BIOXODES SA

Parc d'activités économiques du Wex
Rue de la Plaine, 11
6900 Marche-en-Famenne
BELGIUM

EudraCT N°**: 2019-002305-22**

Clinical Study Protocol N° Clin_IrCPI_101

Biotrial Code**:** 2BIOXO1

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A Phase I, Double Blind, Placebo Controlled, Single Ascending Dose Study of Intravenously Administered Ir-CPI to Evaluate Pharmacokinetics, Pharmacodynamics, Safety and Tolerability in Healthy Male Volunteers.

Investigational Medicinal Product Code:	Ixodes ricinus-Contact Phase Inhibitor (Ir-CPI)
Development Phase:	I

Principal investigator:

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Version: V5.0

Date: 15 OCT 2020

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Protocol Approval Form

The protocol entitled "A Phase I, Double Blind, Placebo Controlled, Single Ascending Dose Study of Intravenously Administered Ir-CPI to Evaluate Pharmacokinetics, Pharmacodynamics, Safety and Tolerability in Healthy Male Volunteers.", version V5.0 dated 15 OCT 2020 has been approved for submission to the Ethics Committee and regulatory authorities (Comité d'Ethique Hospitalo-Facultaire Universitaire de Liège and Federal Agency for Medicines and Health Products) by:

Sponsor's Representative: Edmond Godfroid (Authentication)

Date: Signature numérique de Edmond Godfroid (Authentication) Date : 2020.10.16 14:19:01 +02'00'

EDMOND GODFROID

Principal Investigator's Approval

I, the undersigned, have examined this protocol and agree to conduct this trial according to this protocol, to comply with its requirements, subject to ethical and safety considerations, as set out in this protocol, the International Council for Harmonisation on Good Clinical Practice (EMA/CHMP/ICH/135/1995), the Declaration of Helsinki 1964 (latest revision Fortaleza 2013) and all other applicable laws and regulations on the use of investigational medicinal products.

Principal investigator:

Date: 19 OCT 2020

BERNARD JANDRAIN, MD

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1. SYNOPSIS

Name of Company:

BIOXODES SA

Parc d'activités économiques du Wex ; Rue de la Plaine, 11; 6900 Marche-en-Famenne; BELGIUM

Name of Finished Product: NA

Name of active ingredient: Ixodes ricinus-Contact Phase Inhibitor (Ir-CPI)

Title of Study:

A Phase I, Double Blind, Placebo Controlled, Single Ascending Dose Study of Intravenously Administered Ir-CPI to Evaluate Pharmacokinetics, Pharmacodynamics, Safety and Tolerability in Healthy Male Volunteers.

Principal Investigator:

Bernard Jandrain, MD

Study centre:

ATC SA, Clinical Pharmacology Unit, CHU of Liège, B35, Route 124, 4000 Liège, BELGIUM

Publication (reference):

NA

Clinical Phase:

Phase I

Rationale:

Ixodes ricinus-Contact Phase Inhibitor (Ir-CPI) is under development for the prevention of thrombosis in hospital procedures in which blood is exposed to non-biological surfaces known to activate the intrinsic pathway of the coagulation cascade (e.g. percutaneous endovascular interventions, cardiopulmonary bypass), especially under clinical circumstances where the use of classical anticoagulation is not adequate due to heparin intolerability and/or high risk of bleeding. As Ir-CPI has to be administered by intravenous (IV) infusion and as it has a half-life of a few hours, it is intended for specialised use in a hospital setting.

This study will be the first-in-human study of Ir-CPI. This study will provide an initial assessment of the safety, tolerability, pharmacokinetics (PK), and pharmacodynamics (PD) of Ir-CPI after a single administration of a 6-hour infusion at ascending doses without (Part 1) and with a loading dose (Part 2) to healthy adult participants.

The doses to be evaluated are based on the results obtained in pharmacology studies. Once PK results are obtained in Part 1 of the study (increasing single doses of Ir-CPI in healthy participants upon 6-hour infusion), a combination of rapid (30 minutes) and slow (5.5 hours) infusions will be administered to healthy participants in Part 2 of the study. These results will be used thereafter in Proof of Concept (POC) efficacy studies in human where there is a need for reliable anticoagulation, such as – but not limited to – coronarography procedures and arterio-venous shunting. In the end, part 2 of the study was not performed.

This design will be used to define the experimental condition(s) of administration allowing to rapidly reach and maintain steady state Ir-CPI concentration for up to 6 hours.

Objective(s) and associated endpoints:

	Objectives	Endpoints
Primary	Part 1: Assess the safety and tolerability of Ir-CPI following a single ascending 6-hour intravenous infusion in healthy male participants.	Safety parameters [adverse events (AEs), physical examinations, vital signs, telemetry including SpO_2, weight, Hemoccult® test, supine 12-lead ECG, serum chemistry, haematology, standard coagulation aPTT, urinalysis, and monitoring for concomitant medications].
	Part 2*: Assess the safety and tolerability of Ir-CPI following a combination of rapid (30 minutes) and slow (5.5 hours) intravenous infusions over a total of 6 hours in healthy male participants.	

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Secondary	Part 1: Assess the pharmacokinetics (PK) of Ir-CPI following single ascending 6-hour intravenous infusion in healthy male participants.	Maximum plasma concentration (C_{max}), time to reach maximum plasma concentration (t_{max}), Area under the plasma concentration-time curve from time zero to 6h (AUC_{0-6}), Area under the plasma concentration-time curve from time zero to time of infinity (AUC_{inf}), apparent terminal elimination rate constant (λz), terminal elimination half-life ($t_{1/2}$), volume of distribution (Vd), total body clearance (CL).
	Part 2*: Assess the PK of Ir-CPI following a combination of rapid (30 minutes) and slow (5.5 hours) intravenous infusions over a total of 6 hours in healthy male participants.	
	Investigate the preliminary Pharmacodynamics (PD) of Ir-CPI in healthy male participants using a GCLP validated method according to FDA guidelines 2018 for activated Partial Thromboplastin Time (aPTT). Inhibition of Factor XI (FXI) and Factor XII (FXII) procoagulant activities will also be assessed to support the aPTT dynamics.	Change from baseline in aPTT and residual FXI and FXII activities.
Exploratory	Investigate the potential of Ir-CPI to induce the formation of anti-drug antibodies (ADA), including their capability to neutralize drug activity.	Immunogenicity assessed by ADA plasma levels.
	Investigate the effect of Ir-CPI on markers related to the coagulation system	D-dimers, fibrinogen and prothrombin time (PT) levels in whole blood.
	Investigate the effect of Ir-CPI on biomarkers for renal injury.	***Blood assay:*** serum cystatin C (CysC). ***Urinary assay:*** urinary microalbumin, Kidney Injury Molecule-1 (KIM-1), urinary neutrophil gelatinase-associated lipocalin (NGAL).
	Investigate the effect of Ir-CPI on inflammatory markers.	tumor necrosis factor alpha (TNFα), high sensitivity C-reactive protein (hsCRP) levels in serum.
	Assess the PK of Ir-CPI in urine.	Total amount excreted in urine (Ae), Ae%, volume of urine excreted (Vol_UR), Maximal excretion rate (C_{max} rate), Time to reach maximum excretion (T_{max}) rate and Renal Clearance (CL_R).
	PK-PD relationship.	No new endpoint: only graphical investigation of PK and PD parameters.

*: because part 2 was not performed, these objectives are not applicable.

Design:

This is a Phase 1, double-blind, placebo controlled, single dose escalation study of Ir-CPI in healthy male participants. The terms ***panel*** and ***cohort*** can be used interchangeably. The study will consist of two parts:

Part 1: an Ir-CPI or matching placebo single ascending dose administration in healthy male participants using a continuous 6-hour intravenous infusion.

Part 2: an Ir-CPI or matching placebo single ascending dose administration in healthy male participants using a single ascending 30-minute loading dose infusion followed by a fixed dose of continuous 5.5-hour

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intravenous infusion (maintenance infusion). In the end, part 2 was not performed.

In both parts, a screening visit will take place from Day (D)-28 to D-2.

In Cohorts A, B and C, participants will then remain in-house between D-1 and D2. Consecutively, ambulatory visits will be planned every day until 96h postdose (D5, postdose refers to the beginning of infusion).

In Cohort D and subsequent cohorts, participants will then remain in-house from D-1 and will remain hospitalised until the investigator is able to review the 24h aPTT assay results. If, for each participant, the results are less than or equal to 2X aPTT level at baseline (i.e. D1 predose), then the participants may leave the clinic. They will return for the ambulatory visits on D3 (48h post infusion start), D4 (72h), D5 (96h) and D7 (144h). If the 24h safety aPTT assay of one participant is above 2X the participant's baseline aPTT, then all participants will remain hospitalised until the completion of the D3 (48h) assessments and an additional aPTT sample will be drawn at 36h. If the 36h aPTT assay result of a participant is more than 2X baseline aPTT of this participant, the investigator will decide the most appropriate procedures for the follow-up of this participant. Participants will return for the ambulatory visits on D4 (72h), D5 (96h) and D7 (144h).

The dosing period will be followed by a discharge visit held on D10 ± 2 days. An extra ambulatory visit will be planned approximately on D30 (1 month ± 3 days) and on D90 (3 months ± 7 days) after dosing for immunogenicity testing. AEs occurring between the discharge visit and the 2 extra ambulatory visits will be recorded during the 2 extra ambulatory visits. Concomitant treatment after the discharge visit (D10 ± 2 days) will be recorded only if an AE is recorded between the discharge visit and the extra ambulatory visits. At the extra ambulatory visits, laboratory samples will be taken to look for immunogenicity (presence of ADAs and neutralizing antibodies if applicable). If ADAs are detected at the D90 extra ambulatory visit, a supplemental ambulatory visit will be planned on D180 (6 months ± 7 days) after dosing.

Supplemental ADA samplings ("long-term follow-up of ADAs"):

If ADAs are detected at the D180 ambulatory visit, supplemental samplings will be performed every 3 months (±14 days) for the first year after the D180 visit and then every 6 months (±30 days) for the following 4 years (i.e. follow-up of immunogenicity testing for a maximum of 5 years after D180). The samplings will be performed until the participants reach a negative status, until they show the same ADA titre result for 3 samplings in a row, or up to a maximum of 5 years after D180, whichever comes first.

Details of timing of each clinical visit, and the assessments to be performed at each visit are summarised in the schedule of activities (SOA).

Number of participants:

Part 1: Approximately forty (40) healthy male participants will be enrolled in 5 sequential panels. Each panel will have 8 participants (6 active: 2 placebo). The fifth group will be optional depending on the PK and PD data obtained in the previous groups.

Part 2: Approximately twenty-four (24) healthy male participants will be enrolled in 3 sequential panels. Each panel will have 8 participants (6 active: 2 placebo).

In the end, the optional fifth group in part 1, and part 2 were not performed.

Rationale for Number of Participants:

The number of participants is not based on statistical power considerations. A sample size of 8 participants per cohort (i.e., 6 active and 2 placebo) was chosen based on the design of similar studies and is considered adequate to provide an initial assessment of the safety and tolerability profile of Ir-CPI.

Number of study centres:

1

Duration of treatment:

The study duration for each participant for the main study part (from the screening visit until the discharge visit) will be approximately 6 weeks. Participants will be followed up until D90 (or D180) for immunogenicity testing. The total study duration will be approximately 18 weeks in total for a given

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participant, and up to 31 weeks in case of D180 visits.

Supplemental ADA samplings ("long-term follow-up of ADAs"):

Additional samplings for immunogenicity testing may be performed until the participants reach a negative status, until they show the same ADA titre for 3 samplings in a row, or up to 5 years after the end of the study in participants with positive ADA results at D180 whichever comes first.

Study products, dose and mode of administration:

Ir-CPI will be supplied frozen, as 5 mL PBS solution containing 25 mg/mL Ir-CPI in glass vials (10 mL). The vials will be thawed at room temperature under gentle agitation. The matching placebo will consist of NaCl 0.9 % solution for intravenous administration. All doses are expressed in mg/kg, and the body weight of each participant at the screening visit will be used to calculate the exact amount of study drug needed to prepare the allocated total dose for each participant.

In **Part 1**, single doses of Ir-CPI or its matching placebo will be administered to the participants using a continuous 6-hour intravenous infusion.

In **Part 2**, single doses of Ir-CPI or its matching placebo will be administered to the participants using a single ascending 30-minute loading dose infusion followed by a fixed dose of continuous 5.5-hour intravenous infusion. In the end, part 2 was not performed.

Fasting conditions are required for the blood sample collection at the selection visit, on D3 (ambulatory or inpatient), for the ambulatory visits [D4/D5/D7 (D7 for Cohort D and subsequent cohorts only)/Discharge visit] and for the D1 predose blood sample.

Water intake is allowed during the infusion. Meals will be standardised across the different panels during the in-patient period of the study. Following the D1 predose blood sample collection, a light breakfast (2 slices of bread with ham or cheese and jam, glass of water) / snack can be given between 2h and 1h before the start of the infusion. Lunch will be served after completion of the study drug infusion.

A light breakfast / snack will also be given after the D1H24 (D2) blood sample collection and after the blood sample collections performed on D3 (ambulatory or inpatient), at the ambulatory visits on D4, D5 and D7 (D7 for Cohort D and subsequent cohorts only) and at the Discharge visit.

Participants must not be in fasting condition for the safety aPTT 36h sample and the extra ambulatory visits.

Details of the infusion rates, doses and suggested total dose administered are provided below:

Study Part	***Group***	***Infusion rate (mg/kg/h)*****	***Infusion Duration (h)***	***Total dose (mg/kg)***
1	A	0.25	6	1.5
1	B	0.75	6	4.5
1	C	1.50	6	9
1	D	3.00	6	18
1	E[#]	4.5	6	27
2[##]	F	Rapid infusion*: 6	0.5	11.25*
		Slow infusion*: 1.5	5.5	
2[##]	G	Rapid infusion*:7	0.5	11.75*
		Slow infusion*: 1.5	5.5	
2[##]	H	Rapid infusion*: 8	0.5	12.25*
		Slow infusion*: 1.5	5.5	

**The rapid and the slow infusion rates will be calculated based on Part 1 emerging safety, PD and PK data. The total dose administered by rapid and by slow infusion will not exceed the highest total dose tested in Part 1. The infusion rate (in mg/kg/h) for the slow infusion will not exceed the maximum infusion rate used in Part 1. The dose may also be adjusted.*

***Weight of the participant at the screening visit will be used to calculate the dose of study drug.*

[#]: Cohort E was not performed according to a recommendation from the Ethics Committee.

[##]: Part 2 was not performed because the targeted plasmatic PK concentration was planned in the unperformed group E.

Escalation to the next higher dose will only take place after review of the Investigator Safety Report from the previous dose levels by the PI, in consultation with the Sponsor's representative [called hereafter the Safety

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Review Committee (SRC)]. Dose escalation will be based on any relevant information on participants' demographics/characteristics, medical history, physical examination, concomitant medications, AEs, clinically significant out of ranges safety clinical laboratory tests results (as assessed by the PI), physical examination results (including basic neurological testing for isocoria, light reflexes, gait and balance) including clinical significance for abnormal findings, ECG and vital sign results including clinical significance for abnormal value, and the statement of the PI's recommendation regarding dose decision. Other parameters of interest will be considered as per SRC charter (blinded PK, PD).

Within the planned dose range, a dose lower than the next planned dose may be tested, depending on emerging safety, tolerability and/or other relevant data, such as blinded PK or PD. The final dose to be used for each cohort will be decided based on the results of the previous panel and can be adjusted downward if deemed necessary. If the highest planned dose level is found to be safe and tolerable, also considering the PK and PD data, additional higher doses may be added by amendment.

Within each panel, the first 2 study participants will be designated as sentinel study participants. The sentinel study participants will be randomized 1:1 to receive either placebo or Ir-CPI (1 study participant each). Between the first participants of 2 consecutive panels, a period of at least 7 days will be respected between the administrations of treatment.

The PI and Sponsor medical monitor will review at least 24hrs of post-dose safety data (vital signs, labs, electrocardiograms [ECGs], telemetry and physical examination) for the sentinel study participants. If no safety concerns are identified, the remainder of the cohort will be randomized in a 5:1 ratio and dosed.

The SRC will meet for deciding on the continuation of dosing after intake in at least 6 participants (i.e. a minimum of 4 on active treatment). However, stopping rules will be applicable from dosing of the first sentinel participant.

Diagnosis and main criteria for inclusion / exclusion:

Inclusion:

Participants must satisfy all of the following inclusion criteria before being allowed to enter the study:

1. Have given written informed consent approved by the relevant Ethics Committee (EC) governing the site, indicating that they understand the purpose of and procedures required for the study and are willing to participate in the study.
2. Male participants between 18 and 55 years of age, inclusive at screening.
3. Otherwise healthy with no clinically significant abnormalities as determined by medical history, physical examination, blood chemistry assessments, haematological assessments, coagulation and urinalysis, measurement of vital signs, and ECG. Isolated out-of-range values judged by the PI (or designated physician) to be of no clinical significance can be allowed. This determination must be recorded in the participant's source documents.
4. Have a body weight in the range of 50 to 90 kg inclusive at screening. Have a body mass index (BMI) of 19 to 28 kg/m^2 inclusive at screening.
5. Agree to abstain from alcohol intake 24 hours before administration of study drug, during the in-patient period of the study and 24 hours prior to all other ambulatory visits, up until and including the discharge visit.
6. Agree not to use prescription medications within 14 days prior to study drug administration and through the duration of the study, unless approved by the PI and Sponsor medical monitor.
7. Non-smokers or abstinence from tobacco or nicotine-containing products for at least 3 months prior to screening.
8. Agree not to use over-the-counter (OTC) medications [including decongestants, antihistamines, and herbal medication (including herbal tea and St. John's Wort], within 14 days prior to study drug administration through the discharge visit, unless approved by the PI and Sponsor medical monitor. Occasional use of paracetamol at recommended doses is allowed. Special rules apply for aspirin, corticosteroids and nonsteroidal anti-inflammatory drugs (NSAIDS (see exclusion criteria 6-7-8-9)
9. Venous access (both arms) sufficient to allow blood sampling and study drug administration as per

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protocol.

Inclusion (continued):

10. Participants and their partners of childbearing potential [meaning who are not surgically sterile (tubal ligation/obstruction or removal of ovaries or uterus) or post-menopausal (absence of menstrual periods for at least 12 consecutive months)] must be willing to use 2 methods of contraception:
    - a highly effective method of birth control starting at screening. Highly effective methods of birth control are defined as those that result in a low failure rate (i.e. Pearl Index less than 1% per year) when used consistently and correctly, such as implants, rings, patches, injectable or combined oral contraceptives, intrauterine devices (IUDs), or sexual abstinence (periodic abstinence e.g. calendar, ovulation, symptothermal, postovulation methods, declaration of abstinence for the duration of the trial, and withdrawal are not acceptable methods of contraception) .
    - and a local barrier form of contraception. Acceptable barrier methods are either the participant's use of a condom or the partner's use of an occlusive cap or diaphragm, or spermicides.

    Participants will not donate sperm from the selection visit and up to 90 days after the infusion. In case of sterile or vasectomised participants, no contraception will be required for their partners of childbearing potential.
11. Willing/able to adhere to the study visit schedule and other requirements, prohibitions and restrictions specified in this protocol.

Exclusion:

If any of the following exclusion criteria apply, the participant must not enter the study:

1. Currently have or have a history of any clinically significant medical illness or medical disorders the PI considers should exclude the participant, including (but not limited to) cardiovascular disease, neuromuscular, haematological disease, immune deficiency state, respiratory disease, hepatic or gastrointestinal disease, neurological or psychiatric disease, ophthalmological disorders, neoplastic disease, renal or urinary tract diseases, or dermatological disease.
2. History of personal or familial bleeding disorders; including prolonged or unusual bleeding.
3. History of deficiency in factor XII (FXII) or haemophilia type A (FVII) or type B (FIX) or type C (FXI).
4. History of cerebral bleeding (e.g. after a car accident), stroke and cerebrovascular accident (CVA).
5. Anamnestic history of Lyme disease or tick-borne encephalitis.
6. Use of Acetylsalicylic-Acid (ASA)-containing OTC medications within 1 month prior to screening.
7. Chronic administration of NSAIDs, chronic use of corticosteroids within 1 month prior to screening.
8. Chronic administration of clopidogrel, ticlopidin, dipyridamole, Coumadin-like anticoagulants, new oral anticoagulant dabigatran, rivaroxaban, apixaban or edoxaban within 3 months prior to screening.
9. Administration of unfractionated heparin, low molecular weight heparin, fibrinolytic agents and anti-FXa within 3 months prior to screening.
10. Have an active acute or chronic infection or diagnosed latent infection.
11. Systolic blood pressure (SBP) greater than 150 or less than 90 mmHg, diastolic blood pressure (DBP) greater than 90 or less than 50 mmHg, and heart rate (HR) greater than 100 or less than 40 bpm.
12. Acute clinically relevant illness within 7 days prior to study drug administration or have had a major illness or hospitalisation within 1 month prior to study drug administration.
13. Major or traumatic surgery within 12 weeks of screening.
14. Any participant who plans to undergo elective surgery within 4 weeks prior to study drug administration and through the discharge visit.
15. Positive serology test for HIV antibodies, hepatitis B surface antigen (HBsAg), or hepatitis C virus (HCV) antibodies at screening.

Exclusion (continued):

16. Recent history (within previous 6 months) of alcohol or drug abuse.

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17. Have positive urine toxicology screen at screening or D −1 for substances of abuse including amphetamines, benzodiazepine, cocaine, opiates, barbiturate and cannabinoids.
18. Have a positive alcohol breath test at screening or D-1.
19. Consumes, on average, more than approximately 500 mg/day of caffeine (as contained in 5 cups of tea or coffee or 8 cans of caffeine-containing soda or other caffeinated products per day).
20. Donated blood (i.e. 500 mL) within 3 months before D1.
21. Have a history of active drug and/or food allergy or other active allergic disease requiring the constant use of medications, or a history of severe allergic reaction, angioedema or anaphylaxis.
22. Received any other experimental therapy or new investigational drug within 30 days or 5 half-lives (whichever is longer) of study drug administration.

Concomitant medications and lifestyle restrictions:

Water intake is allowed during the infusion.

Any medication the participant takes during the study, and until the discharge visit, other than the study drugs, including any prescription or OTC drug (including vitamins, herbal and mineral supplements), is considered a concomitant medication. At the extra visits scheduled for ADA assessment (i.e. up to D180), only the medications that are administered in treatment of AEs will be considered concomitant medication.

Participants will be requested to comply with the following restrictions during the study and abstain from:

- Strenuous exercise, as judged by the Investigator, from the selection visit until after the discharge visit.
- Use of prescriptions or OTC medications [including decongestants, antihistamines], and herbal medication (including herbal tea and St. John's Wort), within 14 days prior to study drug administration through the discharge visit, unless approved by the PI and Sponsor medical monitor. Occasional use of paracetamol at recommended doses is allowed. Specific rules apply for aspirin, corticosteroids and NSAIDS (see exclusion criteria 6, 7, 8 and 9).
- Consumption of grapefruit, grapefruit juice, Seville oranges, Seville orange marmalade or other products containing grapefruit or Seville oranges from selection visit until after the discharge visit.
- Consumption of any alcoholic beverages 24 hours before administration of study drug, during the in-patient period of the study and 24 hours prior to all other ambulatory visits, up until and including the discharge visit.
- Smoking and using nicotine-containing products excluded for at least 3 months prior to the screening visit, and until after the discharge visit.
- Use of Acetylsalicylic-Acid (ASA)-containing over-the-counter medications within 1 month prior to screening and until the discharge visit.
- Consumption of any caffeine-containing products (e.g. coffee, tea, chocolate, or Coca-Cola like/ energy drinks): excluded from 24 hours prior to D1 until the end of the in-patient period. Limited to less than 500 mg/day of caffeine (as contained in 5 cups of tea or coffee or 8 cans of caffeine-containing soda or other caffeinated products per day) until the discharge visit.
- Donation of blood (i.e. 500 mL) within 3 months before D1, throughout the study and for 3 months after the end of the study (D90 or D180).

The compliance of the participant to these lifestyle restrictions will be checked at each visit.

Participants and their partners of childbearing potential must be willing to use 2 methods of contraception, one of which must be a barrier method, and participants will not donate sperm, from the selection visit and up to 90 days after the infusion.

<u>Supplemental ADA samplings ("long-term follow-up of ADAs"):</u>

At the additional samplings scheduled for ADA assessment after D180, only treatments given for Serious Adverse Drug Reactions (SADRs) will be considered concomitant medications, and they will be collected only in the Pharmacovigilance (PV) database.

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Study Schedule:

Screening:

Participants will be screened for eligibility within 28 days of admission to D-2.

Written informed consent will be obtained before any study procedure is performed. The screening will consist of: demography, medical and medication history, physical examination, vital signs, weight, height and BMI, supine 12-lead ECG, haematology, safety aPTT, serum chemistry and urinalysis tests, HIV, hepatitis B and hepatitis C serology, urine drug screen, presence of blood in faeces and alcohol breath test, and review of the inclusion / exclusion criteria and prior/concomitant treatments.

The results of screening must be known to the Investigator prior to the participant's admission to the treatment period.

Treatment period (D-1 to D5 for cohorts A-C and D-1 to D7 for cohort D and subsequent cohorts):

In both parts of the study, eligible participants will be admitted to the research facilities on D-1 in the evening, to assess eligibility based on urine drug screen, breath alcohol test, and adherence to the study restrictions. Inclusion and exclusion criteria will be reviewed, and a physical examination will be performed. Participants will be randomised to active treatment or placebo on D1, and study drug administration will be performed on D1. The participants will be hospitalised at the clinical unit until at least 24h after the start of study drug administration (D2). Participants included in Cohort D and subsequent cohorts may remain hospitalised up to 48h after the start of study drug administration (D3), if deemed necessary by the investigator.

Ambulatory visits will be planned on D3* (48h post infusion start), D4 (72h), D5 (96h) and D7 (144h) (D7 for Cohort D and subsequent cohorts only), for safety, PD and PK assessments.

The dosing period will be followed by a study discharge visit on D10 ± 2 days after infusion.

**Note: for Cohort D and subsequent cohorts, D3 may be inpatient.*

Discharge visit (D10 ± 2 days after infusion or early discontinuation):

A discharge visit will take place between D10 ± 2 days after infusion or early discontinuation, and will involve physical examinations, vital signs, weight, supine 12-lead ECG, serum chemistry, haematology, urinalysis, AE monitoring, concomitant medication monitoring, last PD samples for cluster A and cluster B, immunogenicity testing (ADA) and exposure to tick bites.

Following the discharge visit procedures, the participants will be discharged and will return for 2 or 3 extra-ambulatory visits.

Extra ambulatory visits:

An extra ambulatory visit will be planned approximately at D30 (1 month ± 3 days) and at D90 (3 months ± 7 days) after dosing for immunogenicity testing. AEs will be recorded during this visit. A laboratory sample will be taken to look for immunogenicity (presence of anti-drug antibodies, neutralizing antibodies). If ADAs are detected at D90, a supplemental ambulatory visit will be planned on D180 (6 months ±7 days).

Supplemental ADA samplings ("long-term follow-up of ADAs"):

If ADAs are still detected at the D180 ambulatory visit, supplemental samplings will be planned every 3 months (±14 days) for the first year and every 6 months (±30 days) until the participants reach a negative status, until they show the same ADA titre for 3 samplings in a row, or for a maximum of 5 years after the D180 visit whichever comes first. With regards to safety, only SADRs and related information will be reported in the PV database at these sampling time points.

Criteria for evaluation:

Pharmacokinetic Assessments	Blood samples (4.5 mL) will be drawn, for the assay measuring plasma Ir-CPI concentration, at the following time-points: **In Part 1:** D1, pre-dose and at 30min, 60min, 90min, 2h, 4h, 6h, 6h30, 7h, 7h30, 8h, 10h, 12h, 16h, then D2 (24h), D3 (48h), D4 (72h), D5 (96h) and D7 (144h, Cohort D and subsequent cohorts only) post-dose (after start of

Name of Company: BIOXODES SA Parc d'activités économiques du Wex ; Rue de la Plaine, 11; 6900 Marche-en-Famenne; BELGIUM	
Name of Finished Product: NA	
Name of active ingredient: Ixodes ricinus-Contact Phase Inhibitor (Ir-CPI)	
	infusion)**. **In Part 2:** D1 pre-dose and at 15min, 30min, 45min, 60min, 90min, 2h, 4h, 6h, 6h30, 7h, 7h30, 8h, 10h, 12h, 16h, then D2 (24h), D3 (48h), D4 (72h), D5 (96h) and D7 (144h) post-dose**. In the end, part 2 was not performed. *** In case of infusion less than 6 hours, blood sample for Ir-CPI pharmacokinetics will be done at the end of infusion then 30min, 60min, 90min, 2h, 4h, 6h, 10h, 18h, 42h, 66h, 90h and 138h (138h for Cohort D and subsequent cohorts only) after end of infusion.* Urine samples will be collected at the following intervals: **In Part 1**: predose, [0-6h[, [6-12h[and [12-24h] after start of infusion. **In Part 2:** predose, [0-6h[, [6-12h[and [12-24h] after start of infusion. The following parameters will be derived by compartmental or non-compartmental analysis, as appropriate, from the plasma Ir-CPI concentration-time profiles: C_{max}, t_{max}, AUC_{0-6}, AUC_{inf}, λz, $t_{1/2}$, Vd, CL.
<u>Pharmacodynamic Assessments</u>	Blood samples (4.5 mL) will be drawn for the assay of PD aPTT, Factor XI and Factor XII activities at the following time-points: **In Part 1:** The effect of Ir-CPI on aPTT and on FXI/FXII activities (Cluster A sampling times) will be measured on D1: pre-dose, then at 30min, 60min, 90min, 2h, 4h, 6h, 6h30, 7h, 7h30, 8h, 10h, 12h, 16h post-dose, then on D2 (24h), D3 (48h), D4 (72h), D5 (96h), D7 (144h, Cohort D and subsequent cohorts only) and discharge visit**. *** In case of infusion less than 6 hours, blood sample for aPTT and FXI/FXII activities will be done at the end of infusion then 30 min, 60 min, 90 min, 2h, 4h, 6h, 10h, 18h, 42h, 66h, 90h and 138h (138h for Cohort D and subsequent cohorts only) after end of infusion.* *Note: The effect of Ir-CPI on the following exploratory biomarkers (BM) (Cluster B sampling times) will be measured on D1 predose and at 2h, 4h, 6h, 8h, 12h and 24h after start of infusion (D2), then then 48h (D3), and 72h (D4) after start of infusion and at the discharge visit: PT (sec and INR), D-dimers, fibrinogen, hsCRP, TNFα). In case of infusion less than 6 hours, blood sample for PD cluster B parameters will be done at the end of infusion then 2h, 6h, 18h, 42h, 66h after end of infusion and at the discharge visit.* **In Part 2:** The effect of Ir-CPI on aPTT and on FXI/FXII activities (Cluster A sampling times) will be measured on D1 pre-dose and at 15min, 30min, 45min, 60min, 90min, 2h, 4h, 6h, 6h30, 7h, 7h30, 8h, 10h, 12h, 16h, then D2 (24h), D3 (48h), D4 (72h), D5 (96h), and D7 (144h) and discharge visit**. *** In case of infusion less than 6 hours, blood sample for aPTT and FXI/FXII activities will be done at the end of infusion then 30 min, 60 min, 90min, 2h, 4h, 6h, 10h, 18h, 42h, 66h, 90h and 138h after end of infusion.* *Note: The effect of Ir-CPI (Cluster B sampling times) on the following exploratory BM will be measured on D1 predose and at 2h, 4h, 6h, 8h, 12h and 24h postdose (D2), then 48h (D3), 72h (D4) after the start of infusion and at the discharge visit: PT (sec and INR), D-dimers, fibrinogen, hsCRP, TNFα). In case of infusion less than 6 hours, blood sample for PD cluster B parameters will be done at the end of infusion then 2h, 6h, 18h, 42h, 66h after end of infusion and at the discharge visit.* In the end, part 2 was not performed.

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Name of active ingredient: Ixodes ricinus-Contact Phase Inhibitor (Ir-CPI)	
Exploratory Biomarkers (BM) Assessments	**Blood:** The following blood sample volumes will be drawn for the assay of exploratory BM (one sample per group of BM as described below): - For PT, D-dimers and fibrinogen assay: 4.5 mL; - For CysC and inflammatory BM assay (hsCRP and TNFα): 8 mL; The effect of Ir-CPI on exploratory BM will be measured at Cluster B sampling times: **In Part 1:** CysC samples will be drawn at the same timepoints as the safety blood chemistry and haematology samples. The other biomarkers samples will be drawn at D1, predose then at 2h, 4h, 6h, 8h, 12h post-dose, on D2 (24h post-dose), on D3, D4 and at the discharge visit. **In Part 2:** CysC samples will be drawn at the same timepoints as the safety blood chemistry and haematology samples. The other biomarkers samples will be drawn at D1, predose then at 2h, 4h, 6h, 8h, 12h post-dose, on D2 (24h post-dose), D3 and D4 and at the discharge visit. Blood samples will be drawn for immunogenicity testing, i.e. for the detection of anti-drug antibodies (ADA). In both parts, immunogenicity samples will be drawn on D1 (predose), at the discharge visit (D10 ± 2 days) and at the 2 to 3 extra ambulatory visits. Supplemental ADA samplings ("long-term follow-up of ADAs"): A maximum of 12 additional samplings for immunogenicity may be performed for long-term follow-up of ADAs. **Urine:** In both parts, urine biomarkers for renal injury (Urinary microalbumin, KIM-1, NGAL) will be assessed using the urine PK collection. In the end, part 2 was not performed.
Demographic and Safety Assessments	Screening and demography assessment will cover demography (including race and ethnicity), inclusion / exclusion criteria, medical and medication history, drug / alcohol screen and serology. Safety will be evaluated from: reported AEs, physical examinations (the complete examination includes basic neurological testing for isocoria, light reflexes, gait and balance), vital signs, telemetry including SpO_2, weight, presence of occult blood in faeces (Hemoccult® test), supine 12-lead ECG, serum chemistry, haematology, coagulation aPTT (safety aPTT), laboratory safety urinalysis, and monitoring for concomitant medications. Exposure to potential tick bites will be monitored (extra serology sample for archiving at baseline for anti-Borrelia IgG and IgM assays, and question asked to participants on D1, at the discharge visit, and at the extra-ambulatory visits performed for ADA analysis). AEs related to bleeding (e.g. positive Hemoccult® testing, gingival bleeding, bruising/haematoma, conjunctival bleeding, prolonged bleeding after trauma) will be considered as adverse events of special interest (AESI). Also considered as AESI are all infusion site reactions, including hypersensitivity reactions that would occur during or after study drug administration. AEs will be monitored from the screening visit until the last extra-ambulatory visit.
Statistical methods:	Statistical analysis of clinical parameters will be performed under the responsibility of Biotrial.

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PK analysis:

Ir-CPI individual and mean plasma concentrations at each sampling time point will be presented by listings and descriptive summary statistics including means, geometric means, medians, ranges, standard deviations and coefficients of variation. Individual and mean plasma concentrations versus time will be plotted on linear and log-linear scales. Descriptive statistics and graphs will be also performed for the plasma PK parameters.

Relevant plasma PK parameters will be derived for the parent by compartmental methods for plasma and tabulated along with descriptive statistics.

The linear dose-proportionality of C_{max}, AUC_{0-6} and AUC_{inf} (if applicable) will be assessed using an exponential regression model ("power model") in part 1 and part 2.

PD analysis:

Descriptive statistics will be provided on values and changes from baseline for each time-point.

Exploratory PK-PD analysis:

As an exploratory analysis, evaluation of the relationship between doses and concentrations of Ir-CPI and the change from baseline in coagulation parameters will be investigated.

Exploratory Biomarkers Analysis

Descriptive statistics will be provided on values and changes from baseline for each time-point.

Presence of ADA will be described by time-point until D180.

Safety Analysis

All AEs and treatment-emergent AEs will be described overall and by preferred term and system organ class until D180. Summary descriptive statistics will be provided for the other safety parameters.

Supplemental ADA samplings ("long-term follow-up of ADAs"):

ADA results from the additional samplings after D180 will only be listed and appended to the final Clinical Study Report (CSR). SADRs and related information after D180 will only be collected in the PV database.

2. SCHEDULE OF ACTIVITIES (SOA)

2.1. Part 1 SOA: single 6-hour infusion

2.1.1. Part 1 SOA: single 6-hour infusion for Cohorts A, B and C

Assessments	Screening visit [1]	Treatment Period						Discharge Visit	Extra ambulatory visits for ADA analysis	Additional ADA sampling in case of ADAs detected at D180
	Visit 1 (V1) D-28 to D-2	V2 D-1	V3 D1 [2]	V4 D2	V5 D3	V6 D4	V7 D5	V8 D10 ± 2d	V9: D30 (±3d), V10: D90 (±7d) V11: D180 (±7d) [22]	From D270 (±14d), and up to every 3 months (±14d) for the first year and every 6 months (±30d) for the following 4 years [24]
Screening / Administrative / Other Assessments										
Informed consent	X									
Demography [3]	X									
Inclusion / exclusion criteria	X	X								
Medical and medication history	X									
Life restriction monitoring		X			X	X	X	X		
Drug / alcohol screen[4]	X	X								
Serology[5]	X									
Randomisation to treatment			X							
In-patient period		←	—	→						
Safety Assessments										
Physical exam [6]	X	X		X				X		
Brief physical exam [7]			X							
Vital signs [8]	X		X	X	X	X		X		
Telemetry (including SpO_2)[9]			←	—	→					
Height and BMI	X									
Weight	X							X		
Faeces collection (Hemoccult® test)	X			←	—	—	→			

Assessments	**Screening visit [1]**	**Treatment Period**						**Discharge Visit**	**Extra ambulatory visits for ADA analysis**	**Additional ADA sampling in case of ADAs detected at D180**
	Visit 1 (V1) D-28 to D-2	**V2 D-1**	**V3 D1 [2]**	**V4 D2**	**V5 D3**	**V6 D4**	**V7 D5**	**V8 D10 ± 2d**	**V9: D30 (±3d), V10: D90 (±7d) V11: D180 (±7d) [22]**	**From D270 (±14d), and up to every 3 months (±14d) for the first year and every 6 months (±30d) for the following 4 years [24]**
Supine 12-lead ECG [10]	X		X	X	X	X		X		
Serum chemistry[11]	X		X	X		X		X		
Haematology [11]	X		X	X		X		X		
Exposure to tick bites [12]			X					X	X	
Anti-Borrelia immunity (IgG/IgM) [13]			X							
Safety aPTT [14]	X		X	X	X	X		X		
Urinalysis [15]	X		X	X		X		X		
Adverse event monitoring	◄								►	Only SADRs
Concomitant medication monitoring	◄								Only if related to the reported AE	Only if related to SADRs
Study drug Administration / Pharmacokinetic / Pharmacodynamic Assessments										
Study drug administration [16]			X							
PD samples cluster A: PD aPTT (central lab) and FXI/FXII activities [17]			X	X	X	X	X	X		
PD samples cluster B [18]			X	X	X	X		X		
CysC samples [19]	X		X	X		X		X		
Blood PK samples [20]			X	X	X	X	X			
Immunogenicity Testing (ADA) [21]			X					X	X [22]	X**[24]**
Urine collection [23]			◄	►						

NOTE: postdose is defined as « after start of infusion"

1. Screening procedures must occur within 28 days up to 2 days before study drug administration.
2. The results of the analysis of the D1 predose samples are collected to establish baseline references and are not needed before administration.
3. Demography includes: year of birth, age, sex, ethnicity and race.

4. See laboratory safety parameter assessments (section 9.7.6) for list of tests to be run.
5. See laboratory safety parameter assessments (section 9.7.6) for list of tests to be run.
6. Physical Examination (including basic neurological testing for isocoria, light reflexes, gait and balance) should be performed at screening, on D-1, then 24h postdose (D2) and at the discharge visit.
7. An abbreviated physical exam should be performed at 8h postdose on D1.
8. Supine vital signs should be measured after 5 minutes of rest in a supine position and include body temperature, resting pulse rate, and blood pressure: at screening, then from D1 to D4, vital signs should be done predose, 60min, 2h, 4h, 6h, 8h, 10h, 12h, 24h, 48h, 72h postdose, and then at the discharge visit (D10+/-2d).
9. Cardiac monitoring using telemetry (3-lead minimum and including SpO_2) is to be performed from 1h predose up to 12h postdose.
10. ECG should be done at screening, then on D1 (predose, 2h, 4h, 6h, 8h, 12h), 24h (D2), 48h (D3), 72h (D4) postdose and at the discharge visit. Timing can be adjusted based on emerging PK data.
11. See laboratory safety parameter assessments for list of tests to be run (section 9.7.6): screening, then from D1 to D4: Predose (D1), 24h (D2), 72h postdose (D4), and then at the discharge visit.
12. Participants will be asked at baseline if they have been previously exposed to tick bites. At the discharge and extra-ambulatory visits performed for ADA analysis, the participants will be asked if they have been exposed to tick bite since the previous visit (response recorded as yes/no/unknown). If the answer is yes, then the participant will be asked to provide information on treatment (e.g. antibiotics) and on presence of erythema migrans and other signs or symptoms.
13. The baseline chemistry (predose sample) will also contain an extra archive serum sample for anti-Borrelia serology testing (IgG and IgM) as backup to test for anti-Borrelia antibodies due to previous exposure via potential tick bites.
14. Safety aPTT (local lab) at screening, then on D1, predose, then 2h, 4h, 6h, 8h, 12h, D2 (24h), D3 (48h) and D4 (72h) postdose and at the discharge visit.
15. Urinalysis at screening, then predose (D1), 24h (D2), 72h postdose (D4) and at the discharge visit. See laboratory safety parameter assessments for list of tests to be run (section 9.7.6).
16. All participants will receive a single intravenous dose of Ir-CPI/Placebo given as a 6-hour infusion. Post dose is measured after the beginning of infusion. A light breakfast (2 slices of bread with ham or cheese and jam, glass of water) can be given between 2h and 1h before start of infusion. Lunch will be served after completion of the infusion.
17. Cluster A (central lab): the effect of Ir-CPI on PD aPTT (central lab) (not to be confused with safety aPTT) and on FXI/FXII activities will be measured on D1: pre-dose, then at 30min, 60min, 90min, 2h, 4h, 6h, 6h30, 7h, 7h30, 8h, 10h, 12h, 16h post-dose, then on D2 (24h), D3 (48h), D4 (72h), D5 (96h) and discharge visit. In case of infusion less than 6 hours and besides assessment at predose, 30 min, 60 min, 90 min, 2h and 4h after start of infusion, Cluster A (central lab) parameters should be measured at the end of infusion, then 30 min, 60 min, 90 min, 2h, 4h, 6h, 10h, then 18h, 42h, 66h and 90h after the end of infusion and then at the discharge visit.
18. Cluster B (local lab) the effect of Ir-CPI on PT (sec and INR), D-dimers, fibrinogen, hsCRP and TNFα will be measured on D1, predose then at 2h, 4h, 6h, 8h, 12h post-dose, on D2 (24h post-dose), D3 (48h) and D4 (72h) and discharge visit. In case of infusion less than 6 hours and besides assessment on D1 at predose and at 2h and 4h after start of infusion, Cluster B (local lab) parameters will be measured at the end of infusion, then 2h, 6h, 18h, 42h and 66h after the end of infusion and then at the discharge visit.
19. CysC (central lab) will be collected at screening, then from D1 to D4: Predose (D1), 24h (D2), 72h postdose (D4), and then at the discharge visit.
20. Blood samples for Ir-CPI PK will be done on D1, pre-dose and at 30min, 60min, 90min, 2h, 4h, 6h, 6h30, 7h, 7h30, 8h, 10h, 12h, 16h, then D2 (24h), D3 (48h), D4 (72h) and D5 (96h) post-dose (after start of infusion). In case of infusion less than 6 hours, blood sample for Ir-CPI pharmacokinetics will be done at the end of infusion then 30min, 60min, 90min, 2h, 4h, 6h, 10h, 18h, 42h, 66h and 90h after end of infusion.
21. ADA analysis sample: pre-dose on the day of infusion, at the discharge visit, on D30 and on D90.

22. D180 only if ADAs are detected in the D90 samples.

23. Urine collections for PK assay are to be done at these intervals predose, [0-6h[, [6-12h[and [12-24h] after start of infusion. Urinary NGAL, microalbuminuria and KIM-1 will be assessed.

24. Samplings will be performed until the participants reach a negative status, until they show the same ADA titre result for 3 samplings in a row, or up to a maximum of 5 years after D180, whichever comes first. Otherwise, samplings will be performed every 3 months for the first year then every 6 months up to a maximum of 5 years after the D180 ambulatory visit. As the D180 visit was performed on the same day for all participants (and not according to D1 of a given cohort), these additional samplings will be performed in the same time-frame for all concerned participants. If a planned sampling is missed, the subsequent samplings can still be performed.

2.1.2. Part 1 SOA: single 6-hour infusion for Cohorts D and E

Note: Cohort E was not performed according to a recommendation from the Ethics Committee

Assessments	Screening visit [1]	Treatment Period							Discharge Visit	Extra ambulatory visits for ADA analysis	Additional ADA sampling in case of ADAs detected at D180
	Visit 1 (V1) D-28 to D-2	V2 D-1	V3 D1 [2]	V4 D2 [6]	V5 D3 [6]	V6 D4	V7 D5	V8 D7	V9 D10 ± 2d	V10: D30 (±3d), V11: D90 (±7d) V12: D180 (±7d) [23]	From D270 (±14d) and up to every 3 months (±14d) for the first year and every 6 months (±30d) for the following 4 years [25]
Screening / Administrative / Other Assessments											
Informed consent	X										
Demography [3]	X										
Inclusion / exclusion criteria	X	X									
Medical and medication history	X										
Life restriction monitoring		X			X	X	X	X	X		
Drug / alcohol screen[4]	X	X									
Serology[5]	X										
Randomisation to treatment			X								
In-patient period [6]		←	—	—	→						
Safety Assessments											
Physical exam[7]	X	X		X[7]	X[7]				X		
Brief physical exam[8]			X								
Vital signs[9]	X		X	X	X	X			X		
Telemetry (including SpO_2)[10]			↔								
Height and BMI	X										
Weight	X								X		
Faeces collection (Hemoccult® test)	X			↔							
Supine 12-lead ECG[11]	X		X	X	X	X			X		
Serum chemistry[12]	X		X	X		X			X		
Haematology [12]	X		X	X		X			X		
Exposure to tick bites [13]			X						X	X	

Assessments	Screening visit [1]	Treatment Period						Discharge Visit	Extra ambulatory visits for ADA analysis	Additional ADA sampling in case of ADAs detected at D180
	Visit 1 (V1) D-28 to D-2	V2 D-1	V3 D1 [2]	V4 D2 [6]	V5 D3 [6]	V6 D4	V7 D5	V8 D7	V9 D10 ± 2d	V10: D30 (±3d), V11: D90 (±7d) V12: D180 (±7d) [23]	From D270 (±14d) and up to every 3 months (±14d) for the first year and every 6 months (±30d) for the following 4 years [25]
Anti-Borrelia immunity (IgG/IgM) [14]			X								
Safety aPTT[15]	X		X	X	X	X	X	X	X		
Urinalysis[16]	X		X	X		X			X		
Adverse event monitoring	←									→	Only SADRs
Concomitant medication monitoring	←									Only if related to the reported AE	Only if related to SADRs
Study Drug Administration / Pharmacokinetic / Pharmacodynamic Assessments											
Study drug administration[17]			X								
PD samples cluster A: PD aPTT (central lab) and FXI/FXII activities [18]			X	X	X	X	X	X	X		
PD samples cluster B[19]			X	X	X	X			X		
CysC samples [20]	X		X	X		X			X		
Blood PK samples[21]			X	X	X	X	X	X			
Immunogenicity Testing (ADA) [22]			X						X	X[23]	X **[25]**
Urine collection [24]			←	→							

NOTE: postdose is defined as « after start of infusion"

1. Screening procedures must occur within 28 days up to 2 days before study drug administration.
2. The results of the analysis of the D1 predose samples are collected to establish baseline references and are not needed before administration.
3. Demography includes: year of birth, age, sex, ethnicity and race.
4. See laboratory safety parameter assessments (section 9.7.6) for list of tests to be run.
5. See laboratory safety parameter assessments (section 9.7.6) for list of tests to be run.
6. A safety aPTT sample is drawn on D2, 24h after D1 administration. All participants will remain hospitalised until the investigator is able to review the 24h aPTT assay results. If, for each participant, the results are less than or equal to 2X aPTT level at baseline (i.e. D1 predose), then the participants may leave the clinic. They will return for the ambulatory visits on D3 (48h post infusion start), D4 (72h), D5 (96h) and D7 (144h). If the 24h safety aPTT assay of one participant is above 2X the participant's baseline aPTT, then all participants will remain

hospitalised until the completion of the D3 (48h) assessments and an additional aPTT sample will be drawn at 36h. If the 36h aPTT assay result of a participant is more than 2X baseline aPTT of this participant, the investigator will decide the most appropriate procedures for the follow-up of this participant. Participants will return for the ambulatory visits on D4 (72h), D5 (96h) and D7 (144h).

7. A full physical examination (including basic neurological testing for isocoria, light reflexes, gait and balance) should be performed at screening, on D-1, then, at 24h postdose (D2), also at 48h (D3) if hospitalisation is extended (see footnote 6), and at the discharge visit.
8. An abbreviated physical examination will be performed at 8h postdose on D1.
9. Supine vital signs should be measured after 5 minutes of rest in a supine position and include body temperature, resting pulse rate, and blood pressure: at screening, then from D1 to D4, vital signs should be done predose, 60min, 2h, 4h, 6h, 8h, 10h, 12h, 24h, 48h, 72h postdose, and then at the discharge visit (D10+/-2d).
10. Cardiac monitoring using telemetry (3-lead minimum and including SpO_2) is to be performed from 1h predose up to 12h postdose.
11. ECG should be done at screening, then on D1 (predose, 2h, 4h, 6h, 8h, 12h), 24h (D2), 48h (D3), 72h (D4) postdose and at the discharge visit. Timing can be adjusted based on emerging PK data.
12. See laboratory safety parameter assessments for list of tests to be run (section 9.7.6): screening, then from D1 to D4: Predose (D1), 24h (D2), 72h postdose (D4), and then at the discharge visit.
13. Participants will be asked at baseline if they have been previously exposed to tick bites. At the discharge and extra-ambulatory visits performed for ADA analysis, the participants will be asked if they have been exposed to tick bite since the previous visit (response recorded as yes/no/unknown). If the answer is yes, then the participant will be asked to provide information on treatment (e.g. antibiotics) and on presence of erythema migrans and other signs or symptoms.
14. The baseline chemistry (predose sample) will also contain an extra archive serum sample for anti-Borrelia serology testing (IgG and IgM) as backup to test for anti-Borrelia antibodies due to previous exposure via potential tick bites.
15. Safety aPTT (local lab) at screening, then on D1, predose, then 2h, 4h, 6h, 8h, 12h, 24h (D2), 36h (D2, optional sample only drawn if hospitalisation is extended at least until 36h postdose, see footnote 6), D3 (48h), D4 (72h), D5 (96h), D7 (144h) postdose and at the discharge visit.
16. Urinalysis at screening, then predose (D1), 24h (D2), 72h postdose (D4) and at the discharge visit. See laboratory safety parameter assessments for list of tests to be run (section 9.7.6).
17. All participants will receive a single intravenous dose of Ir-CPI/Placebo given as a 6-hour infusion. Post dose is measured after the beginning of infusion. A light breakfast (2 slices of bread with ham or cheese and jam, glass of water) can be given between 2h and 1h before start of infusion. Lunch will be served after completion of the infusion.
18. Cluster A (central lab): the effect of Ir-CPI on PD aPTT (central lab) (not to be confused with safety aPTT) and on FXI/FXII activities will be measured on D1: pre-dose, then at 30min, 60min, 90min, 2h, 4h, 6h, 6h30, 7h, 7h30, 8h, 10h, 12h, 16h post-dose, then on D2 (24h), D3 (48h), D4 (72h), D5 (96h), D7 (144h) and discharge visit. In case of infusion less than 6 hours and besides assessment at predose, 30 min, 60 min, 90 min, 2h and 4h after start of infusion, Cluster A (central lab) parameters should be measured at the end of infusion, then 30 min, 60 min, 90 min, 2h, 4h, 6h, 10h, then 18h, 42h, 66h, 90h and 138h after the end of infusion and then at the discharge visit.
19. Cluster B (local lab) the effect of Ir-CPI on PT (sec and INR), D-dimers, fibrinogen, hsCRP and TNFα will be measured on D1, predose then at 2h, 4h, 6h, 8h, 12h post-dose, on D2 (24h post-dose), D3 (48h) and D4 (72h) and discharge visit. In case of infusion less than 6 hours and besides assessment on D1 at predose and at 2h and 4h after start of infusion, Cluster B (local lab) parameters will be measured at the end of infusion, then 2h, 6h, 18h, 42h and 66h after the end of infusion and then at the discharge visit.
20. CysC (central lab) will be collected at screening, then from D1 to D4: Predose (D1), 24h (D2), 72h postdose (D4), and then at the discharge visit.
21. Blood samples for Ir-CPI PK will be done on D1, pre-dose and at 30min, 60min, 90min, 2h, 4h, 6h, 6h30, 7h, 7h30, 8h, 10h, 12h, 16h, then D2 (24h), D3 (48h), D4 (72h), D5 (96h) and D7 (144h) post-dose (after start of infusion). In case of infusion less than 6 hours, blood sample for Ir-CPI pharmacokinetics will be done at the end of infusion then 30min, 60min, 90min, 2h, 4h, 6h, 10h, 18h, 42h, 66h, 90h and 138h after end of infusion.

22. ADA analysis sample: pre-dose on the day of infusion, at the discharge visit, on D30 and on D90.

23. D180 only if ADAs are detected in the D90 samples.

24. Urine collections for PK assay are to be done at these intervals predose, [0-6h[, [6-12h[and [12-24h] after start of infusion. Urinary NGAL, microalbuminuria and KIM-1 will be assessed.

25. Samplings will be performed until the participants reach a negative status, until they show the same ADA titre result for 3 samplings in a row, or up to a maximum of 5 years after D180, whichever comes first. Otherwise, samplings will be performed every 3 months for the first year then every 6 months up to a maximum of 5 years after the D180 ambulatory visit. As the D180 visit was performed on the same day for all participants (and not according to D1 of a given cohort), these additional samplings will be performed in the same time-frame for all concerned participants. If a planned sampling is missed, the subsequent samplings can still be performed.

2.2. Part 2 SOA: loading dose infusion of 30 minutes followed by fixed dose continuous infusion over 5.5 hours

Note: Part 2 was not performed because the targeted plasmatic PK concentration was planned in the unperformed group E.

Assessments	Screening visit [1]	Treatment Period							Discharge Visit	Extra ambulatory visits for ADA analysis
	V1 D-28 to -2	V2 D-1	V3 D1 [2]	V4 D2 [6]	V5 D3 [6]	V6 D4	V7 D5	V8 D7	V9 D10 (±2d)	V10: D30 (±3d), V11: D90 (±7d) V12: D180 (±7d) [23]
Screening / Administrative / Other Assessments										
Informed consent	X									
Demography [3]	X									
Inclusion / exclusion criteria	X	X								
Medical and medication history	X									
Lifestyle restriction monitoring		X			X	X	X	X	X	
Drug / alcohol screen[4]	X	X								
Serology[5]	X									
Randomization to treatment			X							
In-patient stay [6]		◄	———	——►	►					
Safety Assessments										
Physical exam[7]	X	X		X[7]	X[7]				X	
Brief physical exam[8]			X							
Vital signs[9]	X		X	X	X	X			X	
Telemetry (including SpO_2)[10]			◄►							
Height and BMI	X									
Weight	X								X	
Faeces collection (Hemoccult® test)	X			◄	———	——►				
Supine 12-lead ECG[11]	X		X	X	X	X			X	
Serum chemistry[12]	X		X	X		X			X	
Haematology[12]	X		X	X		X			X	
Exposure to tick bites [13]			X						X	X
Anti-Borrelia immunity (IgG/IgM) [14]			X							
Safety aPTT[15]	X		X	X	X	X	X	X	X	
Urinalysis[16]	X		X	X		X			X	
Adverse event monitoring	◄	———	———	———	———	———	———	———	——►	
Concomitant medication monitoring	◄	———	———	———	———	———	———	——►		Only if related to the reported AE

Assessments	Screening visit [1]	Treatment Period							Discharge Visit	Extra ambulatory visits for ADA analysis
	V1 D-28 to -2	V2 D-1	V3 D1 [2]	V4 D2 [6]	V5 D3 [6]	V6 D4	V7 D5	V8 D7	V9 D10 (±2d)	V10: D30 (±3d), V11: D90 (±7d) V12: D180 (±7d) [23]
Study Drug Administration / Pharmacokinetic / Pharmacodynamic Assessments										
Study Drug administration[17]			X							
PD samples cluster A: PD aPTT (central lab) and FXI/FXII activities [18]			X	X	X	X	X	X	X	
PD samples cluster B [19]			X	X	X	X			X	
CysC sample [20]	X		X	X		X			X	
PK samples [21]			X	X	X	X	X	X		
Immunogenicity Testing (ADA) [22]			X						X	X[23]
Urine collection [24]			←	→						

NOTE: postdose is defined as « after start of infusion"

1. Screening procedures must occur within 28 days up to 2 days before study drug administration.
2. The results of the analysis of the D1 predose samples are collected to establish baseline references and are not needed before administration.
3. Demography includes: years of birth, age at screening, sex, ethnicity and race.
4. See laboratory safety parameter assessments (section 9.7.6) for list of tests to be run.
5. See laboratory safety parameter assessments (section 9.7.6) for list of tests to be run.
6. A safety aPTT sample is drawn on D2, 24h after D1 administration. All participants will remain hospitalized until the investigator is able to review the 24h aPTT assay results. If, for each participant, the results are less than or equal to 2X aPTT level at baseline (i.e. D1 predose), then the participants may leave the clinic. They will return for the ambulatory visits on D3 (48h post infusion start), D4 (72h), D5 (96h) and D7 (144h). If the 24h safety aPTT assay of one participant is above 2X the participant's baseline aPTT, then all participants remain hospitalized until the completion of the D3 (48h) assessments and an additional aPTT sample will be drawn at 36h. If the 36h aPTT assay result of a participant is more than 2X baseline aPTT of this participant, the investigator will decide the most appropriate procedures for the follow-up of this participant. The participant will return for the ambulatory visits on D4 (72h), D5 (96h) and D7 (144h).
7. A full physical examination (including basic neurological testing for isocoria, light reflexes, gait and balance) should be performed at screening, on D-1, then, at 24h postdose (D2), also at 48h (D3) if hospitalization is extended (see footnote 6), and at the discharge visit.
8. An abbreviated physical examination will be performed at 8h postdose on D1.
9. Supine vital signs should be measured after 5 minutes of rest in a supine position and include body temperature, resting pulse rate, and blood pressure: at screening, then from D1 to D4, vital signs should be done predose, 60min, 2h, 4h, 6h, 8h, 10h, 12h, 24h, 48h, 72h postdose, and then at the discharge visit (D10+/-2d).
10. Cardiac monitoring using telemetry (3-lead minimum and including SpO_2) is to be performed as of 1h predose up to 12h postdose.
11. ECG should be done at screening, then at D1 (predose, 2h, 4h, 6h, 8h, 12h), 24h (D2), 48h (D3), 72h (D4) postdose and discharge visit. Timing can be adjusted based on emerging PK data.
12. See laboratory safety parameter assessments (section 9.7.6) for list of tests to be run: at screening, then D1 predose, D2 (24h), D4 (72h postdose), and discharge visit.

13. Participants will be asked at predose visit if they have been previously exposed to tick bites. At the discharge and extra-ambulatory visits performed for ADA analysis, the participants will be asked if they have been exposed to tick bite since the previous visit (response recorded as yes/no/unknown). If the answer is yes, then the participant will be asked to provide information on treatment (e.g. antibiotics) and on presence of erythema migrans and other signs or symptoms.
14. The baseline chemistry (predose sample) will also contain an extra archive serum sample for anti-Borrelia serology testing (IgG and IgM) as backup to test for anti-Borrelia antibodies due to previous exposure via potential tick bites.
15. Safety aPTT (local lab) at screening, then on D1, predose, then 2h, 4h, 6h, 8h, 12h, 24h (D2), 36h (D2, optional sample only drawn if hospitalisation is extended at least until 36h postdose, see footnote 6), D3 (48h), D4 (72h), D5 (96h), D7 (144h) postdose and at the discharge visit.
16. Urinalysis at screening, then D1 predose, D2 (24h) and D4 (72h postdose), and discharge visit. See laboratory safety parameter assessments for list of tests to be run (section 9.7.6).
17. All participants will receive a single intravenous loading dose of Ir-CPI/Placebo given as a 30-minute infusion. A light breakfast (2 slices of bread with ham or cheese and jam, glass of water) can be given between 2h and 1h before start of infusion. Upon completion of the loading dose infusion, all participants will receive a single intravenous dose of Ir-CPI/Placebo given as a 5.5-hour infusion. Postdose is measured after the beginning of infusion.
18. Cluster A (central lab): the effect of Ir-CPI on PD aPTT (not to be confused with safety aPTT) and on FXI/FXII activities will be measured at D1 pre-dose and at D1 15min, 30min, 45min, 60min, 90min, 2h, 4h, 6h, 6h30, 7h, 7h30, 8h, 10h, 12h, 16h, then D2 (24h), D3 (48h), D4 (72h), D5 (96h), D7 (144h) and discharge visit. In case of infusion less than 6 hours and besides assessment at predose, 15 min, 30 min, 45 min, 60 min, 90 min, 2h and 4h after start of infusion, Cluster A (central lab) parameters should be measured at the end of infusion, then 30 min, 60 min, 90 min, 2h, 4h, 6h, 10h, then 18h, 42h, 66h, 90h and 138h after the end of infusion and then at the discharge visit.
19. Cluster B (local lab) the effect of Ir-CPI on PT (sec and INR), D-dimers, fibrinogen, hsCRP and TNFα will be measured on D1, predose then at 2h, 4h, 6h, 8h, 12h post-dose, on D2 (24h post-dose), D3 (48h) and D4 (72h) and at the discharge visit. In case of infusion less than 6 hours and besides assessment on D1 at predose and at 2h and 4h after start of infusion, Cluster B (local lab) parameters will be measured at the end of infusion, then 2h, 6h, 18h, 42h and 66h after the end of infusion and then at the discharge visit.
20. CysC (central lab) will be collected at screening, then from D1 to D4: Predose (D1), 24h (D2), 72h postdose (D4), and then at the discharge visit.
21. Blood samples for Ir-CPI pharmacokinetics will be done D1 pre-dose and at 15min, 30min, 45min, 60min, 90min, 2h, 4h, 6h, 6h30, 7h, 7h30, 8h, 10h, 12h, 16h, then D2 (24h), D3 (48h), D4 (72h), D5 (96h) and D7 (144h) post-dose (after start of infusion). In case of infusion less than 6 hours, blood sample for Ir-CPI pharmacokinetics will be done at the end of infusion then 30min, 60min, 90min, 2h, 4h, 6h, 10h, 18h, 42h, 66h, 90h and 138h after end of infusion.
22. ADA analysis sample: pre-dose on the day of infusion, at the discharge visit, on D30 and on D90.
23. D180 only if ADAs are detected in the D90 samples. As this study part was not performed in the end, no additional samplings after D180 are applicable.
24. Urine collections for PK assay are to be done at the following intervals: predose, [0-6h[, [6-12h[and [12-24h]. Urinary NGAL, microalbuminuria and KIM-1 will be assessed.

LIST OF ABBREVIATIONS

ADA	Anti-Drug Antibodies
ADME	Absorption, distribution, metabolism and excretion
AE	Adverse Event
Ae	Total amount excreted in urine
AESI	Adverse Event of Specific Interest
Ag	Antigen
ALT	Alanine Amino Transferase
AMM	Autorisation de Mise sur le Marché (French Drug Marketing Licence)
ANOVA	Analysis of Variance
API	Active Pharmaceutical Ingredient
aPTT	activated Partial Thromboplastin Time
AST	Aspartate Amino Transferase
AUC	Area Under the Curve
AUC_{inf}	Area under the plasma concentration time curve from time 0 to the infinity
BMI	Body Mass Index
CA	Competent Authorities
CL	Clearance
CL_R	Renal Clearance
C_{max} rate	Maximal excretion rate
CONSORT	Consolidated Standards of Reporting Trials
CPB	CardioPulmonary Bypass
CPK	Creatinine Phospho Kinase
CRO	Contract Research Organization
CRF	Case Report Form
CSR	Clinical Study Report
CTA	Clinical Trial Application
CTD	Clinical Trial Document
CV	Coefficient of variation
CysC	Cystatin C
DBP	Diastolic Blood Pressure
DRD	Daily Recommended Dose
ECG	Electrocardiogram
ECMO	Extracorporeal membrane oxygenation

EMEA	European Medical Agency
EudraCT	European Clinical Trials Database
EU-GMP	European Union-Good Manufacturing Practice
FAMHP	Federal Agency for Medicines and Health Products (Belgium's CA)
FIH	First-In-Human
FXI	Factor XI
FXIa	Activated FXI
FXII	Factor XII
FXIIa	Activated FXII
FU	Follow-up visit
GGT	Gamma Glutamyl Transferase
GCP	Good Clinical Practice
GLP	Good Laboratory Practice
HBc	Hepatitis B core
HBs	Hepatitis B surface Antigen
HCV	Hepatitis C Virus
HED	Human Equivalent Dose
HIT	Heparin-induced Thrombocytopenia
HIV	Human Immunodeficiency Virus
HR	Heart Rate
hsCRP	High sensitivity C-reactive protein
IB	Investigator Brochure
ICF	Informed Consent Form
ICH	International Council on Harmonization
IEC	Independent Ethics Committee
Ig	Immunoglobulin
IgE	Immunoglobulin E
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IMP	Investigational Medicinal Product
IND	Investigational New Drug
IRB	Institutional Review Board
Ir-CPI	*Ixodes ricinus*-Contact Phase Inhibitor
KIM-1	Kidney Injury Molecule 1
LC-MS/MS	Liquid chromatography coupled to tandem Mass Spectrometry

LPLV	last patient last visit
MABEL	minimum anticipated biological effect level
MedDRA	Medical Dictionary for Regulatory Activities
MRSD	Maximum Recommended Starting Dose
MTD	Maximum Tolerated Dose
NCS	Non Clinically Significant
NGAL	neutrophil gelatinase-associated lipocalin
NOAEL	No Observed Adverse Effect Level
NOEL	No Observed Effect Level
OTC	Over the Counter
PD	Pharmacodynamics
PK	Pharmacokinetics
POC	Proof of Concept
pp	Per protocol
PV	Pharmacovigilance
QA	Quality Assurance
SADR	Serious Adverse Drug Reaction
SAE	Serious Adverse Event
SBP	Systolic Blood Pressure
SOP	Standard Operating Procedure
SpO2	Peripheral Capillary Oxygen Saturation
SRC	Safety Review Committee
SUSARs	Suspected Unexpected Serious Adverse Reactions
SVT	Supraventricular Tachycardia
$t_{1/2}$	Half-life
$T_{max\ rate}$	Time to reach maximum excretion rate
TEAE	Treatment Emergent Adverse Event
TNFα	tumor necrosis factor alpha
UFH	Unfractionated Heparin
ULN	Upper Limit of Normal
Vol_UR	Volume of urine excreted
WHODDRUG	World Health Organization Drug Dictionary
VT	Ventricular Tachycardia
WMA	World Medical Association

3. INTRODUCTION

3.1. Study Rationale

Ixodes ricinus-Contact Phase Inhibitor (Ir-CPI) is under development for the prevention of thrombosis in hospital procedures in which blood is exposed to non-biological surfaces known to activate the intrinsic pathway of the coagulation cascade (e.g. percutaneous endovascular interventions, cardiopulmonary bypass).

Inhibitors specific to factor XI and factor XII (FXI/FXII) of the intrinsic pathway are expected to have preventive antithrombotic effects with lower risk of bleeding compared to the currently available anticoagulants. They would particularly be indicated for preventing clotting on blood-contacting medical devices or extracorporeal circuits. Indeed, exposure of blood to polyanionic artificial surfaces induces a highly procoagulant condition through the activation of the intrinsic pathway of the coagulation.

Potential indications for Ir-CPI will be the prevention of thrombotic events associated to the use of contact phase activating devices, e.g. percutaneous endovascular interventions, extracorporeal circulation during cardiac surgical interventions (CPB), especially under clinical circumstances where the use of classical anticoagulation is not adequate due to heparin intolerability (HIT, Heparin-induced Thrombocytopenia) and/or high risk of bleeding.

As Ir-CPI has to be administered by intravenous (IV) infusion and as it has a half-life of a few hours, it is intended for specialised use in a hospital setting. The infusion of Ir-CPI would have to be initiated prior to and last until the end of the procedure. In the context of a CPB, the infusion would last for 4-6 hours, depending on the duration of the intervention. In the context of an endovascular intervention, the infusion would last around 2 hours. Due to the possible anti-drug antibody (ADA) generation, intended indications will be medical prevention in acute situation, with no repeated or chronic use of Ir-CPI.

This study will be the first-in-human study of Ir-CPI. This study will provide an initial assessment of the safety, tolerability, pharmacokinetics (PK), and pharmacodynamics (PD) of Ir-CPI after intravenous administration as 6 hour-infusion at different doses without [single ascending dose (SAD) part 1] or with a loading dose [single ascending dose (SAD) part 2] to healthy adult male participants.

A pivotal good laboratory practice (GLP) toxicity study in monkey was designed to support the First-In-Human (FIH) clinical trial design, by mimicking as much as possible the route and type of administration foreseen, by testing an appropriate safety margin (3, 10 and 30-fold) in regards to the first human clinical dose and by choosing a continuous infusion duration largely exceeding the one foreseen in the FIH clinical trial (i.e. 6 hours). This toxicity study was performed with Ir-CPI administered by continuous IV infusion during 24h at 3 dose levels (mid, high, super high) with systemic exposure to Ir-CPI up to at least 30-fold the expected therapeutic concentration in humans. The 24h infusion was followed by a recovery period (24h or 14 days) at high and super high doses. No clinical sign or macroscopic findings was observed, except an increase in kidney weight in males at the super high dose (2/3 animals). Microscopic examination showed adverse toxicity (kidneys) in both females and males only at the super high dose in the early scheduled euthanasia animals (24h after ending infusion). Immuno-histochemical staining suggested that there was no formation of immune complexes (high and super high doses tested), either for the early or late scheduled euthanasia animals, suggesting a direct effect of Ir-CPI at the highest dose in the renal tissue. No effect related to ADAs was observed, even though ADAs were detected 9

(one animal) or 11 (all animals) days after ending infusion in the high and super high dose groups. A loss of proportionality in Ir-CPI plasma concentration was observed between the high and the super high dose. During post-infusion, a decrease of Ir-CPI clearance was only observed at the super high dose with a possible link to renal toxicity. Considering these results, the no-observed-adverse-effect-level (NOAEL) was set at 201.6 mg/kg/day. This NOAEL was used to define the Maximal Recommended Starting Dose (MRSD) for this FIH clinical study.

The doses to be evaluated in this study are based on the results obtained in the GLP toxicity studies (2-week infusion study in rats and monkeys; 24-hour infusion study in monkeys), the 2-hour safety pharmacology study in monkeys and additional non-GLP pharmacology studies performed.

Once PK results are obtained at increasing doses of Ir-CPI in healthy participants upon 6-hour infusion, a combination of rapid (30 minutes) and slow (5.5 hours) infusions will be administered to healthy participants as it will be used thereafter in Proof of Concept (POC) efficacy studies in human such as coronarography and later in patients undergoing a Cardiopulmonary Bypass (CPB) intervention.

This design will be used to define the experimental condition(s) of administration allowing to rapidly reach and to maintain steady state Ir-CPI concentration for up to 6 hours.

The participants participating in the study will be followed up during a 24h hospitalisation combined with daily ambulatory visits thereafter (up to 96h after dosing). The proposed 24 hours is deemed sufficient because this is a single intravenous infusion where it is expected that the most severe and acute reactions will occur during or very short after the infusion. Based on animal data, the pharmacodynamics effect and potential side effects are expected to be related to plasma levels that will decline rapidly after ending the IV infusion. The discharge from the research facility at 24h will be at the discretion of the Investigator and will be based on the available safety parameters [including – but not limited to – activated Partial Thromboplastin Time (aPTT)]. Although the combination of the presence of a relevant biomarker measuring study drug activity, daily visits to the research facility and the proposed safety testing is considered to be sufficient safety coverage to allow a discharge of participants at 24h after drug administration, the investigator may extend the hospitalisation period up to 48h if deemed necessary (Cohort D and subsequent cohorts only).

3.2. Background

Ir-CPI, a non-glycosylated recombinant protein of 67 amino acids, is a first-class candidate targeting the intrinsic pathway of the coagulation cascade. It is to be administered intravenously for the prevention of thrombosis in procedures in which blood is exposed to polyanionic artificial surfaces known to activate the intrinsic pathway of the coagulation cascade (e.g. percutaneous endovascular interventions, extracorporeal circulation during cardiac surgical interventions [CPB]).

Ir-CPI is a serine protease inhibitor, originally isolated from the saliva of the tick *Ixodes ricinus*. Ticks are well known to produce salivary substances capable of counteracting the haemostatic system of the host. Initially produced by chemical synthesis (synth Ir-CPI), Ir-CPI is now produced in the yeast *Pichia (Komagataella) pastoris* under GMP conditions (rec Ir-CPI).

Ir-CPI is able to inhibit the intrinsic coagulation pathway (contact phase) through inhibition of the activation of FXI into FXIa by FXIIa and FXII into FXIIa by FXIa.

This results in an increase of the clotting time in the *in vitro* assay aPTT, which is specific to the intrinsic coagulation pathway. However, Ir-CPI has no effect on both prothrombin and thrombin time, assays which measure the effect of the molecule on the extrinsic or common pathway.

Mechanistic data accumulated so far indicate that Ir-CPI reduces the generation of FXIa by interfering with upstream activation of FXI by FXIIa and reciprocal activation of FXII by FXIa while it has no effect on activation of FIX by FXIa. Ir-CPI was proven to be antithrombotic in two experimental models involving blood contact with non-biological surfaces (arteriovenous shunt in rabbits; CPB with open-heart surgery in sheep). In the CPB model, Ir-CPI was as efficient as unfractionated heparin (UFH). Ir-CPI was shown to be markedly safer than UFH in an experimental model of bleeding induced by liver punch biopsies in pigs.

Pharmacokinetic data available in mouse, rat, rabbit, dog and monkey fit with a bi-compartmental model. This is characterized by a high volume of distribution (V_d: 1.98-8.55 L/Kg), a rapid half-life of distribution ($t_{1/2\alpha}$: 14-72 min), a slower terminal elimination half-life ($t_{1/2\beta}$: 3.9-13.7 h), giving a rapid clearance (CL: 0.17-1.51 L/kg/h) with V_d and CL respectively increasing and decreasing with the size of the animal species. Ir-CPI plasma concentrations increase proportionally to the administered dose (bolus or infusion) as was verified in monkeys and rats. A rapid decline in the plasma concentration was observed after the arrest of continuous IV infusion performed on course of toxicokinetic studies (rat and monkey). Absorption, distribution, metabolism and excretion (ADME) studies conducted in rats indicate that Ir-CPI is rapidly cleared by metabolism/catabolism in the liver and the kidneys. Ir-CPI is excreted in the urine as a degraded product.

GLP toxicity studies in rats and cynomolgus monkeys were first performed with Ir-CPI administered by continuous IV infusion (24h/24h) during 14 days at 3 dose levels (low, mid, high) with systemic exposure to Ir-CPI up to at least 10-fold the expected therapeutic concentration in humans. This 2-week infusion was followed by a 2-week recovery period (high dose). In these two-week IV infusion studies, toxicity was only observed at high dose in rats (kidneys) and monkeys (vascular and inflammatory changes in various organs, with the highest severity observed at high dose). Clinical signs were observed for monkeys but not in rats. In rats, Ir-CPI plasma concentrations were stable during 14 days. In monkeys, Ir-CPI plasma concentrations were stable during the first 7 days of infusion and approximately proportional to the administered doses, but an abnormal increase was documented at Days 14-15. Anti-drug antibodies (ADAs) were detected in plasmas from monkeys but not from rats. Immune complexes were detected by immunohistochemistry (co-localization of Ir-CPI, IgM and complement [C3/C3b]), in kidneys, at high dose in all tested monkeys. All findings were considered to be most likely related to ADA-mediated immune complex formation, deposition, and systemic activation of complement. Abnormal increase in Ir-CPI plasma concentration was also considered as most likely related to the presence of ADAs. The NOAEL was defined at 60 mg/kg/day in monkeys and 192 mg/kg/day in rats.

A pivotal GLP toxicity study in monkey was then designed to support the FIH clinical trial design, by mimicking as much as possible the route and type of administration foreseen, by testing an appropriate safety margin (3, 10 and 30-fold) in regards to the first human clinical dose and by choosing a continuous infusion duration largely exceeding the one foreseen in the FIH clinical trial (i.e. 6 hours). This toxicity study was performed with Ir-CPI administered by continuous IV infusion during 24 h at 3 dose levels (mid, high, super high) with systemic exposure to Ir-CPI up to at least 30-fold the expected therapeutic concentration in humans. The 24 h infusion was followed by a recovery period (24 h or 14 days) at high and

super high doses. No clinical signs or macroscopic findings were observed, except an increase in kidney weight in males at the super high dose (2/3 animals). Microscopic examination showed adverse toxicity (kidneys) in both females and males only at the super high dose in the early scheduled euthanasia animals (24h after ending infusion). Immunohistochemical staining suggested that there was no formation of immune complexes (high or super high doses tested), either for the early or late scheduled euthanasia, suggesting a direct effect of Ir-CPI at the highest dose in the renal tissue. No effect related to ADAs was observed, even though ADAs were detected 9 (one animal) or 11 (all animals) days after ending infusion in the high and super high dose groups. A loss of proportionality in Ir-CPI plasma concentration was observed between the high and the super high dose. During post-infusion, a decrease of Ir-CPI clearance was only observed at the super high dose with a possible link to renal toxicity. Considering these results, the NOAEL was set at 201.6 mg/kg/day. This NOAEL was used to define the MRSD for the FIH clinical study.

Note that in all toxicity studies, a dose-related moderate (rats, monkeys) to marked (monkeys) and reversible prolongation in aPTT was observed in all animals and at all dose levels, showing that Ir-CPI was present and under an active form.

In order to investigate ADA appearance and effect on product efficacy and safety, an *in vivo* study was conducted in 3 cynomolgus monkeys receiving 3 repeated administrations (Day 1, 15, 36) of 6-h continuous IV infusion (duration of infusion corresponding to the clinical development of Ir-CPI). ADAs (IgM, IgG but no IgE) developed in monkeys from Day 14 with a rather classical immune memory response profile in terms of Ig sub-class switch and antibody titre evolution, although interindividual variation is observed. *In vitro*, ADAs showed neutralizing effects on aPTT, but unexpectedly not *in vivo* as aPTT remained similar upon the 3 repeated administrations. Noteworthy, although ADAs were present in animal body from the second infusion, the repeated administrations of Ir-CPI were well tolerated and did not induce any macroscopic or microscopic changes in organs and no formation of immune complexes in tissues.

In silico and *in vitro* cellular experiments suggest that the risk of immunogenicity of Ir-CPI is moderate in humans.

An ex vivo human immunogenicity study was performed by analysing the presence of ADAs in human blood samples collected from healthy volunteers or Lyme disease patients (Belgium + US). In the Belgian population, 2/50 healthy volunteers and 1/50 Lyme disease patients were ADA positive (with an optical density close to the cut-point). All positive samples were collected from donors having been bitten by ticks. None of US donors (15 Lyme and 50 healthy) were ADA positive. Data are considered as informative due to the limited size of samples.

Safety Pharmacology studies indicate that Ir-CPI did not affect cardiovascular and respiratory functions in cynomolgus monkeys (telemetry) (at low and mid doses through a combined rapid and low infusion, during 0.5h and 2h, respectively) and neurobehavioral parameters (FOB) in the rat (single IV administration at 10, 30 or 60 mg/kg).

The estimation of the MRSD was performed based on safety data provided from toxicity studies in animals (NOAEL) (see Section 5.6).

Further detailed information can be found in the Investigator's Brochure [1].

3.3. Benefit/Risk Assessment

No evidence available at the time of the completion of this study protocol indicated that special warnings or precautions were appropriate, other than those noted in the Investigator's Brochure.

As this study involves volunteers who are not suffering from any disease, no therapeutic benefit is expected for the healthy participants taking part in this study.

Given the new mechanism of action, the nature of the compound and the intended route of administration, dose staggering will be applied in this FIH trial for all panels. This dose staggering will be as such that 2 participants will be dosed at least 24 hours before the remainder of the panel. These 2 sentinel participants will be randomised as such that there will be one placebo and one *verum*. After the review of AEs, vital signs and ECGs collected over 24 hours postdose (up to D2), the remaining participants of the panel will receive the same dose level. Following completion of the initial 24-hour safety period for the sentinel participants, the decision to proceed with the remaining participants in that panel will be mutually discussed/agreed upon between Investigator (or his designee) and the Sponsor and the decision to dose will be formally confirmed by the Sponsor. The proposed minimal 24 hours between the sentinel and the remainder of the cohort is deemed sufficient because this is a single intravenous infusion where it is expected that the most severe and acute reactions will occur during or very short after the infusion. Based on animal data, the pharmacodynamics effect and potential side effects are expected to be related to plasma levels that will decline rapidly after ending the IV infusion. In addition to this, aPTT, which is a reliable biomarker available for drug activity, will be followed up at specific time-points. The results of this biomarker will be available for dose escalation decisions.

At each dose level, after completion of study drug administration in at least 6 participants (i.e. a minimum of 4 on active treatment), the Investigator will provide a comprehensive Investigator Safety Report which will include (but not limited to) the following content:

- Relevant information on participants' demographics/characteristics, medical history, physical examination, concomitant medications;
- List of all AEs, including severity, time of onset related to study drug administration, duration, clearly highlighting adverse events of special interest (AESIs) and Serious Advert Events (SAEs) and relatedness/causality of AEs;
- Any clinically significant out of ranges safety clinical laboratory tests results (as assessed by the Investigator);
- Physical Examination (including basic neurological testing for isocoria, light reflexes, gait and balance) results including clinical significance for abnormal findings;
- ECG and Vital Sign results including clinical significance for abnormal values;
- Statement of the Principal Investigator's recommendation regarding dose decision.

Escalation to the next higher dose or a modification to the dose will only take place after review of the Investigator Safety Report from the previous dose levels by the Investigator, in consultation with the Sponsor's representative [called hereafter the Safety Review Committee (SRC)].

Dose escalation will be based on any relevant information on participants' demographics/characteristics, medical history, physical examination, concomitant medications, AEs, clinically significant out of ranges safety clinical laboratory tests results (as assessed by the PI), physical examination results (including basic neurological testing for

isocoria, light reflexes, gait and balance) including clinical significance for abnormal findings, ECG and vital sign results including clinical significance for abnormal value, and the statement of the PI's recommendation regarding dose decision. Other parameters of interest will be considered as per SRC charter (blinded PK, PD).

Within the planned dose range, a dose lower than the next planned dose may also be tested, depending on emerging safety, tolerability and/or other relevant data, such as blinded PK or PD. If the highest planned dose level is found to be safe and tolerable, also considering the PK and PD data, additional higher doses may be added by amendment.

Between the first participants of 2 consecutive panels, a period of at least 7 days will be respected between the administrations of treatment.

The SRC will meet for deciding on the continuation of dosing after drug administration in at least 6 participants (i.e. a minimum of 4 on active treatment). However, stopping rules (see Section 7.5) will be applicable from dosing of the first sentinel participant on.

The risks for the individual participant due to treatment with Ir-CPI or study related procedures are considered minimal because of the choice of the doses, of careful monitoring of all critical safety and tolerability parameters. Therefore, the risks are outweighed by the gain of additional knowledge on Ir-CPI and the possibility to develop a potent new treatment for the prevention of thrombosis.

4. OBJECTIVES AND ENDPOINTS

Objectives	Endpoints
Primary	
Part 1: Assess the safety and tolerability of Ir-CPI following a single ascending 6-hour intravenous infusion in healthy male participants.	Safety parameters [adverse events (AEs), physical examinations, vital signs, telemetry including SpO_2, weight, Hemoccult® test, supine 12-lead ECG, serum chemistry, haematology, coagulation aPTT, urinalysis, and monitoring for concomitant medications].
Part 2*: Assess the safety and tolerability of Ir-CPI following a combination of rapid (30 minutes) and slow (5.5 hours) intravenous infusion over a total of 6 hours in healthy male participants.	
Secondary	
Part 1: Assess the PK of Ir-CPI following single ascending 6-hour intravenous infusion in healthy male participants.	Maximum plasma concentration (C_{max}), time to reach maximum plasma concentration (t_{max}), Area under the plasma concentration-time curve from time zero to 6h (AUC_{0-6}), Area under the plasma concentration-time curve from time zero to time of infinity (AUC_{inf}), apparent terminal elimination rate constant (λz), terminal elimination half-life ($t_{1/2}$), volume of distribution (Vd), total body clearance (CL).
Part 2*: Assess the pharmacokinetics (PK) of Ir-CPI following a combination of rapid (30 minutes) and slow (5.5 hours) intravenous infusion over a total of 6 hours in healthy male participants.	
Investigate the preliminary PD of Ir-CPI in healthy male participants using a GCLP validated method according to FDA guidelines 2018 for activated Partial Thromboplastin Time (aPTT). Inhibition of Factor XI (FXI) and Factor XII (FXII) procoagulant activities will also be assessed to support the aPTT dynamics.	Change from baseline in aPTT and residual FXI and FXII activities.
Exploratory	
Investigate the potential of Ir-CPI to induce the formation of anti-drug antibodies (ADA), including their capability to neutralize drug activity.	Immunogenicity assessed by ADA plasma levels.
Investigate the effect of Ir-CPI on markers related to the coagulation system.	D-dimers, fibrinogen and prothrombin time (PT) levels in whole blood.
Investigate the effect of Ir-CPI on biomarkers for renal injury.	***Blood assay:*** serum cystatin C (CysC). ***Urinary assay:*** urinary microalbumin, Kidney Injury Molecule (KIM-1), urinary neutrophil gelatinase-associated lipocalin (NGAL).

Objectives	**Endpoints**
Investigate the effect of Ir-CPI on inflammatory markers	tumor necrosis factor alpha (TNFα), high sensitivity C-reactive protein (hsCRP) levels in serum.
Assess the PK of Ir-CPI in urine	Total amount excreted in urine (Ae), Ae%, volume of urine excreted (Vol_UR), Maximal excretion rate (C_{max} rate), Time to reach maximum excretion (T_{max}) rate and Renal Clearance (CL_R).
PK PD relationship	No new endpoint: only graphical investigation of PK and PD parameters.

*: because part 2 was not performed, these objectives are not applicable.

5. STUDY DESIGN

5.1. Overall Design

This study is a First-In-Human (FIH) Phase 1, double-blind, placebo controlled, single dose escalation study of Ir-CPI in healthy male participants.

The study will consist of two parts:

- Part 1: an Ir-CPI or matching placebo single ascending dose administration in healthy male participants using a continuous 6-hour intravenous infusion.
- Part 2: an Ir-CPI or matching placebo single ascending dose administration in healthy male participants using a single ascending 30-minute loading dose infusion followed by a fixed dose of continuous 5.5-hour intravenous infusion (maintenance infusion).

In all the following descriptions, the term ***postdose*** is defined as « after start of infusion ».

The terms ***panel*** and ***cohort*** can be used interchangeably.

In the end, part 2 was not performed.

5.1.1. Part 1

Five (5) panels (A to E), each consisting of eight (8) healthy male participants will be randomised to receive either Ir-CPI or matching placebo: first, 2 sentinel participants (1:1 active / placebo) then for the rest of the panel, a 5:1 ratio (active / placebo). The first four panels will be administered Ir-CPI/Placebo in a sequential group design. Depending of the PK and PD results obtained for the first 4 panels, a fifth panel (E) may be performed thereafter.

Participants will be recruited on the basis of their medical history and health status as judged by the Investigator. Participants will be admitted in the evening of the day before dosing (D-1) to assess eligibility based on urine drug screen, breath alcohol test, adherence to the study restrictions, review of inclusion and exclusion criteria and physical examination. The participants will be hospitalised at the clinical unit until at least 24h after the start of study drug administration (D2) (Cohorts A, B and C).

Participants included in Cohort D and subsequent cohorts will remain hospitalised until the investigator is able to review the 24h aPTT assay results. If, for each participant, the results are less than or equal to 2X aPTT level at baseline (i.e. D1 predose), then the participants may leave the clinic. They will return for the ambulatory visits on D3 (48h post infusion start), D4 (72h), D5 (96h) and D7 (144h). If the 24h safety aPTT assay of one participant is above 2X the participant's baseline aPTT, then all participants will remain hospitalised until the completion of the D3 (48h) assessments and an additional aPTT sample will be drawn at 36h. If the 36h aPTT assay result of a participant is more than 2X baseline aPTT of this participant, the investigator will decide the most appropriate procedures for the follow-up of this participant. Participants will return for the ambulatory visits on D4 (72h), D5 (96h) and D7 (144h).

Ambulatory visits will be planned on D3 (48h postdose, may also be inpatient in the case of Cohort D and subsequent cohorts), D4 (72h), D5 (96h) and D7 (144h, Cohort D and subsequent cohorts only), for safety, PD and PK assessments. The treatment period (from D-1 to D5 for Cohorts A to C, and from D-1 to D7 for Cohort D and subsequent cohorts) will be followed by a discharge visit corresponding to a post study safety evaluation on D10 ± 2 days, after infusion. Extra ambulatory visits will be planned D30 ± 3 days and D90 ± 7 days

after dosing for immunogenicity testing (ADA). Any new AE will be recorded during these visits, and when applicable, the concomitant medications prescribed. A laboratory sample will be taken to look for immunogenicity (presence of anti-drug antibodies, neutralizing antibodies). If ADAs are detected at D90 a supplemental ambulatory visit will be planned D180 ± 7 days after analysis. Participants will also be asked at the ADA analysis visits if they are aware of an exposure to tick bites since the previous visit.

The suggested doses of Ir-CPI for dose escalation in Part 1 are provided in Table 1.

The final dose to be used for each cohort will be decided based on the results of the previous panel and can be adjusted downward if deemed necessary.

Fasting conditions are required for the blood sample collection at the selection visit, on D3 (ambulatory visit or inpatient), for the ambulatory visits [D4/D5/D7 (D7 for Cohort D and subsequent cohorts only) /Discharge visit] and for the D1 predose blood sample.

Water intake is allowed during the infusion.

Meals will be standardized across the different panels during the in-patient period of the study (D-1 evening to D2). Following the D1 predose sample collections, a light breakfast (2 slices of bread with ham or cheese and jam, glass of water) / snack can be given between 2h and 1h before the start of the infusion. Lunch will be served after completion of the study drug infusion. A light breakfast / snack will also be given after the D1H24 (D2) blood sample collection and after the collections performed on D3 (may be inpatient in the case of Cohort D and subsequent cohorts) and at the ambulatory visits on D4, D5, D7 (D7 for Cohort D and subsequent cohorts only) and Discharge visit.

Participant must not be in fasting condition for the safety aPTT 36h sample and the extra ambulatory visits for ADA blood sample collection.

Table 1: Suggested Doses of Ir-CPI for Dose Escalation in Part 1

Group	Infusion rate* (mg/kg/h)	Infusion Duration (h)	Ir-CPI target plasma concentration (µg/mL) at 6 hours	Total dose (mg/kg)
A	0.25	6	1.4	1.5
B	0.75	6	4.1	4.5
C	1.50	6	8.3	9
D	3.00	6	16.6	18
E**	4.5	6	24.8	27

**Weight of the participant at the screening visit will be used to calculate the dose of study drug.*

***: Cohort E was not performed according to a recommendation from the Ethics Committee.*

Supplemental ADA samplings ("long-term follow-up of ADAs"):

If ADAs are still detected at the D180 ambulatory visit, supplemental samplings will be performed every 3 months (± 14 days) for the first year and then every 6 months (± 30 days) up to a maximum of 5 years after the D180 visit. The samplings will be performed until the participants reach a negative status, until they show the same ADA titre for 3 samplings in a row, or up to a maximum of 5 years after the D180 visit, whichever comes first.

5.1.2. Part 2

Part 2 was not performed because the targeted plasmatic PK concentration was planned in the unperformed group E.

Three (3) panels F-G-H, each consisting of eight (8) healthy male participants will be randomized to receive either Ir-CPI or matching placebo. First, 2 sentinel participants (1:1 active / placebo) then for the rest of the group, a 5:1 ratio (active / placebo). The three panels will be administered Ir-CPI/Placebo in a sequential group design.

Participants will be recruited on the basis of their medical history and health status as judged by the Investigator. During each period, participants will be admitted on the evening of the day before dosing (D-1) to assess eligibility based on urine drug screen, breath alcohol test, adherence to the study restrictions, review of inclusion and exclusion criteria and physical examination. The participants will be hospitalised at the clinical unit until the investigator is able to review the 24h aPTT assay results. If, for each participant, the results are less than or equal to 2X aPTT level at baseline (i.e. D1 predose), then the participants may leave the clinic. They will return for the ambulatory visits on D3 (48h post infusion start), D4 (72h), D5 (96h) and D7 (144h). If the 24h safety aPTT assay of one participant is above 2X the participant's baseline aPTT, then all participants will remain hospitalised until the completion of the D3 (48h) assessments and an additional aPTT sample will be drawn at 36h. If the 36h aPTT assay result of a participant is more than 2X baseline aPTT of this participant, the investigator will decide the most appropriate procedures for the follow-up of this participant.

Ambulatory visits will be planned on D3 (48h postdose, D3 may also be inpatient), D4 (72h), D5 (96h) and D7 (144h) for safety, PD and PK assessments. The treatment period (from D-1 to D7) will be followed by a discharge visit corresponding to a post study safety evaluation on D10 ± 2 days, after infusion.

An extra ambulatory visit will be planned D30 ± 3 days and D90 ± 7 days after dosing for immunogenicity testing. Any new AE will be recorded during these visits, and when applicable, the concomitant medications prescribed. A laboratory sample will be taken to look for immunogenicity (presence of anti-drug antibodies, neutralizing antibodies). If ADAs are detected at D90 a supplemental ambulatory visit will be planned D180 ± 7 days (month 6) after dosing. Participants will also be asked at the ADA analysis visits if they are aware of an exposure to tick bites since the previous visit.

The suggested doses of Ir-CPI for dose escalation in Part 2 are provided in Table 2.

Fasting conditions are required for the blood sample collection at the selection visit, on D3 (inpatient or ambulatory), at the ambulatory visits (D4/D5/D7/Discharge visit) and for the D1 predose blood sample.

Water intake is allowed during the infusion.

Meals will be standardized across the different panels during the in-patient period of the study (D-1 evening to D2). Following the D1 predose sample collections, a light breakfast (2 slices of bread with ham or cheese and jam, glass of water) / snack can be given between 2h and 1h before start of infusion. Lunch will be served after completion of the study drug infusion. A light breakfast / snack is also given after the D1H24 (D2) blood sample collection and after the collections performed on D3 (inpatient or ambulatory), and at the ambulatory visits on D4, D5, D7 and Discharge visit.

Participants must not be in fasting condition for the safety aPTT 36h sample and the ADA blood sample collection visits.

Table 2: Suggested Doses of Ir-CPI for Dose Escalation in Part 2

Group	Infusion rate** (mg/kg/h)	Time (h)	Ir-CPI target plasma concentration (µg/mL) between 30 min and 6 hours	Total dose (mg/kg)
F	Rapid infusion*: 6	0.5	8	11.25*
	Slow infusion*:1.5	5.5		
G	Rapid infusion*:7	0.5	8	11.75*
	Slow infusion*:1.5	5.5		
H	Rapid infusion*8	0.5	8	12.25*
	Slow infusion*:1.5	5.5		

** The rapid and the slow infusion rates will be calculated based on Part 1 emerging safety, PD and PK data. The total dose administered by rapid and by slow infusion will not exceed the highest total dose tested in Part 1. The infusion rate (in mg/kg/h) for the slow infusion will not exceed the maximum infusion rate used in Part 1. The dose may also be adjusted.*

***Weight of the participant at the screening visit will be used to calculate the dose of study drug*

In groups F, G and H, dose levels for rapid infusion and slow infusion could be adjusted upward / downward based on emerging safety / tolerability and pharmacokinetic / pharmacodynamic data. The total dose administered by rapid and by slow infusion will not exceed the highest total dose tested in Part 1. The infusion rate (in mg/kg/h) for the slow infusion will not exceed the maximum infusion rate tested in Part 1. In Part 2, the aim is to reach a plasma concentration of about 8 µg/mL at the end of the rapid infusion and to maintain, with the slow infusion, this plasma concentration until the end of the infusion. As predicted with PK modelling (Figure 1), an infusion rate of 1.5 mg/kg/h is expected in the slow infusion part to reach a plasma concentration of 8 µg/mL. The total doses and infusion duration to be used will be decided on the results of the previous panels and can be adjusted upward/downward if needed to reach a plasma concentration of 8 µg/mL between 30 min and 6 hours.

5.2. Number of Participants

In any part of this trial, if it is decided that additional data are needed to better define the safety and tolerability or PK/PD of Ir-CPI, additional panels of healthy male participants may be enrolled per amendment. For example, a given dose level may be repeated in newly enrolled participants, or a lower or intermediate dose may be administered based on emerging data.

Participants who drop out for safety and tolerability reasons will not be replaced; participants withdrawing for other reasons might be replaced. Replacement participants will be assigned to the same treatment group as the participants they are replacing (as shown in Table 4).

Randomised participants who discontinue after having been exposed to the study drug may be replaced, if this discontinuation is not related to safety issues. The decision to replace participants will be made on a case-by-case basis.
The data collected on the withdrawn participant prior to withdrawal will be included in the statistical analyses.

5.2.1. Part 1

Approximately forty (40) healthy male participants will be enrolled in five (5) sequential panels (A-E). Each panel will have eight (8) participants (6 active: 2 placebo). The fifth group will be optional depending on the PK and PD data obtained in the previous groups. In the end, this group was not performed.

5.2.2. Part 2

Approximately twenty-four (24) healthy male participants will be enrolled in three (3) sequential panels (F-H). Each panel will have eight (8) participants (6 active: 2 placebo). In the end, this part was not performed.

5.3. Number of Study Centres

The study will be performed at 1 investigational site in Belgium.

5.4. End of Study Definition and Duration of Participation

The end of the study is defined as the date of the last visit of the last participant in the study.

Therefore, the end of the study is defined as either the date on which the last study participant completes the Month 3 visit (D90) for immunogenicity testing or, if for a given participant ADAs are detected, the date on which the last study participant completes the supplementary Month 6 visit (D180), whichever is later.

The study duration for each participant for the main study part (from the screening visit until the discharge visit) will be approximately 6 weeks. Participants will be followed up until D90 (or D180) for immunogenicity testing. The total study duration will be approximately 18 weeks in total for a given participant, and up to 31 weeks in case of D180 visits.

Following the completion of the study, a 3-month exclusion period will apply to the participant before they are allowed to take part in another clinical study.

Supplemental ADA samplings ("long-term follow-up of ADAs"):

Extra ambulatory samplings that will be performed after D180 in participants positive for ADAs at D180 will be considered as occurring after the end of the study: these samplings are required for long term follow-up of the potential impact of ADAs, but based on the clinical data accumulated in this study, no SAE or AE or AESI has been reported as associated with the occurrence of ADAs. The results of these additional samplings will be not collected in the eCRF but in source documents only, and will be added to the final Clinical Study Report (CSR) as an appended additional listing.

Participants followed up after D180 for ADA analysis will be authorised to participate in other clinical trial studies except for clinical trials with biologicals (e.g. antithrombotic molecule acting on FXI and FXII).

5.5. Scientific Rationale for Study Design

The study design and data analysis follow the recommendations issued from the following guidance for industry:

- EMA Guideline on strategies to identify and mitigate risks for first-in-human and early clinical trials with investigational medicinal products, Jul. 2017 [3]
- FDA Guideline for Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers, Jul. 2005. [4].
- FDA Guideline for Immunogenicity Testing of Therapeutic Protein Products — Developing and Validating Assays for Anti-Drug Antibody Detection, JAN 2019.

The participants' race will be collected, as it constitutes a source of variability in drug metabolism and drug activity; for example, Caucasians from southern Europe and from

Middle Eastern countries display a more frequent Sickle cell trait than other Caucasians, which can be associated with different reactions to anticoagulants.

5.6. Justification for Dose

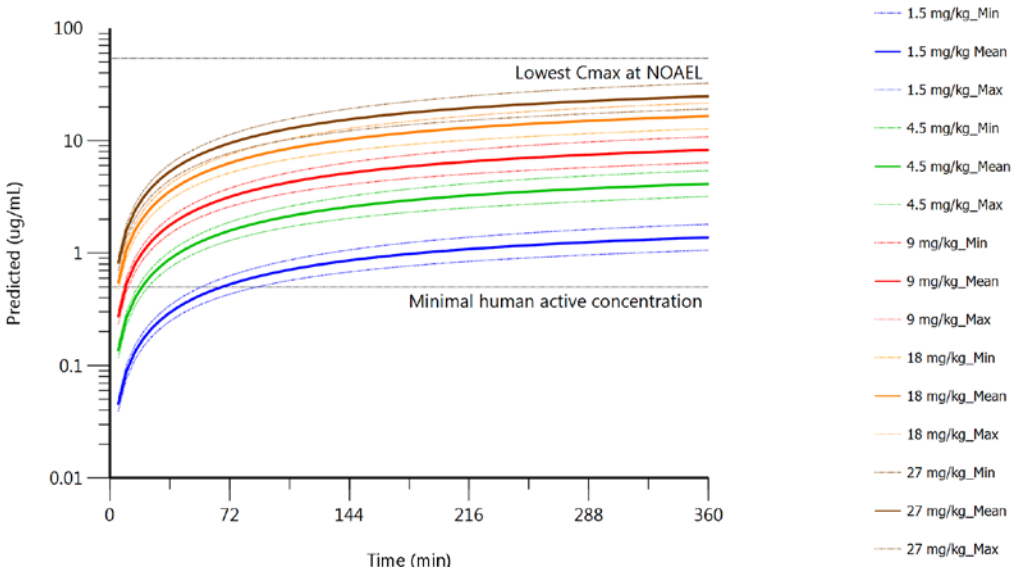
The estimation of the MRSD was performed either based on safety data provided from toxicity studies in animals (NOAEL) or based on pharmacological data provided from *in vitro* functional assay on human plasma [the minimum anticipated biological effect level (MABEL)].

PK modelling

Simulated Ir-CPI dose levels and plasma concentrations in human, based on allometric method are illustrated in Figure 1.

Kinetics constants (K12, K21, K10, α, β, A, B) and distribution volume (V1) in human were extrapolated from linear regression of the Log (K10), Log (K21), Log (K12), Log (V1) as function of the Log (Body weight) of 5 animal species for which pharmacokinetic data are available (mice, rat, dog, rabbit and monkey).

Figure 1: Simulation of evolution of plasma Ir-CPI concentration upon single infusion (6 hours) of ascending doses of Ir-CPI



Note: Plasma concentrations were predicted in human using allometric method from 5 species. The inter-individual variability observed in monkey was assumed to be similar in human and three different human bodyweights (50, 80 and 100 kg) were used for prediction. The lowest C_{max} values at the NOAEL (53.7 µg/mL) and the minimal human active concentration (0.5 µg/mL) were added as reference lines.

MRSD calculation based on NOAEL (Toxicology data)

To determine the safe starting dose in human, the No Observed Adverse Effect Level (NOAEL) obtained in the most sensitive model (monkey species, 24h-infusion acute study) is used as the reference. In the acute 24-hour monkey toxicity study, the NOAEL is defined as the High-dose (i.e. 201.6 mg/kg/day or 8.4 mg/kg/h).

By using allometric scaling relating body weight to body surface, the Human Equivalent Dose (HED) corresponding to the NOAEL is calculated as a 1/3rd of the NOAEL in monkeys. The HED will hence be 65 mg/kg/day or 2.7 mg/kg/h.

By using PK modelling deduced from analysis of the clearance in 5 different species, extrapolated human clearance is estimated as 80 mL/h/kg. The HED would be 88 mg/kg/day or 3.7 mg/kg/h (Dose = AUC x CL; using the AUC at the NOAEL in the 24h toxicity study in monkey).

The HED calculated using allometric scaling (2.7 mg/kg/h) is lower than what is calculated by modelling (3.7 mg/kg/h) and therefore is used to define the MRSD. Applying a safety factor of 10 leads to a starting dose of 0.27 mg/kg/h.

MRSD calculation based on MABEL: pharmacological data

- *In vitro* increase of aPTT in human plasma of 25 % at ± 0.5 µg/mL.
- *In vitro* inhibition of FXII procoagulant activity on human plasma in vitro of 15 % inhibition at ± 0.5 µg/mL.
- *In vivo* thrombosis model of arteriovenous shunt in rabbits (most sensitive): thrombus inhibition of ± 30 % at the dose of 1 mg/kg = 45 min post-administration, corresponding to Ir-CPI plasma concentration of 1.0 µg/mL. Considering that the in vitro response to Ir-CPI is more sensitive (± 2.5-fold) in human plasma than the response in rabbit plasma, a concentration of 1.0 µg/mL would correspond to ± 0.4 µg/mL (human equivalent active concentration).

Therefore, using these different approaches, minimal human active concentration is estimated at ± 0.5 µg/mL. Based on the modelling analysis (PK parameters in humans calculated from the knowledge of PK in animals), the human dose required to reach a plasma concentration of 0.5 µg/mL (0-6 hrs of infusion) would be 0.1 mg/kg/h.

When using the MABEL approach, the HED of 0.1 mg/kg/h, would yield a plasma concentration of 0.5 µg/mL, compatible with an increase in aPTT of 25 %. This increase will not be detectible in real-life due to the variability in the assay results within the normal physiological range. In order to induce detectable changes in aPTT in humans, the starting dose according to the MABEL approach should be at least 0.2 mg/kg/h, enabling a big enough effect to measure with the day-to-day clinical testing of aPTT.

In conclusion

The starting dose, as calculated from the HED corresponding to the NOAEL of the 24-hour toxicity study in monkeys and applying a safety factor of 1/10, is 0.25 mg/kg/h, with an expected Ir-CPI plasma concentration of 1.4 µg/mL.

Based on our preclinical work (*in vitro* and *in vivo*), it is estimated that the proposed MRSD (1/10th of the NOAEL in monkeys) will yield an estimated maximal plasma concentration of 1.4 µg/mL after 6 hours of infusion. This concentration would induce at maximum an increase of aPTT between 25 % and 50 % from baseline, resulting in aPTT values at or slightly above the Upper Limit of Normal. Such elevations in aPTT are not known to be associated with adverse events. Therapeutic ranges for aPTT are generally accepted to be 150 % - 250 % of the Upper Limit of Normal, which can be equivalent to a 350 % increase from baseline values.

A relevant GCLP validated according to FDA guidelines 2018 pharmacodynamic biomarker (aPTT) is available to follow-up this anticoagulant activity and will be assessed alongside

pharmacokinetics at various time-points during the study. In addition – for safety – the aPTT will be followed up "bedside" via regular sampling at predefined time-points. These clinical aPTT results will be readily available to the Principal Investigator for safety follow-up during and after the study drug administration.

Based on the presence of a relevant clinical biomarker for study drug activity, a biomarker that can be measured "bedside", the preclinical data – including the elaborated assays in human plasma (effector compartment), the mode of administration (slow intravenous infusion) and the preclinical toxicology results, the sponsor proposes to use 0.25 mg/kg/h as the MRSD – a safety factor of 10x compared with the NOAEL in the most sensitive species (monkey) and only slightly above the starting dose that would be used when considering MABEL.

5.7. Identification of Source Data

All source documents must be accurate, clear, unambiguous, permanent, and capable of being audited. They should be made using some permanent form of recording (ink, typing, printing, optical disc). They should not be obscured by correction fluid or have temporary attachments (such as removable self-stick notes). All data reported on the eCRF should be supported by source documents, unless otherwise specified.

Electronic Data Capture (EDC) via eCRFs will be used for this study, as described in Section 10.1.1. The source data will not be directly collected in the eCRF but will be captured in supportive documentation: laboratory parameters and clinical interpretation of the values on the analytical laboratory print outs, ECG records and clinical significance of observations (when applicable) on the ECG print-outs. The other investigated parameters are collected in the Investigator's source data book.

The data collected on source documents will be entered by the study personnel into the eCRFs in a timely manner.

Supplemental ADA samplings ("long-term follow-up of ADAs"):

Data collected after D180 (extra ambulatory samplings for ADA analysis) will not be collected in the eCRF but only in source documents, and will be added to the final CSR as an appended additional listing. With regards to safety, only Serious Adverse Drug Reactions (SADRs) and any associated information will be reported after D180, and they will only be reported in the Pharmacovigilance (PV) database and not the study database.

6. STUDY POPULATION

Each participant must participate in the informed consent process and read, sign and date the informed consent form (ICF) before any procedures specified in this protocol are performed.

If necessary and after agreement of the sponsor, in order to minimize the constraints on the participants, screening results for a participant could be obtained from the results of any examinations identical to those planned for screening in this protocol and already undergone by the participant within the timelines given for the screening examination. In such a case, the participant must also sign an IEC-approved generic ICF before any such procedure is performed.

6.1. Inclusion criteria

Participants will be required to fulfil all of the following inclusion criteria:

1. Have given written informed consent approved by the relevant Ethics Committee (EC) governing the site, indicating that they understand the purpose of and procedures required for the study and are willing to participate in the study.
2. Male participants between 18 and 55 years of age, inclusive at screening.
3. Otherwise healthy with no clinically significant abnormalities as determined by medical history, physical examination, blood chemistry assessments, haematological assessments, coagulation and urinalysis, measurement of vital signs, and ECG. Isolated out-of-range values judged by the PI (or designated physician) to be of no clinical significance can be allowed. This determination must be recorded in the participant's source documents.
4. Have a body weight in the range of 50 to 90 kg inclusive at screening. Have a body mass index (BMI) of 19 to 28 kg/m^2 inclusive at screening.
5. Agree to abstain from alcohol intake 24 hours before administration of study drug, during the in-patient period of the study and 24 hours prior to all other ambulatory visits, up until and including the discharge visit.
6. Agree not to use prescription medications within 14 days prior to study drug administration and through the duration of the study, unless approved by the PI and Sponsor medical monitor.
7. Non-smokers or abstinence from tobacco or nicotine-containing products for at least 3 months prior to screening.
8. Agree not to use over-the-counter (OTC) medications [including decongestants, antihistamines, and herbal medication (including herbal tea and St. John's Wort], within 14 days prior to study drug administration through the discharge visit, unless approved by the PI and Sponsor medical monitor. Occasional use of paracetamol at recommended doses is allowed. Special rules apply for aspirin, corticosteroids and nonsteroidal anti-inflammatory drugs (NSAIDS (see exclusion criteria 6, 7, 8 and 9)
9. Venous access (both arms) sufficient to allow blood sampling and study drug administration as per protocol.
10. Participants and their partners of childbearing potential [meaning who are not surgically sterile (tubal ligation/obstruction or removal of ovaries or uterus) or post-menopausal (absence of menstrual periods for at least 12 consecutive months)] must be willing to use 2 methods of contraception:
    - a highly effective method of birth control starting at screening. Highly effective methods

of birth control are defined as those that result in a low failure rate (i.e. Pearl Index less than 1% per year) when used consistently and correctly, such as implants, rings, patches, injectable or combined oral contraceptives, intrauterine devices (IUDs), or sexual abstinence (periodic abstinence e.g. calendar, ovulation, symptothermal, postovulation methods, declaration of abstinence for the duration of the trial, and withdrawal are not acceptable methods of contraception) .
- and a local barrier form of contraception. Acceptable barrier methods are either the participant's use of a condom or the partner's use of an occlusive cap or diaphragm, or spermicides.
Participants will not donate sperm from the selection visit and up to 90 days after the infusion. In case of sterile or vasectomised participants, no contraception will be required for their partners of childbearing potential.

11. Willing/able to adhere to the study visit schedule and other requirements, prohibitions and restrictions specified in this protocol.

6.2. Exclusion criteria

If any of the following exclusion criteria apply, the participant must not enter the study:

1. Currently have or have a history of any clinically significant medical illness or medical disorders the PI considers should exclude the participant, including (but not limited to) cardiovascular disease, neuromuscular, haematological disease, immune deficiency state, respiratory disease, hepatic or gastrointestinal disease, neurological or psychiatric disease, ophthalmological disorders, neoplastic disease, renal or urinary tract diseases, or dermatological disease.
2. History of personal or familial bleeding disorders; including prolonged or unusual bleeding.
3. History of deficiency in factor XII (FXII) or haemophilia type A (FVII) or type B (FIX) or type C (FXI).
4. History of cerebral bleeding (e.g. after a car accident), stroke and cerebrovascular accident (CVA).
5. Anamnestic history of Lyme disease or tick-borne encephalitis.
6. Use of Acetylsalicylic-Acid (ASA)-containing OTC medications within 1 month prior to screening.
7. Chronic administration of NSAIDs, chronic use of corticosteroids within 1 month prior to screening.
8. Chronic administration of clopidogrel, ticlopidin, dipyridamole, Coumadin-like anticoagulants, new oral anticoagulant dabigatran, rivaroxaban, apixaban or edoxaban within 3 months prior to screening.
9. Administration of unfractionated heparin, low molecular weight heparin, fibrinolytic agents and anti-FXa within 3 months prior to screening.
10. Have an active acute or chronic infection or diagnosed latent infection.
11. Systolic blood pressure (SBP) greater than 150 or less than 90 mmHg, diastolic blood pressure (DBP) greater than 90 or less than 50 mmHg, and heart rate (HR) greater than 100 or less than 40 bpm.

12. Acute clinically relevant illness within 7 days prior to study drug administration or have had a major illness or hospitalisation within 1 month prior to study drug administration.
13. Major or traumatic surgery within 12 weeks of screening.
14. Any participant who plans to undergo elective surgery within 4 weeks prior to study drug administration and through the discharge visit.
15. Positive serology test for HIV antibodies, hepatitis B surface antigen (HBsAg), or hepatitis C virus (HCV) antibodies at screening.
16. Recent history (within previous 6 months) of alcohol or drug abuse.
17. Have positive urine toxicology screen at screening or D −1 for substances of abuse including amphetamines, benzodiazepine, cocaine, opiates, barbiturate and cannabinoids.
18. Have a positive alcohol breath test at screening or D-1.
19. Consumes, on average, more than approximately 500 mg/day of caffeine (as contained in 5 cups of tea or coffee or 8 cans of caffeine-containing soda or other caffeinated products per day).
20. Donated blood (i.e. 500 mL) within 3 months before D1.
21. Have a history of active drug and/or food allergy or other active allergic disease requiring the constant use of medications, or a history of severe allergic reaction, angioedema or anaphylaxis.
22. Received any other experimental therapy or new investigational drug within 30 days or 5 half-lives (whichever is longer) of study drug administration.

During the time window authorised for the screening visit (i.e. between 28 and 2 days before admission to the first treatment period), the Investigator may order a re-test of any parameter evaluated during the initial screening visit if they need to evaluate the evolution of said parameter(s) or to confirm the value observed.

6.3. Lifestyle restrictions

Any medication the participant takes during the study, and until the discharge visit, other than the study drugs, including any prescription or OTC drug (including vitamins, herbal and mineral supplements), is considered a concomitant medication. At the extra visits scheduled for ADA assessment (i.e. up to D180), only the medications that are administered in treatment of AEs will be considered concomitant medication. Participants will be requested to comply the following restrictions during the study and abstain from:

- Strenuous exercise, as judged by the Investigator, from the selection visit until after the discharge visit.
- Use of prescriptions or OTC medications [including decongestants, antihistamines], and herbal medication (including herbal tea and St. John's Wort), within 14 days prior to study drug administration through the discharge visit, unless approved by the PI and Sponsor medical monitor. Occasional use of paracetamol at recommended doses is allowed. Specific rules apply for aspirin, corticosteroids and NSAIDS (see exclusion criteria 6, 7, 8 and 9).
- Consumption of grapefruit, grapefruit juice, Seville oranges, Seville orange marmalade or other products containing grapefruit or Seville oranges from selection visit until after the discharge visit.

- Consumption of any alcoholic beverages 24 hours before administration of study drug, during the in-patient period of the study and 24 hours prior to all other ambulatory visits, up until and including the discharge visit.
- Smoking and using nicotine-containing products excluded for at least 3 months prior to the screening visit, and until after the discharge visit.
- Use of Acetylsalicylic-Acid (ASA)-containing over-the-counter medications within 1 month prior to screening and until the discharge visit.
- Consumption of any caffeine-containing products (e.g. coffee, tea, chocolate, or Coca-Cola like/ energy drinks): excluded from 24 hours prior to D1 until the end of the in-patient period. Limited to less than 500 mg/day of caffeine (as contained in 5 cups of tea or coffee or 8 cans of caffeine-containing soda or other caffeinated products per day) until the discharge visit.
- Donation of blood (i.e. 500 mL) within 3 months before D1, throughout the study and for 3 months after the end of the study (D90 or D180).

The compliance of the participant to these lifestyle restrictions will be checked at each visit.

Participants and their partners of childbearing potential must be willing to use 2 methods of contraception, one of which must be a barrier method, and participants will not donate sperm, from the selection visit and up to 90 days after the infusion.

Water intake is allowed during the infusion.

Meals will be standardized across the different panels. A light breakfast (2 slices of bread with ham or cheese and jam, glass of water) can be given between 2h and 1h before start of infusion. Lunch will be served after completion of the study drug infusion.

Fasting conditions are required for the blood sample collection at the selection visit, on D3 (may be inpatient in the case of Cohort D and subsequent cohorts), at the ambulatory visits [D4/D5/ D7 (D7 for Cohort D and subsequent cohorts only) /Discharge visit] and for the D1 predose blood sample.

On D1, lunch will be served after completion of the study drug infusion. A light breakfast / snack is also given after the D1H24 (D2) blood sample collection and after the collections performed on D3 (may be inpatient in the case of Cohort D and subsequent cohorts), at the ambulatory visits on D4, D5, D7 (D7 for Cohort D and subsequent cohorts only) and Discharge visit.

Participants must not be in fasting condition for the safety aPTT 36h sample and the ADA blood sample collection visits.

<u>Supplemental ADA samplings (“long-term follow-up of ADAs”):</u>

All randomised participants will be asked to participate in an additional informed consent process and read, sign and date an additional ICF to authorise the use of already collected plasma samples for method development in the framework of Ir-CPI development and follow-up of ADA. For participants with positive ADA results at D180, this ICF will also authorise the collection of additional ADA samples for up to 5 years after D180. At the additional samplings scheduled for ADA assessment after D180, only treatments given for SADRs will be considered concomitant medications, and they will be collected only in the PV database.

6.4. Screen Failures

Screen failures are defined as participants who consent to participate in the clinical study but are not subsequently randomised/included. A minimal set of screen failure information is required to ensure transparent reporting of screen failure participants to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to respond to queries from regulatory authorities: date of ICF signature by participant, demographics and reason for screen failure will be entered in the eCRF.

Individuals who do not meet the criteria for participation in a given panel (screen failure) may be rescreened for a consecutive panel. Reserve participants may be rescreened (if outside of the protocol window) for a consecutive panel. Rescreened participants will be assigned a new screening number. For the new screening visit, the participant must undergo all screening evaluations planned in the protocol.

7. IMP

7.1. Investigational Medicinal Products Administered

Table 3 provides an overall summary of the investigational medicinal product (IMPs) administered during the study, further information is provided in the Investigator's Brochure [1] and in the IMPD [2].

Table 3: IMPs

Study Treatment Name:	**Ir-CPI**	**Placebo**
Active ingredients	*Ixodes ricinus*-Contact Phase Inhibitor as a non-glycosylated recombinant protein produced in the yeast *Pichia Pastoris*	NA
Excipients	Phosphate Buffered Saline (PBS)	NA
Dosage formulation:	10 mL vials: 25 mg/mL non-glycosylated recombinant protein produced in the yeast *Pichia Pastoris*, in 5 mL extractable PBS solution. To be stored at -20°C ± 5°C.	NaCl 0.9% for IV infusion
Unit dose strength(s)/Dosage level(s):	***Part I:*** 1.5 mg/kg, 4.5 mg/kg, 9 mg/kg, 18 mg/kg and 27 mg/kg. ***Part II:*** three doses will be investigated in Part II, the total daily dose of Ir-CPI will be determined based on the emerging safety, tolerability and pharmacokinetic data from Part 1.	NA
Route of Administration	IV infusion	IV infusion
Dosing instructions:	Refer to Section 7.3	Refer to Section 7.3
Packaging and Labelling	Study Treatment will be provided in boxes containing 30 x 10 R glass vials. Each box and vial will be labelled as required per country	

Study Treatment Name:	Ir-CPI	Placebo
	requirement. Details of labelling are provided in Section 7.6.	
Manufacturer	Halix B.V.	Commercially available source

7.2. Method of Treatment Assignment

The randomisation list will be generated by an independent statistician, who will be different from the study statistician, using SAS® software. This list will be transmitted to the unblinded pharmacist of the clinical site in a secure way in order to maintain the blinding for the other staff members involved in this trial.

Two sets of individual sealed decoding envelopes will be also generated by the independent statistician: one set of the sealed decoding envelopes will be provided to the investigational site and one set will be provided to the pharmacovigilance contact for the trial.

For this double blind, placebo controlled, ascending dose trial of single dose infusions of Ir-CPI/Placebo, the participants will be randomised taking into account the specific requirements for the sentinel groups:

In Part 1:

Five (5) panels (A to E), each consisting of eight (8) healthy male participants will be randomised to receive either Ir-CPI or matching placebo: first, 2 sentinel participants (1:1 active / placebo) then for the rest of the group, a 5:1 ratio (active / placebo). The first four (4) panels will be administered Ir-CPI/Placebo in a sequential group design. Depending of the PK and PD results obtained for the first 4 panels, a fifth panel might be performed thereafter. In the end, this fifth panel was not performed.

In Part 2:

Three (3) panels F-G-H, each consisting of eight (8) healthy male participants will be randomized to receive either Ir-CPI or matching placebo: there will be 2 sentinel participants per group (one active treatment and one placebo), the rest of the group follows a 5:1 randomization ratio. The three (3) panels will be administered Ir-CPI/Placebo in a sequential group design. In the end, part 2 was not performed.

Table 4: Randomisation

Study Part	Group	Randomisation number	Replacement randomisation number	Randomisation ratio Active/Placebo
1	A	1101 to 1108	5101 to 5108	2 sentinels (1 active, 1 placebo) then for the rest of the group, 5:1 ratio
1	B	1201 to 1208	5201 to 5208	2 sentinels (1 active, 1 placebo) then for the rest of the group, 5:1 ratio
1	C	1301 to 1308	5301 to 5308	2 sentinels (1 active, 1 placebo) then for the rest of the group, 5:1 ratio
1	D	1401 to 1408	5401 to 5408	2 sentinels (1 active, 1 placebo) then for the rest of the group, 5:1 ratio
1	E	1501 to 1508	5501 to 5508	2 sentinels (1 active, 1 placebo) then for the rest of the group, 5:1 ratio
2	F	2101 to 2108	6101 to 6108	2 sentinels (1 active, 1 placebo) then for the rest of the group, 5:1 ratio
2	G	2201 to 2208	6201 to 6208	2 sentinels (1 active, 1 placebo) then for the rest of the group, 5:1 ratio
2	H	2301 to 2308	6301 to 6308	2 sentinels (1 active, 1 placebo) then for the rest of the group, 5:1 ratio

Participants withdrawn from the study retain their randomisation number, if already given. New participants must always be allotted a new randomisation number/treatment number (replacement treatment number): once assigned, randomisation numbers are never reused within the study site. Note that participants will be identified by their randomisation numbers, throughout the entire course of the study.

7.2.1. Blinding

The pharmacist and his/her attendant, the unblinded monitor who reviews the IMP-related documentation, and the bioanalytical staff (external lab) will be the only personnel to have access to the randomisation list in order to prepare the drug for administration. All Sponsor and Investigator site staff involved in the study will be blinded to the IMP assignment.

In order to maintain blinding, preparation of the dosing solution will be completed by the Good Distribution Practice (GDP) Pharmacy. All documents related to the Pharmacy should be kept in a locked and secure area in order to prevent any unblinding of the staff involved in the trial.

For participants who are randomised to active treatment, the solution of Ir-CPI supplied in vials at 25 mg/mL may be diluted in 0.9 % w/v sodium chloride to a final concentration calculated to achieve the correct dose according the participants weight and according dose assigned to the cohort.

Placebo treatment (0.9 % w/v sodium chloride) will be prepared. The GDP Pharmacy will prepare the IV infusions according to the randomization list and label them with a subject-specific label. Intravenous infusion of the IMP solution will be administered by the blinded study staff according to local SOPs. If needed, additional precautions (suitable ancillaries

such as coloured syringes and infusion lines) will be taken to ensure the blinding of the participant and Investigator.

Emergency code break procedure

In case of an emergency, when knowledge of the investigational product assignment is required for the medical management of an individual participant, the treatment for that participant may be unblinded. The Investigator must notify the Sponsor and study monitor within 24 hours after determining that it is necessary to unblind the treatment assignment for that participant only.

The Sponsor Medical Monitor should be consulted before unblinding, whenever possible.

This documentation must include the name of the individual breaking the blind, the date on which the blind was broken, and a description of the event that led to the unblinding. The Investigator must also indicate in source documents and in the eCRF that the blind was broken and provide the date, time, and reason for breaking the blind. Any adverse event (AE) or serious adverse event (SAE) associated with breaking the blind must be recorded and reported as specified in this protocol.

Unblinded monitors will routinely check the integrity of the envelopes that are stored at the site. All code envelopes (opened or not opened) will be collected from the site prior to study close-out and will be returned to the Sponsor.

7.3. Dose administration

The investigational products will be administered only to participants randomized in this study following the procedures set out in the present clinical study protocol.
The start and the stop time as well as the total volume of drug administered for each participant will be recorded.

7.3.1. IMP preparation

Ir-CPI will be supplied frozen, as a 5 mL PBS solution containing 25 mg/mL Ir-CPI in glass vials (10 mL) to be stored between -15°C and – 25°C.

The total doses administered to the participants are presented in Table 5 and Table 6 for Part 1 and Part 2 respectively.

All doses are expressed in mg/kg, and the body weight of each participant at screening will be used to calculate the exact amount of study drug needed to prepare the allocated total dose for each participant.

The detailed instructions for preparing and calculating the doses of the IMP for administration are provided in the Pharmacy Manual.

7.3.2. Doses administered in Part 1

In Part 1, single doses of Ir-CPI or its matching placebo will be administered to the participants using a continuous 6-hour intravenous infusion.

Table 5: Part 1 Suggested Doses of Ir-CPI for Dose Escalation

Group	Infusion rate* (mg/kg/h)	Infusion Duration (h)	Ir-CPI target plasma concentration (µg/mL) at 6 hours	Total dose (mg/kg)
A	0.25	6	1.4	1.5
B	0.75	6	4.1	4.5
C	1.50	6	8.3	9
D	3.00	6	16.6	18
E**	4.5	6	24.8	27

**Weight of the participant at the screening visit will be used to calculate the dose of study drug.*

***: in the end, group E was not performed.*

7.3.3. Doses administered in Part 2

In the end, part 2 was not performed.

In Part 2, single doses of Ir-CPI or its matching placebo will be administered to the participants using a single ascending 30-minute loading dose infusion followed by a fixed dose of continuous 5.5-hour intravenous infusion

Table 6: Part 2 Suggested Doses of Ir-CPI for Dose Escalation

Group	Infusion rate** (mg/kg/h)	Time (h)	Ir-CPI target plasma concentration (µg/mL) between 30 min and 6 hours	Total dose (mg/kg)
F	Rapid infusion*: 6	0.5	8	11.25*
	Slow infusion*:1.5	5.5		
G	Rapid infusion*:7	0.5	8	11.75*
	Slow infusion*:1.5	5.5		
H	Rapid infusion*8	0.5	8	12.25*
	Slow infusion*:1.5	5.5		

** The rapid and the slow infusion rates will be calculated based on Part 1 emerging safety, PD and PK data. The total dose administered by rapid and by slow infusion will not exceed the highest total dose tested in Part 1. The infusion rate (in mg/kg/h) for the slow infusion will not exceed the maximum infusion rate used in Part 1. The dose may also be adjusted.*

***Weight of the participant at the screening visit will be used to calculate the dose of study drug*

7.3.4. Dosing and meals

Dosing is described in Section 5.1. Meals and food intake are described in Section 6.3.

7.4. Dose Escalation Rules and Dose Staggering Approach

Given the new mechanism of action, the nature of the compound (peptide) and the intended route of administration, dose staggering will be applied in this FIH trial for all panels. This dose staggering will be as such that 2 participants will be dosed at least 24 hours before the remainder of the cohort. These 2 sentinel participants will be randomised as such that there will be one placebo and one *verum*, as shown in Table 4. After the review of adverse events, vital signs, safety labs and ECGs collected over 24 hours postdose (D2), the remaining participants of the cohort will receive the same dose level. Following completion of the initial 24-hour safety period for the sentinel participants, the decision to proceed with the remaining participants in that cohort will be mutually discussed/agreed upon between Investigator (or his designee) and the Sponsor and the decision to dose will be formally confirmed by the

Sponsor. The proposed minimal 24 hours between the sentinel and the remainder of the cohort is deemed sufficient because this is a single intravenous infusion where it is expected that the most severe and acute reactions will occur during or very short after the infusion. Based on animal data, the pharmacodynamic effect and potential side effects are expected to be related to plasma levels that will decline rapidly after ending the IV infusion.

At each dose level, after completion of Investigational Medicinal Product (IMP) administration in at least 6 participants (i.e. a minimum of 4 on active treatment), the Investigator will provide a comprehensive Investigator Safety Report which will include (but not limited to) the following content:

- Relevant information on participants' demographics/characteristics, medical history, physical examination, concomitant medications;
- List of all AEs, including severity, time of onset related to study drug administration, duration, clearly highlighting AESI and Serious Advert Events (SAEs) and relatedness/causality of AEs;
- Any clinically significant out of ranges safety clinical laboratory tests results (as assessed by the PI);
- Physical Examination (including basic neurological testing for isocoria, light reflexes, gait and balance) results including clinical significance for abnormal findings;
- ECG and Vital Sign results including clinical significance for abnormal values;
- Statement of the Principal Investigator's recommendation regarding dose decision.

Escalation to the next higher dose will only take place after review of the Investigator Safety Report from the previous dose levels by the Investigator, in consultation with the Sponsor's representative [called hereafter the Safety Review Committee (SRC)]. Dose escalation will be based on emerging safety and tolerability data as defined by adverse events, laboratory results, ECGs, vital signs, and telemetry assessments. Other parameters of interest will be assessed (blinded pharmacokinetics, blinded pharmacodynamics) per SRC charter and section 7.5 criterion n°5, before dosing in the next cohort can start.

Within the planned dose range, a dose lower than the next planned dose may be tested, depending on emerging safety, tolerability and/or other relevant data, such as blinded pharmacokinetics or pharmacodynamics. The final dose to be used for each cohort will be decided based on the results of the previous panel and can be adjusted downward if deemed necessary.
If the highest planned dose level is found to be safe and tolerable, also considering the PK and PD data, additional higher doses may be added by amendment.

Between the first patients (sentinels) of two consecutive panels, a period of at least 7 days will be respected.

7.5. Stopping Rules

The SRC will meet for deciding on the continuation of dosing after drug administration in at least 6 participants [i.e. a minimum of four (4) on active treatment]. However, stopping rules will be applicable from dosing of the first sentinel participant on. These decisions will be based on the following:

1. If a Serious AE, which is considered drug-related, occurred in one (1) participant or if a Severe AE, which is considered drug-related, occurred in two (2) participants in the same cohort, the dosing in the trial should be temporarily stopped in order to review additional

safety data. If after review of the additional data and unblinding of the randomisation for that participant the drug-relatedness assessment changes, the study can resume without substantial amendment.

2. If 1 sentinel participant (out of 2) or at least two (2) out of eight (8) participants in a cohort experience a Dose Limiting Toxicity (DLT) that is related to Ir-CPI administration, the dosing in the trial should be temporarily stopped in order to review additional safety data. If after review of the additional data and the unblinding of the randomisation for that participant, the drug-relatedness assessment changes, the study can resume without substantial amendment;
3. If at least one (1) DLT is reported, the SRC recommendation will depend on the DLT profile (i.e. identical DLTs in different participants) or the frequency and severity of DLTs in the same participant and on the recommendation made by the Investigator;
4. If no DLT is reported, the planned dose escalation may proceed with the next cohort or part, unless the Investigator has any other safety concern;
5. In any case, administered dose of Ir-CPI will be limited to a dose predicted to generate upper 95 % confidence interval (CI) C_{max} value being not higher than the lowest C_{max} values at the NOAEL (53.7 µg/mL) in the most sensitive species [24h-infusion acute study in monkeys at NOAEL 201.6 mg/kg/day]. PK data will be monitored, so that preliminary PK parameters of the 1.5 mg/kg panel (A) should be available before dosing the 4.5 mg/kg panel (B), 4.5 mg/kg panel (B) PK parameters before administration of 9 mg/kg panel (C) etc.

All decisions will be documented in writing and communicated to the study team in a timely manner, before dosing in the next cohort can start.

Definition of the DLT:

- Serious AE drug-related in one (1) participant (in the study);
- Severe AE drug-related in two (2) participants in one cohort;
- ECG: prolongation of QTcF to >500 ms or of >60 ms over baseline drug-related in two (2) participants in one cohort;
- Safety laboratory tests: Increase in ALT/AST ≥ 5 x ULN in 2 participants in the study;
- Safety laboratory tests: Increase in ALT/AST ≥ 3 x ULN and TBIL ≥ 2x ULN in one (1) participant in the study;
- Other clinically significant changes in safety clinical laboratory tests, ECG, VS or other safety parameters deemed as a significant safety concern by the Investigator;
- aPTT increase > 5x ULN at the 24h postdose time point in two (2) participants in one cohort;
- Hypersensitivity reaction occurring during study drug administration or within 6h after end of study drug administration accompanied by symptomatic bronchospasm, with or without urticaria; allergy-related oedema/angioedema; hypotension in one participant.

If no further dosing is planned due to the stopping criteria as described above, previous randomised and dosed participants will continue the study as planned. Safety assessments will be performed as planned. Pharmacokinetic assessments will be performed if deemed to be relevant and agreed by the Investigator and Sponsor without compromising the safety of a participant.

7.6. Supply, Packaging and labelling of the investigational products

A sufficient quantity, to be defined by the Sponsor, of IMPs will be supplied under proper storage condition (temperature record) as well as an acknowledgement of receipt form. The Sponsor's representative will provide a certificate of analysis and a batch release certificate for those batches of IMP used in the study.

The investigational products will be bearing the following information on the primary packaging labels:

Table 7: IMP packaging labels

	Secondary packaging (boxes of 30 vials)	**Primary packaging (10 R glass vials)**
Sponsor's name, address and telephone number	X	X
Pharmaceutical dosage form, route of administration, name of the product and dosage, quantity of dosage units	X	X
Batch and/or code number (kit or vial's number)	X	X
Study code	X	X
Participant number		X
Investigator's name	X	
Investigator's site	X	
Instructions for use	X	
Statement "For clinical trial use only"	X	
Storage conditions	X	X
Expiry date	X	X

The Investigator, or designee, will only dispense IMPs to eligible participants who are included in the study. Each participant will only be given the IMP carrying his number. The dispensing for each participant will be documented in the source documents and in the participant's case report form (eCRF).

7.7. Storage of the investigational products

Prior to the preparation of individual doses, all IMPs will be stored frozen between -15°C / -25°C in a secure and locked storage area of limited access, with continuous temperature control and monitoring.

The pharmacist at ATC pharma will be responsible for the correct storage and handling of the IMPs.

Once an individual dose has been prepared it will be stored prior to administration as detailed in the Pharmacy Manual.

Deviations from the storage requirements, reporting and any actions taken, must be documented.

7.8. Accountability, reconciliation and return of the investigational products

The Investigator or his designee must maintain a complete and current dispensing and inventory record. The Investigator or his designee is accountable for all test articles supplied by the Sponsor. The dispenser uses this information to maintain an accurate and complete dispensing and inventory record. The designated copies of the completed dispensing and inventory record will be returned to the Sponsor.

Regulatory agencies require accounting for the disposition of all investigational drugs received by the clinical site. Information on drug disposition required by law consists of the date received, date administered, quantity administered, and the participant to whom the drug was administered.

Supplies are shipped to the investigational site as needed. Drug accounting will be reviewed by the unblinded monitor during routine pharmacy monitoring visits. Each time a dose is prepared for a participant, the following information should be recorded: the participant's randomisation number, the total dose prepared, the volume of IMP used, the number of the batch from which the dose was prepared, the date (and time) of preparation/administration and the initials of the persons preparing and checking the dose.

At defined timepoints during the study, a drug accountability review and reconciliation will be performed, and at the completion or termination of the study, a final drug accountability review and reconciliation must be completed. Any discrepancies must be investigated and their resolution documented. After each round of drug accountability review and reconciliation, partially full and full containers of IMP can be returned to the Sponsor. The Sponsor will review the accountability form before authorising the destruction of empty containers on site. For this purpose, the site will provide a destruction certificate.

7.9. Treatment compliance

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

The detailed definitions of overdoses, medication errors, abuse and misuse are provided in Appendix 2: overdoses, medication errors, abuse or misuse will be collected as part of IMP dosing information and/or as a protocol deviation, as required.

7.10. Prior Treatments

In addition to the compliance with the inclusion and exclusion criteria, reasonable efforts will be made to determine all relevant treatments received by the participant within 2 weeks before IMP administration. All relevant information must be recorded on the participant's medical records and eCRF.

As listed in sections 6.1 and 6.2, the following treatments are banned:

- ***Within three (3) months prior to screening:***
    - chronic administration of clopidogrel or ticlopidin or dipyridamole, Coumadin-like anticoagulants, new oral anticoagulant dabigatran or rivaroxaban or apixaban or edoxaban;
    - unfractionated heparin, low molecular weight heparin, fibrinolytic agents and anti-FXa.

- ***Within one (1) month prior to screening:***
  - Acetylsalicylic-Acid (ASA)-containing over-the-counter medications;
  - chronic administration of nonsteroidal anti-inflammatory Drugs (NSAIDs) (COX-2 inhibitors excluded);
  - chronic use of corticosteroids, defined as > 5 consecutive days (including topical application).
- ***Within 30 days prior or 5 half-lives (whichever is longer) prior to study drug administrations***
  - Any other experimental therapy or new investigational agent

- ***Within 14 days prior to study drug administrations:***
  - Prescription medications or OTC medications (including decongestants and antihistamines),
  - Herbal medication (including herbal tea, St. John's Wort),
  - Occasional use of paracetamol at recommended doses is allowed.

7.11. Concomitant Treatments

Any medication the participant takes during the study and until the discharge visit, other than the study drugs, including any prescription or over-the counter drug (including vitamins, herbal and mineral supplements), is considered a concomitant medication. At the additional visits scheduled for ADA assessment, only the medications that are administered in treatment of AEs will be considered concomitant medications. As listed in sections 6.1, 6.2, and 6.3, concomitant treatment is not permitted throughout the study. No medication other than the study products is allowed during the study unless absolutely required for treatment of adverse events. Occasional use of paracetamol at recommended doses is allowed.

For any participant, if the use of any concomitant treatment becomes necessary (e.g. the treatment of an adverse event), the treatment must be recorded in the source documents and in the eCRF, including the reason for treatment, the generic name of the drug, the dosage, the route, and the date of administration. If a treatment is administered, the Sponsor's medical monitor must be promptly notified in order to assess the participant's eligibility for continuing study participation.

Supplemental ADA samplings ("long-term follow-up of ADAs"):

At the additional samplings scheduled for ADA assessment after D180, only treatments given for SADRs will be considered concomitant medications, and they will be collected only in the PV database.

8. DISCONTINUATION CRITERIA AND RELATED PROCEDURES

8.1. Withdrawal criteria

Any participant may be withdrawn from the study at the discretion of the Investigator. The participant is also free to terminate his participation at any time. However, if the participant has been dosed with study medication, it is recommended to ask the participant to remain in contact with the centre, and the investigational site should make every effort to convince the participant to return for a safety visit (corresponding to the discharge visit) and a laboratory sample taken to look for immunogenicity (presence of anti-drug antibodies and neutralizing antibodies if applicable).

The Investigator will also undertake to obtain more detailed information about any participant who is lost to follow-up.

Participants who are withdrawn from the study must not be re-included.

The eCRF must document the primary reason for withdrawal.

Investigators should contact the Medical Monitor, whenever possible, to discuss the withdrawal of a participant in advance.

Supplemental ADA samplings ("long-term follow-up of ADAs"):

These procedures do not apply for the additional samplings performed after D180.

8.2. Discontinuation of Study Treatment (Infusion)

In case of discontinuation or interruption of the study drug regardless the reason (AE, technical problem …), every effort should be made to perform PK/PD sampling as per SoA.
Any safety assessments will be performed as originally planned.
In case of interruption of the infusion:

- Infusion should never be restarted in case of paravenous infusion or other catheter site problems
- Infusion can be restarted only in case of equipment failure and if this failure can be resolved within 10 minutes of the failure occurring.

8.3. Discontinuation of Study

In case of premature discontinuation, please refer to the Schedule of Assessments (SoA) for data to be collected at the discontinuation visit (corresponding to the discharge visit and to a laboratory sample taken to look for immunogenicity, i.e., presence of anti-drug antibodies, and neutralizing antibodies if applicable).

8.4. Unscheduled Visit

At the Investigator's discretion, an unscheduled Visit may be completed at any time during the study, but before the Discharge Visit, if deemed necessary for the subject's safety and well-being.

8.5. Lost to Follow Up

A participant will be considered lost to follow-up if he fails to return for scheduled visits and does not respond to the study site's attempts to contact them.

The following actions must be taken if a participant fails to return to the clinic for a required study visit:

- The site must attempt to contact the participant and reschedule the missed visit as soon as possible and counsel the participant on the importance of maintaining the assigned visit schedule and ascertain whether or not the participant wishes to and/or should continue in the study.
- In cases in which the participant is deemed lost to follow up, the investigator or designee must make every effort to regain contact with the participant (where possible, 3 telephone calls and 1 written letter to the participant's last known mailing address or local equivalent methods). These contact attempts and a copy of the written letter should be documented in the participant's medical record.
- Should the participant continue to be unreachable, they will be considered to have withdrawn from the study with a primary reason of lost to follow-up.

Supplemental ADA samplings ("long-term follow-up of ADAs"):

These procedures do not apply for the additional samplings performed after D180. However, if a sampling is missed, the next sampling can still take place and the participant will be encouraged to come back for this next sampling.

8.6. Withdrawn participant data collection

The principal Investigator and/or other Investigator involved in the study will document on the termination page of the eCRF and in the participant's medical records the primary reason for the participant's withdrawal as follows:

- AE: an AE form must be completed;
- Protocol deviation;
- Withdrawal by participant;
- Lost to follow-up;
- Study terminated by the Sponsor;
- Death;
- Other: if none of the above-mentioned reasons are applicable, then the reason will be specified.

All withdrawals will be reported to the Sponsor.

For all withdrawals, the end-of-study visit will be arranged scheduled within 10 days after the day of withdrawal, and will document the progress of their condition. In every case, the eCRF must be filled out up to the last visit performed.

Supplemental ADA samplings ("long-term follow-up of ADAs"):

These procedures do not apply for the additional samplings performed after D180.

8.7. Replacement of participants

The Investigator and the Sponsor will decide whether participants who withdraw from the study for any reason other than safety will be replaced.

If replacement of participants is necessary, the replacement participants will be assigned to the same treatment as the participants they are replacing.

In case of moderate or severe adverse event causing the participant to withdraw, then the participant will not be replaced.

9. PROCEDURES

9.1. Investigational schedule

The schedule of assessments is described in Section 2, Schedule of Activities (SoA).

When several procedures are scheduled at the same theoretical time, the 12-lead ECG will be obtained first and within 15 minutes of the scheduled time, followed by vital signs.

The PK and PD blood samples will be collected at the scheduled time within the windows noted in Section 9.2.

The Investigator cannot perform any other biological assay but those described in the protocol, except when investigating an AE or suspected AE requires additional samples for the participant's safety.

9.2. Total volume of blood collected

Less than 550 mL of blood will be collected from selection visit until extra ambulatory visits for ADA analysis on D30. Less than 20 mL of blood will be collected for extra ambulatory visits for ADA analysis on D90 and D180. Such a blood sample is safe provided it is not followed or preceded by other important blood samples [(blood donations within 3 months before D1, throughout the study and for 3 months after the end of the study (D90 or D180)]. Blood samples for PK and PD analysis should be collected at the requested times but a window for sample collection will be allowed as outlined in Table 8. The exact actual time of collection should be noted on the source data and in the eCRFs and used for the calculations.

All collected samples will be stored for up to 5 years [starting from the last patient last visit (LPLV)], unless local rules, regulations or guidelines require different timeframes or different procedures.

Table 8: Time windows for sampling collection

Time point	Window for taking sample (min)
Pre-dose	anytime within 60' predose except otherwise specified (e.g. samples in fasted conditions taken before breakfast)
15 min	± 3 minutes
30 min	± 5 minutes
45 min	± 5 minutes
1 h	± 5 minutes
90 min	± 10 minutes
2 h	± 10 minutes
3 h	± 10 minutes
4 h	± 15 minutes
6 h	± 30 minutes
8 h	± 30 minutes
12 h	± 30 minutes
24 h	± 60 minutes
32 h	± 60 minutes
36 h	± 60 minutes
48 h	± 120 minutes
72 h	± 120 minutes
96 h	± 120 minutes

Time point	Window for taking sample (min)
144 h	± 120 minutes

Supplemental ADA samplings ("long-term follow-up of ADAs"):

Less than 30 mL of blood will be collected at each additional sampling performed after D180 for ADA analysis.

9.3. Pharmacokinetic Evaluation

Samples will not be labelled with information that directly identifies the patients but will be coded with the identification number for the participant.

9.3.1. Pharmacokinetic Assessment Methods and Timing

Plasma PK Samples

- Blood samples will be collected in 4.5 mL sodium 3.2 % citrate (0.109M) tubes with citrate volume/blood volume equal to 1/9. These samples will be collected at the approximate times indicated in the study schedule
- The detailed process for handling and preparing plasma PK samples is provided in the laboratory manual.

Urine PK Samples (exploratory)

- Participants should be instructed to void at the beginning and end of each collection period and to collect all urine that is passed between the start and end of the urine collection intervals. Urine samples (all urine voided between the time-points) will be collected in polypropylene jugs and should be stored refrigerated (between 2 and 8°C) until each collection period is completed.
- The detailed process for handling and preparing urine PK samples is provided in the laboratory manual

9.3.2. PK Sample handling and labelling

Refer to laboratory manual.

9.3.3. PK Sample shipment and storage

The samples will be shipped in a container filled with enough dry ice to ensure that the samples are kept frozen.

Study samples will be shipped to Eurofins | ADME Bioanalyses.

9.3.4. PK Bioanalytical method

A validated sandwich ELISA method will be used for the quantification of Ir-CPI in human citrate sodium plasma. The lower limit of quantification is 5 ng/L.

9.3.5. Pharmacokinetic Criteria

The pharmacokinetic parameters will be determined using Phoenix® WinNonlin® version 8.1 or higher (Certara USA, Inc., Princeton, NJ).

The following plasma pharmacokinetic parameters will be calculated for Ir-CPI, by compartmental or non-compartmental methods, as appropriate for those participants with sufficient plasma concentration data:

- C_{max}: Maximum plasma concentration,
- t_{max}: time to reach maximum plasma concentration,
- AUC_{0-6}: Area under the plasma concentration-time curve from time zero to 6h,
- AUC_{inf}: Area under the plasma concentration-time curve from time zero to time of infinity,
- λ_z: apparent terminal elimination rate constant,
- $t_{1/2}$: terminal elimination half-life,
- V_d: volume of distribution,
- CL: total body clearance.

The list of PK parameters could be adjusted if needed during the analyses.

9.4. Pharmacodynamic Evaluation

9.4.1. Pharmacodynamic outcome measurements

- ***PD aPTT – Factor XI and Factor XII inhibition:*** measured by the change from baseline in PD aPTT and residual FXI and FXII activities (central lab).

9.4.2. Pharmacodynamic Assessment Methods and Timing

For PD variables (PD aPTT, Factor XI and Factor XII), blood sampling and preparation of human plasma for PD analyses (avoiding activation of the contact phase of the coagulation) will be collected in 4.5 mL sodium citrate 3.2 % (0.109M) tubes with citrate volume/blood volume equal to 1/9. The detailed process for collection, preparation, handling and transportation of the samples is provided in the laboratory manual.

9.4.3. PD Sample handling and labelling

Refer to laboratory manual.

9.4.4. PD Sample shipment and storage

The samples will be shipped in a container filled with enough dry ice to ensure that the samples are kept frozen.

Study samples will be shipped to Eurofins | Biomnis Clinical Trials.

9.4.5. PD Bioanalytical method

Qualified methods were used to measure the activity of Ir-CPI in human citrated plasma in PD aPTT, an assay measuring the intrinsic pathway of coagulation and in an aPTT-based method using FXI or FXII deficient plasma to measure the residual activities of FXIa or FXIIa, respectively.

9.5. Exploratory Biomarkers Evaluation

Collection of samples for other biomarker research is also part of this study. Blood samples and urine samples will be collected at visits as specified in the SoA (Section 2).

If the data permit, biomarker samples will be tested to evaluate their association with the observed clinical responses to Ir-CPI.

9.5.1. Exploratory outcome measurements

In Blood:

- D-dimers, fibrinogen, prothrombin time PT (expressed in seconds and as INR),
- TNFα,
- hsCRP,
- cystatin C (CysC),
- ADA.

In Urine:

- Urinary microalbumin,
- Kidney Injury Molecule (KIM-1),
- Urinary Neutrophil Gelatinase-Associated Lipocalin (NGAL).

9.5.2. Exploratory Assessment Methods and Timing

The detailed process for collection, preparation, handling and transportation of each type of sample is provided in the laboratory manual.

- For PT, D-dimers and fibrinogen, blood sample will be collected in 3 mL sodium citrate 3.2 % (0.109M) tubes. After blood collection, store / transport the tubes upright, at room temperature and transport the tubes to the local lab.
- For TNFα, blood samples will be collected in 8 mL SST tubes and transport the tubes to the local lab.
- For hsCRP, 3.5 mL tube without anticoagulant will be taken. After blood collection, store the tubes in 2-8°C and transport it to the local lab.
- For serum Cystatin assay, blood samples will be collected in 8 mL SST tubes.
- For Anti-Drug Antibodies (ADA), blood samples will be collected in 4.5 mL sodium 3.2 % citrate (0.109M) tubes with citrate volume/blood volume equal to 1/9.

Urine samples for exploratory biomarkers, 10 mL tubes will be collected pre-dose and after each collection period, centrifuged and stored at -20°C.

<u>Supplemental ADA samplings ("long-term follow-up of ADAs"):</u>

Less than 30 mL of blood will be collected at the additional samplings performed after D180 in order to analyse ADAs and develop a sensitive ADA neutralizing assay, using different concentrations of plasma and different concentrations of Ir-CPI.

9.5.3. Exploratory Biomarkers Sample handling and labelling

The labelling used to identify each aliquot of whole blood / plasma / serum / urine is provided in the Laboratory Manual.

9.5.4. Exploratory Biomarkers Sample shipment and storage

Study samples will be stored in frozen conditions until they are shipped in a container filled with enough dry ice to ensure that the samples are kept frozen.

9.5.5. Exploratory Biomarkers Bioanalytical method

The concentrations of the exploratory biomarkers will be evaluated using the methods applicable in each of the analytical laboratories in charge of each assay.

9.6. Adverse events and treatment emergence

The definitions of an AE, adverse events of special interest (AESI) or SAE can be found in Appendix 2: Adverse event and serious adverse event definitions.

AE will be reported by the participant (or, when appropriate, by a caregiver, surrogate, or the participant's legally authorised representative).

AESIs: for the purpose of this study, AEs related to bleeding (e.g. positive Hemoccult® testing, gingival bleeding, bruising/haematoma, conjunctival bleeding, prolonged bleeding after trauma) will be considered as AESIs.

In case of extreme bleeding or emergencies, Fresh Frozen Plasma can be tried to mitigate the bleeding. Dialysis will eliminate the protein from the body.

Also considered as AESI are all infusion site reactions, including hypersensitivity reactions that would occur during or after study drug administration.

The Investigator and any designees are responsible for detecting, documenting, and reporting events that meet the definition of an AE, AESI or SAE and remain responsible for following up AEs that are serious, considered related to the study treatment or the study, or that caused the participant to discontinue the study (see Section 8).

Supplemental ADA samplings ("long-term follow-up of ADAs"):

Detection, documentation, follow-up and reporting described below do not apply for samplings performed after D180, where only SADRs (either expected or unexpected [i.e. Suspected Unexpected Serious Adverse Reactions (SUSARs)]) and related information will be collected and followed-up if applicable in the PV database only, and not in the eCRF.

9.6.1. Documentation and Treatment of Adverse Events by the Investigator

All AEs, including AESIs and SAEs, occurring within the period of observation for the clinical study must be recorded.

The **period of observation** for the collection of AEs extends from the time when the participant gives informed consent until the last study visit. For all participants, this period will be extended to follow-up on all on-going AEs after the end of the treatment period, until all AEs are finally resolved or it is medically justifiable to stop further follow-up (e.g., a chronic condition has been reached, i.e. stabilization of symptoms).

There is no time limit on the collection of SAEs that are considered related to IMP. If the investigator detects an SAE in a study participant after the end of the period of observation, and considers the event possibly related to prior study treatment or procedures, he or she must contact the Pharmacovigilance (PV) contact of the Sponsor to determine how the SAE should be documented and reported.

The description of the AEs (including date and time of onset and resolution when applicable) must be documented as soon and as completely as possible on the "Adverse Events" pages in the eCRF. Follow-up information must be entered as soon as available.

The following will also be specified:

- Seriousness including criteria for seriousness;
- Severity
- Action taken;
- Relationship to treatment;
- Additional therapy;
- If it was the cause of study discontinuation;
- Outcome.

AEs that occur during the study should be treated by established standards of care that will protect the life and health of the participants. If such treatment constitutes a deviation from the protocol, the participants should be withdrawn from the study and the reason must be documented in the eCRF.

9.6.2. Method of Detecting AEs, AESIs and SAEs

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

9.6.3. Follow-up of AE, AESI and SAE

After the initial AE/AESI/SAE report, the Investigator is required to proactively follow each participant at subsequent visits/contacts. All SAEs, and non-serious AESIs (as defined in Appendix 2: Adverse event and serious adverse event definitions) will be followed until resolution, stabilization, until the event is otherwise explained, or the participant is lost to follow-up. Further information on follow-up procedures is given in Appendix 2.

9.6.4. Regulatory Reporting of Serious Adverse Events

Procedure for SAE declaration by Investigator to Sponsor:

The Sponsor's PV contact for the study is:

BIOTRIAL

Email: PV.BIOTRIAL@biotrial.com

The Principal Investigator (PI), or designee, is responsible for providing notification to the Sponsor's PV contact of any SAE, whether deemed IMP-related or not, that a participant experiences during his participation in the study within 24 hours of becoming aware of the event. For all SAE Report Forms faxed or emailed, the PI must sign and date the form.

In addition, within one business day of receipt by Sponsor's PV contact any other requests for clarification of reported SAE data, and as appropriate, a list of any source documentation to be requested from the site (e.g. admission/discharge summaries, relevant to the events reported, lab/radiology/procedure reports, death certificate, autopsy report, etc.) are sent to the Sponsor.

Follow up information must be reported to the Sponsor's PV contact and within 24 hours of the study site staff becoming aware of new information.

The Sponsor's PV contact will be responsible for reporting all expedited reports like SAEs, expected SADRs and SUSARs occurring in this study to competent authorities, FAMHP (Federal Agency for Medicines and Health Products), Ethics Committee and European Medicines Agency (EMA) according to applicable guidelines.

If an AESI occurs, the PI (or designee) will not be required to fill out a form, but must record the AE in the eCRF. The Sponsor will assess the AEs on a case-by-case basis to determine whether an AE is considered an AESI and, if necessary, will request that the PI (or designee) fill out a PV report form to document the event. The BIOTRIAL PV contact will then perform any further actions that may be required.

9.6.5. Treatment of Overdose

There is no known antidote in case of excessive dosing (beyond that prescribed in the protocol and including overdose). A symptomatic/supportive treatment will be provided.

Excessive dosing will be recorded in the eCRF. Any SAE or AE associated with excessive dosing must be followed as any other SAE or AE. These events are only considered AEs or SAEs if there are associated clinical signs and symptoms or if the act of taking the excess medicine itself is an AE or SAE.

9.7. Safety Assessment

9.7.1. Physical Examination

The timing of the complete physical examinations is provided in Section 2, SoA.

The physical examinations will be performed by the Investigator or his representatives. A complete physical examination will include, at a minimum, assessments of the cardiovascular, respiratory, gastrointestinal, dermatological, neurological (including basic neurological testing for isocoria, light reflexes, gait and balance), musculoskeletal and lymphatic systems, in addition to head, eyes, ears, nose, throat, and neck. The physical examination will not include rectal examinations.

Further examination of other body systems may be performed in case of evocative symptoms at the Investigator's discretion.

Any abnormality identified at baseline should be recorded as medical history.

At subsequent visits (or as clinically indicated), limited, symptom-directed physical examinations should be performed (abbreviated physical examination). Changes from baseline abnormalities should be recorded in participant's notes. New or worsened clinically significant abnormalities should be recorded as adverse events on the eCRF.

9.7.2. Weight, height and BMI

The timing of the body weight and height measurements is provided in Section 2, SoA.

Height will only be measured at screening, and used for calculating BMI at that visit.

9.7.3. Vital Signs

9.7.3.1. Parameters

Measured parameters: Supine systolic blood pressure (SBP), diastolic blood pressure (DBP) and heart rate (HR), body temperature.

9.7.3.2. Method of assessment

Qualified staff members will perform blood pressure (BP) measurements. If possible, BP measurements will be taken from the arm used for the infusion (opposite of the arm that is used for blood sample collection) by an automated BP monitor using the oscillometric method (e.g., Dinamap®). If there is a clinically important change in BP from the previous recording, measurements will be repeated immediately to confirm the change.

BP and HR will be measured using an automatic device for each participant, after at least 5 minutes at rest in the supine position.

The timing of the assessments is summarised in Section 2, SoA.

The following normal ranges will apply:

Table 9: normal ranges for vital signs

Systolic blood pressure (mmHg)	**Diastolic blood pressure (mmHg)**	**Heart rate (bpm)**	**Tympanic temperature (°C)**
$90 \leq SBP \leq 150$	$50 \leq DBP \leq 90$	$40 \leq HR \leq 100$	$36.0 \leq t° \leq 37.5$

9.7.4. Telemetry monitoring

9.7.4.1. Parameters

SpO_2, non-artifact events [arrhythmias, bradycardia, tachycardia, pause, ventricular tachycardia (VT), supraventricular tachycardia (SVT)].

9.7.4.2. Method of assessment

A centralised, continuous and automatic evaluation of the ECG signal is carried out. The telemetry traces will be monitored in real time. Alerts are triggered as soon as an abnormal event occurs. Alerts are reviewed by the investigator and considered for safety management of the participants. In the treatment period, cardiac telemetry monitoring will begin 1 hour prior to study drug administration and continue until 12 hours postdose.

9.7.5. Standard 12-lead ECG

9.7.5.1. Parameters

- Measured parameters: HR, PR interval, QRS duration, QRS axis, QT interval;
- Derived parameters: two corrections of the QT interval will be investigated: Fridericia's correction (QTcF) and Bazett's correction (QTcB).
- Observations and comments on the quality of the trace (if needed), on normality or abnormality.

9.7.5.2. Method of assessment

The timing of the assessments is summarised in Section 2, SoA.

The measurements will consist of 12-lead digital ECGs. Participants must rest in the supine position for at least 5 minutes before the ECG recording is started. The ECG may be recorded during the period of rest required before the measurements of supine blood pressure and pulse. A qualified physician will review the ECGs promptly and any clinically important finding will be recorded on the appropriate CRF. The Investigator is responsible for providing the interpretation of all ECGs.

Table 10: normal ranges for ECG parameters

PR (ms)	QRS (ms)	QTc_F(ms)	Heart rate(bpm)
110 ≤ PR ≤ 220	QRS ≤ 120	QTc ≤ 450 ms	40 ≤ HR ≤ 100

9.7.6. Laboratory safety parameters

9.7.6.1. Serology, drug screen and alcohol breath test

Blood tests will be carried out to test for the presence of HIV antibodies, hepatitis B surface antigen (HBsAg), and hepatitis C virus (HCV) antibodies.

Urinary screening will be carried out to test for amphetamines, benzodiazepine, cocaine, opiates, barbiturate and cannabinoids.

An alcohol breath test will be performed.

The timing of the assessments is summarised in Section 2, SoA.

9.7.6.2. Laboratory Safety

9.7.6.2.1. Blood safety analysis

The following parameters will be determined:

- **Haematology:**
  - Haematocrit, haemoglobin, red blood cell count, WBC count, WBC differential count, platelets counts.
- **Safety coagulation:**
  - activated Partial Thromboplastin Time (aPTT).
- **Blood chemistry:**
  - fasting glucose, creatinine, urea, sodium, potassium, calcium, chloride, phosphorus, bicarbonate.
  - AST, ALT, GGT, alkaline phosphatase.
  - total bilirubin (fraction of direct bilirubin in case > 1.25 x ULN).
  - albumin, total protein.
  - LDH, CK (and fraction of CK-MB if > 1.5 x ULN).

NB: The baseline chemistry sample (predose sample) will also contain an extra archive serum sample for anti-Borrelia serology testing as backup to test for anti-Borrelia antibodies suggestive of previous exposure to **tick bites** in case of outlying results for PK/PD.

9.7.6.2.2. Urinalysis parameters

The following parameters will be investigated by microscopic examination of the sediment: cylinders, erythrocytes, leucocytes.

9.7.6.2.3. Method of assessment

Samples of blood will be collected in fasted conditions at the visits summarised in Section 2, SoA.

For all clinically significant abnormal laboratory test values identified after investigational product administration, testing will be repeated until the values return to normal or baseline. If laboratory values do not return to normal or baseline within a reasonable period, the aetiology should be identified and the Sponsor notified.

Samples of urine will be collected at the visits summarised in Section 2, SoA.

9.7.6.2.4. Laboratory safety determinations

Laboratory tests will be performed by Clinical Biology Department of CHU of Liège, Avenue Hippocrate, 15, B35, Route 52, 4000 Liège, Belgium.

All clinical laboratory analyses will be performed at the local laboratory. Alcohol test, urine drug screen, will be performed at the clinical site by study personnel.

Serology (HIV and Hepatitis B and C testing) will be performed only at the Screening Visit;

Urine drug screen and alcohol breath test will be performed at the Screening Visit and D-1.

9.7.7. Occult Blood in Faeces

9.7.7.1. Parameters

Absence/presence of occult blood in faeces.

9.7.7.2. Method of assessment

Faeces samples will be collected at the timepoints listed in the SOA, section 2, Hemoccult® testing will be performed to investigate any presence of occult blood in faeces.

Positive findings will be recorded as AEs, and also analysed as AESIs.

9.7.8. Tick bite exposure monitoring

9.7.8.1. Parameters

Absence/presence of exposure to tick bites.

9.7.8.2. Method of assessment

As highlighted in Section 9.7.6.2.1, an extra sample for serology testing as backup to test for anti-Borrelia antibodies (IgG and IgM) suggestive of previous exposure to **tick bites** is drawn at baseline.

At the extra-ambulatory visits and samplings performed for ADA analysis, the participants will be asked if they have been exposed to tick bite since the previous visit (response recorded as yes/no/unknown). If the answer is yes, then the participant will be asked to

provide information on treatment (e.g. antibiotics) and on presence of *erythema migrans* and other signs or symptoms.

10. DATA MANAGEMENT AND STATISTICS

10.1. Data entry and management

10.1.1. Data collection

Electronic Data Capture (EDC) via eCRFs will be used for this study. For each participant who was exposed to study drug, an eCRF must be completed and signed electronically by the investigator or co-investigator. This also applies to those participants who fail to complete the study. If a participant withdraws from the study, the reason must be noted on the eCRF. eCRFs are to be completed on an ongoing basis.

Study data will be collected by the investigator or dedicated study personnel. Data will be collected on source documents and entered by the study personnel into the eCRFs in a timely manner.

All the documents must be archived for a minimum of 25 years or according to the Sponsor's procedures, whichever is longer.

Supplemental ADA samplings ("long-term follow-up of ADAs"):

ADA analysis data (including tick bite information if applicable) collected at the additional samplings after D180 will be collected in source documents and not in the eCRF. The results will be forwarded to the Sponsor after each time-point, and at the end of the follow-up period, individual ADA data will be forwarded to Biotrial.

10.1.2. Data coding

Biotrial will ensure coding for adverse events, prior and concomitants medications and medical history up to D180 in compliance with the Medical Dictionary for regulatory activities (MedDRA) and the World Health Organization Drug Dictionary (WHODDRUG), using the most recent versions of MedDRA and WHODDRUG.

10.1.3. Data validation

Biotrial will prepare the data validation document.

For each part, Biotrial will organise a Blind Review of the data before database locking.

Supplemental ADA samplings ("long-term follow-up of ADAs"):

The data lock-point date will be the D180 visit and the database will not be unlocked to add ADA data after this date. All data are unblinded from that point onwards. No influence of breaking the blind is anticipated on the dynamics of ADA.

10.2. Statistical considerations

10.2.1. Sample size

The number of participants is not based on statistical power considerations. A sample size of 8 participants per panel (i.e., 6 active and 2 placebo) was chosen based on the design of similar SAD studies and is considered adequate to provide an initial assessment of the safety and tolerability profile.

10.2.2. Statistical methods

The statistical analyses will be performed by the Biostatistics Unit of Biotrial Biometrics using SAS® software version 9.4 or higher release (SAS Institute Inc., Cary, NC, USA).

Pharmacokinetic data will be analyzed by Biotrial using Phoenix® WinNonlin® version 8.1 or higher (Certara USA, Inc., Princeton, NJ) and SAS® software Version 9.4 or higher release (SAS Institute Inc., Cary, NC, USA).

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

- Quantitative variable: sample size, arithmetic mean, standard deviation (SD), standard error of the mean (SEM), minimum, median and maximum, and quartiles if necessary (with geometric mean, arithmetic and geometric coefficients of variation (CV), and quartiles for PK parameters).
- Qualitative variable: sample size, absolute and relative frequencies per class

The statistical analysis will be performed separately for each part of the study.

In each part of the study, all listings will be presented by treatment group and participant.

All listings will be presented by treatment group and participant.

No interim analysis is planned.

Further details on statistical analysis will be described in the Statistical Analysis Plan, agreed by the Sponsor.

10.2.3. Description of data sets

10.2.3.1. Definition of data sets

In each part of the study, the following data sets will be defined:

The Included set will be defined as all included participants who have signed an ICF.

The Randomised set will be defined as all randomised participants.

The Safety set will be defined as all included participants who have received at least a partial dose of IMP (Ir-CPI or placebo).

The Pharmacodynamic set will be defined as all the included participants who have completed the study without any protocol deviation affecting PD evaluation and with at least one available post-baseline PD data.

The Pharmacokinetic set will be defined as all the included participants who have been administered a complete infusion of IMP without major protocol deviation affecting PK evaluation. The inclusion of the subjects with incomplete PK profile(s) or incomplete infusion will be discussed before the PK concentration dataset is locked.

The analysis sets will be validated during the blind-review meeting.

10.2.3.2. Description of the sets

A summary table with the description of the number of included participants, the number of randomised participants, the number of participants who completed the study, the number of participants who discontinued classified by reason of withdrawal, the number of participants in each analysis set will be performed by treatment group and overall. Corresponding individual listings will be prepared.

Listings with analysis sets, end of study status and visit dates will also be carried out. A specific listing with discontinued participants will be also prepared as well as a listing of participants excluded from analysis sets.

10.2.4. Demographic and baseline characteristics

The following analysis will be performed on the Randomised set.

The participants' demographic characteristics and baseline characteristics will be summarised by treatment group and overall and listed.

Tables by treatment group and overall with the number of participants having at least one medical or surgical history and a corresponding listing will be prepared.

Tables by treatment group and overall with number of participants having at least one previous treatment will be prepared. The same table will be prepared for concomitant treatments.

Details of drug dosing and meals will be listed.

10.2.5. Protocol deviations

A summary table by treatment group and overall of protocol deviations and the corresponding listing will be prepared.

10.2.6. Pharmacokinetic analysis

The analysis will be performed on the Pharmacokinetic Set.
Relevant PK parameters will be calculated for Ir-CPI by non-compartmental or compartmental methods, as appropriate for those participants with sufficient plasma and urine concentration data (as described in Section 9.3).

10.2.6.1. Plasma concentration and PK parameters

Plasma concentrations of Ir-CPI will be summarised over time by treatment group and corresponding listings will be prepared.

Time profile plots will be prepared on linear and log-linear coordinates for each participant, as well as arithmetic mean (±SEM) by treatment group.

PK parameters will be summarised by treatment group and corresponding listings will be prepared.

Scatter plots and/or box whisker plots will be generated for the comparison of C_{max}, AUC_{0-6} and AUC_{inf}.

For each part, the linear dose-proportionality of C_{max}, AUC_{0-6} and AUC_{inf} (if applicable) will be assessed for parent (and metabolites if appropriate) using an exponential regression model ("power model"). The log-transformed PK parameter will be compared among the dose groups by using a 1-factor analysis of variance (ANOVA), with log-transformed dose as a fixed effect and participant as random effect.

10.2.7. Pharmacodynamic analysis

10.2.7.1. Criteria

- PD aPTT

- Factor XI and Factor XII inhibition

10.2.7.2. Statistical methodology

Analysis of pharmacodynamic parameters will be performed on the Pharmacodynamic set. For each parameter, descriptive statistics will be provided by treatment group on values and changes from baseline for each timepoint.

All pharmacodynamic parameters will be listed.

10.2.8. Exploratory Pharmacokinetic / Pharmacodynamic analysis

As an exploratory analysis, evaluation of the relationship between doses and concentrations of Ir-CPI and the change from baseline in coagulation parameters defined above will be graphically investigated and displayed.

10.2.9. Exploratory biomarkers' analysis

10.2.9.1. Criteria

- Serum cystatin C (CysC),
- TNFα,
- hsCRP,
- D-dimers,
- Fibrinogen,
- PT,
- ADA,
- Urinary microalbumin, Kidney Injury Molecule (KIM-1), urinary neutrophil gelatinase-associated lipocalin (NGAL).

10.2.9.2. Statistical methodology

Analysis of exploratory parameters will be performed on the Pharmacodynamic set.

For each parameter, descriptive statistics will be provided by treatment group on values and changes from baseline for each timepoint. Graphs over time by treatment group will be also produced.

Presence of ADAs will be described by treatment group and timepoint until D180.

All exploratory parameters will be listed.

Supplemental ADA samplings ("long-term follow-up of ADAs"):

Results after D180 will be listed when applicable and the listing will be appended to the final CSR.

10.2.10. Safety analysis

10.2.10.1. Criteria

- Physical examination, brief physical examination,
- Vital signs and temperature
- Weight,
- Telemetry (including SpO_2),
- Occult blood in faeces (Hemoccult® test),
- Supine 12-lead ECG,

- Serum chemistry, safety coagulation, haematology, urinalysis,
- AE (including AESI),
- Exposure to tick bites.

10.2.10.2. Statistical methodology

Analysis of safety parameters will be performed on the Safety set.

AEs (including AESIs) will be considered treatment-emergent according to the rule set out in Appendix 2: Adverse event and serious adverse event definitions.

AEs (including AESIs) will be summarised by system organ class and preferred term in tables with:

- The number of participants with at least one AE and the number of adverse events by treatment group and overall,
- The number of participants with at least one treatment-emergent AE and the number of treatment-emergent AEs for each treatment group.

Analyses taking into account intensity and drug relationship to treatment will be also carried out. All AEs will be listed and coded using the Medical Dictionary for Regulatory Activities (MedDRA®) and characterized as either pre-treatment or treatment-emergent, according to the intake of the IMP.

For other parameters, the description will be performed by treatment group and measurement time.

For laboratory data, vital signs, temperature and ECG parameters, potentially clinically significant abnormalities (PCSA) will be specified in the Statistical Analysis Plan and participants with PSCA values will be summarised. The presence of orthostatic hypotension and abnormal HR increase will be derived and the number and percentage of participants with at least one orthostatic hypotension/ abnormal HR increase will be presented by treatment group and measurement time as well as a corresponding listing.

All safety parameters will be listed.

Supplemental ADA samplings (“long-term follow-up of ADAs”):

SADRs and their related information, if applicable, after D180 will only be collected in the PV database and will not be analysed.

11. REFERENCES

1. Investigator's Brochure, current version.

2. IMPD, current version.

3. EMEA Guideline on strategies to identify and mitigate risks for first-in-human and early clinical trials with investigational medicinal products; EMEA/CHMP/SWP/28367/07 Rev. 1; Jul 2017.

4. Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers, July 2005, accessed from http://www.fda.gov/downloads/Drugs/Guidances/UCM078932.pdf, on 14th May 2019

12. APPENDICES

Appendix 1: Regulatory and legal considerations

RIGHT OF ACCESS TO DATA AND SOURCE DOCUMENTS

Monitoring

The Investigator will allow the representative of the Sponsor and the study Monitor:

- To inspect the site, the facilities and the material used for the study,
- To meet all members of the team involved in the study,
- To consult all the documents relevant to the study,
- To check that the eCRFs have been correctly completed,
- To have direct access to source documents for comparison of data therein with the data in the eCRFs,
- To check that AE have been documented,
- To verify that the study is carried out in compliance with the protocol

- This study will be monitored at regular intervals, by mutual agreement of the investigator and Monitor. All or part of the data will be monitored at periodic visit to the study site, according to a monitoring plan. All entries in the e-CRFs, corrections and alterations are to be made by the responsible Investigator or his/her designee.
- Once monitored and cleaned, the paper-based data (either source or transcribed data) that have been selected for populating the database will follow a single entry process. Electronic data will be directly entered in the database.
- Details of all data management procedures, from the initial planning to the archiving of final datasets / documents following database freeze/lock will be documented in appropriate data management and validation plan(s). Among others, these procedures will also describe quality control checks, data handling process for any missing, unused or spurious data, as well as coding procedures for AE's, medical history, physical examination and medications.

All information dealt with during these visits will be treated as strictly confidential.

The Investigator will provide the Sponsor with the following:

- Screen logs and AE logs at regular intervals,
- Adequately completed eCRFs

Audit- Inspection

The Investigator will be informed that an audit will be carried out, at the request of the Sponsor, before, during or after the study.

The Investigator will be informed that the Regulatory Authorities may also carry out an inspection. In this case, the Investigator must inform the Sponsor the CRO as soon as he receives the notification of inspection.

The Investigator must allow the representatives of the Regulatory Authorities and persons responsible for the audit to:

- Inspect the site, facilities and material used for the study,
- Meet all members of his team involved in the study,
- Have direct access to study data and source documents,
- Consult all the documents relevant to the study.

QUALITY CONTROL AND QUALITY ASSURANCE

Written procedures describing data flow, data entry or electronic capture, data cleaning and processing, and required quality control must be utilized.

Trained site personnel must enter data manually into the EDC system.

The Investigator is responsible for prompt reporting of accurate, complete, and legible data in the eCRFs and in all required reports.
Any change or correction to the eCRF after saving must be accompanied by a reason for the change.
Corrections made after the Investigator's review and approval (by means of a password/electronic signature) will be reapproved by the Investigator.

The Investigator should maintain a list of personnel authorised to enter data into the eCRF.

To ensure accurate, complete, and reliable data, the Sponsor or its representative will provide instructional material to the study site(s), as appropriate. Training session will be given during a start-up/initiation meeting for instructions on the completion/data entry of any source data.

The entered data will be systematically checked by CRO (Biotrial) staff, either by using error messages printed from validation programs or by manual checks carried out based on database listings. Data checks for data consistency and plausibility must be defined and implemented in the EDC system. Queries are raised and resolved electronically within the EDC system. The Investigator or his/her designee must verify that all data entries in the e-CRFs are accurate and correct. If certain information is Not Done, Not Applicable, the Investigator must enter: ND or NA, respectively, in the appropriate field.

Every effort should be made to ensure that all safety evaluations are completed by the same individual who made the initial baseline determination.

After the completion of the data cleaning and data review process and full resolution of the data queries, the database will be locked. Any changes to the database after that time may only be made by joint written agreement between the Clinical Pharmacologist and the Investigator.

The Investigator must guarantee the safety of the study data in the medical files by implementing security measures to prevent unauthorised access to the data and to the computer system.

The Investigator should not deviate from the protocol. However, the Investigator should take any measure necessary in deviation from or not defined by the protocol in order to protect

study participants from any immediate hazard to their health and safety. In this case, this action should be taken immediately, without prior notification of the regulatory authority, IEC, or Sponsor.
After implementation of such measure, the Investigator must notify Biotrials PM and the Sponsor within 24 hours and follow any local regulatory requirements.

Participants will be assigned a unique identifier by the sponsor. Any participant records or datasets that are transferred to the sponsor will contain the identifier only; no names will be transferred and records will use unique identifier with no information that reveals the identity of the participant. The participant must be informed that his/her personal study-related data will be used by the Sponsor in accordance with local data protection law. The level of disclosure must also be explained to the participant. The participant must be informed that his/her medical records may be examined by Clinical Quality Assurance auditors or other authorised personnel appointed by the Sponsor, by appropriate IRB/IEC members, and by inspectors from regulatory authorities.

Investigator's Regulatory Obligations

All clinical work under this protocol will be conducted according to GCP rules. This includes that the study may be audited at any time by a Quality Assurance personnel designated by the Sponsor, or by regulatory bodies. The Investigator must adhere to the GCP principles in addition to any applicable local regulations.

If requested, the Investigator will provide the Sponsor, applicable regulatory agencies, and applicable Ethics Committee with direct access to any original source documents.

The Investigator should demonstrate due diligence in recruitment and screening of potential study participants. The enrolment rate should be sufficient to complete the study as agreed with the Sponsor. The Sponsor should be notified of any projected delays, which may impact the completion of the study.

Record Keeping

The Investigator will keep records of all original source data. This might include laboratory tests, medical records, and clinical notes.

Depending on the data collected and the type(s) of data, records will be kept for 25 years following storage, and archiving procedures agreed by the Sponsor and the Investigator site and any other parties involved in this study, and in accordance to any regulations that are applicable in the country where the study is conducted.

Regulatory Considerations

This study will be conducted in accordance with applicable laws and regulations, GCPs, GDPR and the ethical principles that have their origin in the Declaration of Helsinki.

All or some of the obligations of the Sponsor will be assigned to a Clinical Research Organisation (CRO).

An identification code assigned to each participant will be used in lieu of the participant's name to protect the participant's identity when reporting AEs and/or other trial-related data.

Final Report Signature

The Investigator or designee will sign the CSR for this study, indicating agreement with the analyses, results, and conclusion of the report.

Insurance

The Sponsor declares that an insurance policy will be in place covering the participants in respect to risks involved in the study.

Financial Agreement

A financial agreement between the Sponsor and the Investigator will be signed prior to initiating the study.

STUDY SUSPENSION, TERMINATION, AND COMPLETION

The Sponsor may suspend or terminate the study or any part of the study at any time for any reason, and has an obligation to report stopping of the study to both EC/CA.

If the Investigator suspends or terminates the study, the Investigator will promptly inform the Sponsor, the IEC and the regulatory authorities and provide them with a detailed written explanation. The Investigator will also return all test articles, test article containers, and other study materials to the Sponsor.

Upon study completion, the Investigator will approve and will provide the Sponsor and IRB/IEC with final reports and summaries as required by regulations.

Additional conditions, besides the discovery of an unexpected, relevant, or unacceptable risk to the participants enrolled in the study, that may warrant termination of the study include, but are not limited to:

- Other ethical issues
- Inaccurate or incomplete data recording
- Noncompliance
- Unsatisfactory enrolment with respect to quality or quantity
- A decision of the Sponsor to suspend or discontinue development of the IMP

If the study is prematurely discontinued, all study data must be returned to the Sponsor. In addition, arrangements will be made for all unused investigational product(s) in accordance with the Sponsor’s applicable procedures for the study. Financial compensation to the investigator and participant indemnification will be in accordance with the agreement established between the investigator and the Sponsor.

ETHICS AND REGULATORY ASPECTS

Current texts

The investigator will ensure that this study is conducted in full conformity with the current revision of the Declaration of Helsinki, with the International Conference for Harmonisation Good Clinical Practice (ICH-GCP) regulations and guidelines, and with local rules and regulations of the country where the study is conducted, whichever affords the greater protection to the patient. In particular, the study will be carried out in accordance with:

- The most recent recommendations of the World Medical Association (WMA).
- The ICH recommendations: Good Clinical Practice [E6 (R2)], (CPMP/ICH/135/95), 2016.
- Directive 2001/20/EC translated in Belgian Law of 07 May 2004 with regards to the experiments on the human person.
- The Belgian Law of 8 December 1992 relative to the protection of privacy in relation to the processing of personal data, the Belgian Law of 22 August 2002 on patient rights and the GDPR (EU) 2016/679.
- European directive 2005/28/EC translated in Belgian Law of 18 May 2006 setting the GCP for biomedical research on drugs for human use.
- EMEA Guideline on strategies to identify and mitigate risks for first-in-human and early clinical trials with investigational medicinal products; EMEA/CHMP/SWP/28367/07 Rev. 1; Jul 2017.

The Investigator will supply the following documents to the relevant Ethics Committee for review and approval to conduct the study (this list may not be exhaustive):

- Protocol approved by the Sponsor,
- Currently applicable Investigator's Brochure or package labelling and updates during the course of the study,
- Informed consent document(s),
- Relevant Investigator's curriculum vitae,
- Participant information and informed consent form,
- Recruitment materials (advertisements).

The EC will review all submission documents as required, and the EC decision on the conduct of the study will be made in writing to the Investigator. This document must be dated and clearly identify the version number and date of the documents submitted.

The study may begin at the investigative site only after receiving the dated and signed documentation of the EC approval of the protocol and the informed consent documents.

During the study the following documents will be sent to the EC for their information, or for review and approval: (1) update(s) to the Investigator's Brochure; (2) reports of SAEs; (3) all substantial protocol amendments and revised informed consent(s), if any.

At the end of the study, the Investigator will notify the EC about the study completion.

Participant Information and Consent

An unconditional prerequisite for a participant's participation in the trial is his written informed consent. The participant's written informed consent to participate in the trial must be given before any trial-related activities are carried out.

Participants will be verbally informed by an investigator of all pertinent aspects of the trial: the nature of the study, its aim, its possible risks and restrictions, its duration and the fee that they will receive. The protocol will be explained during a meeting prior to the study and each participant must be informed that participation in the study is voluntary and that they may withdraw from the study at any time. At this meeting, an information sheet will be given to each participant. The language used in doing so must be chosen so that the information can be fully and readily understood by lay persons.

The Investigator is responsible for ensuring that the participant understands the potential risks and benefits of participating in the study, including answering, orally and/or in writing, to any questions the participant may have throughout the study and sharing any new information that may be relevant to the participant's willingness to continue his or her participation in the study in a timely manner.

The participant information sheet and consent document will be used to explain the potential risks and benefits of study participation to the participant in simple terms before the participant is entered into the study, and to document that the participant is satisfied with his or her understanding of the study and desires to participate. The participant information sheet and consent document will also contain information related to Article 13 of the General Data Protection Regulation (GDPR) to comply with the transparency agreement. The Investigator is ultimately responsible for ensuring that the EC-approved informed consent is appropriately signed and dated by each participant prior to the performance of any study procedures. Informed consent obtained under special circumstances may occur only if allowed by local laws and regulations.

The completed "participant screen log" will be signed by the Investigator to attest that consent has been obtained from all participants.

Whenever important new information becomes available that may be relevant to the participant's consent, the written participant information sheet and any other written information provided to participants will be revised by the Sponsor and be submitted again to the IEC/IRB for review and favorable opinion/authorisation. The agreed, revised information will be provided to each participant in the trial for signing and dating. The Investigator will explain the changes from the previous version.

Participant card

A participant card will be provided to each participant that is registered in the study. The information to be provided on the participant card will include the contact details of the treating physician/hospital and any other relevant study-related information

Submission to the authorities

Each clinical trial protocol is identified at European level with a unique number. This number has to be asked by the requesting company in the EudraCT database before submission of any demand. The European application form, which is part of the dossier, has also to be completed in the EudraCT database.

A clinical trial can only start after receiving a favourable opinion from the recognized Ethics Committee and if the relevant authority (FAMHP: R&D) has not indicated any major

insufficiency within the legal timeframe provided for in the law dated 7th May 2004 related to experiments on human people.

An application for the FAMHP is a full electronic file and a cover letter. The application must be sent via CESP.

DATA PROCESSING AND ARCHIVING OF DOCUMENTS AND DATA RELATIVE TO THE RESARCH

After the study, the Investigator will keep all information relevant to the study for 25 years, or according to the Sponsor's procedures, whichever is longer.
The Investigator will contact the Sponsor for authorization prior to the destruction of any study records or in the event of accidental loss or destruction of any study records.
The Investigator will also notify the Sponsor should he/she relocate or move the study-related files to a location other than that specified in the Sponsor's study master file.

CONFIDENTIALITY AND AGREEMENTS

Before starting the study, the Investigator must confirm receipt of adequate documentation from the Sponsor so as to be able to decide whether or not to perform the study.

All documents and information given to the Investigator by the Sponsor with respect to the study are strictly confidential.

The Investigator and his colleagues agree to use them only with the framework of this study, in order to carry out the protocol. This agreement is binding as long as the confidential information has not been disclosed to the public by the Sponsor.

The Investigator may use the technical protocol to obtain the informed consent of study participants. It must not be disclosed to other parties without the written authorisation of the Sponsor.

The Investigator keeps a confidential participant identification list for the study to permit easy identification of each participant during and after the study. This document will be reviewed by the sponsor site contact for completeness.

The participant identification and enrollment log will be treated as confidential and will be filed by the investigator in the study file. To ensure participant confidentiality, no copy will be made. All reports and communications relating to the study will identify participants by initials and/or assigned number only.

The Investigator must also complete a participant-screening log, which reports on all participants who were seen to determine eligibility for inclusion in the study.

The Investigator must maintain source documents for each participant in the study.

Data on participants collected in CRFs during the study will be documented in an anonymous fashion. All information in the eCRFs must be traceable to these source documents.

REPORT AND PUBLICATION

Report

The results of the study will be reported in a CSR. This report will be prepared by Biotrial according to existing Standard Operating Procedures (ICH Biotrial or Sponsor own format).

In compliance with the regulations, the final report will be produced within one year of completing the study and in agreement with the work order. The final CSR will include the data up to the D180 visit date, and when all results from the additional ADA samplings are collected, the final CSR will be amended to add the ADA results as an additional appended listing.

The final report will be provided to all Investigators having included participants in the study.

The Clinical Study Report will be provided as Word and PDF files. Also, SAS transfer files of the data will be provided electronically. All data will be presented CTD-compliant.

Publication

The Investigator will only use the information in the context of the study. The information cannot be used without the Sponsor's authorisation. Hence, all or part of the information should only be divulged, submitted for publication or claimed for industrial proprietary act with the written consent of the Sponsor.

Appendix 2: Adverse event and serious adverse event definitions

Definitions

- **Adverse Events**

An adverse event (AE) is any untoward medical occurrence in a participant to whom a medicinal product is administered and which does not necessarily have a causal relationship with this treatment.

An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of a medicinal product, whether related to the medicinal product or not.

An AE includes, but is not limited to, the following:

- Any clinically significant worsening of a pre-existing condition,
- An AE occurring from overdose of an IMP, whether accidental or intentional. Overdose is a dose greater than specified in the protocol,
- An AE occurring from abuse (e.g., use for nonclinical reasons) of an IMP,
- An AE that has been associated with the discontinuation of the use of an IMP.

Laboratory/ECG/vital signs abnormalities should not be documented as AEs unless they are considered clinically relevant, require treatment, fulfil any serious AE (SAE) criterion, or cause the participants to change the study schedule.

In the case of laboratory/ECG abnormalities that are a sign of a medical condition, the condition should be reported as an AE and not the sign.

Events occurring in participants in the course of a clinical study during treatment-free periods or on treatment with placebo or a comparative medicine are also to be considered AEs.

- **Adverse Event of Specific Interest**

For the purpose of this study, AEs related to bleeding (e.g. positive Hemoccult® testing, gingival bleeding, bruising/haematoma, conjunctival bleeding, prolonged bleeding after trauma) will be considered as Adverse Events of Specific Interest (AESIs).

In case of extreme bleeding or emergencies, Fresh Frozen Plasma can be tried to mitigate the bleeding. Dialysis will eliminate the protein from the body.

Also considered as AESI are all infusion site reactions, including hypersensitivity reactions that would occur during or after study drug administration.

- **Adverse Drug Reactions**

An Adverse Drug Reaction (ADR) is a response to a drug that is noxious and unintended and that occurs at doses normally used in man for prophylaxis, diagnosis, or therapy of disease or for modification of physiological function.

All AEs judged by either the reporting Investigator or the Sponsor as having a reasonable causal relationship to a medicinal product qualify as an ADR.

All AEs for which the judgement of relationship to study medication is “possible” or higher will be considered ADRs.

- **Treatment-Emergent Adverse Events**

A treatment-emergent AE is an AE that occurs after IMP dosing or that was present prior to dosing but becomes exacerbated between dosing during a treatment period and the end of the study.

- **Abuse, Misuse, Medication Error and Overdose**

Abuse is defined as persistent or sporadic, intentional excessive use of IMP which is accompanied by harmful physical or psychological effects.

Misuse is defined as situations where an IMP is intentionally and inappropriately used not in accordance with the terms of the study protocol.

Medication error is defined as an unintended failure in the IMP administration process that leads to, or has the potential to lead to, harm to the participant.

Overdose is defined as intake of a quantity of an IMP given per administration or cumulatively which is above the maximum dose recommended in the study protocol.

Serious Adverse Events or Serious Adverse Drug Reactions

- **General definitions**

A Serious Adverse Event (SAE) is any untoward medical occurrence that at any dose:

- results in death,
- is life threatening,
- requires inpatient hospitalisation or prolongation of existing hospitalisation*,
- results in persistent or significant disability/incapacity,
- is a congenital anomaly/birth defect, or
- is an important medical event that requires intervention to prevent one of the above.

** "Inpatient hospitalisation" is defined as 24hours in the hospital or an overnight stay.*

Additionally, a Serious Adverse Drug Reaction (SADR) is a SAE that occurs after IMP administration and is considered to have any potential causal relationship with the IMP according to the Investigator or the Sponsor.

Additionally, important medical events that may not result in death, be life-threatening, or require hospitalisation may be considered SAEs when, based on appropriate medical judgment, they may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in hospitalisation, or development of drug dependency or drug abuse.

Life-threatening refers to immediate risk of death as the event occurred per the reporter. A life-threatening experience does not include an experience, had it occurred in a more severe form, might have caused death, but as it actually occurred, did not create an immediate risk of death. For example, hepatitis that resolved without evidence of hepatic failure would not be considered life-threatening, even though hepatitis of a more severe nature can be fatal. Similarly, an allergic reaction resulting in angioedema of the face would not be life-threatening, even though angioedema of the larynx, allergic bronchospasm, or anaphylaxis can be fatal.

Hospitalisation is official admission to a hospital. Hospitalisation or prolongation of hospitalisation constitutes criteria for an AE to be serious; however, it is not in itself considered a serious adverse event (SAE). In absence of an AE, hospitalisation or prolongation of hospitalisation should not be reported as an SAE. This is the case in the following situations:

- The hospitalisation or prolongation of hospitalisation is needed for a procedure required by the protocol.
- The hospitalisation or prolongation of hospitalisation is part of a routine procedure followed by the centre (e.g., stent removal after surgery). This should be recorded in the study file.

In addition, hospitalisation for a pre-existing condition that has not worsened does not constitute an SAE.

Disability is defined as a substantial disruption in a person's ability to conduct normal life functions.

If there is any doubt about whether the information constitutes an SAE, the information is treated as an SAE.

Other Reportable Information: Certain information, although not considered an SAE, must be recorded, reported, and followed up as indicated for an SAE. This includes:

- Overdose of an investigational product as specified in this protocol with or without an AE.
- Inadvertent or accidental exposure with or without an AE.

- **Expected and unexpected adverse events**

Expected AEs with the IMP are those described in the current Investigator's brochure [1]. An unexpected AE is an experience not previously reported in or the nature or severity of which is not consistent with the current prescribing information. **Suspected Unexpected Serious Adverse Reactions (SUSARs)** are unexpected SAEs having a reasonable possibility of a causal relationship with the study drug.

- **Adverse Event of Special Interest**

Adverse Events of Special Interest (AESIs) are AEs (serious or non-serious) that need to be monitored, documented, and managed in a pre-specified manner. For the tested drug, AEs related to bleeding (e.g. positive Hemoccult® testing, gingival bleeding, bruising/haematoma, conjunctival bleeding, prolonged bleeding after trauma), and all infusion site reactions, regardless of causality, including hypersensitivity reactions that would occur during or after study drug administration, will be considered as AESIs.

Participants that report an AESI will be followed until stabilisation of the symptoms.

- **Severity**

The maximum intensity of an AE during a day should be graded according to the definitions below and recorded in details as indicated on the eCRF. If the intensity of an AE changes over a number of days, then separate entries should be made having distinct onset dates.

1. Mild: AEs are usually transient, requiring no special treatment, and do not interfere with patient's daily activities.
2. Moderate: AEs typically introduce a low level of inconvenience or concern to the patient and may interfere with daily activities, but are usually ameliorated by simple therapeutic measures.
3. Severe: AEs interrupt a patient's usual daily activity and traditionally require systemic drug therapy or other treatment.

- **Causal relationship with trial medication**

The relationship of an AE to the investigational product is a clinical decision by the Investigator based on all available information at the time of the completion of the eCRF and is graded as follows:

1. **Not related (no reasonable possibility):** a reaction for which sufficient information exists to indicate that the etiology is unrelated to the IMP; the participant did not receive the study medication or the temporal sequence of the AE onset relative to administration of the study medication is not reasonable or the event is clearly related to other factors such as the participant's clinical state, therapeutic intervention or concomitant therapy.
2. **Unlikely (reasonable possibility):** a clinical event, including a laboratory test abnormality, with a temporal relationship to drug administration which makes a causal relationship improbable and in which other drugs, chemicals or underlying disease provide plausible explanations.
3. **Possible (reasonable possibility):** a clinical event, including a laboratory test abnormality, with a reasonable time sequence to administration of the drug but which could also be explained by concurrent disease or other drugs or chemicals, information on drug withdrawals may be lacking are unclear.
4. **Probable (reasonable possibility):** a clinical event, including a laboratory test abnormality, with a reasonable time sequence to administration of the drug, unlikely to be attributed to concurrent disease or other drugs or chemicals and which follows a clinically reasonable response on withdrawal (de-challenge): re-challenge information is not required to fulfil this definition.
5. **Definite (reasonable possibility):** a reaction that follows a reasonable temporal sequence from administration of the drug or in which the drug level has been established in body fluids or tissues, that follows a known or expected response pattern to the suspected drug, and that is confirmed by improvement on stopping or reducing the dosage of the drug, and reappearance of the reaction on repeated exposure (re-challenge).