed prior to admission to treatment period.

9 From Day 4 and Day 18 givinostat 50 mg as oral suspension will be administered twice a day, in the morning and in the evening. On Day 19, only the morning dose will be administered.

10 Blood samples for PK assessment of midazolam and 1-hydroxymidazolam following intravenous midazolam will be collected on Days 1, 6 and 17 at pre-dose and at 2 minutes, 0.25, 0.5, 0.75, 1, 2, 3, 4, 6, 9, 12 and 24 hours after the administration of intravenous midazolam. Blood samples for PK assessment of midazolam and 1-hydroxymidazolam following oral midazolam will be collected on Days 2, 7 and 18 at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12 and 24 hours post-dose. The 24-hour blood collection following intravenous midazolam will be assumed as the pre-dose sampling for oral midazolam.

11 Blood samples for PK assessment of dabigatran (total and free) will be collected on Days 1, 6 and 17 at pre-dose and at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 24, 36, 48 and 72 hours post-dose.

2.2 Study Diagram – Part 1



3 ADMINISTRATIVE INFORMATION

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4 SIGNATURE PAGES

4.1 Clinical Research Unit

We agree to conduct the study in compliance with this Protocol, Declaration of Helsinki, Good Clinical Practice (GCP) guidelines and the applicable regulatory requirements.

Marlene Fonseca, MD
Principal Investigator
PPD
mfonseca@blueclinical.pt

..... Signature ..... Date

PPD , MD, PhD
Clinical Pharmacology Director
PPD
PPD

..... Signature ..... Date

4.2 Sponsor's Representative/Sponsor Medical Expert

On behalf of the Sponsor, I agree to comply with the procedures and Sponsor's responsibilities defined in the Protocol, which has been compiled in observation of the Declaration of Helsinki, Good Clinical Practice (GCP) guidelines and applicable regulatory requirements.

PPD
Clinical R&D Director
Italfarmaco S.p.A.
PPD

..... Signature ..... Date

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5.2 List of Abbreviations

ADR	Adverse Drug Reaction
AE	Adverse Event
ALP	Alkaline Phosphatase
ALT	Alanine Aminotransferase
Amt_{CUM}	Cumulative amount of drug excreted in urine
ANOVA	Analysis of Variance
aPTT	Activated Partial Thromboplastin Time
AST	Aspartate Aminotransferase
ATC	Anatomic Therapeutic Class
AUC	Area Under the Plasma Concentration-Time Curve
AUC_{0-24}	AUC from time zero to 24 hours
AUC_{0-72}	AUC from time zero to 72 hours
AUC_{0-t}	AUC from Time Zero to Last Sampling Time with Quantifiable Concentrations
$AUC_{0-\tau,ss}$	AUC during a Dosing Interval at steady state
$AUC_{0-\infty}$	AUC from Time Zero to Infinity
$\%AUC_{extrap}$	Residual Area or Percentage of Extrapolated Part of $AUC_{0-\infty}$
$AURC_{0-tlast}$	Area under the urine excretion curve from time zero to last observed concentration
β-hCG	Beta Human Chorionic Gonadotropin
BMD	Becker Muscular Dystrophy
BMI	Body Mass Index
BP	Blood Pressure
CEIC	Comissão de Ética para a Investigação Clínica (Portuguese Ethics Committee for Clinical Research)
CI	Confidence Interval
CL_{ss}/F	Apparent total body clearance at steady state
C_{max}	Maximum Observed Plasma Concentration
$C_{max,ss}$	Maximum Observed Plasma Concentration at steady state
Cr_{CL}	Creatinine Clearance
CL_R/F	Apparent renal clearance
CSR	Clinical Study Report
CTCL	Cutaneous T Cell Lymphoma
C_{trough}	Pre-dose Plasma Concentration
CV%	Coefficient of Variation
CYP	Cytochrome P450
DBP	Diastolic Blood Pressure
DDI	Drug-Drug Interaction
DMD	Duchenne Muscular Dystrophy
DNA	Deoxyribonucleic Acid
ECG	Electrocardiogram
eCRF	Electronic Case Report Form
eCTD	Electronic Common Technical Document
eGFR	Estimated Glomerular Filtration
EoS	End-of-Study
EMA	European Medicines Agency
FDA	Food and Drug Administration
FSH	Follicle Stimulating Hormone
GCP	Good Clinical Practice
GGT	Gamma-Glutamyltransferase
GLP	Good Laboratory Practice

GMP	Good Manufacturing Practice
GMR	Geometric Least Square Means Ratio
HBsAg	Hepatitis B Surface Antigen
HCV	Hepatitis C Virus
HDAC	Histone Deacetylases
HDL-C	High-Density Lipoprotein Cholesterol
HIV	Human Immunodeficiency Virus
ICH	International Council for Harmonisation
IMP	Investigational Medicinal Product
INFARMED	Autoridade Nacional do Medicamento e Produtos de Saúde I.P. (Portuguese National Authority of Medicines and Health Products)
INR	International Normalized Ratio
ISCV%	Intrasubject Coefficient of Variation
LDH	Lactate Dehydrogenase
LDL-C	Low-Density Lipoprotein Cholesterol
LLN	Lower Limit of the Normal
LLOQ	Lower Limit of Quantification
MCH	Mean Corpuscular Hemoglobin
MCHC	Mean Corpuscular Hemoglobin Concentration
MCV	Mean Corpuscular Volume
MedDRA	Medical Dictionary for Regulatory Activities
mRNA	Messenger Ribonucleic Acid
OTC	Over-The-Counter
PCP	Phencyclidine
PCR	Polymerase Chain Reaction
P-gp	P-glycoprotein
PK	Pharmacokinetic
PT	MedDRA Preferred Term
PTCL	Peripheral T-cell Lymphoma
PR	Pulse Rate
QTcF	Corrected QT interval by Fredericia
RBC	Red Blood Cell
REC%	Percentage of drug recovered in urine
R_{max}	Maximum urinary excretion rate
RR	Respiratory Rate
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus 2
SBP	Systolic Blood Pressure
SD	Standard Deviation
SE	Standard Error
SOC	MedDRA System Organ Class
SOP	Standard Operating Procedure
SUSAR	Suspected Unexpected Serious Adverse Reaction
TEAE	Treatment-Emergent Adverse Events
TMF	Trial Master File
t_{max}	Time to Maximum Observed Concentration
$t_{max,ss}$	Time of occurrence of $C_{max,ss}$
tu_{max}	Time to R_{max}
$t_{1/2}$	Apparent Terminal Elimination Half-Life
UAR	Unexpected Adverse Reaction
ULOQ	Upper Limit of Quantification
ULN	Upper Limit of Normal

V_D/F	Apparent volume of distribution
WBC	White Blood Cell
λ_z	Apparent Terminal Elimination Rate Constant

6 INTRODUCTION

ITF2357 (Givinostat hydrochloride monohydrate) is an orally active hydroxamic acid derivative that is being developed by Italfarmaco S.p.A. in the treatment of Duchenne Muscular Dystrophy (DMD) and Becker Muscular Dystrophy (BMD) as an oral suspension formulation.

6.1 Background on HDAC Inhibitors

Zinc ion dependent histone deacetylases (HDACs) are a class of 11 isoenzymes associated with numerous nuclear repressor complexes that, once recruited to specific sites of euchromatin, maintain nucleosome histones in a state of deacetylation so that DNA remains tightly bound and inaccessible to transcription factors for gene expression. In contrast, inhibition of HDAC results in hyperacetylation of these histones and allows the unraveling of DNA sufficient for the binding of transcription factors and the synthesis of mRNA. However, histone deacetylases also deacetylate non-histone substrates in the cytosol. For instance, regulation of protein functions through reversible lysine acetylation involves a growing number of proteins. A report indicates that more than 3,600 critical lysine residues are acetylated in more than 1,750 proteins involved in the regulation of both nuclear and cytoplasmic functions of the cell [Choudhary 2009].

Agents that inhibit HDAC activity vary in structure and examples of selective inhibitors of specific HDACs are also reported in the literature [Wang 2009; Pan 2012].
Investigations of inhibitors of HDAC have focused mainly in malignant cells. Most tumor cells survive and proliferate by suppressing expression of pro-apoptotic genes and this mechanism is reversed by inhibition of HDAC. Furthermore, oncogenic proteins are able to efficiently recruit HDAC enzymes forming multi-protein complexes acting as repressors on those promoter regions normally controlled by the wild-type transcription factor.
The finding that aberrant HDAC activity occurs widely in cancer has provided a rationale for using HDAC inhibitors as a potential treatment with therapeutic benefits and an unprecedented low toxicity for normal cells and selectivity for tumor cells [Khan O 2012; Marks 2010].
A pan HDAC inhibitor, Vorinostat (Zolinza; suberoyl anilide hydroxamic: SAHA) is already approved for cutaneous T cell Lymphoma (CTCL). Romidepsin (Istodax) an inhibitor with different specificity for HDAC isoenzymes, and of different chemical class compared to Vorinostat, has also been approved for CTCL and for peripheral T-cell lymphoma (PTCL). More recently Belinostat (Beleodaq) and Panobinostat (Farydak), other HDAC inhibitors, have been approved for relapsed or refractory PTCL and for the treatment of relapsed and refractory multiple myeloma in combination with Velcade (Bortezomib) and Dexamethasone (Decadron), respectively.
Givinostat is an orally active hydroxamic acid derivative possessing a potent HDAC inhibitory activity and possibly the anti-tumoral properties typical of this class of inhibitors. Therefore, its use for the treatment of malignancies in general and the hematological ones in particular appears to be well justified.

In vitro, the concentrations of HDAC inhibitors required to induce cell death are in the micromolar or higher range; in animals, similar circulating concentrations are needed for tumor regression. An unexpected and seemingly paradoxical property of HDAC inhibition is the reduction of inflammation, particularly in models of autoimmune diseases and inflammation. This additional effect is exerted at concentrations/doses which are significantly inferior to the tumoricidal ones and is due to the inhibition of pro-inflammatory cytokine gene expression [Leoni 2002; Nishida 2004]. Several studies *in*

vitro and *in vivo* support the concept that inhibition of HDAC is also a strategy to reduce the production and activity of pro-inflammatory cytokines [Suliman 2012]. The anti-inflammatory activities of a number of HDAC inhibitors, among them Vorinostat (SAHA), givinostat and Dacinostat (LAQ824) have been described [Leoni ; Brogdon 2007; Leoni 2005; Carta 2006]. Recent evidence also indicates that the inhibition of HDAC enzymes leads to profound changes in the epigenetic programme of immune cells. Thus, the deregulated reactivity and lineage commitment of cells of the innate and adaptive immune system found in a variety of inflammatory-related diseases can be reverted to normal by HDAC inhibition [Sweet 2012; Dinarello2011]. The aforementioned anti-inflammatory and immune-modulating activities are the basis of the potential use of givinostat in a number of diseases, such as rheumatological disorders, Graft Versus Host Disease, and selected oncohematological conditions. Finally, the anti-inflammatory properties of givinostat associated with its anti-fibrotic and pro-regenerative activities are the basis for the development of this compound in DMD [Minetti 2006; Colussi 2008; Colussi 2009; Iezzi 2004; Mal 2001; Nebbioso 2009].

6.2 Background on Givinostat

Givinostat has been tested in a number of clinical studies. Three major indications have been explored so far with givinostat in completed or ongoing trials: (i) inflammatory diseases, (ii) oncology and (iii) muscular dystrophies.
Clinical experience with givinostat in humans includes 21 sponsored closed trials and compassionate use program and 4 ongoing trials. The total exposed population is 655 patients in givinostat and 230 patients in givinostat or placebo (ongoing double-blind trials).

Givinostat is well absorbed after oral administration and plasma concentrations increase in a dose proportional manner. The apparent half-life after oral administration is approximately 6-8 hours with a time of occurrence of maximum observed plasma concentration (t_{max}) at 2-3 hours post dose. Steady state levels are achieved after 5-7 days of continuous dosing.
Body weight was identified as covariate of clearance. An increase of 10 kg in body weight in patients weighing from 10 kg to 30 kg resulted in reduction of maximum observed plasma concentration (C_{max}) and area under the curve (AUC) between 20 and 30% on average, while in patients weighing from 30 kg to 70 kg the reduction was between 10-15%. A high fat standard meal slightly affected the pharmacokinetics of givinostat resulting in increased plasma concentrations (about 40% increase in givinostat AUCs and about 20% increase in C_{max}) and in a slight delay in absorption.

The most common adverse events (AEs) observed were thrombocytopenia as well as gastrointestinal toxicities. AEs were generally mild to moderate and reversible upon discontinuation of study drug. The maximum administered dose was a single dose of 600 mg in healthy volunteers and up to 400 mg once per week in patients with multiple myeloma. Doses up to approximately 100 mg b.i.d. were generally well tolerated in adults. At higher doses of givinostat, transient reductions in hematological parameters (particularly platelets), diarrhea, nausea and vomiting were observed.
No serious adverse events (SAEs) occurred throughout the study period in any of the studies with healthy volunteers.
Overall, treatment with givinostat of both healthy volunteers and patients did not show clinically meaningful changes for any of the measured chemistry parameters, with the exception of a transient and slight increase in serum creatinine levels observed in only one study in Crohn's disease patients.
There was no evidence of general systemic toxicity reflected by changes in vital signs in

the studied populations. Isolated cases of palpitation and tachycardia have been reported in givinostat treated patients. There is no indication that those might be causally associated with givinostat. Finally, despite some episodes of QTc prolongation were reported, no events of clinical concern or dose related trend were seen.
In a dedicated QT/QTc (TQT) study, preliminary data suggest that after givinostat administration in single doses of 100 mg (therapeutic dose) or 300 mg (supratherapeutic dose) to healthy subjects, givinostat exerted a dose-dependent effect on heart rate, which was clinically relevant for the supratherapeutic dose (300 mg) only. The effect seen on 100 mg is of no clinical concern. Givinostat caused prolongation of the QTc interval, for the supratherapeutic dose (300 mg) only. The peak effect was seen at 5 hours post-dose for both givinostat tested doses. Givinostat did not have a clinically relevant effect on the heart rate or QRS intervals.
Overall, no subjects reported QTcF > 480 ms or ΔQTcF > 60 ms. There was only one subject administered with the supratherapeutic dose (300 mg) of givinostat with QTcF >450 and ≤480 ms at one time point.

Further details on givinostat, including a summary of the results of the nonclinical and clinical studies already available, are presented in the respective Investigator's Brochure [Givinostat IB].

6.3 Study Rational

6.3.1 Rational for the Study Design

In vitro data showed that givinostat may have a potential for drug-drug interaction (DDI) at level of cytochrome P450 (CYP)3A-mediated metabolism and P-glycoprotein (P-gp) transport.
In the current study, the perpetrator DDI potential of givinostat as inhibitor and inducer of CYP3A and P-gp activity will be evaluated in Part 1, by determining the impact of givinostat on the pharmacokinetics of midazolam (sensitive index CYP3A substrate) and dabigatran etexilate (P-gp substrate). The perpetrator DDI potential of givinostat at the level of CYP3A activity will be evaluated following oral (effect at both intestinal and hepatic CYP3A) and intravenous (mainly hepatic CYP3A) midazolam administration.

6.3.2 Rational for Dose Selection and Treatment Duration

Part 1

Rationale for Givinostat Dose and Treatment Duration as Perpetrator

According to the FDA Guidance on Clinical Drug Interaction Studies [FDA Guidance 2020], the doses of the perpetrator drug used in DDI studies should maximize the possibility of identifying a DDI. The use of the maximum dose and the shortest dosing interval of the perpetrator under the intended conditions is recommended. According to Givinostat Investigator's Brochure [Givinostat IB], this corresponds to 50 mg twice-daily. The treatment duration shall allow appropriate inhibitory effect (usually near steady-state) and induction effect (i.e., about 2 weeks of daily drug administration, according to the FDA Guidance on DDI studies [FDA Guidance 2020]. Thus, the inhibitory effect will be assessed on Days 6 to 9 and the induction effect will assessed on Days 17 to 20.
To maintain adequate inhibition/induction of P-gp and CYP3A, givinostat should be continued for 4–5 half-lives of the substrate with longer half-life (dabigatran), i.e., at least up to 48 hours after dabigatran etexilate administration on Day 17.

Rationale for Substrates Dose

According to the FDA Guidance on DDI studies [FDA Guidance 2020], the therapeutic dose most likely to demonstrate a DDI should be used.
Intravenous midazolam is usually administered in doses up to 2 mg intravenous as a substrate of hepatic CYP3A [Fuhr, 2019]. In the present study, a 1 mg intravenous dose is planned.
Midazolam in the form of oral solution is usually administered in doses up to 5 mg as a substrate of intestinal CYP3A [Fuhr, 2019]. In the present study, a 2.5 mg single dose is planned.
Dabigatran is usually administered in doses up to 150 mg as a probe for intestinal P-gp activity [Fuhr, 2019]. In the present study, a 75 mg single dose is planned.

Rationale for Perpetrator and Substrates Administration Schedule:

DDI studies evaluating the effect of potential inhibitors and inducers on substrate exposure have used simultaneous dosing of perpetrator and substrate/victim, as well as staggered dosing, with the substrate being administered between 1 and 4 hours after the perpetrator dose on the day of coadministration. As a general rule, substrates shall be administered in a timing that aims to capture maximal P-gp and CYP3A inhibition by the perpetrator.
Midazolam oral solution is absorbed rapidly and completely, achieving C_{max} at 0.5 hours [Buccolam® SmPC]. Dabigatran etexilate pharmacokinetic profile in plasma is characterized by a rapid increase in concentrations with C_{max} attained within 0.5 to 2.0 hours [Pradaxa® SmPC].
Considering a T_{max} around 2 hours for givinostat and the pharmacokinetic profile of the substrates, the administration of the substrates will occur 1 hour after the givinostat morning dose on Days 6, 7, 17 and 18.

6.4 Benefit-Risk Assessment

As this clinical pharmacology study is not conducted in the target population for givinostat, there are no anticipated clinical benefits for participants beyond the thorough medical check-up which each subject will undergo prior to receiving the investigational products and at the end of the study.

The previous clinical experience indicates that givinostat is well tolerated at the tested doses in healthy volunteers or patients with chronic inflammatory diseases, or with DMD where dose-related platelets count reduction and diarrhea were the most frequently reported AEs.
As far as patients with onco-hematological conditions are concerned, givinostat seems to have more profound effects on bone marrow cell-lines. Overall, there has been no other major safety concern in the patient population tested.
There is extensive experience with midazolam and dabigatran as probes and with clarithromycin as perpetrator in DDI studies with healthy subjects. The planned doses and treatment duration follow a standard approach and are expected to be safe and well-tolerated by healthy subjects and to allow adequate demonstration of DDI.

Subjects will be kept under medical supervision during the study. Safety assessments will include physical examination, vital signs, 12-lead electrocardiogram (ECG) and clinical laboratory safety tests. Due to the known effects of givinostat on bone-marrow cell-lines, hematology parameters will be frequently monitored during the study.
Subject's total volume of blood withdrawn during the study, including the volume

required for safety tests, will be approximately 477 mL in Part 1. The total blood donation may be higher if repeat blood samples are required.

7 STUDY OBJECTIVES

7.1 Primary

1. To assess the potential inhibitory and inducing effect of oral givinostat on the single dose pharmacokinetics of intravenous and oral midazolam (Part 1).
2. To assess the potential inhibitory and inducing effect of oral givinostat on the single dose pharmacokinetics of oral dabigatran etexilate (Part 1).

7.2 Secondary

1. To assess the safety and tolerability of concomitant administration of givinostat plus midazolam and dabigatran etexilate (Part 1).

8 INVESTIGATIONAL PLAN

8.1 Study Design

This is a phase I, open-label, 3-part, fixed-sequence, non-randomized study in healthy male and female subjects.
A summary of procedures and a study design diagram are presented in Section 2 (Study Flow-Chart and Study Design Diagram).
Study parts may be conducted concomitantly.

8.2 Study Plan

8.2.1 Screening Procedures

The healthy status will be determined by the following pre-study assessments:

- Collection of demographic data, medical history, and medication history.
- Complete physical examination, weight and height and vital signs [blood pressure (BP), pulse rate (PR), respiratory rate (RR), and body temperature].
- 12-lead ECG.
- Fasting safety laboratory assessments:
  - Hematology [complete blood count: red blood cell (RBC), white blood cell (WBC) (including differential count), hemoglobin, mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular volume (MCV), red cell distribution width, hematocrit, platelets and mean platelet volume].
  - Biochemistry [total bilirubin, direct and indirect bilirubin, alkaline phosphatase (ALP), amylase, alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), cystatin C, C-reactive protein (CRP), gamma-glutamyltransferase (GGT), creatine kinase, total protein, albumin, uric acid, triglycerides, total cholesterol, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), sodium, potassium, chloride, calcium, magnesium,

glucose, creatinine, urea and thyroid-stimulating hormone (TSH)].
  - Coagulation [prothrombin time, prothrombin rate, international normalized ratio (INR) and activated partial thromboplastin time (aPTT)].
  - Urinalysis.
- Viral serology [anti-Human Immunodeficiency virus 1 and 2 antibodies (anti-HIV-1Ab and anti-HIV-2Ab), Hepatitis B surface antigen (HBsAg) and anti-Hepatitis C virus antibodies (anti-HCVAb)].
- Serum pregnancy test.
- Urine cotinine, alcohol and drug screen test.
- Follicle stimulating hormone (FSH) test.

8.2.2 Admission to Treatment Period

In each part, the following procedures will be performed during admission to treatment period (Day -1):

- Collection of body weight and physical examination.
- Vital signs measurements (BP, PR, RR and body temperature).
- 12-lead ECG.
- Safety laboratory assessments [hematology, biochemistry and coagulation (only on Part 1)].
- Urine drug and alcohol screen.
- Urine cotinine test.
- Urine pregnancy test for all female subjects.
- SARS-CoV-2 test.

8.2.3 Part 1 - Inhibitory and Inducing Effect of Oral Givinostat on the Single Dose Pharmacokinetics of Intravenous and Oral Midazolam

Subjects will be confined PPD from Day –1 to Day 20.

On Days 1, 6 and 17, single doses of midazolam 1 mg i.v and dabigatran etexilate 75 mg, will be administered 1 hour after the planned morning time of givinostat administration.
On Days 2, 7 and 18, a single oral dose of midazolam 2.5 mg oral solution will be administered 1 hour after the planned morning time of givinostat administration.
From Day 4 to Day 18, givinostat 50 mg as oral suspension will be administered twice a day, in the morning and in the evening. On Day 19, only the morning dose will be administered.
Midazolam and dabigatran etexilate will be administered following an overnight fasting of at least 8 hours and subjects will remain fasted until at least 3 hours post-dose. Oral midazolam and dabigatran etexilate will be administered with 150 mL of water. Except for water given with the investigational products, no fluids will be allowed from 1 hour before midazolam and dabigatran dosing until 2 hours post dose. Water will be provided ad libitum at all other times.
Midazolam and dabigatran etexilate will be administered with the subjects in a semi-recumbent position. Subjects will remain semi-recumbent until at least 3 hours post-dose.

The following assessments will be performed [see Section 2.1 (Study Flow-Chart – Part 1)]:

- Blood collection for pharmacokinetic analysis on Days 1 to 4, 6 to 9 and 17 to 20.
- Vital signs measurements (BP, PR and RR) on Days 1, 2, and 4 to 19.
- 12-lead ECG on Days 3 to 19.

- Blood collection for laboratory tests (hematology, biochemistry and coagulation on Days 4 and 9 and hematology on Days 6, 12, 15 and 18).

Subjects will be discharged from PPD in the morning of Day 20 if allowed by the investigator based on their medical condition. The following procedures will be performed:

- Physical examination.
- Collection of body weight.
- Vital signs measurements (BP, PR, RR and body temperature).
- 12-lead ECG.
- Safety laboratory assessments (hematology, biochemistry, coagulation and urinalysis).
- Serum pregnancy test for all female subjects.

Subjects will return to PPD 10 to 14 days after the end of study to undergo additional assessments as required per protocol.

The total duration of Part 1 for each subject will be up to approximately 8 weeks from Screening to Follow-up visit, divided as follows:

- Screening: up to 21 days
- Treatment Period: Days 1 to 20
- Safety follow-up visit: 12±2 days

8.2.4 Follow-up Visit

In each study part, a follow-up visit will occur 12 ± 2 days after the end of study or premature study discontinuation:

- to perform safety laboratory assessments including hematology, biochemistry and coagulation (only in Part 1), vital signs (BP, PR, RR and body temperature), 12-lead ECG and, if female, urine pregnancy test
- to monitor ongoing AEs or to check for possible AEs.

8.3 Study Restrictions

The study conditions will be standardized in order to minimize the variability of all factors involved except that of the being tested.

To avoid possible negative effects on the measurement of plasma drug concentrations and/or on subjects' safety, several restrictions will be implemented, as described in the next sections.

8.3.1 Lifestyle and Dietary Restrictions

While confined, participants will receive a standardized diet at scheduled times that do not conflict with other study-related activities.
Food and fluid intake will be controlled for all subjects as described in Section 8.2.3 (for Part 1).

Subjects will be requested to abstain from consuming pineapple, Seville orange, pomelo, pomegranate, starfruit or grapefruit products (fresh, canned, or frozen) from 7 days prior to admission until the end-of-study.

Chewing gum will not be allowed during confinement.

Subjects will be advised that they should, within 48 hours prior to admission abstain from consuming any food or beverages containing poppy seeds, because it may render positive the urine drugs-of-abuse tests. Subjects will also be advised to avoid indoor spaces contaminated with tobacco or cannabinoids smoke, because it may render positive the drugs-of-abuse tests.
Use or the consumption of any methylxanthines-containing products (e.g., coffee, tea, chocolate, or cola) is prohibited from 48 hours prior to admission until the end-of-study, i.e. until the last sample collection for pharmacokinetic assessment.

Use or the consumption of alcoholic beverages is prohibited from 72 hours prior to admission and until the end-of-study. Throughout the study, in case of any doubt about alcohol consumption, an alcohol test may be performed if requested by the investigator.

Use of tobacco products is not allowed from 3 months prior to screening until the end-of-study.

Subjects will be advised to abstain from strenuous physical activity within 48 hours prior to blood collection for clinical laboratory tests.

8.3.2 Previous and Concomitant Medication

A previous medication is any medication for which the end date is prior to first dosing.
A concomitant medication is any medication ongoing or initiated after first dosing.

The use of any medications including Over-the-Counter (OTC) products (including herbal medicines such as St John's Wort, homeopathic preparations, vitamins, and minerals) is forbidden from 28 days or within 5 half-lives of the medicinal product, whichever is longer, prior to admission up to last sample for pharmacokinetics assessment, except for medications for the treatment of adverse events (AEs).

In study Part 1, the use of anticoagulants is forbidden within 28 days or 5 drug half-lives, whichever is longer, prior to admission until the end of study.

Any medication (previous or concomitant) taken within the period of medication restriction must be recorded in the electronic case report form (eCRF). If concomitant medication is ongoing at the follow-up visit, no end date will be provided in the eCRF.

8.3.3 Contraception Requirements

Only nonpregnant females can be admitted to the study and women must not become pregnant during the study and until at least 90 days after the last study drug administration.

A woman is not considered of childbearing potential if she:

- Is in a post-menopausal state.
  NOTE: A post-menopausal state is defined as 12 consecutive months with no menses without an alternative medical cause, confirmed by a FSH test.
- Pre-menopausal female with documented hysterectomy, bilateral salpingectomy or bilateral oophorectomy.

Female subjects of childbearing potential will have to take appropriate measures to

prevent pregnancy from at least 28 days prior to Screening until 90 days after the last study drug administration.
It is the participant's responsibility to notify the clinical research unit if a pregnancy occurs from the end of their study participation until 90 days after the last study drug administration.

The acceptable contraceptive measures are the following:
- Intrauterine device.
- Bilateral tubal occlusion.
- Total abstinence of heterosexual intercourse, in accordance with the lifestyle of the subject.
- Vasectomized partner, who has received medical assessment of the surgical success, or clinically diagnosed infertile partner

The not acceptable contraceptive measures are the following:
- Hormonal contraception.
- Male or female condom with or without spermicide.
- Cap, diaphragm or sponge with spermicide.
- Periodic abstinence (e.g., calendar, ovulation, symptothermal, post-ovulation methods).
- Withdrawal (coitus interruptus).

Male subjects who are sexually active with a female partner of childbearing potential (pregnant or non-pregnant) must use contraception (condom) from investigational product administration up to at least 90 days following the last study drug administration.
Male subjects must ensure that his non-pregnant female partner of childbearing potential agrees to consistently and correctly use for the same period one of the following highly effective methods of contraception:
- Progestogen-only hormonal contraception associated with inhibition of ovulation (oral, injectable, implantable).
- Combined (estrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation (oral, intravaginal, transdermal).
- Intrauterine device.
- Intrauterine hormone-releasing system.
- Bilateral tubal occlusion.

Male subjects must be willing not to donate sperm until 90 days following the last study drug administration.

8.4 Measures to Minimize Bias

8.4.1 Randomization

This is a fixed sequence study. Randomization is not applicable.

8.4.2 Blinding

The study will be conducted as open label. Blinding procedures are not applicable.

9 STUDY POPULATION

9.1 Recruitment and Screening Procedures

Study subjects will be recruited PPD 's pool of healthy volunteers, according to the applicable standard operating procedures (SOPs).

Subjects will be screened between Days -21 to -3 (both inclusive) of each study part to confirm that they meet the subject selection criteria.

Prior to any screening assessment, the Investigator (or an appropriate delegate) will obtain informed consent from each subject in accordance with the procedures described in Section 17.1.2 (Subject Information and Consent). The assessments to be performed at Screening are described in Section 8.2.1).
The results of tests performed at screening must be known by the investigator prior to subject's admission to the clinical research site.

Subjects who meet all the inclusion criteria and none of the exclusion criteria at the screening visit of each study part may be eligible for participation in the respective study part. Continued eligibility will be assessed upon admission to the clinical research site, prior to the first study drug administration.

Subjects will receive a unique subject number upon enrolment in the study. Subject numbers will be allocated sequentially in the order in which the subjects are enrolled. The subject number will ensure identification until the Follow-up visit. Subjects will be identified on all study documentation by their subject number only. A list identifying the subjects by subject number will be kept in the Site Master File.

9.2 Inclusion Criteria

Subjects must meet the following criteria to be eligible for enrolment into the trial.
For all subjects:

1. Subject's written informed consent obtained prior to any study-related procedure.
2. Male or female subject, ≥18 and ≤55 years of age, at the time of signing the informed consent.
3. Body mass index (BMI) of 18.5 to 32.0 kg/m^2 inclusive, and body weight ≥55 kg and ≤100 kg for females and body weight ≥60 kg and ≤110 kg for males.
4. Non-smoker or ex-smoker (i.e. someone who abstained from using tobacco- or nicotine-containing products for at least 3 months prior to Screening).
5. No clinically relevant diseases.
6. No major surgery within 4 weeks prior to dosing.
7. No clinically relevant abnormalities on physical examination.
8. No clinically relevant abnormalities on 12-lead ECG.
9. No clinically relevant abnormalities on clinical laboratory tests.
10. Negative test results for anti-Human Immunodeficiency virus 1 and 2 antibodies (anti-HIV-1Ab and anti-HIV-2Ab), Hepatitis B surface antigen (HBsAg) and anti-Hepatitis C virus antibodies (anti-HCVAb).
11. Female subjects are eligible if they are of non-childbearing potential or agree to use a non-hormonal highly effective contraceptive method from 28 days prior to Screening until at least 90 days after the last study drug administration. Nonchildbearing potential female is defined as:
    a) Menopausal, i.e. no menses for ≥ 12 months without an alternative medical cause other than menopause, and a high FSH level.
    b) Pre-menopausal female with documented hysterectomy, bilateral salpingectomy and/or bilateral oophorectomy.

A non-hormonal effective contraceptive method is defined as:
a) Intrauterine device.
b) Bilateral tubal occlusion.
c) Total abstinence of heterosexual intercourse, in accordance with the lifestyle of the subject.
d) Vasectomized partner, who has received medical assessment of the surgical success, or clinically diagnosed infertile partner.

12. Male subjects who are sexually active with a female partner of childbearing potential (pregnant or non-pregnant) must use contraception (condom) from investigational product administration up to at least 90 days following the last study drug administration.
13. Male subjects must ensure that his non-pregnant female partner of childbearing potential agrees to consistently and correctly use for the same period a highly effective method of contraception (see Section 8.3.3).
14. Male subjects must be willing not to donate sperm until 90 days following the last study drug administration.
15. Willingness and capability to comply with the requirements of the study and ability to understand the study procedures and the risks involved.

9.3 Exclusion Criteria

Subjects to whom any of the following criteria apply will be excluded from the study:

At Screening

1. Previous use of givinostat.
2. History of anaphylaxis reaction or clinically significant drug hypersensitivity reaction (e.g., angioedema, Stevens-Johnson syndrome, acute generalized exanthematous pustulosis, drug-induced hypersensitivity syndrome, drug-induced neutropenia).
3. Known history of hypersensitivity and/or allergic reactions to givinostat, histone deacetylases (HDAC) inhibitors or to any excipient in the formulation.
4. History of sorbitol intolerance, sorbitol malabsorption or fructose intolerance.
5. Any medical condition (e.g. gastrointestinal, renal or hepatic, including peptic ulcer, inflammatory bowel disease or pancreatitis) or surgical condition (e.g. cholecystectomy, gastrectomy) that may affect drug pharmacokinetics (absorption, distribution, metabolism or excretion) or subject safety.
6. Systolic blood pressure lower than 90 or over 140 mmHg, diastolic blood pressure lower than 60 or over 90 mmHg, or pulse rate lower than 50 or over 100 bpm.
7. QTcF >450 msec.
8. Subjects with history of cardiac arrhythmias (documented), family history of sudden cardiac death or history of additional risk factors for torsades-de-pointes (e.g. heart failure, hypokalemia, long QT syndrome).
9. Having an estimated glomerular filtration (eGFR) < 80 mL/min, based on creatinine clearance calculation by the Cockcroft-Gault formula and normalized to an average surface area of 1.73 m^2.
10. Any of the following abnormal laboratory test values:
    a) Platelet count below the lower limit of the normal range (LLN)
    b) Total white blood cells count below the LLN
    c) Hemoglobin below the LLN
    d) Triglycerides above the upper limit of normal range (ULN)
    e) Potassium or magnesium below the LLN
11. Positive urine alcohol, drugs-of-abuse or cotinine screen tests.
12. Positive serum pregnancy test.

13. If woman, she is breast-feeding.
14. History of alcohol abuse within 1 year prior to screening or regular use of alcohol within 6 months prior to the screening visit (i.e. more than 14 units of alcohol per week for males or more than 7 units for females).
15. History of drug abuse within 1 year prior to screening or use of soft drugs (such as marijuana) within 3 months prior to the screening visit or hard drugs [such as cocaine, phencyclidine (PCP), crack, opioid derivatives including heroin, and amphetamine derivatives] within 1 year prior to screening.
16. Participation in any clinical trial within the previous 2 months.
17. Participation in more than 2 clinical trials within the previous 12 months.
18. Blood donation or significant blood loss (≥ 450 mL) due to any reason or had plasmapheresis within the previous 2 months.
19. Veins unsuitable for intravenous puncture on either arm.
20. Difficulty in swallowing capsules, tablets or suspensions.
21. Any reason which, in the opinion of the Investigator, would prevent the subject from participating in the study.

At Admission to Treatment Period

22. Any clinically relevant abnormalities on clinical laboratory tests.
23. Positive urine alcohol, drugs-of-abuse or cotinine screen tests.
24. Positive urine pregnancy test.
25. Positive or inconclusive SARS-CoV-2 test prior to admission.
26. Use of prescription or non-prescription medicinal products within the previous 28 days or within 5-half-lives of the medicinal product, whichever is longer.
27. Any reason which, in the opinion of the Investigator, would prevent the subject from participating in the study.

For Part 1 subjects:

At Screening

28. Known history of hypersensitivity and/or allergic reactions to midazolam, other benzodiazepines, dabigatran etexilate or to any excipient of the formulations.
29. Clinically relevant history of impaired respiratory function, obstructive sleep apnea, myasthenia gravis, respiratory arrest and/or cardiac arrest.
30. History of glaucoma.
31. Presence of respiratory failure.
32. Presence of active clinically significant bleeding.
33. Lesion or condition considered to pose a significant risk factor for major bleeding including, but not limited to: current or recent gastrointestinal ulceration, malignant neoplasms, recent brain or spinal injury, recent brain, spinal or ophthalmic surgery, recent intracranial hemorrhage, known or suspected esophageal varices, arteriovenous malformations, vascular aneurysms or major intraspinal or intracerebral vascular abnormalities.
34. Presence of a medical condition requiring anticoagulant treatment.

At Admission to Treatment Period

35. Treatment with anticoagulants within 28 days or 5 drug half-lives, whichever is longer, before admission.

10 SUBJECT'S PREMATURE STUDY DISCONTINUATION AND STUDY TERMINATION

10.1 Subject's Premature Discontinuation

According to the Declaration of Helsinki and the Good Clinical Practice (GCP) requirements issued by the International Conference on Harmonization (ICH), a subject may withdraw the consent and abandon the study at his/her request, at any time, irrespective of the reason.

The investigator or Sponsor may withdraw a subject at any time if it is determined their continuing in the study may result in a significant safety risk to the subject or if their behaviour is deleterious to the study environment.
Over the course of the study the Investigator may withdraw any subject for the following reasons:

- Occurrence of a SAE.
- Clinically significant study treatment-related changes in safety parameters that are considered unacceptable by the Investigator and/or the Sponsor.
- Significant protocol violation.
- Subject received/required any concomitant medication at a timeframe in which, according to the Investigator, may interfere with the pharmacokinetics or safety of study drugs.
- Positive pregnancy test (if woman).
- Difficulty in collecting blood.
- Any other condition that in the Investigator's and/or Sponsor's opinion no longer justifies or permits a safe participation of the subject in the study.

As soon as subject's withdrawal is confirmed, blood sampling for pharmacokinetic assessments should be stopped.

In the event of a premature withdrawal after dosing, the subject will be requested to complete a safety assessment at the time of discontinuation (or as soon as possible after the time of discontinuation). Subjects withdrawn for safety reasons will be asked to remain in the clinical site until the Investigator agrees that the subject can be safely discharged.

Reasons for a premature withdrawal must be clearly documented in the subject's medical records and in the eCRF. In case of multiple reasons, safety related issues should be indicated as the primary reason if applicable. Investigators must attempt to contact subjects who fail to attend scheduled visits by telephone, e-mail, etc., to exclude the possibility of an AE being the cause. Should this be the case, the AE must be documented, reported and followed up as described in this Protocol.

In case of withdrawal due to AE, the Investigator will institute the appropriate follow-up investigations in accordance with the accepted standards of medical care, including tests at the time of withdrawal. Any subject withdrawn during the study due to any AE will be followed up wherever possible until resolution or until the investigator believes that there will be no further change. This may involve additional visits, whose information must be clearly documented in the subject's medical records.

Subjects withdrawn after the first dose administration will not be replaced.

11 INVESTIGATIONAL PRODUCTS

11.1 Identity of Investigational Products

Givinostat
Name of the Product: ITF2357/Givinostat* 10 mg/mL oral suspension (140 mL/bottle)
*Givinostat is used to indicate the whole study drug name givinostat hydrochloride monohydrate. The dosages/concentrations of the study drug are expressed as givinostat hydrochloride monohydrate
Route/mode of administration: Orally, without water
Manufacturer: Italfarmaco SpA, viale F. Testi 330 – 20126 Milan, Italy (final labelling/packaging and release for clinical use).

Midazolam (IV)
Name of the Product: Midazolam Aurobindo 1 mg/ml solution for injection/infusion or rectal administration
Route/mode of administration: Intravenously
Marketing Authorization Holder: Aurobindo Pharma B.V.

Midazolam (Oral)
Name of the Product: Buccolam® 2.5 mg oromucosal solution
Route/mode of administration: Orally with 150 mL of water
Marketing Authorization Holder: Laboratorios Lesvi, S.L.

Dabigatran etexilate
Name of the Product: Pradaxa® 75 mg hard capsules
Route/mode of administration: Orally with 150 mL of water
Marketing Authorization Holder: Boehringer Ingelheim International GmbH

The batch number of each product will be included in the final Clinical Study Report (CSR). Also, the Givinostat certificate of analysis will be included in the final CSR.

11.2 Investigational Products Supplies

The Sponsor will be responsible for ensuring that givinostat is manufactured under Good Manufacturing Practice (GMP) and is provided in adequate quantities for the study.

Givinostat will be supplied by the Sponsor as follows: labeled and sealed white cartoon box each containing one labeled multidose bottle of 140 mL Givinostat 10 mg/mL oral suspension. The label will include at least the following information: name of the study drug, batch number, and expiry date.
At PPD I, and according to the applicable SOPs, the Pharmacist will prepare the individual doses of Givinostat for each subject and dosing time using 5 mL oral syringes. The single containers will be labeled in accordance with the applicable laws and regulations.
A sample of the label will be provided in the CSR.

Midazolam and dabigatran will be obtained from the commercial sources.
At PPD , the Pharmacist will label, prepare and dispense the respective

individual doses for each subject.

11.3 Storage and Return of Investigational Products

The pharmacist is responsible for safe and proper handling and storage of the investigational products at PPD in an appropriate lockable room. For givinostat, the requirements provided by the Sponsor should be followed, while for products obtained from the commercial sources the requirements reported on the commercial boxes or public literature (e.g. Summary of Product Characteristics) should be followed. Only the pharmacist or his/her designee may handle the investigational products.

Upon receipt of the investigational products, the responsible pharmacist must inspect the external package and verify the completeness and integrity of the treatments at a bulk level (e.g. IMP packages as received by the Pharmacists, including those supplied by the Sponsor and those obtained from the commercial circuit). Subsequently, he/she must immediately return the enclosed acknowledgement of receipt form, duly completed and signed (the date of receipt must be noted).

The supplies and inventory must be available for inspection by the monitor. The Treatment Accountability Log(s) will be collected upon completion of the study for archiving in the TMF.
On termination of the study, all unused investigational product material (medication and secondary packaging) must undergo an investigational product accountability check by the monitor.

The pharmacist will be responsible for the inventory and accountability of all clinical supplies, exercising accepted pharmaceutical practices. An accurate, timely record of the clinical study supply must be maintained.

On termination of the study, and upon confirmation by the Sponsor:

- the Givinostat oral suspension bottles will be returned PPD to the Sponsor and destroyed by the Sponsor
- the unused Givinostat individual doses will be destroyed PPD

The products obtained from the commercial sources and any packaging materials used at the clinical site will be destroyed PPD

11.4 Investigational Products Administration

The pharmacists will prepare the investigational products individual doses as described in the Pharmacy Manual and will dispense them to the investigator or a person under his/her direct supervision.

The investigator or a person under his/her direct supervision must administer the investigational product(s) to the subjects as described in Section 8.2.3 (for Part 1), Under no circumstances must the investigator allow the investigational product to be used otherwise than as directed by this clinical study protocol.

At least the date and time of administration of the investigational product and the dose administered must be documented in the eCRF.

11.4.1 Treatment Compliance

Administration of Investigational Medicinal Products (IMPs) will only be performed by authorized clinical research staff and, in case of givinostat, oral midazolam, and dabigatran will be followed by an oral cavity and hand (where applicable) check in order to confirm the swallowing of the product.

Upon dispensing medication, the clinical research staff will record the related information on the applicable source documents, to allow investigational product accountability and evaluation of subject compliance.

12 STUDY ASSESSMENTS

Subjects who have voluntarily signed written informed consent may start the following trial procedures according to the flow-chart (Section 2). All the results will be reported in the appropriate CRF sections and all source documents will be archived in the subject file at the trial site.

12.1 Pharmacokinetic Assessments

12.1.1 Blood Sampling for Pharmacokinetic Assessments

Part 1

A total of 117 blood samples will be collected as follows:

- Thirteen (13) blood samples of 3 mL each will be collected in K_2-EDTA collection tubes at pre-dose and at 2 minutes, 0.25, 0.5, 0.75, 1, 2, 3, 4, 6, 9, 12 and 24 hours after the administration of intravenous midazolam, on Days 1, 6 and 17, for the determination of midazolam and 1-hydroxymidazolam plasma concentrations.
- Eleven (11) blood samples of 3 mL each will be collected in K_2-EDTA collection tubes at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12 and 24 hours after the administration of oral midazolam, on Days 2, 7 and 18, for the determination of midazolam and 1-hydroxymidazolam plasma concentrations. The 24-hour blood collection following intravenous midazolam will be assumed as the pre-dose sampling for oral midazolam.
- Fifteen (15) blood samples of 4 mL each will be collected in K2-EDTA collection tubes at pre-dose and at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 24, 36, 48 and 72 hours after the administration of dabigatran etexilate, on Days 1, 6 and 17, for the determination of total and free dabigatran plasma concentrations.

In each study part, while subject is confined at the clinical research unit, blood samples will be taken preferably via an indwelling cannula placed in a vein of an upper limb of the subject. During ambulatory visits, blood samples will be taken by direct venipuncture.

The actual time of all pharmacokinetic blood draws will be recorded and reported for all subjects. The post-dose blood samples will be collected within ±3 minutes from the scheduled sampling time. Greater deviations will be reported as a protocol deviation and its cause will be recorded.

In case blood sampling for pharmacokinetics and other procedures coincide in time, blood draws will have priority unless other procedures are necessary for assuring subject's safety.
The date and actual time of urine collection start and completion will be recorded and reported for all subjects.

12.1.2 Sample Processing, Storage and Transfer

Blood and urine samples for pharmacokinetic analysis will be collected and processed as per the analytical methodology instructions provided by the bioanalytical laboratory.

Samples will be labeled with self-adhesive pre-printed labels suitable to withstand freezing temperatures. Labels should bear the following minimum information:

- Labels of blood sample tubes: protocol code, subject number, sample number, study day, sample timepoint, study part, matrix type, and one of the statements "for IV midazolam and metabolite assay"/ "for oral midazolam and metabolite assay"/"for dabigatran total and free assay" (Part 1).
- Labels of plasma aliquots: protocol code, subject number, sample number, study day, sample timepoint, aliquot series, study part, matrix type, and one of the statements "for IV midazolam and metabolite assay"/ "for oral midazolam and metabolite assay"/ "for dabigatran total and free assay" (Part 1).
- Labels of urine collection containers: protocol code, subject number, study day/day interval, scheduled sample interval, matrix type and statement.
- Labels of urine aliquots: protocol code, subject number, study day/day interval, scheduled sample interval, aliquot series, matrix type (urine) and statement.

At agreed times, samples will be transferred to the bioanalytical laboratory, with each series of aliquots in separate shipments. The blood samples will be packed in dry ice for transport and no interruption of the freeze cycle is allowed. The temperature in the box during the transport will be monitored. Once the bioanalytical laboratory confirms receipt of the first shipment, the second series of aliquots will be sent, if required. For all shipments, the laboratory should acknowledge in writing the receipt of samples in good condition.

12.1.3 Bioanalytical Methods

The plasma or urine levels of each analyte will be determined by the bioanalytical laboratory identified in Section 3 in accordance with the applicable principles of Good Laboratory Practice (GLP), using previously validated analytical methods.

The lower (LLOQ) and upper (ULOQ) limits of the quantification range will be provided in the analytical method documentation.

Details of the methods used and the results obtained will be given in the bioanalytical report.

12.1.4 Incurred Samples Reanalysis

In order to establish the reproducibility of the assay, 10% of the first 1000 samples and 5% of the number of samples exceeding 1000 samples will be reanalyzed [EMA Guideline 2011; FDA Guidance 2018]. Both the original and replicate values will be presented in the bioanalytical report, with the percent difference between the two values. The original value will be the one used for pharmacokinetic analysis.

12.2 General Safety Assessments

Subjects' safety will be monitored during the study. A summary of safety procedures is presented in Section 2.1 (*Study Flow-Chart – Part 1*).

Safety assessments will include pre-study medical history, physical examination, vital signs, 12-lead ECG, clinical laboratory tests and AE monitoring. Additional safety measurements may be performed at the discretion of the investigator for reasons related to subject safety.

12.2.1 Medical History

Medical history will cover all relevant past or present information related to subject's health at the time of informed consent signature.
Medical history at screening will include past or present relevant cardiovascular, respiratory, renal, genitourinary, gastrointestinal, hepatic, hematological, immunological, endocrine, dermatological, musculoskeletal, neurological, psychiatric, drug and surgical history, or any other diseases or disorders.

12.2.2 Physical Examination

Physical examination at screening and end-of-study will include: general appearance; skin; head and neck; thorax and abdomen; pulmonary auscultation; cardiac auscultation; abdomen palpation; limbs; brief neurological examination.

12.2.3 Weight, Height and Body Mass Index

The body height and weight values as well as the BMI will be recorded in the eCRF, and will be determined at Screening. Only body weight will be determined also at admission and end-of-study.

The subjects' body weight will preferably be measured using the same weighing scale for all subjects and throughout the study. The weighing scale should have a precision of at least 0.5 kg.

12.2.4 Vital Signs

Vital signs will be performed as scheduled in Section 2.1 (*Study Flow-Chart – Part 1*), and include rest systolic blood pressure (SBP), diastolic blood pressure (DBP), PR, RR and body temperature.
When vital signs recordings coincide with a blood draw, they should preferably be performed before the blood collection.

12.2.5 12-Lead Electrocardiogram

12-lead ECG will be performed as scheduled in Section 2.1 (*Study Flow-Chart – Part 1*).
When ECG recordings coincide with a blood draw, they should preferably be performed before the blood collection.

12.2.6 Laboratory Safety Tests

Laboratory safety test will be performed as scheduled in Section 2.1 (*Study Flow-Chart –*

Part 1). Additional or unscheduled laboratory safety tests may be performed if deemed necessary for an appropriate subject's safety management.

12.2.6.1 Samples and Results Management

PPD Portugal) will perform the hematology, biochemistry, coagulation, viral serology, urinalysis and serum pregnancy tests. Alcohol, drugs-of-abuse, cotinine and pregnancy urine tests will be performed at PPD SARS-CoV-2 diagnostic test using polymerase chain reaction (PCR) technology will be performed at one of the PPD Portugal facilities.

All samples will be properly labeled for correct identification. The reference ranges will be provided in the study files (e.g. TMF and IF) together with the description of the laboratory methods used.

The investigator will assess the clinical significance of results of laboratory investigations outside the normal ranges. All changes from screening that are observed at any time during the study and meet the requisites for clinical significance will be recorded as AEs.

12.2.6.2 Safety Laboratory Variables

Hematology:

- RBC count, WBC count with differential (neutrophil, eosinophil, basophil, lymphocyte and monocyte), hemoglobin, MCV, MCH, MCHC, RBC distribution width, hematocrit, platelets and mean platelet volume.

Coagulation:

- Prothrombin rate, prothrombin time, INR, aPTT.

Biochemistry:

- Total, direct and indirect bilirubin, ALP, amylase, AST, ALT, LDH, cystatin C, C-reactive protein, GGT, CK, total protein, albumin, uric acid, triglycerides, total cholesterol, LDL-C, HDL-C, sodium, potassium, chloride, calcium, magnesium, glucose, creatinine, urea and TSH.

  NOTE: Creatinine clearance (Cr_{CL}) will be estimated at screening by using the Cockcroft-Gault formula: Cr_{CL} male = [(140-age) x (weight in kg)] / [(serum Cr mg/dL) x (72)]; Cr_{CL} female = 0.85 x (CrCL male) and normalized to an average surface area of 1.73 m^2: Cr_{CL} normalized = Cr_{CL} x 1.73/BSA.
  Body Surface Area (BSA) = ($weight^{0.425}$ [in kg] × $height^{0.725}$ [in cm]) × 0.007184

Viral serology:

- anti-HIV-1Ab, anti-HIV-2Ab, HBsAg and anti-HCVAb.

Pregnancy test:

- β-hCG serum pregnancy test will be performed by automated immunoassay using the biochemistry blood sample.
- Urine pregnancy test will be performed using a qualitative chromatographic immunoassay β-hCG kit.

Urinalysis:

- pH, specific gravity, protein, hemoglobin, glucose, ketones, bilirubin, nitrites, urobilinogen and microscopy.

Cotinine, alcohol and drug screen test (cannabinoids, opiates, cocaine, amphetamines, and benzodiazepines).

FSH test will be performed to confirm the post-menopausal status of female of non-childbearing potential.

12.3 Total Blood Volume

Part 1

The total volume of blood to be taken per subject during Part 1 is as follows:

Procedure	Sample	Blood volume per sample (mL)	Number of blood samples per subject	Total volume per subject (mL)
Laboratory tests	Hematology	3	10	30
	Coagulation	1.8	6	10.8
	Biochemistry (including pregnancy and FSH tests, if female, and viral serology)	5	6	30
	Cystatin C	5	2	10
Bioanalysis	PK dabigatran (total and free)	4	45	180
Bioanalysis	PK midazolam and metabolite (IV)	3	39	117
Bioanalysis	PK midazolam and metabolite (oral)	3	33	99
Total volume of blood collected				476.8

Total volume of blood collected could be different if laboratory test re-checks are performed for safety reasons.

13 ADVERSE EVENTS DEFINITIONS AND REPORTING REQUIREMENTS

The adopted definitions and the methods of assessment, documentation and management of adverse events can be found in Appendix 1.

13.1.1 Expedited Reporting

Any SAE that occurs after a subject has signed the Informed Consent Form and up to the follow-up visit (regardless of relationship to study drug/comparator) must be reported by the Investigator to the Sponsor (Drug safety Manager) and PPD -within 24 hours of learning of its occurrence.
The Investigator must notify the Sponsor (Drug Safety Manager) and PPD - of the SAE by completing the word

version of the SAE reporting form and e-mailing the pdf version to the addresses specified below.
Serious adverse event reports must be made whether or not the Investigator considers the event to be related to the investigational drug.
The Investigators are required to complete the SAE Form. Sufficient details must be provided to allow for a complete medical assessment of the SAE and independent determination of possible causality. The Investigators are obliged to pursue and provide additional information as requested by Italfarmaco S.p.A. or its designee and PPD. The notification must be directed to:

PPD

And

Sponsor's Drug Safety Manager:
Italfarmaco S.p.A.
Corporate Drug Safety
Via dei Lavoratori, 54
20092 Cinisello Balsamo (MI), Italy
Phone: +39 02 6443 2506
Fax: +39 02 6610 6538
Fax (back-up): +39 02 6443 2955
Mobile: +39 39 333 2140004
e-mail: drug-safety@italfarmaco.com

The same procedure must be applied to the SAE follow-up information.

All Suspected Unexpected Serious Adverse Reactions (SUSARs) shall be expeditely reported to INFARMED and CEIC. Expeditely means that any fatal or life-threatening SUSAR will be reported within 7 days of knowledge (i.e., 7 days after the Sponsor - Drug Safety Manager and PPD was first aware of the reaction) and all other SUSARs will be reported within 15 days of knowledge.

Sponsor - Drug Safety Manager - will be responsible for the expediting report of SUSARs to INFARMED via EudraVigilance clinical trials module (EVCTM), and for providing PPD with the respective copy of the Council for International Organizations of Medical Sciences (CIOMS) form. PPD will be responsible for reporting SUSARs to CEIC via email (ceic@ceic.pt).

14 STUDY ENDPOINTS

14.1 Primary Endpoints

1. Midazolam and dabigatran plasma concentrations and thereof derived pharmacokinetic

parameters alone and in combination with Givinostat (Part 1).

14.2 Secondary Endpoints

1. Incidence and severity of adverse events (AEs); changes in vital signs, physical examination, electrocardiogram (ECG) and clinical laboratory tests following administration of midazolam and dabigatran etexilate alone and in combination with givinostat (Part 1).

15 STATISTICAL METHODOLOGY AND ANALYSIS

15.1 Sample Size Determination

Part 1

Midazolam: Assuming an Intrasubject Coefficient of Variation (ISCV%) of ≤23% for midazolam C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ [Epistatus® SmPC], a true geometric means ratio (midazolam with *vs* without givinostat) of 1.00, a significance level (alpha error) of 5%, and a default no-effect boundary of 80.00% to 125.00%, a sample of 20 evaluable subjects results in at least 80% power. Accounting for early-termination subjects, a sample size of 26 subjects will be enrolled.

Dabigatran: Assuming an ISCV% of approximately 30% for dabigatran C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ [Li, Xin, et al. 2020], a true geometric means ratio (dabigatran with *vs* without givinostat) of 1.00, a significance level (alpha error) of 5%, and that the two-sided 90% confidence interval (CI) for the geometric means ratio of C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ will lie within 74.62% to 134.02%, a sample of 20 evaluable subjects results in at least 80% power. Accounting for early-termination subjects, a sample size of 26 subjects will be enrolled.

15.2 Statistical Analysis Plan

A detailed Statistical Analysis Plan (SAP) will be prepared and finalized prior to the analysis.

Estimation of pharmacokinetic parameters and statistical analyses on pharmacokinetic data will be conducted using Phoenix® WinNonlin® 8.2 or higher (Certara USA Inc, Princeton, NJ) and SAS® 9.4 or higher. The other analyses will use SAS® 9.4 or higher.

15.3 Analysis Populations

15.3.1 Safety Population

All subjects who received at least one dose of investigational product will constitute the safety population.

Safety data analysis will be performed for all subjects in the safety analysis population.

15.3.2 Pharmacokinetic Analysis Population

For each study part, a Pharmacokinetic Analysis Population will be defined.
Each Pharmacokinetic Analysis Population will include all subjects who are expected to provide evaluable pharmacokinetic data for at least one IMP, without deviations affecting pharmacokinetic interpretation.

15.3.3 Drug-Drug Interaction Comparable Bioavailability Analysis Population

For each comparation of interest to assess a potential drug-drug interaction, a Drug-Drug Interaction Comparable Bioavailability Analysis Population will be defined.
Each Drug-Drug Interaction Comparable Bioavailability Analysis Population will include all subjects from the Pharmacokinetic Analysis Population who are expected to provide evaluable pharmacokinetic data for an IMP administered alone and co-administered with another IMP, without deviations affecting pharmacokinetic interpretation.

15.4 Subjects' Characteristics

Subject disposition will be summarized. The demographic, background and baseline data will be presented descriptively for each study population.

The protocol violations will be listed per subject, describing the nature of the violation. Subjects failing to complete the study (as well as the time and reasons for discontinuation) will be displayed.

Medical history will be referred in accordance with the Medical Dictionary for Regulatory Activities (MedDRA) 24.1 or higher. Medications will be mentioned according to the anatomic therapeutic class (ATC) classification system.

15.5 Pharmacokinetic Analysis

For the subjects included in the pharmacokinetic analysis population, descriptive statistics [number of observations (n), geometric mean (G_{mean}), arithmetic mean (A_{mean}), standard deviation (SD), geometric SD (GSD), coefficient of variation (CV%), geometric CV% (GCV%), two-sided 95% CI of the arithmetic and geometric means, median, minimum and maximum] of the plasma concentrations will be presented by investigational product.

Individual plasma concentration time profiles will be displayed graphically on a linear-linear scale and semi-logarithmic scale. G_{mean} plasma concentrations-time curves including 95% CIs will be displayed by treatment period.

Other PK parameters may be calculated for the plasma concentration-time profiles, if considered appropriate and justified at the time of the PK analysis.

For the subjects included in the pharmacokinetic analysis population, individual pharmacokinetic parameters and descriptive statistics (n, G_{mean}, A_{mean}, SD, GSD, CV%, GCV%, two-sided 95% CI of the arithmetic and geometric means, median, minimum and maximum) will be presented by investigational product.

15.5.1 Part 1

Appropriate descriptive statistics will be used to summarize plasma concentrations and PK

parameters. Formal statistical analysis of PK data will be performed to characterize the PK interactions:

- Analysis of the effect of givinostat on midazolam PK: C_{max} and AUC_{0-24} of midazolam and hydroxy-midazolam when it is administered alone and co-administered with givinostat will be the primary pharmacokinetic parameters for the assessment of the effect of givinostat on midazolam PK.
- Analysis of the effect of givinostat on dabigatran PK: C_{max} and AUC_{0-72} of total and free dabigatran when dabigatran etexilate is administered alone and co-administered with givinostat will be the primary pharmacokinetic parameters for the assessment of the effect of givinostat on dabigatran.

An analysis of variance (ANOVA) will be performed on the *ln*-transformed primary pharmacokinetic parameters. A linear mixed effects model will be applied, using Treatment as fixed effects, assessed at a two one-sided 5% significance level ($\alpha = 0.05$). Subject will be included as random effect. Geometric means ratios (GMR) and corresponding 90% CI will be calculated for the ln-transformed primary pharmacokinetic parameters, using substrates alone as the reference.
Wilcoxon signed rank test will be used to test for the difference in t_{max}.

15.6 Safety Data Analysis

Safety and tolerability data will be listed and summarized descriptively by study part, treatment and subject number.

At each time point, absolute values of safety variables and change from baseline will be summarized with mean, median, SD, SE, minimum, and maximum values. The number of available observations and absolute out-of-range values will be presented. Values outside the investigator's normal range will be flagged in the listing.

The occurrence of clinical AEs will be monitored throughout the study.
Clinically significant abnormalities in laboratory safety tests, vital signs, ECG and physical examination will be reported as AEs.

AEs will be tabulated and summarized according to the MedDRA 24.1 or higher, and classified by system organ class (SOC) and preferred term (PT). The following information recorded or computed is used for the description of the AEs: reported Term; SOC and PT by MedDRA coding; start date, start time, end date and end time; seriousness; severity; relationship (causality); action taken; concomitant medication; outcome; most recent study treatment taken; last dosing date.

A separate listing of SAEs will be presented, if applicable.

Incidence and frequency of treatment-emergent adverse events (TEAEs) will be summarized descriptively by SOC and PT for Test and Reference products and overall, for all subjects who were dosed (Safety population).

16 DATA COLLECTION, MONITORING AND RETENTION

16.1 Data Collection

16.1.1 Case Report Forms

Standardized eCRFs will be produced using Viedoc™ software package (Viedoc Technologies AB, Uppsala, Sweden). All study data will be recorded into the eCRFs, except for plasma drug concentrations, which will be directly imported into the final study database.
The record in eCRF is performed by the clinical research staff, except for safety laboratory results which will be imported to eCRF by the PPD s Clinical Data Management department.

eCRFs will be completed for each subject who provided informed consent according to the applicable SOPs and the study eCRF completion guidelines. For subjects who met the selection criteria at screening and admission, data will be fully recorded into the eCRF, including end-of-study safety assessments. For subjects who have signed the informed consent but did not meet the selection criteria, data will only be partially recorded into the eCRFs.

Clinical research staff will perform quality control of data entered into the eCRFs prior to study monitoring activities. eCRFs will be electronically signed off by the Principal Investigator after the database is considered clean.

16.1.2 Source Documents

Study data will be recorded on source documents. Source documents will be verified for completeness and accuracy by PPD . Data from source documents will be transcribed, where necessary, into the eCRFs by the delegated clinical research staff.
The clinical research unit will retain all source documents.
The source documents must be kept in order and up-to-date so they always reflect the latest observations on the subjects enrolled in the study.

Data on subjects collected on eCRFs during the trial will be documented in an anonymous fashion and the subject will only be identified by the subject number and by his/her partial date of birth. All the information required by the protocol should be provided and any omissions require explanation. All eCRFs must be completed expeditiously after the subject's visit. The CRO will provide the study site with eCRF and the guidelines for the eCRF compilation for each patient. The investigator must maintain source documents for each subject in the study. All information on eCRFs must be traceable to these source documents, which are generally maintained in the subject's file.

16.2 Quality Control and Quality Assurance

16.2.1 Quality Control

The study will be conducted and submitted to quality control to assure data integrity, in accordance with applicable SOPs and ICH GCP requirements. These SOPs require quality control on the following processes: medical writing, source documents and eCRF data entry completion and accuracy, data management and study reporting.

16.2.2 Monitoring

The monitoring will be done at the trial site by an independent study monitor. A site initiation visit will be held prior to initiation of subject enrolment. During the study, the

study monitor will visit the site regularly, to check the completeness of subject records, the accuracy of entries on the CRFs, the adherence to the protocol and to GCP, the progress of enrolment, and also to ensure that study medication is being stored, dispensed and accounted for according to specifications. The investigator and key trial personnel must be available to assist the monitor during these visits. The investigator must give the monitor access to clinical records, to confirm their consistency with the CRF entries. No information in these records about the identity of the subjects will leave the study centre. Monitoring standard procedures require full verification for the presence of informed consent, adherence to the inclusion/exclusion criteria, documentation of SAEs and the recording of primary efficacy and safety variables. The investigator is responsible for completing the CRFs expeditiously to capture all the relevant information, while the monitor is responsible for reviewing them and clarifying any data queries.

16.2.3 Source Document Verification

The study monitor will have conditional direct access to source data recorded on source documents for the purpose of source document verification.

16.2.4 Auditing and Inspection

The Sponsor may appoint an Auditor to verify if the activities regarding this study were performed according to the study protocol, the Sponsor internal SOPs, when applicable, CRO and/or their delegates SOPs, ICH GCP, European Medicines Agency (EMA) regulations, and the Portuguese laws and regulations.
A Regulatory Authority may also wish to conduct an inspection (during the study or even after its completion). If an inspection is requested by a Regulatory Authority, the Investigator must inform the Sponsor immediately that this request has been made.

The Principal Investigator will allow the verification of records (source data) during audits or inspections by national or foreign regulatory bodies, in case such authorities require a regulatory inspection.

16.3 Retention and Archiving of Documents

The Principal Investigator must maintain adequate records to enable the conduct of the study to be fully documented.

All source documentation and study records and other documents pertaining to the conduct of the study must be retained according to the current ICH GCP and applicable laws. All essential documents and records will be maintained PPD for a period of at least 25 years. Study essential documents and records should not be destroyed prior written agreement between the Sponsor's representatives and the Principal Investigator.

If the Principal Investigator withdraws from research unit or retires, the responsibility for maintaining the records may be transferred to another person, with Sponsor's previous notice and agreement.
The sponsor shall archive the content of the clinical trial master file for at least 25 years after the end of the clinical study

16.4 Final Study Report

At the end of the study, an integrated CSR will be prepared following the ICH E3 and the

European electronic Common Technical Document (eCTD) format specifications and in accordance with the applicable SOPs. The pharmacokinetic analysis will be included in the integrated CSR.

17 GENERAL STUDY ADMINISTRATION

17.1 Regulatory and Ethical Aspects

17.1.1 Review and Approval by the Independent Ethics Committee

This protocol, informed consent forms, details of subject compensation and any subject recruiting materials will be submitted to CEIC – Ethics Committee for Clinical Research (Avenida do Brasil, Lisboa, Portugal) for evaluation and approval, prior to the start of the study. The approved documents must be filed in the study files (TMF and IF).
The study will not start until the CEIC has approved the documents. The letter of the CEIC approval will be appended to the final CSR.

17.1.2 Subject Information and Consent

Written informed consent will be obtained from each prospective subject in writing prior to the start of the study.

Each prospective participant will receive a full explanation of the objectives, procedures, restrictions and potential hazards of the study by the Investigator. Once this information is provided to the subject, the prospective participant is required to read the informed consent form and ask any questions about its contents. After the physician in charge has the conviction that the subject is aware of the implications of participating in the study, the subject will confirm his/her willingness to participate by signing and dating the informed consent form.
Subjects will receive a copy of subject information and signed informed consent form and will be assured that they may discontinue from the study at any time and for any reason without any prejudice. A subject screening log to document the subjects entering the screening phase must be maintained at trial site and archived in the Investigator File.
In case of amendments to the informed consent form during the study, a renewed written and signed consent must be obtained from each subject still participating in the study.

Subject Information and Consent for SARS-CoV-2 testing

SARS-CoV-2 testing will be performed prior to admission to treatment period and whenever deemed appropriate throughout the study. A specific written informed consent will be obtained from each prospective subject in writing.

17.1.3 Review and Approval by Regulatory Authorities

The study will be submitted to the review and approval of INFARMED, I.P. (Portuguese National Competent Authority) according to rules in force. The letter of the INFARMED approval will be appended to the final CSR.

17.1.4 Insurance, Indemnity and Compensation

It is the Sponsor's responsibility to guarantee sufficient insurance coverage for this study should any serious events or deaths result directly or not from the execution of the present

protocol.
The present article is not to be interpreted as engaging the Sponsor's responsibility in the event of fault or negligence of the subjects, investigators, or any persons or employees under the control of PPD

17.1.5 Compliance Statement

The study will be conducted according to this protocol, the current version of the Declaration of Helsinki, ICH GCP, EMA regulations and applicable Portuguese laws and regulations.

17.1.6 Investigator Statement

FDA 1572 form, Statement of Investigator (Title 21, CFR Part 312), completed and signed by the Principal Investigator, will be kept on file and will be available upon request.

17.1.7 Delegation of Investigator Duties

The Principal Investigator is responsible to assure that all staff involved in the study are adequately qualified, trained and informed about the protocol, any protocol amendment, the study treatments, and all their assigned study-related duties.
The Principal Investigator must maintain a document with the identification of sub-investigators and other personnel to whom he delegates significant study-related duties.

17.2 Conditions for Modifying/Amending the Protocol

All substantial protocol modifications must be submitted to CEIC and INFARMED before they can be implemented. Amendment that eliminates an apparent immediate risk to subjects can be implemented before approval, but CEIC and INFARMED have to be informed about such modification as soon as possible.
Administrative changes (e.g., replacement of a study monitor, address change) that have no impact on the study conduct and/or safety of the volunteers should be notified to CEIC and INFARMED but do not require approval.

17.3 Documents for Study Initiation

The following documents, at minimum, have to be available prior to screening: curricula vitae of all the clinical staff, CEIC approval of the final version of the Clinical Study Protocol, informed consent form and written subject information, reference safety information, written approval by INFARMED, study insurance policy, and list of normal ranges of clinical laboratory tests.

17.4 Ownership of data, disclosure and confidentiality

The Investigator/PPD may not publish the data collected during this study in any form, except with the written permission of the Sponsor. In case of publication, confidentiality of the study volunteers will be maintained.
By signing the protocol, the investigators and their co-workers accept to submit any intended communication (abstract, paper or oral presentation) to the Sponsor reasonably in advance (at least 30 working days for an abstract or oral presentation and 60 working days for a manuscript). This is to allow the Sponsor to review the communications for accuracy

and confidentiality, to provide any relevant supplementary information and to allow establishment of co-authorship and in no way has to be intended as a restriction of the sponsor to the investigators' right to publish the results of the study. In case the Sponsor identifies specific need/opportunity to patent any of the study findings, the Investigator will allow a six month time-window between his submission to the Sponsor and the intended publication and actual submission/communication to third parties, in order to allow the Sponsor to undertake appropriate patenting steps.

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19 APPENDICES

19.1 Appendix 1 - Adverse Events and Pregnancy Considerations

19.1.1 Adverse Events - Definitions

The definitions are in accordance with the ICH-E2A, Directive 2001/20/EC, and the European Commission Detailed Guidance 2011/C 172/01.

Adverse Event (AE) - any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have to have a causal relationship with this treatment.
An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

Examples of events that meet AE definition:
- Any sign/symptom presented during the course of the study or reported within a reasonable time after last drug administration.
- Any abnormal laboratory test results or other safety assessments considered clinically significant in the medical judgment of the investigator.
- Increase in frequency/severity of a pre-existing condition.
- Any new condition detected/diagnosed during the study conduction, including those previously present but unknown until then.
- Any condition resulting from a suspected drug-drug interaction.
- Overdose, misuse, and abuse of the study treatment. Study treatment errors must be documented in the study treatment page of the eCRF.

Adverse Drug Reaction (ADR) - all noxious and unintended responses to a medicinal product related to any dose administered.
The definition implies a reasonable possibility of a causal relationship between the event and the IMP. This means that there are facts (evidence) or arguments to suggest a causal relationship.

Unexpected Adverse Reaction (UAR) - an adverse reaction, the nature or severity of which is not consistent with the applicable product information (Reference Safety Information: Investigator's Brochure or SmPC).

Serious Adverse Event (SAE) - any untoward medical occurrence or effect, that, at any dose:
- results in death;
- is life threatening;
  NOTE: The term refers to an event in which the patient was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.
- requires hospitalization or prolongation of existing hospitalization;
  NOTE: This applies if hospital admission or prolongation of existing hospitalization is a result of the adverse event.
- results in persistent or significant disability/incapacity;
  NOTE: This means a substantial disruption of a person's ability to conduct normal life functions, i.e. the adverse event results in a significant, persistent or permanent change, impairment, damage or disruption in the study participant's

body function/structure, physical activities and/or quality of life.

- is a congenital anomaly/birth defect;
  NOTE: To be considered if there is a suspicion that the exposure to a medicinal product prior to conception or during pregnancy may have resulted in an adverse outcome in the child. Not all congenital anomalies have clinical consequence and can be considered even normal variants. Furthermore, not all genetic conditions have a drug related etiology. To confirm if a congenital anomaly or a genetic condition is to be classified as SAE, the Important Medical Event (IME) list[1] should be consulted.
- is an important medical event that requires intervention to prevent one of the above.
  NOTE: Medical and scientific judgment should be exercised in important medical events that may not be immediately life threatening, result in death or hospitalization, but may jeopardize the subject or may require intervention to prevent one of the above listed. As above, the IME list should be consulted.

A pre-planned hospitalization should not be considered an SAE.

A Suspected Unexpected Serious Adverse Reaction (SUSAR) *is referred to an ADR that complies with both the definitions of "serious" and "unexpected."*

Overdose
In general, a drug overdose in a clinical study is defined as the accidental or intentional use of a drug or medicine in an amount exceeding the protocol defined dose. The Investigator must immediately notify the Sponsor (Drug Safety Manager) and PPD - MaterioVigilance and Pharmacovigilance Unit - of any occurrence of overdose with Investigational Medicinal Products.
Any instance of overdose (suspected or confirmed, with and without an AE) must be reported to the Sponsor (Drug Safety Manager) and PPD - MaterioVigilance and Pharmacovigilance Unit within 24 hours and, only in case of AEs, it must be fully documented as a SAE following the modality report at paragraph 13. Details of any signs or symptoms and their management should be recorded in the SAE Form including details of any antidote or systematic treatment administered. Any signs or symptoms of over-dosage will be treated symptomatically.
Any other situations putting the subject at risk of an adverse reaction, such as misuse and abuse, medication errors, suspect of transmission of infective agents must be reported to the Sponsor (Drug Safety Manager) and PPD - MaterioVigilance and Pharmacovigilance Unit - within 24 hours and be fully documented as a SAE following the modality report at paragraph 13.

19.1.2 Procedures for Recording, Evaluation, Documentation and Reporting

The period of observation for the collection of medical occurrences extends from the time when the subject gives Informed Consent until the follow-up visit. Medical occurrences reported from screening until the first study drug administration should be reported as "pre-treatment adverse events".

TEAEs are defined as AEs not present prior to first administration of investigational

[1] European Medicines Agency (EMA). Inclusion/exclusion criteria for the "Important Medical Events" list, Doc. Ref.: EMA/126913/2021, 18 March 2021, accessed at: https://www.ema.europa.eu/documents/other/eudravigilance-inclusion/exclusion-criteria-important-medical-events-list_en.pdf

product, or AEs present before first administration of investigational product that worsen after the subject receives the first dose of investigational product. TEAEs that occur after administration of investigational product during the washout of a given period will be assigned to the treatment administered in that period.

Follow-up of AEs and SAEs still ongoing at end-of-study or 30 days after premature study discontinuation for a given subject (if no end-of-study has been performed) will be extended until final resolution or until it is medically justifiable to stop further follow-up (e.g. a chronic condition has been reached) or, for SAEs, until stabilization or until the event is otherwise explained.

If the investigator becomes aware of an SAE in a study subject after the end of the period of observation, he/she must communicate this to the Sponsor *(Drug Safety Manager) and* PPD - *MaterioVigilance and Pharmacovigilance Unit* - in order to mutually agree on further measures and appropriate reporting. Related SAEs MUST be collected and reported regardless of the time elapsed from the last study drug administration, even if the study has been closed.

All AEs, including SAEs, will be collected into the eCRFs. The outcome of all AEs that are ongoing at the end-of-study evaluation will be recorded in the eCRF as recovering/ resolving, not recovered/not resolved or unknown. The follow-up information of these AEs will only be recorded in the source documents. For SAEs, the follow-up information will also be communicated to the Sponsor *(Drug Safety Manager) and* PPD - *MaterioVigilance and Pharmacovigilance Unit* - for the purpose of updating the drug safety database.
Data collected in the source documents for all AEs after the end-of-study evaluation will not be reported in the CSR.

All AEs should be recorded in the eCRF after being fully documented by the investigator, in terms of:

a) Seriousness: yes or no (see criteria for SAE above)

b) Severity

The investigator will categorize the severity of the AE as follows:

- *Mild:* An event that is asymptomatic or, if symptomatic, is easily tolerated by the subject and does not interfere with daily activities; medication not usually indicated.
  Note: Self-medication should be assessed on the appropriateness of its clinical indication.
- *Moderate:* An event that causes discomfort and interferes with daily activities; medication possibly indicated.
- *Severe:* An intolerable event that prevents usual daily activities; medication usually indicated.

If an AE has multiple aspects, the one with the highest severity will be graded.

c) Outcome:

Event outcome should be assessed as follows:

- *Fatal*: Death as a result of an adverse event.
- *Not Recovered / Not Resolved*: The event has not improved or recovered.
- *Recovered / Resolved*: The event has improved or recuperated.

- *Recovered / Resolved with Sequelae*: The subject has recovered but retained pathological conditions resulting from the prior disease or injury.
- *Recovering / Resolving*: The event is improving.
- *Unknown*: Not known, not observed, not recorded, or refused.

d) Relationship to the investigational product (or causality assessment)

Medical judgment should be used by the investigator to determine the causality based on all available information and considering (i) temporal relationship with medicinal product administration, (ii) pattern of reaction/biological plausibility, (iii) de-challenge and re-challenge, (iv) confounding factors (e.g. concomitant medication, relevant medical history).

Causality will be assessed as:

- *Reasonably Possible*: There are facts, evidence or arguments that suggest a causal relationship.
  A reasonable temporal relationship exists between the AE onset and investigational product administration that cannot be readily explained by the subject's clinical state or concomitant therapies. Furthermore, the AE appears with some degree of certainty to be related, based on the known therapeutic and pharmacologic actions or AE profile of the investigational product. In case of cessation or reduction of the dose the AE may abate or resolve, and it may reappear upon re-challenge.
- *Not Reasonably Possible:* Evidence exists that the AE has an etiology other than the investigational product.

  The event is clearly/most probably caused by other etiologies such an underlying condition, therapeutic intervention or concomitant medication; or the delay between drug administration and the onset of the adverse event is incompatible with a causal relationship; or the event started before the first dose.

  For SAEs, an alternative cause must be provided (e.g., preexisting condition, underlying disease, intercurrent illness, or concomitant medication).

Arguments for *Reasonably possible*	**Arguments for *Not reasonably possible***
• A plausible time between drug exposure and adverse event onset. • The event is consistent with the known pharmacology of the drug. • The event can be attributed to the drug class. • Evidence that the event is reproducible when the drug is re-introduced. • An indication of dose-response (i.e. greater adverse effect if the dose is increased, smaller if dose is diminished). • The event is typically drug-related and infrequent in the general population not exposed to drugs (e.g.	• No plausible time between drug exposure and adverse event onset (e.g. pre-treatment cases, diagnosis of cancer or chronic disease within days/weeks of drug administration; an allergic reaction weeks after discontinuation of the drug). • Continuation of the event despite the withdrawal of the medication, considering the pharmacological properties of the compound (e.g. after 5 half-lives) and the nature of the event. • A medically sound alternative etiology (e.g. situations where other drugs or underlying diseases appear to provide a more likely explanation

hypersensitivity reactions like Stevens-Johnson syndrome). • No medically sound alternative etiologies that could explain the event (e.g. preexisting conditions, or co-medication).	for the observed event than the drug concerned).

The investigator may change his opinion of causality in light of follow-up information and update causality assessment.

- *Unknown:* Indicates there is not a reasonable suspicion that the adverse event is associated with the use of the investigational product and at the same time there is not the existence of a clear alternative explanation or non-plausibility. In this case, Investigator has to collect all possible information in order to assess the relationship with the IMP, particularly in case of Serious Adverse Events.

e) Action taken on investigational product:

Action taken with study treatment should be graded as follows:

- *Dose Increased*: A medication schedule was modified by addition; either by changing the frequency, strength or amount.
- *Dose Not Changed*: A medication schedule was maintained.
- *Dose Rate Reduced*: A medication schedule was modified by reducing the rate at which the dose was given, without reducing the total dose administered.
- *Dose Reduced*: A medication schedule was modified by subtraction; either by changing the frequency, strength or amount.
- *Drug Interrupted:* A medication schedule was modified by temporarily terminating a prescribed regimen of medication.
- *Drug Withdrawn*: -A medication schedule was modified through termination of a prescribed regimen of medication.
- *Not Applicable:* Action is not relevant in the current context.
- *Unknown:* Not known, not observed, not recorded, or refused.

The **expected/unexpected status** should be evaluated and assessed by the Sponsor *(Drug Safety Manager)*, based on the reference safety information (RSI) available for Givinostat since expectedness in Pharmacovigilance refers strictly to the information listed or mentioned in the applicable reference safety information and not to events that might be anticipated from knowledge of the pharmacological properties of a substance or because it was foreseeable due to the health status (e.g., age, medical history) of the study subjects.

19.1.3 Pregnancy

The investigator must report any pregnancy to the Sponsor *(Drug Safety Manager) and* PPD *- MaterioVigilance and Pharmacovigilance Unit* -within the same timelines as the SAE, but a pregnancy is not *per se* a SAE.
Pregnancy in a volunteer or in a male volunteer's partner shall be followed-up to determine its outcome, including spontaneous or voluntary termination, details of birth, presence or absence of any birth defects, congenital anomalies, or maternal and/or newborn complications. If the outcome of the pregnancy meets the criteria for immediate classification of a SAE (e.g. spontaneous or elective abortion – any congenital anomaly detected in an aborted fetus is to be documented; stillbirth; neonatal death; congenital anomaly), the investigator will report the event by completing a SAE form.

Any subject who becomes pregnant during the study will be immediately withdrawn.
In case of pregnancy of a female volunteer or of the partner of a male volunteer, volunteer should be instructed to notify the investigator if, after completion of the study, it is determined that they became pregnant during the treatment phase or through 3 months after the last dose of study drug.
Note 1: To confirm if a congenital anomaly or a genetic condition is to be classified as SAE, the IME list[2] should be consulted.
Note 2: An induced abortion without medical reasons is not considered an SAE.

[2] European Medicines Agency (EMA). Inclusion/exclusion criteria for the "Important Medical Events" list, Doc. Ref.: EMA/126913/2021, 18 March 2021, accessed at: https://www.ema.europa.eu/documents/other/eudravigilance-inclusion/exclusion-criteria-important-medical-events-list_en.pdf