BZ-371 – SUPPORTIVE THERAPY TO MAINTAIN PENILE INTEGRITY IN PROSTATE CANCER PATIENTS SUBMITED TO RADICAL PROSTATECTOMY

BZ371CLI003 STUDY
(NCT05332340)

"Phase 1 Clinical Trial to evaluate safety and pharmacokinetic of BZ371A in a gel form applied in the genitalia of healthy men and women”

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VERSION CONTROL

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IR	1.0	11/09/2021	Document revision
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IR	2.0	05/26/2022	Review according to ANVISA requirements: addition of new inclusion and exclusion criteria, method for evaluating causality, explanation and information about the code that will be generated for participants, a section on special interest adverse events, notification of serious adverse events to ANVISA, a subsection on regulatory agency audits, and clarification on the qualitative analysis of adverse events.
IR	3.0	08/30/2022	Update of the analytical laboratory hired to perform pharmacokinetic analysis; revision according to suggestions from the principal investigator: better description of the procedures and timepoints for blood collection regarding laboratory and pharmacokinetic exams; update of an exclusion criteria (item 1) by adding pregnant women (confirmed by β-HCG test); addition of β-HCG test at visit V-1; correction of the visit number in which the ECG will be collected after application of the IP; better definition of vital sign measurement times; description addition regarding unscheduled visits; and addition of a section regarding participant's feeding.
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IR: Internal Revision

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I have read this project, the experimental protocol and the Informed Consent Form and I agree to conduct this study in compliance with the protocol, Resolution nº 466 of December 12, 2012, Resolution nº 251 of August 7, 1997 of the National Health Council - Ministry of Health, and according to the Helsinki Declaration (1964) and the revisions of Tokyo (1975), Venice (1983), Hong Kong (1989), Somerset West (1996), Edinburgh (2000), Tokyo (2004) and Seoul (2008).

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PHASE I SYNOPSIS

TITLE	Phase 1 clinical study to evaluate the pharmacokinetics and safety of BZ371A in gel form applied to the genitals of healthy men and women.
Study Identification	BZ371CLI003
Study description	Single dose, single center, non-blinded
Dosage and Administration	BZ371A, at a concentration of 5 mg/mL, applied to the genital area (entire penis including glans, clitoris, labia majora and minora) in a volume of 1.5 mL. The genital area should be washed with water and mild soap beforehand, followed by drying. The product should be applied using gloves.
Objectives	• Determine safety and tolerability of BZ371A qhen topically Applied to genitália of healthy men and women • Evaluate systemic presence of BZ371A, or its fragments, through a pharmacokinetics study using blood, when BZ371A is topically applied.
Outcomes	• Safety ○ Adverse Events Report ○ Physical Exam in the genital region ○ Vital signs, including heart rate and systolic blood pressure ○ Electrocardiogram (ECG) ○ Laboratorial Exam, including (i) complete blood count, including platelets; (ii) coagulation profile, including prothrombin time (PT) and activated partial thromboplastin time (aPTT); (iii) biochemistry, including glucose, sodium, potassium, urea, creatinine, chloride, total calcium, phosphorus, total proteins and fractions (albumin and globulin), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase, gamma-glutamyl transferase (GGT), total cholesterol, HDL cholesterol, triglycerides, pH; (iv) urinalysis for abnormal elements and sediment (AES). • PK analysis ○ Detection of BZ371A and its fragments in the blood at the following collection times: pre-dose, 15, 30, 60, 180, and 360 minutes. ○ If detected, determine the pharmacokinetic parameters: maximum concentration (Cmax), half-life (t1/2), area under the curve (AUC), clearance (CL), distribution volume (Vd).
Study Population	Healthy research participants, without any injuries that could compromise the study results. Inclusion Criteria: 1. Men or Woman older than 18 years old; 2. Body Mass Index $\geq$ 19 and $\leq$ 28,5 Kg/m^2; 3. Ability to understand the Informed Consent Form (ICF). Exclusion Criteria: 1. Women who are menstruating or pregnant (confirmed by a positive β-HCG test); 2. Diseases that interfere with drug absorption, distribution and excretion, such as a history or presence of liver or kidney diseases; 3. Presence of genital lesions or active sexually transmitted diseases (such as herpes, gonorrhea, candidiasis, HPV, among others) that impair local adverse effects analysis in the genital area; 4. Presence of lesions that may compromise the study outcome or diseases that may compromise their safety; 5. Use of topical medications in the genital area that may interfere with the evaluation of the experimental drug; 6. History of symptomatic hypotension or diseases that increase the risk of symptomatic hypotension, such as patients with heart disease (including a history of angina and/or heart failure) and kidney disease; 7. Findings on ECG and/or laboratory tests that, at the investigator's discretion, are considered significant and pose a risk to the research volunteer's participation or may interfere with the study's analyses;

	8. Blood pressure (BP) outside the safe limits: systolic BP less than 90 mmHg or greater than 140 mmHg; or diastolic BP less than 60 mmHg or greater than 90 mmHg, except in situations such as "white coat syndrome". 9. Any disease or finding in which the investigator considers significant, and/or that increases the volunteer risk on participating in the trial or that could interfere with the results. The research participant will be eligible if they meet all inclusion criteria and none of the exclusion criteria.
Number of participants	Total of 12 research participants, 6 men and 6 women.
Study duration	The study will have three visits and 7 days of duration for each participant.

ABREVIATIONS

°C: degrees celsius

ADR: Adverse Drug Reaction

AE: Adverse events

AES: Abnormal Elements and Sediments

ALT: pyruvate transaminase

ANVISA: *Agência Nacional de Vigilância Sanitária*

API: Active Pharmaceutical Ingredient

Art.: Article

AST: oxalacetic transaminase

AUC: area under the curve

BMI: body mass index

BP: Blood pressure

CL: *clearance*

Cmax: maximum concentration

CONEP: *Comissão Nacional de Ética em Pesquisa*

CONITEC: *Comissão Nacional de Incorporação de Tecnologias no Sistema Único de Saúde*

CPC: Chinese Peptide Company

CRF: Clinical Report File

D0: Study initiation day

D-1: Screening day

D7: Safety evaluation after study (7 after study initiation)

DBP: Diastolic Blood Pressure

EC: Ethics Committee

ECG: Electrocardiogram

ED: Erectile Dysfunction

GCP: Good Clinical Practice

GMP: Good Manufacture Practice

GOT: glutamic oxaloacetic transaminase

GT: glutamyl transpeptidase

HDL: high density lipoprotein

HPV: Human Papillomavirus

HR: heart rate

ICF: Informed Consent Form

ICH: International Council Harmonization

INCA: *Instituto Nacional do Câncer*

iNOS: inducible nitric oxide synthase

IP: investigational product

IU: international units

Kg: kilogram

m: meter

mg: milligram

ml: milliliter

mM: millimolar

mmHg: milligrams of mercury

nNOS: neuronal nitric oxide synthase

NO: nitric oxide

NOS: nitric oxide synthase

PDE5i: phosphodiesterase 5 inhibitor

PK: pharmacokinetics

POT: piruvic oxaloacetic transaminase

PTT: Part time tromboplastine

RDC: *Resolução da Diretoria Colegiada*

RP: radical prostatectomy

RR: respiratory rate

SAE: Serious Adverse Events

SBP: Systemic Blood Pressure

SMC: smooth muscle cells

SOP: Standard Operational Procedure

STD: sexually transmitted diseases

T ^{o}C: temperature

t1/2: half-life

TAP: prothrombin activation time

V0: study visit

V1: post study safety visit

V-1: screening visit

Vd: distribution volume

WHO: world health organization

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1. INTRODUCTION

1.1 Medical need and study justification

Apart from non-melanoma skin cancer, which is of very low aggressiveness, prostate cancer is the most prevalent cancer in men. In Brazil, an estimated 65,840 new cases are expected each year, corresponding to an estimated risk of about 63 new cases per 100,000 men (INCA, 2021). This number has been progressively increasing due to higher diagnosis rates and longer life expectancy. The advancement of early diagnosis and treatment, both medicinal and surgical, has resulted in relatively low mortality, despite significant prevalence. In Brazil, there were about 16,000 deaths in 2019 (INCA, 2021). This means that the risk of a man having prostate cancer in Brazil is 16%, but the risk of dying from this cancer is 3.4% (CONITEC, 2018). However, this technological advancement and longer patient survival has also resulted in significant morbidity.

One of the most recent technological advances refers to the radical prostatectomy (RP) techniques. This surgical procedure is the complete removal of the prostate, including the prostatic urethra, seminal vesicles and *ampullae* of the *vas deferens*, with or without bilateral lymphadenectomy. It is currently considered the gold standard for the treatment of non-metastatic prostate cancer (CONITEC, 2018). The surgical approach can be perineal, retropubic, or laparoscopic; and can also be robotically assisted or not (CONITEC, 2018).

RP, although curative for prostate cancer, can result in damage to the cavernous nerves and thus, result in erectile dysfunction (ED). The cavernous autonomic nerves travel posterolateral to the prostate, entering the penis, and regulating blood flow and consequently erection.

In the past, virtually all men undergoing RP had permanent ED. With the introduction of the "nerve-sparing" technique, possibly depending on the size and local invasion of the tumor, this number has decreased. However, in any type of RP, even robotically assisted, the surgical manipulation of the neurovascular bundles causes a neuropraxia, and the vast majority of men lose complete sexual function immediately after RP, regardless of whether the nerves were spared or not. The return of the erectile function will be determined by a series of factors such as age, type of surgery, sexual function pre-surgery (Gabrielsen, 2018; Salonia, 2016).

Some authors consider it almost impossible for men to return to the same preoperative erectile function, except in rare cases - only 6.7% of patients undergoing RP report that their erection was comparable to before the surgery (Vaznesensky, 2016). However, even inferior to preoperative performance, some can recover erectile function, which is defined as the ability to have an erection sufficient for penetration. This recovery is slow, usually occurring between 6 and 48 months. However, 30 to 60% of RP patients never regain sexual function (Salonia, 2016; Rambhatla, 2008).

The definitive loss of sexual function is explained by the loss of penile mass, caused by the lack of blood flow. The preservation of nocturnal erections is a good sign of returning to some sexual function.

However, no existing treatment method is able to preserve penile integrity. Due to the lack of therapeutic options, phosphodiesterase 5 inhibitors (PDE5i), such as Viagra® and Tadalafil®, have been used, but without definitive scientific evidence to support their use. PDE5i acts by maintaining the relaxation of smooth muscle cells (SMCs) (and consequently blood flow) initiated by nitric oxide (NO). With the neuropraxia caused by RP, NO is not released and, therefore, PDE5i are not able to act.

BZ371A is a topical agent that increases nitric oxide synthase (NOS), an enzyme responsible to produce NO. BZ371A is a synthetic peptide composed of 19 amino acids, derived from the venom of the Brazilian spider *Armadeira* (Phoneutria nigriventer), found near banana trees (Silvia, 2015). When injected into small boys, the venom of this spider causes priapism, a strong and painful involuntary erection (Shigehara 2016). However, in addition, the natural venom causes vascular congestion in various organs. Based on bioinformatics study, BZ371A was developed and modified to exclude deleterious effects, maintain the vasodilator effect, and at the same time make its degradation more difficult.

BZ371A increases the expression of inducible NOS (iNOS), an enzyme that produces NO and is present in all cells, including the smooth muscle cells of the corpus cavernosum, which need to be distended to achieve an erection. It also increases the action of neuronal NOS (nNOS), present in the nerve endings of the penis. By acting independently of the autonomic nerves and the integrity of the pelvic vessels, BZ371A can act in prostatectomized patients, increasing local NO production and preventing penile mass loss. This combined dual action allows for a rapid onset and long-lasting effect necessary for the treatment of prostatectomized patients.

In addition, by increasing NO, BZ371A will present a synergistic effect with PDE5i, allowing this class of drugs to perform their action of maintaining blood flow, once stimulated by NO.

According to preclinical studies, BZ371A is a gel, considered safe, practical, and tolerable for topical use. In these studies, when applied topically, BZ371A did not show systemic bioavailability and, therefore, did not cause typical side effects of systemic vasodilators, such as arterial hypotension.

The aim of this study is to confirm whether BZ371A topically applied, is safe, tolerable, and does not show systemic bioavailability in humans.

2. STUDY GOALS

2.1 Primary goals

The objectives of this study are:

- To determine the safety and tolerability of BZ371A when topically applied to the genitals of men and women;

- To determine the systemic presence of BZ371A and/or its fragments, through a pharmacokinetic study in the blood, when topically applied to the genitalia of men and women.

2.2 Endpoints

For safety and tolerability analysis of BZ371A, the following outcomes were considered:

- Report of adverse events (AE);
- Local physical examination of the genital area;
- Vital signs, including systemic blood pressure (BP) and heart rate (HR);
- Electrocardiogram (ECG);
- Laboratory tests, including (i) complete blood count, including platelets; (ii) coagulation profile, including prothrombin time (PT) and activated partial thromboplastin time (aPTT); (iii) biochemistry, including glucose, sodium, potassium, urea, creatinine, chloride, total calcium, phosphorus, total protein and fraction (albumin and globulin), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase, gamma-glutamyl transferase (GGT), total cholesterol, HDL cholesterol, triglycerides, and pH; (iv) urinalysis for abnormal elements and sediments (EAS).

For pharmacokinetics analysis of BZ371A, the following outcomes will be considered:

- Detection of BZ371A and its fragments in the blood at the following timepoints: pre-dose, 15, 30, 60, 180, and 360 minutes;
- If detected, determination of the pharmacokinetic parameters: maximum concentration (Cmax), half-life (t1/2), area under the curve (AUC), clearance (CL), volume of distribution (Vd).

3. STUDY PLAN

3.1 General study design

Open-label study, conducted at a single center, with the aim of being the first human trial with the new BZ371A formulation in order to guide future clinical studies such as to understand safety, tolerability, and systemic bioavailability of the peptide topically applied.

The study will comprise only one group, consisting of 6 men and 6 women.

The formulation will be administered in a single dose, topically, followed by medical and laboratory analyses for safety assessment, and blood collections for pharmacokinetic analysis.

The concentration of BZ371A and its fragments in the blood will be measured by an appropriate analytical method, developed for this purpose and previously validated. The collected blood will be

analyzed by the company *Unidade Integrada de Farmacologia e Gastroenterologia* (UNIFAG), the same company that validated the analytical method, and will not be stored or used for any other purpose.

The visits and procedures are illustrated in the table below:

CHART 1. VISITS AND BZ371CLI003 STUDY PROCEDURES

Visit	V-1	V0	V1
Day	D-1	D0	D7
Interval	-7 a 0	0	+2 a -2
Visit Name	Screening	PK	Safety revision
ICF	X		
Demogrphic data	X		
Medical history	X	X	
Physical exam	X		X
Ganitalia exam	X	X / X (*)	X
Antropometric data	X		
Vital Signs	X	X / X (**)	X
Concomitant medication	X	X	X
Blood collection for laboratory analysis	X	X (*)	
Urine collection	X	X (*)	
Electrocardiogram	X	X (*)	X
Elegibility	X	X	
Blood sample collection for PK		X	
BZ371A application		X	
AE collection		X	X
Next visit schedueling	X	X	
Study dismissal			X

*: after 360 minutes after IP application

**: after IP application at 30, 60, 180 and 360

At the screening visit (V-1), the first procedure will be the administration of the Informed Consent Form (ICF). Subsequently, procedures that will support determining participant eligibility will be performed, including the collection, recording of demographic, medical history, and concomitant medication data; physical examination including genital examination, anthropometric data and vital signs; collection of blood and urine for laboratory analysis; and ECG. The participant will be scheduled for the next visit, which must occur within one week.

At visit V0, the actual study visit, participant eligibility for the study will be confirmed by reanalyzing medical history and concomitant medication history. Before administration of the investigational product (IP), vital signs should be collected and recorded. After administration of the IP, serial collections for pharmacokinetic analysis will be performed; blood and urine will be collected for laboratory analysis; an electrocardiogram will be performed; vital signs and genital region analysis will be

conducted. Any adverse events (AEs) should be recorded. The participant will be scheduled for the next visit, which should occur within 5 to 9 days after V0.

Visit V1 aims to evaluate participant safety after participation in the study. At this visit, a physical examination, including genital examination and vital signs, should be performed. In addition, it should also be confirmed the use of medication used during study period and AEs should be registered. If there are no complaints, they should be instructed of the end of the study.

Unscheduled visits may be made for orientation, treatment, and evaluation of discontinuation criteria, at the Investigator's discretion in the event that a participant presents any significant sign or symptom, as well as AEs, that result in a return to the Research Center before regular visits. Unscheduled visits should be numbered according to the last regular study visit, for example, if one of these visits is performed after Visit 1, it will be considered as Visit 1.1, and so on until the next regular visit is conducted. All information collected during an unscheduled visit should be recorded in source documents and in the individual Clinical Record of each research participant. If the unscheduled visit results in discontinuation of the participant from the clinical protocol study or interruption of the use of the investigational product, it will be considered a study discontinuation visit and all procedures related to the final study visit should be performed.

3.2 Study location

The study will be conducted at a single center, at the headquarters of Azidus Brasil Pesquisa Científica e Desenvolvimento, located in the city of Valinhos. Azidus Brasil is a Clinical Research Center that has a highly qualified team with 20 years of experience in developing drugs and health products. It has certified and equipped units for conducting Phase I clinical research studies. Participants will receive specialized assistance and care throughout the treatment period of this protocol.

4. STUDY POPULATION

4.1 Participant eligibility

The study group will consist of 12 healthy adults, 6 males and 6 females, with no restrictions on ethnicity, social class, or group. Participants should not have injuries that could compromise the study results, nor diseases that could compromise their safety.

4.1.1 Inclusion Criteria

1. Men or women above 18 years of age;
2. Body mass index (BMI) > 19 and < 28.5 kg/m2;

3. Ability to understand the informed consent form.

4.1.2 Exclusion Criteria

1. Women who are menstruating or pregnant (confirmed by a positive β-HCG test);
2. Diseases that interfere with the absorption, distribution, and excretion of drugs, such as a history or presence of liver or kidney diseases;
3. Presence of genital lesions or active sexually transmitted diseases (STDs) (such as herpes, gonorrhea, candidiasis, HPV, among others) that impair the analysis of local adverse effects on the genitalia;
4. Presence of lesions that may compromise the study results, or diseases that may compromise their safety;
5. Use of topical medications in the genital region that may interfere with the evaluation of the experimental medication;
6. History of symptomatic hypotension, or diseases that increase the risk of symptomatic hypotension, such as patients with heart disease (including a history of angina and/or heart failure) and kidney disease;
7. Findings on ECG and/or laboratory tests that, at the investigator's discretion, are considered significant and pose a risk to the research participant's participation or may interfere with the study's analyses;
8. Blood pressure (BP) outside the considered safe limits: systolic BP less than 90 mmHg or greater than 140 mmHg; or diastolic BP less than 60 mmHg or greater than 90 mmHg, except in situations such as "white coat syndrome."
9. Any disease or condition or physical finding that the investigator considers significant and that increases the risk of the research participant's participation or may interfere with the results.

The research participant will be eligible if they meet all inclusion criteria and no exclusion criteria.

4.2 Screening and recruitment

The study participants will be recruited from the healthy volunteer database from the Clinical Research Center. They will be invited to come to the center, where the first mandatory procedure must be the explanation of the research and all activities related to it, and all participant's doubts must be clarified. Those who agree to participate must sign the Informed Consent Form (ICF) (ANNEX 1).

5. STUDY PROCEDURES

5.1 Research participants identity preservation

In order to maintain participants' right of privacy, a unique identification code will be assigned to each participant during the screening visit, which must be used in all study documentation until the end of the study. The identification code will be generated according to the order of participant inclusion in an ascending order. This code will start with the identification of the clinical study (BZCLI003) followed by participant's entry order into the study.

Example: Participant 1: BZCLI003P01; Participant 2: BZCLI003P02; Participant 3: BZCLI003P03; etc.

5.2 Safety analysis and tolerability procedures

5.2.1 Clinical and physical evaluation

Demographic data, medical history, current medication, and adverse events will be collected through direct interviews with the research participant and/or their family members when necessary. The use of any concomitant medication should be recorded, including its indication.

5.2.2 Vital signs and anthropometric

The collection of vital signs aims to detect the presence of any AE, especially hypotension. In order to evaluate safety, the following parameters should be collected and recorded: systolic blood pressure (SBP), diastolic blood pressure (DBP), heart rate (HR), respiratory rate (RR) and axillary temperature (T°C).

Before evaluation period, participants should remain at rest (sitting and without physical effort) for at least 5 minutes in a room at room temperature. Blood pressure measurement should be obtained in only one of the participant's arms (right or left), which should be selected as a reference for subsequent measurements. The examiner should perform three measures with an interval of one minute between them; the first measurement should be discarded and the real blood pressure that should be considered is the average between the last two measurements.

The normal values are: SBP between 90-140 mmHg and DBP between 60-90 mmHg. Values outside these ranges will be considered as medical history or AE (whether before or afterIPadministration) and should be reported as such if there is clinical significance in the physician's opinion.

Vital signs should be collected at V-1, V0, and V1. At V0, they will be collected before IP administration (concomitant with medical evaluation); and in 30, 60, 180, and 360 minutes after IP administration.

Arterial hypotension should be recorded as an AE. Its occurrence should be associated with the following values: 20 mmHg reduction in SBP, 10 mmHg reduction in DBP, and/or 20% increase in HR. These values should all be related to the data recorded on the same day, before IP administration.

Weight, height, and body mass index (BMI) will be measured only at V-1 for eligibility and characterization of the evaluated sample.

5.2.3 Physical and genitalia exam

In order to be completed, physical examination must be performed at V-1 and V1 to capture diseases and AEs, respectively. The assessment includes a general inspection (skin, oropharynx, and appendages), and cardiovascular, pulmonary, digestive, musculoskeletal, peripheral, and neurological exams.

Physical examination of the male or female genitalia must be performed at all visits: at V-1, for eligibility assessment; at V0, before and 360 minutes afterIPadministration, for AE assessment; and at V1, for AE assessment.

5.2.4 Blood and urine sample collection

In order to evaluate the systemic safety of the PSI, blood and urine will be collected before (at V-1) and after the application of theIP(at V0 at the 360-minute time point), for analysis of the following laboratory tests: (i) complete blood count, including platelets; (ii) coagulogram, including PT and PTT; (iii) biochemistry, including glucose, sodium, potassium, urea, creatinine, chloride, total calcium, phosphorus, total and fractionated proteins (albumin and globulin), TGO, or AST, TGP or ALT, alkaline phosphatase, gamma GT, total cholesterol, HDL cholesterol, triglycerides, pH; (iv) urinalysis.

In addition, blood will be collected from women during the V-1 visit for the β-HCG test and confirmation of absence of pregnancy, allowing the participant to be included in the study.

Approximately 20 ml of blood will be collected per evaluation (V-1 and V0), totaling 40 ml of blood for laboratory tests.

Any difference between the baseline and post-application of theIPshould be evaluated by the examiner and, if clinically significant, should be noted as an AE.

5.2.5 Electrocardiogram

In order to evaluate the cardiac safety of PSI, an ECG examination will be performed at all visits. At V0, it should be performed 360 minutes after the administration of PSI, with a window of +/- 30 minutes. At V-1 and V1, the cardiac evaluation can be performed concurrently with the clinical evaluation.

The standard 12-lead ECG examination will be performed and an analytical report must be provided descriptively and/or conclusively in accordance with the Guidelines of the Brazilian Society of Cardiology on Electrocardiographic Analysis and Reports. The interpretation of the tracing should be performed by a physician and clinically significant abnormalities (and/or those related to the current health condition) should be recorded as relevant medical history and/or current conditions during the screening visit, which should be analyzed by the Investigator physician as a risk or not for inclusion in the study. Other clinically significant abnormalities found after the administration ofIPshould be reported and evaluated by the Investigator physician as EA. All ECG reports should be kept as part of the study documentation.

5.2.6 Participants food

In order to maintain standardization of blood collection for pharmacokinetics, unbalanced feeding will follow the same pattern for all participants.

- Fluids: water intake is free during the study.
- Food: At visit V-1, participants must fast for at least 6 hours and a maximum of 10 hours before blood collection for laboratory tests. After this collection, participants can eat without restrictions. At visit V0, participants can receive a light breakfast before starting blood collection for pharmacokinetics and after 2 hours ofIPapplication.

5.3 Pharmacokinetics analysis procedure

5.3.1 Blood sample collection procedure

The collection of blood samples will be done through a heparinized catheter inserted into the superficial vein of the volunteer's forearm. After each blood collection, the catheter will be washed with 1 mL of sodium heparin solution (5000 IU/mL in 0.90% sterile NaCl). Samples will be collected at the following times: immediately before the application of PSI, and 15, 30, 60, 180, and 360 minutes after the application of PSI.

In total, 6 samples of approximately 4.9 mL (5 mL tube) of blood will be collected from each participant for the BZ371A dosage. Thus, approximately 29.40 mL of blood will be used for the pharmacokinetic study. In addition, adding to the 40 mL for laboratory tests before and after the application of PSI, a total of approximately 69.40 mL of blood will be collected during the study period for each participant.

The absolute real time of each blood sample collection should be recorded in the Clinical Record Forms (CRF) using a 24-hour clock. The absolute real time of the IP application should also be recorded,

so that the actual time interval between the application of IP and the respective collections can be computed. Any loss of sample collection should also be recorded. The collected samples should allow for the unequivocal identification of the study, research subject (identification code), collection period, and time.

In this study, yellow tubes with clot activator and gel separator will be used for blood sample collection. These two components cause the blood to clot more quickly, and after clotting, the gel performs the physical separation between the cellular and liquid (serum) portions. After collection, the samples should be kept in an ice bath at 4ºC ± 2ºC from collection until centrifugation.

5.3.2 Blood sample processing and storage

The blood samples will be centrifuged at approximately 3,000 rpm for 10 minutes at 4°C (±2ºC). Immediately after centrifugation, a minimum of 1.2 mL of serum from each sample will be collected and aliquoted into 3 transparent Eppendorf LoBind tubes with a maximum capacity of 1.5 mL. The minimum volume of serum collected will be 1.2 mL and will be divided equally among the 3 tubes (e.g., if exactly 1.2 mL of serum is collected, each Eppendorf LoBind tube will have approximately 400 µL of volume). The remaining serum will be transferred to another cryogenic tube as a reserve sample.

Lo Bind Eppendorf tubes will be stored in polypropylene boxes labeled with the study name, study code, period, and subject number. These boxes will be stored at a temperature < -70°C ± 5ºC in a specific freezer for the storage of biological samples, at the research center.

The period between sample collection, centrifugation, and storage in the freezer should not exceed 30 minutes.

5.3.3 Blood sample transportation and analysis

The transportation of samples from the storage unit to the analytical unit will be carried out in thermal boxes containing dry ice and a digital thermometer where the maximum and minimum temperatures reached are recorded.

The company *Unidade de Integração Farmacologia e Gastroenterologia* (UNIFAG) is the analytical company that will quantify BZ371A and its fragments in the samples.

If the peptide or its fragments are detected, the following pharmacokinetic parameters must be determined: maximum concentration (Cmax), half-life (t1/2), area under the curve (AUC), clearance (CL), volume of distribution (Vd).

6. STUDY MEDICATION

6.1 Medication preparation

The PSI, named BZ371A, will be incorporated into a gel formulation. The formulation will be produced and stored according to good manufacturing practices (GMP), following the RDC 301 of August 21, 2019. The pharmaceutical characteristics of theIPare listed in the table below:

CHART 2. IP IDENTIFICATION

Active ingrediente	BZ371A
Commercial name	Non Aplicable
Batch	21051801
Therapeutci class	Nitric Oxide Synthase Enhancer
Administration method	Topical
Pharmaceutical form	Gel
Concentration	5 mg/ml
Presentation	Seringes with single dose
Dosage regimen	1,5 ml, single dose
API fabrication	CPC (*Chinese Peptide Company*)
IP production	BIOLAB Farmacêutica
Responsible company	Biozeus Biopharmaceutical S.A.

The active pharmaceutical ingredient (API) was produced in accordance with good manufacturing practices (GMP) by the company CPC - Chinese Peptide Company, lot 21051801, and stored in a -20°C freezer. The API will be transported in thermal boxes containing dry ice and a digital thermometer where the maximum and minimum temperatures reached are recorded.

BIOLAB, a Brazilian pharmaceutical company, will receive the API and produce the formulation also in accordance with GMP. The finished product formulation has the following components:

CHART 3. COMPOSITION OF THE FINISHED PRODUCT

Compounds	Quantity
Sodium Acetate Buffer 20mM	60%
Propilenoglycol	30%
Pluronic F-127	10%
BZ371A Acetate (Batch 21051801)	5 mg/ml

The choice of BZ371A dose for this clinical study was based on physicochemical studies that determined the maximum viable concentration of the compound in this formulation. In addition, in vivo

studies of pharmacodynamics, biodistribution, and toxicology confirmed the efficacy and safety of this formulation, respectively.

The study drugs will be sent by the sponsor, along with all relevant documentation, in an adequate quantity for the study, including an additional quantity for sample retention and potential losses.

6.2 Packaging and labelling

TheIPwill be available in 3ml syringes containing 1.5ml of the formulation, for single-dose administration. All syringes will be provided in individual laminated bags, of the stand-up pouch type weighing 250 grams, with the label containing the necessary information for clinical research according to the regulations in force in the country.

The label model to be used will contain all the labeling information required by ANVISA for products intended for clinical research, as shown in Figure 1 below:

Sponsor:
Study Protocol
Responsible investigator (name, Regional Counsil identification number):
Responsible pharmacist (name, Regional Counsil identification number):
Pharmaceutical form:
Batch:
Fabrication date (month/year):
Expiration date (month/year):
Weight:
Storage conditions:
How to use it:
Administration Route:

ATENTION!
Product exclusively destinated for the Clinical Research.
This product should not be comercialized.
Should be kept out of children's reach.
This product should not be used in other people. Exclusive use for whom is destinated to.
This packaging should not be discarted and should be given back in the next visit, together with the product, even thoggh it have not been entirely used.
This product should be used as medical orientation.
In case of any doubts, please contact the Clinical Research facility and ask fo Investigators Name and telephones

FIGURE 1. LABEL MODEL FOR PRODUCTS INTENDED FOR CLINICAL RESEARCH (ANVISA)

6.3 Medication storage

TheIPmust be stored in a secure location equipped with locked cabinets and restricted access, protected from light and moisture, and at room temperature between 20 to 25ºC. Only authorized personnel will have access to the products, and a document controlling access to the storage area must be available for inspection during possible monitoring and/or audits.

Deviation from storage requirements, including any actions taken, must be documented and immediately reported to the Sponsor. Once the deviation is identified, theIPshould be placed in quarantine, and it should not be used until the Sponsor provides specific documentation permitting its use.

All drug packaging (used or unused) should be stored at the Research Center, so that at the end of the study, they can be sent for final disposal, which is the responsibility of the Sponsor.

6.4 Receiving, dispensing and accounting of the IP

The IP for the study will be transported to the Research Center accompanied by an invoice and a shipping form, which should contain all the necessary information to fill in the receipt form and check by the Research Center. If the quantity of the medication sent is inconsistent with the quantity described on its shipping invoice or form, or if the product is damaged in any way, the sponsor must be immediately contacted, and the products segregated until a favorable opinion is issued by the sponsor.

A designated pharmacist from the Research Center will be responsible for both receiving and storing the study medications, as well as dispensing, collecting, and accounting for those dispensed to research participants, through the completion of the IP Dispensing and Return Control Form provided by the Sponsor. In case of discrepancies, these must be properly documented in a specific field of the form, as well as the reason why they occurred. The dispensing of study medications will be carried out in sufficient quantities, as allocated in the research.

During monitoring visits, checks will be made between the information contained in the dispensing and control forms and the physical quantity of study medications. All medications used or unused, as well as empty packaging, during the study must be returned to the Sponsor at the end of the study to be destroyed in accordance with current legislation.

6.5 Investigational Product application procedure

At visit V0, prior to the application of PSI, an analysis of the genital area and registration of vital signs should be done. The genital area should be washed beforehand with water and neutral soap, followed by drying. TheIPshould be applied with gloves on the entire surface of the genital organ: from the glans to the base of the penis in the case of men, and on the clitoris and labia majora in the case of women.

The application will be performed by a trained nursing professional. The characteristics of irritation and sensitivity on the applied skin (AE of the formulation on the skin) will be observed and recorded from this moment on.

7. SAFETY

7.1 AE definition

An AE is defined as "any unfavorable medical occurrence in a patient, or clinical trial participant, to whom a pharmaceutical product has been administered, which does not necessarily have a causal relationship with the treatment." All AEs observed after the signing of the ICF, regardless of the possible causal relationship with the investigational product, must be reported on the AE page(s) of each individual participant's CRF. AEs can be considered as the following:

- Suspected adverse drug reactions (SADRs);
- AEs due to medication’s quality deviations;
- AEs due to unapproved use of medication;
- Medication interactions;
- Therapeutic ineffectiveness, total or partial;
- Medication-related intoxications;
- Medication abuse;
- Potential and actual medication errors.

If the research participant has a relevant previous medical history, the Investigator must specify in the participant's individual CRF the date of diagnosis, history of follow-up, and the treatment followed. Pre-existing conditions will not be considered as AE, except when there is/are:

- Decompensation of the controlled chronic disease before starting the clinical study;
- Symptoms Increased frequency or intensity of symptoms of the disease described in the medical history related to the use of the study medication;
- Need for discordant treatment compared to the treatment usually used for the disease described in the medical history;
- Specified in the clinical study protocol.

The following will not be considered AE:

- Planned elective procedures before the start of the clinical study, even when performed during its duration, except for any complications that occur during the procedure.

The Sponsor will be responsible for all expenses related to examination procedures, especially those for diagnosis, treatment, hospitalization of the participant, and other necessary actions for the resolution of AEs related to the clinical trial (Art. 12, ANVISA RDC 09/2015).

The safety of treatments will be evaluated throughout the study by the incidence and classification of AEs occurring between treatments in terms of type, frequency, and intensity.

7.2 AE classification according to predictability

For the classification of predictability of AEs reported during the clinical trial, the Investigator will use the Investigator's Brochure (IB) as the reference safety document.

- **Unexpected**: an AE whose nature or intensity is not consistent with the information provided in the Investigator's reference safety document for the investigational product, meaning it is unexpected based on the product's characteristics;
- **Expected**: an AE whose nature or intensity is consistent with the information provided in the Investigator's reference safety document for the investigational product.

The expected AEs are referred to as "AEs of interest" because they are actively sought during clinical trials. They have been defined based on the results of previous animal studies and exploratory studies in humans. The expected AEs of the investigational product can be divided according to their origin (Table 1).

TABLE 1. POSSIBLE AE FROM THE IP

Origin	Potential side effects
Local allergy reaction or sensibility due to formulation components	Pruritus, erythema, urticaria, skin rash, edema.
Outcomes based on systemic vasodilation	Headache, hypotension
Outcomes based on excessive local vasodilation	Priapism
Outcomes resulted from peptide excretion by the kidneys	Tubulopathy
Other effects, with no clear rationale, detected in preclinical studies.	Diarrhea

Among the potential risks described above, two main effects were observed during preclinical studies: i) the development of renal tubulopathies, observed only in a study in rats with high systemic doses of BZ371A; ii) and diarrhea, observed in female pigs with topical application of IP. In addition to these AEs, in exploratory studies, pruritus was the only effect reported by study participants, with only one case of mild severity and complete spontaneous resolution identified.

Therefore, for safety monitoring during the clinical trial development, the evaluation of risks related to the proposed formulation containing BZ371A will be carried out through (I) clinical and physical assessment (demographic data, medical and current history, concomitant medications); (II) vital signs

(SBP, DBP, HR, RR and T oC) and anthropometric measurements; (III) complete physical examination, including genitalia; and (IV) laboratory analysis (Table 4).

CHART 4. IP MONITORING DURING CLINICAL STUDIES

Possible AE	Monitoring
Unkown side effects	AE report and general physical exam; Complete blood count analysis, biochemistry, and urinalysis.
Local reaction: pruritus itching, redness, urticaria, skin rash, edema	Local physical evaluation (genitália)
Hypotension (or arrhythmia caused by hypotension)	BP, HR (ECG) analysis
Priapism	Local physical evaluation (genitália)
Tubulopathy	Biochemistry and urinalysis
Diahrrea	AE report

7.3 AE classification according to intensity

The classification of the intensity of AEs will be performed according to the World Health Organization's grading of intensity for health conditions described below:

- Mild: a problem is present less than 25% of the time, with an intensity that a person can tolerate and that rarely occurs in the last 30 days.
- Moderate: means that a problem is present less than 50% of the time, with an intensity that interferes with people's daily life and that occasionally occurs in the last 30 days.
- Severe: means that a problem is present more than 50% of the time, with an intensity that partially alters people's daily life and that frequently occurs in the last 30 days.
- Complete impairment: means that a problem is present more than 95% of the time, with an intensity that completely alters the person's daily life and occurs every day in the last 30 days.
- Not specified: means that there is not enough information to specify the intensity.
- Not applicable: means that it is inappropriate to use a grading (e.g. menstrual functions).

7.4 AE classification according to frequency

- Once: the AE occurred only once;
- Continuous: AE persists or worsens, without resolution between treatment courses or cycles;
- Intermittent: AE occurs and resolves during one treatment cycle/course, and then occurs again in another treatment cycle/course.

7.5 AE classification according to intensity

The AEs will be considered as serious (SAEs), regardless of the administered medication dose, in situations that result in any of the following clinical outcomes:

- Deaths;
- Life-threatening; there is a risk of death at the time of the event;
- Hospitalization or prolongation of existing hospitalization: hospitalization will be considered as hospital care requiring participant admission, as well as their stay for a period less than 24 hours in a hospital unit;
- Significant or persistent disability: substantial interruption of a person's ability to perform normal life functions;
- Congenital anomaly and birth defect;
- Any suspicion of transmission of an infectious agent through a drug;
- Clinically significant event: any event resulting from drug use that requires medical intervention to avoid death, life-threatening situations, significant disability, or hospitalization.

All situations that do not result in the clinical outcomes mentioned above will be considered as non-serious AEs.

7.6 AE classification according to causality

To determine causality, the Investigator should use the World Health Organization's (modified) system. In this way, three criteria will be evaluated to define the causal relationship:

- Temporal relationship - The AE occurred after the use of the investigational product (IP), within at least 10 times its half-life;
- Other causes - Strong presence of other causes that may be related to the AE;
- Known effect - The effect is known or physiologically related to any of the components of the IP.

CHART 5. MONITORING OF THE IP THROUGHOUT THE STUDY

Classification	Time related	Other causes	Known effect
Improbable	No	NA	NA
	Yes	Yes	No
Possible	Yes	No	No
	Yes	Yes	Yes
Probable	Yes	No	Yes

- Probable: a reaction that follows a reasonable temporal sequence between medication administration and the appearance of the AE. It follows a known pattern of response to the suspected medication. It cannot be reasonably explained by the participant's known clinical state characteristics.

- Possible: a reaction that follows a reasonable temporal sequence between medication administration and the appearance of the AE. It follows a known pattern of response to the suspected medication, but it could have been produced by the participant's clinical state or other therapies administered concomitantly.
- Unlikely: a reaction that does not follow a reasonable temporal sequence between medication administration and the appearance of the AE; or that does not follow a known pattern of response to the IP, and can be explained by the participant's known clinical state characteristics.

If there is not enough data available to correctly establish the causality of the AE (due to insufficient evidence, conflicting data, or poor documentation), the Investigator should classify it as **non-evaluable**.

AEs classified as "Probable" and "Possible" will be considered "related" to the PSI, and those classified as "Unlikely" will be considered "not related".

7.7 Information regarding the action taken regarding the IP

The investigator must report in both source documents and the participant's CRF what action was taken regarding the use of theIPat the time they became aware of the AE. The investigator must report if there was a need for clinical, pharmacological, or surgical treatment, as well as any other medical management required to resolve the presented AEs.

7.8 Information regarding participants development

The investigator must report the evolution of the condition (clinical outcome), considering the following progression:

- Recovered: when the AE is no longer present;
- Recovering: when the AE is present but improving compared to its initial state;
- Not recovered: when the AE is present and/or getting worse;
- Recovered with sequelae: when the AE is no longer present, but the patient presents associated sequelae;
- Ignored: when the condition of the AE is unknown;
- Death: when the AE in question has led the patient to death.

In cases of death, it is essential to record the date related to cause of death, to provide a death certificate, or medical autopsy report, when possible.

7.9 AE reports follow-up

The Investigator shall monitor the symptoms presented, whether or not they are related to the IP. This monitoring should be continued until the symptoms disappear or if they persist, the Investigator should initiate classical medical procedures to treat the symptom and evaluate the participant's continued participation in the study. A follow-up report shall be submitted to the Sponsor, with data regarding the monitoring of these reports, in accordance with current regulations.

7.10 Ethics Committee notification

The research Ethics Committee (EC) must be notified of all Serious Adverse Events (SAEs) occurring with the research participant from the center under its responsibility within a maximum period of 24 hours, after the center becomes aware of the event through its responsible investigators.

7.11 ANVISA notification

The sponsor must ensure that all relevant information about unexpected serious adverse events that are fatal or life-threatening occurring in the national territory are documented and notified to ANVISA, through an electronic form within a maximum of 7 (seven) consecutive days from the date of knowledge of the case by the sponsor. In addition, additional information about the follow-up of these adverse events must be included in the form within 8 (eight) consecutive days from the date of notification. All other serious unexpected adverse events, whose causality is possible, probable, or definite in relation to the investigational products, must be reported to ANVISA within 15 (fifteen) consecutive days from the date of knowledge of the case by the sponsor.

7.12 Clinical Research Center notification to Sponsor

Non-serious AE reports should be forwarded to the Sponsor's Clinical Research Department through the AE pages in the CRF by the clinical research monitor after monitoring has been conducted.

Serious AE reports should be sent to the Sponsor within 24 hours of the Research Center's knowledge via the specific AE reporting form provided by the Study Sponsor. This form should be sent to the Clinical Study Monitor and to the Sponsor.

This submission to the Sponsor's Clinical Research Department is independent of the relationship between the AE and the investigational product.

7.13 Consistency of the data shared from the Clinical Research Center to the Sponsor

If there are any inconsistencies in the information about the AE in the CRFs or forms, a clarification note will be sent by email directly to the Investigator responsible for recording the AE for updates. A follow-up will be scheduled with the Investigator to monitor the progress of the condition.

7.14 Financing and Insurance

The Investigator, the Sponsor, and the institutions and/or organizations involved in the different phases of the research must provide immediate assistance, as well as take responsibility for the comprehensive care of the research participants regarding the complications and damages arising from the research.

8. ETHICAL ASPECTS / HUMAN PROTECTION

This research protocol was designed according to resolution 466/12 and subsequent regulations. Researchers must ensure that this study is conducted in full compliance with the principles of the "Helsinki Declaration", the principles described in the "Guidelines for Good Clinical Practice" of the International Conference on Harmonization (ICH), and the regulations of resolution 466/12.

8.1 Ethical Committee

The clinical study must be previously approved in writing, according to applicable legislation, by an Ethics Committee (EC) indicated by the National Commission for Ethics in Research (CONEP) through the issuance of a Substantiated Opinion. The submission of the clinical study protocol to theECis the responsibility of the Principal Investigator, as well as the monitoring of the status of this evaluation. No activity involving research participants may be initiated before the approval of this clinical protocol by the EC.

Any changes to the clinical protocol as well as to the informed consent form (ICF), or any other written information provided to research participants, must be previously approved by the CEP, with the new approval of this documentation being forwarded to the Sponsor. Records of review and approval of all documents related to the study must be filed by the Investigator, since they may be requested in inspections carried out during and after the study.

8.2 ICF

The Investigator will protect the integrity of the participants, following all applicable regulations.

It is the responsibility of the researcher, or a person designated by the researcher, to obtain the signed informed consent form (ICF) from each patient, before any study-related procedures are performed. The participant must be informed of all pertinent aspects of the study and the elements of the ICF, including the objectives, methods, anticipated benefits, and potential risks of the study. The participant should have sufficient time to ask questions and think about the decision to participate or not. If the participant agrees to voluntarily participate in the study, they must sign and date two copies of the same approved version of the ICF provided by the EC.

The researcher or designated person must also explain that patients are entirely free to refuse to be included in the study or to withdraw from it at any time, for any reason.

The ICF used during the study must be reviewed by the Sponsor, approved by the EC, and made available for inspection. If new safety information results in significant changes in the risk/benefit assessment, the ICF must be reviewed and updated, if necessary. All patients (including those already being treated) must be informed of the new information, provided with a copy of the revised form, and give their consent to continue in the study.

8.3 Participant confidentiality

Considering the privacy of the research participant, all data related to the participant will be identified only by the initials of their name and the corresponding code for the clinical study phase. All personal data will be omitted in any analysis of results.

However, it is necessary for the Investigator to allow the study monitor (in the presence or absence of the Investigator) to review the part of the research participant's records related to the clinical study. This should include all documentation containing the research participant's medical history to verify eligibility, laboratory test results, diagnoses, summaries of the research participant's admission or non-admission to the hospital during the period of the clinical study, as well as death certificates and/or autopsy reports.

8.4 Confidential Information and Publications

Confidential Information is considered for any and all information that has not been previously published and provided to the Investigator and members of their team by the Sponsor. This includes, but is not limited to, the investigator's brochure, basic scientific data, clinical research protocol, amended protocols/amendments (when applicable), case report forms, and experimental methods. Any data obtained during the course of the clinical study is also considered confidential. All confidential information is the property of the Sponsor, however, it will be available to government health authorities.

At the end of the clinical study, after data analysis and submission to regulatory authorities, the results will be published by the Sponsor following the current regulations in the country.

8.5 Risk/benefit analysis

8.5.1 Expected benefits

This is a phase 1 study in healthy individuals, so individual benefits are not expected. This study may benefit many patients in the future if the proposed intervention proves to be safe and effective.

8.5.2 Risks related to the intervention

Pre-clinical studies did not demonstrate any occurrence of local or systemic side effects related to the IP. Therefore, possible adverse effects for IP are limited to the possibility of local reactions such as allergy, irritation or sensitivity, which may manifest as skin rash, urticaria and edema; and effects based on vasodilation resulting from products that act on the NO pathway, such as hypotension, headache and priapism.

Regarding procedures, blood collection may cause bruising. The participant will fast for blood collection at V-1.

8.5.3 Contraceptives methods

To avoid any safety issues for research participants, the following contraceptive methods will be considered acceptable when used correctly: oral contraceptives, contraceptive patches and/or injections, male condoms, diaphragms or cervical caps with spermicide, contraceptive vaginal ring, intrauterine device, surgical sterilization (bilateral tubal ligation, vasectomy), or sexual abstinence.

The Investigator and the participant will decide on the appropriate contraceptive method to be used.

8.6 Safety monitoring

8.6.1 Study Suspension

The Sponsor may suspend or terminate a study, or part thereof, at any time, for any reason. After the decision to suspend or cancel, the Sponsor must notify the Ethics Committee within a maximum of 15 calendar days, except in cases of temporary suspension as a safety measure, when the deadline is 7 calendar days from the date of suspension. It should be noted that cancellations under RDC 09/2015 are final and apply only to ongoing clinical trial protocols, with no possibility of reactivation later.

If the Investigator suspends or terminates the study, he/she must immediately inform the Sponsor and the Ethics Committee and provide them with detailed written information. The Investigator shall also return to the Sponsor all investigational products, their containers, and other study materials.

Upon completion of the study, the Investigator shall provide the Sponsor and the Ethics Committee with final reports and summaries, as required by applicable regulations.

8.6.2 Participants removal

Request by the participant to withdraw from the study at any time:

- Participant does not wish to continue in the study for personal reasons (or even without reason);
- Participant does not wish to continue in the study due to AEs of the investigational drugs (undesirable effects possibly related to the investigational drugs);
- Participant does not wish to continue for reasons other than AEs, for example, unavailability, intolerance to study procedures, or even personal will.

The researcher may withdraw the participant from the study for one of the following reasons:

- Positive response to reevaluation of any of the exclusion criteria, from admission to the first treatment period or on a subsequent occasion;
- Non-adherence to the protocol requirements;
- AEs, symptoms, or signs of possible toxicity;
- Intercurrent illness requiring medication;
- Any other condition that, in the researcher's judgment, is in the participant's best interest for maintaining health.

The details and reasons for the participant's withdrawal from the study will be recorded in the CRF and must be reported to the sponsor.

8.6.3 Criteria for participants discontinuation

In order to ensure the well-being of the participants, the study may be interrupted if, at the discretion of the Principal Investigator, or the Sponsor, it is established that the risks to which the participants are being subjected to, are greater than initially anticipated, or even at any time by the participant's own decision.

Subjects may have their participation in the study prematurely terminated based on the withdrawal criteria described above.

8.7 Criterions for change in the approved Protocol

8.7.1 Amendments

Defined as changes that alter the potential safety risk to the participants and/or the study plan, such as changes in dosage regimen, alteration of the number of participants, or additional blood samples to be collected. Amendments can occur before and/or after the study commencement.

Any change to the study plan requires an amendment to the protocol. The Investigator must not make any changes to the study without the approval of the Ethics Committee (EC) and the Sponsor, except when necessary to eliminate immediate obvious risks to the participants. An amendment to the protocol aimed at eliminating an immediate obvious risk to the participants can be promptly implemented, but the change must then be documented in an amendment, notified to the EC within 5 business days, and submitted to the appropriate regulatory agency within the required timeframe. All protocol amendments must be reviewed and approved following the same process as the original protocol.

8.7.2 Corrections

Defined as changes before the study commencement that do not alter the potential safety risk to the participants and/or the study plan.

Any necessary corrections to this protocol after its initial version approved by the Ethics Committee (EC) should be made with the approval of the sponsor.

8.8 Protocol deviations and violations

The procedures described in the protocol must be followed in full, taking appropriate measures to prevent protocol deviations. However, in the event of any complications, it is the responsibility of the researchers to analyze and evaluate the situation, as well as take appropriate actions accordingly.

Protocol deviation is defined as any failure to comply with the procedures or requirements defined in the version of the protocol approved by the Ethics Committee (EC) without significant implications for the study integrity, data quality, or participant rights and safety. On the other hand, protocol violation refers to a deviation from the protocol that may affect data quality, compromise the integrity of the study, or impact the safety or rights of the participants. In general, a significant deviation from the protocol is considered a protocol violation, while a minor deviation is considered a protocol deviation.

Any deviation from the plan outlined in this protocol must be described, justified, and included in the Final Report.

9. STATISTICAL ANALYSIS PLAN

The statistical analyses will aim to describe the pharmacokinetic parameters. Adverse events (AEs) will be analyzed qualitatively. Therefore, every detected adverse event will be described in the results, characterized by: (i) identification, if possible, as a medical entity (sign, symptom, or disease); (ii) intensity (mild, moderate, or severe); (iii) severity, whether it is a serious adverse event (resulting in death, abortion, immediate risk of death, hospitalization or prolongation of hospitalization, or disability); (iv)

duration; (v) resolution (complete, partial, or unresolved); (vi) mode of resolution (spontaneous or requiring medicinal treatment); and (vii) causality with the investigational product (IP).

Participants in whom the IP has been administered will be included in the analysis.

A sample size of 12 participants is considered adequate for a pharmacokinetic curve.

10. DATA QUALITY CONTROL MANAGEMENT

The quality of the conducted clinical study will be ensured in accordance with the Standard Operating Procedures (SOPs) of the Research Center, which comply with regulatory requirements of the ICH (International Council for Harmonization), Good Clinical Practice (GCP) guidelines, and current national regulations. The study data management will be conducted in accordance with the applicable regulations.

10.1 Source document

The source documents are all original documents in which the first record of collected data was made, including but not limited to:

- Medical records;
- Informed consent forms;
- Questionnaires;
- Dispensation control records and accounting of investigational product.

The completion of source documents is the responsibility of the principal investigator and should be performed by either the principal investigator or a designated member of their team. Availability of the source documents for verification, by the sponsor's assigned monitor during monitoring visits, is mandatory.

All documents related to the clinical study, including but not limited to source documents and the physical study file, should be archived under the responsibility of the Research Center for a period of 10 years from the close-out visit of the clinical study. These documents are subject to auditing by the sponsor as well as regulatory authorities at any time within the aforementioned period. In the case archiving outside the Research Center, the storage location must be informed to the sponsor at the time of close-out through a formal declaration.

10.2 Data acquisition - Clinical file

The raw data generated during the conduct of the study will be directly recorded in the study's paper Case Report Form (CRF). All data related to the study will be the responsibility of the Principal Investigator until its archiving.

Clinical data will be collected by the investigator or delegated responsible party(ies), and the CRF should document each phase throughout the clinical study. All screened research participants will be recorded in the CRF, regardless of their randomization to the clinical protocol, ensuring traceability of the records for participants considered as selection failures.

10.3 Data validation

Data verification will be carried out by the data management department, based on cross-checking between different fields of the CRF. The development of this process takes into account each field of the CRF and the cross-referencing of fields within the same visit and between visits, allowing for verification of all fields considered essential for statistical analysis and obtaining study results. This verification aims to ensure the quality of the collected data**.**

10.4 Audit

During the study or after its completion, the conduct of the study and/or relevant documentation may be audited by the Sponsor or Regulatory Agencies through an independent visit, or by the Research Center through routine quality control audits. These audits aim to assess the conduct of the clinical study and compliance with the clinical protocol, SOPs, GCPs, and applicable regulatory requirements.

11. REFERENCES

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