PROTOCOL

COMPARATIVE STUDY ON THE PHARMACOKINETICS AND PHARMACODYNAMICS BETWEEN TWO AGALSIDASE BETA FORMULATIONS IN A SINGLE DOSE OF 1 MG/KG AGALSIDASE (BIOSIDUS) AND FABRAZYME® (SANOFI GENZYME) BY INTRAVENOUS INFUSION IN HEALTHY MALE VOLUNTEERS

Protocol Number: RD-BIO-AGA-01/03/21

Date of Protocol:

Version: Amendment 2 – September 2, 2021

Sponsor: Biosidus S.A.

SIGNATURES AND OTHER PARTICIPANTS

Protocol Nº RD-BIO-AGA-01/03/21

APPROVAL

Sponsor and Principal Investigator ´s signatures confirm the approval of this protocol and ensure that the study will be conducted in accordance with the clinical and administrative sections of the protocol and the CRF book, including all confidentiality agreements.

The Principal Investigator is informed of his/her responsibilities and of the Good Clinical Practice and local Regulations, and agrees to conduct this study in accordance with these standards and cooperate with the staff involved.

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Version History

Number of substantial amendment	Final version involved	Nature of the amendment
1	17Aug2021	Request for changes by the Regulatory Authority –Communication #01
		Dose selection is accounted for Changes were made to the window periods for COVID19 vaccines for the first and second doses The possibility of voluntarily withdrawing from the study is made explicit to the volunteer given the immediate possibility of receiving COVID19 vaccine The number of volunteers to be hospitalized is clarified
2	02Sept2021	Response to stopclock by ANMAT dated 13 September, 2021
		Rationale was extended to justify the dose The possibility of receiving a COVID vaccine was added as a criterion for withdrawal from the study The number of volunteers to be admitted was changed The window of time in which the volunteer may receive COVID-19 vaccine was clarified in an inclusion criterion

Amendment Version 1 – 17Aug2021
Reviews for version dated July 20, 2021 are the following:
<u>Justification for dose selection:</u> Section # 7 DOSING REGIMEN AND ADMINISTRATION, the following statement was added to account for the selected dose: "A dose of 1mg/kg is the one that did not cause adverse events in preclinical animal studies, conducted with the same Biosidus molecule, which defines this dose as the NOAEL (Non observed adverse event level)."
<u>Changes in the exclusion criteria:</u> New Exclusion Criterion #7 (*Exclusion Criterion #7: Reception of live or dead virus, bacteria vaccines, including COVID-19 vaccine (In this case, patients who have received a single dose of vaccine in the month prior to recruitment will be excluded.*) It was divided into two parts to clearly state the window periods for the COVID-19 vaccine): Exclusion Criterion #7: Reception of live or dead virus, bacteria vaccines within fifteen days prior to visit 1 or 2. The following Exclusion Criterion is added: Exclusion Criterion #8: In the case of COVID vaccines, a window period longer than 15 days must be observed before visits 1 or 2. This period is contemplated based on the recommendations by the National Ministry of Health published on 5-29-2021.

Possibility of withdrawing from the study due to the potential for receiving COVID-19 vaccine:

In the section STUDY COURSE AND PROCEDURES the following statement was added related to COVID-19 Vaccine:

“Note: The participant may withdraw from the study at any time if they decide to receive the first or second dose of the COVID vaccine during the study.”

Number of volunteers to be hospitalized:

The following clarification regarding the number of volunteers to be hospitalized was added to the item VISIT II (Hospitalization):

“The rest of the volunteers will be hospitalized in three groups of 6 volunteers (in compliance with the number of volunteers for bioequivalence studies according to authorizing provision, see Annex F) as described in the section Study course and Procedure - Visit II (Hospitalization).”

Amendment Version 2 – 02Sept2021

Revisions for Amendment 1 dated August 17, 2021 are the following:

Dose justification:

The rationale was extended to justify the recommended dose of 1 mg/kg /body weight of agalsidase beta.

Withdrawal from the study due to COVID vaccine:

The following text was removed: “Note: the participant may withdraw from the study at any time if they decide to receive the first or second dose of the COVID vaccine during the study”. The text was modified and included as a criterion for study withdrawal: "Participant who has the possibility of receiving the first or second dose of the COVID vaccine during the study”

Number of patients to be hospitalized:

In relation to the site authorization for the development of phase 1 clinical trials and bioequivalence by Provision No. 5344/2019, as it is a phase I trial, the use of up to 2 simultaneous beds is authorized. For this reason, the hospitalization of the volunteers was adapted as authorized and a staggered hospitalization will be carried out (see Visit II (Hospitalization)).

Clarification for exclusion criterion:

The following text was added to exclusion criterion #8: “Volunteer may receive the vaccine 35 days after receiving the investigational product.” to make clear the time window in which the volunteer may receive the COVID-19 vaccine.

PROTOCOL SUMMARY

TITLE	COMPARATIVE STUDY ON THE PHARMACOKINETICS AND PHARMACODYNAMICS BETWEEN TWO AGALSIDASE BETA FORMULATIONS IN A SINGLE DOSE OF 1 MG/KG AGALSIDASE (BIOSIDUS) AND FABRAZYME® (SANOFI GENZYME) BY INTRAVENOUS INFUSION IN HEALTHY MALE VOLUNTEERS
CODE	RD-BIO-AGA-01/03/21
SPONSOR	Biosidus S.A. Constitución 4234 (1254) Buenos Aires Phone number: 54-11-4909-8049 / 8000 FAX 54-11-4924-3601
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CO – INVESTIGATOR	Hernán Taconelli, MD
REGULATORY HEAD	Pablo Liuboschitz
SITE	Sanatorio Nuestra Señora del Pilar Av. Gaona 3979 (B1702API) Ciudadela, Provincia de Buenos Aires - Argentina
LABORATORIES	• **Enzyme Activity Assessment:** Biosidus Develpment Department - Constitución 4234 - C1254ABX Buenos Aires, Argentina - Phone.4909 8000 - Fax 4909 8055 • **Immunogenicity Assessment:** Biosidus Develpment Department - Constitución 4234 - C1254ABX Buenos Aires, Argentina - Phone.4909 8000 - Fax 4909 8055 • **Pharmacokinetic Assessment:** Shin Nippon Biomedical Laboratories, Ltd., 2438, Miyanoura, Kagoshima 891-1394, Japan, Phone: +81-99-294-2600; fax: +81-99-294-3619
PRIMARY OBJECTIVE	Compare the pharmacokinetic behavior of Biosidus Agalsidase beta and Fabrazyme® (Genzyme) after administration by intravenous infusion of 1 mg/kg over a period of 5 hours of one of the following formulations: - Fabrazyme®, by Sanofi Genzyme (Reference Formulation, “R”) - Agalsidase beta, biosimilar developed by Biosidus SA (Test Formulation, “T”)

SECONDARY OBJECTIVE	Evaluation of enzyme activity, product safety, incidence of adverse events and tolerance. Evaluation of antibody occurrence.
STUDY DESIGN	Open, randomized, parallel-arms, balanced, single-dose, and sequential
DISCONTINUATION SCHEDULE	The sponsor and investigators may independently discontinue the study at any time at the sole possibility or occurrence of a serious adverse event or situation that could affect the volunteer's health.
NUMBER OF VOLUNTEERS	A total of 20 (twenty) male volunteers will initially be included. As it is a sequential design, if the statistical analysis on the first 20 volunteers does not demonstrate bioequivalence, an additional number of healthy volunteers to be determined will be included to verify the similarity criterion with adequate power.
SCREENING CRITERIA	**INCLUSION CRITERIA:** 1) Male subjects aged between 18 and 40 years. 2) Body mass index (BMI) between 19 and 25 kg/m². 3) Volunteers whose investigations (ECG, chest X-ray, blood and urine, PCR for COVID-19), performed prior to their inclusion and are within normal limits and/or without clinical significance at the investigator's discretion. 4) Subjects with systolic blood pressure higher than 110 mm Hg and lower than 139 mm Hg, a diastolic pressure higher than 70 mm Hg and lower than 89 mm Hg with a heart rate higher than 50 and lower than 90 beats per minute after being 5 minutes in a sitting position and then in a standing position (outliers included). 5) Volunteer being willing to participate in the study and who has signed the informed consent approved before the study initiation. **EXCLUSION CRITERIA:** 1) History of clinically significant allergies (except asymptomatic untreated seasonal allergies). 2) ≥ 20 mm Hg drop in systolic pressure or ≥ 10 mm Hg in diastolic pressure in the first 3 minutes of postural change. 3) Volunteers who are or have been taking other drugs (prescription or over-the-counter) within two weeks prior to the study. 4) Volunteers with a history of autoimmune diseases. 5) Organic CNS mental and/or psychiatric conditions: bipolar disorder, severe depression, insomnia, changes in personality. 6) Active or chronic infections

	7) Reception of live or dead virus, bacteria vaccines within fifteen days prior to visit 1 or 2. 8) In the case of COVID vaccines, a window period longer than 15 days must be observed before visits 1 or 2. This period is contemplated based on the recommendations by the National Ministry of Health published on 5-29-2021. The volunteer may receive the vaccine 35 days after receiving the investigational product. 9) Known allergy to any of the components of the formulations. 10) Active smoker of more than 10 cigarettes/day. 11) Current clinical evidence of severe digestive disorders, digestive tract surgeries (except appendectomy) 12) Current clinical evidence of kidney disease. 13) Current evidence of liver disorders 14) Current clinical evidence of respiratory and cardiac diseases. 15) Presence of diabetes mellitus, thyroid dysfunction or other endocrine disorder. 16) Evidence of active gastroduodenal disease. 17) History of peripheral thrombotic phenomena. 18) Underlying neurological disease 19) Current presence of any progressive chronic disease 20) History of drug or alcohol abuse or addiction during the last three years. 21) Participation in a clinical study within the last three months. 22) Use of any drug within fourteen days prior to the study initiation that, at the principal investigator´s discretion, may interfere with the drug biodistribution. 23) Subject who donated or experienced blood loss within the last twelve weeks prior to the study initiation, or willing to donate blood within three months after the end of the study. 24) Excessive drinker of tea, cocoa, mate, coffee and/or beverages containing caffeine (>5 cups/day) or wine (>0.5 L/day) or alcoholic drinks (>50 ml/day). 25) Significant abnormalities in the electrocardiogram 26) PCR positive for COVID-19.

	27) Serology positive for HIV, hepatitis B or hepatitis C. 28) Abnormal clinical laboratory results (which, at the principal investigator´s discretion, are considered clinically significant) 29) Non-cooperative volunteer
STUDY DURATION PER VOLUNTEER	Duration of the clinical study 56 days per volunteer: 21 days (screening) and 35 days (follow-up until end-of-study visit)
STUDY VISITS	• **Visit I**: Selection of volunteers, obtaining informed consent and completion of entrance exams. • **Visit II**: It will consist of a 24-h hospitalization for the administration of the R or T product, assigned according to the volunteer randomization. Sampling times for the investigational product pharmacokinetic quantification for each volunteer will be: pre-dose (0 h), 1 h, 3 h, 5 h, 5.5 h, 6 h, 7 h, 8 h, 10 h and 12 h. h from the beginning of the infusion. For the assessment of enzymatic activity, the following assessment times will be taken: pre-dose (0 h), 1 h, 3 h, 5 h, 5.5 h, 6 h, 7 h, 8 h, 10 h and 12 h. For the assessment of immunogenicity, the following evaluation times will be taken: pre-dose (0 h) and 12 h. • **Visit III, IV, V and VI (telephone contact)**: The principal investigator or the delegated medical staff will contact the volunteer by telephone after Visit II to ask about medication tolerance and the presence of adverse events to the days 3, 7 and 14 respectively. • **Visit VII (end of study visit)**: It will be carried out 35 days after Visit I, to evaluate the general clinical and biological status of volunteers. The same studies performed at the initial visit will be repeated. For the assessment of immunogenicity, samples will be taken to be evaluated at 35 days.
INVESTIGATIONAL PRODUCT/S DOSAGE AND ROUTE OF ADMINISTRATION	A single dose of 1 mg/kg of agalsidase beta will be administered intravenously in an accurate strength to each volunteer for 5 h. The following formulations will be used: • Sanofi Genzyme Fabrazyme® (Reference Formulation, “R”) supplied as 35 mg and 5 mg lyophilized powder to be reconstituted for infusion. • Agalsidase beta, a biosimilar developed by Biosidus S.A. (Test Formulation, “T”) supplied as 35 mg and 5 mg lyophilized powder to be reconstituted for infusion.
COMPARATIVE ASSESSMENT OF	From the results of the serum concentration of agalsidase beta, at the different sampling times, the following variables will be estimated:

PHARMACOKINETICS AND BIOLOGICAL ACTIVITY BETWEEN FORMULATIONS	- AUC_{0-12h}: area under the serum concentration curve between time 0 and 12 hours. - $AUC_{0-\infty}$: area under the serum concentration curve, as a result of the extrapolation between time 0 and infinite time. - C_{max}: maximum serum concentration of agalsidase beta. - T_{max}: time to maximum serum concentration for agalsidase beta. The difference between the T_{max} of the test formulation and that of the reference will be analyzed, and the drug elimination rate constant (Kel) and half-life (t1/2) in the body will be calculated. Summary measures (arithmetic and geometric means, standard deviation, CV% and range) for each formulation, as well as the individual results of each volunteer will be presented.
STATISTICAL ANALYSIS:	SAMPLE SIZE CALCULATION: The sample size (n: 20) was established based on data from the literature. As it is a sequential design, if the statistical analysis on the first 20 volunteers does not demonstrate bioequivalence, additional volunteers will be included for the eventual demonstration of bioequivalence with adequate power.
ANALYSIS PLAN	COMPARATIVE PHARMACOKINETIC ANALYSIS BETWEEN FORMULATIONS - ABC_T / ABC_R: Ratio between the area under the curve for the Test formulation and the area under the curve for the Reference formulation. For log transformation, the 90% confidence interval of the AUC ratio (for AUC_{0-120h} and $AUC_{0-\infty}$) should be within the range of 0.80-1.25. - $C_{max\ T}$ / $C_{max\ R}$: Ratio between the Cmax of the Test formulation and Cmax of the Reference formulation. For logarithmic transformation, the 90% confidence interval of the Cmax ratio should initially be within the range of 0.80-1.25. COMPARATIVE ENZYME ACTIVITY ANALYSIS BETWEEN FORMULATIONS - Variation in the enzymatic activity for agalsidase beta in the sample at the end of infusion (5 h) compared to baseline (pre-infusion). For logarithmic transformation, the 90% confidence interval of the C_{max} ratio should initially be within the range of 0.80 -1.25. For an estimation of the pharmacokinetic parameters, specific pharmacokinetic computer softwares will be used (WinNonlin

	Professional Edition, version 5.2.1 (2008). Possible inter-subject and inter-treatment differences will be studied using ANOVA. Outliers: for determination of possible outliers, a selective statistical method will be applied. COMPARATIVE IMMUNOGENIC ANALYSIS BETWEEN FORMULATIONS The presence of anti-agalsidase beta antibodies, and their eventual titer, will be determined in the two groups of volunteers, using the biological activity neutralization technique.

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ABBREVIATIONS

Abbreviation	Meaning
AE	Adverse Event
ANMAT	Administración Nacional de Medicamentos, Alimentos y Tecnología Médica (*National Administration of Drugs, Food and Medical Technology*)
AUC	Area Under the Curve
BMI	Body Mass Index
CHO	Chinese Hamster Ovary
CRF	Case Report Form
CRO	Contract Research Organization
DNA	deoxyribonucleic acid
ECG	Electrocardiogram
FDA	Food and Drug Administration
Gamma GT	gamma glutamyl transpeptidase
GCP	Good Clinical Practice
GMP	Good Manufacturing Practice
HIV	Human Immunodeficiency Virus
ICH	International Council for Harmonization
INAME	Instituto Nacional del Medicamento (*National Drug Institute*)
IRR	Infusion-related reactions
KPTT	Partial thromboplastin time with kaolin
r.p.m	Revolutions per minute
RAD	Adverse Drug Reaction
r-hαGAL	Recombinant human α-galactosidase A
RIPA	Radioimmune precipitation
SAE	Serious Adverse Event
SNBL	Shin Nippon Biomedical Laboratories
SNSDP	Sanatorio Nuestra Señora del Pilar
USP	U.S. Pharmacopeia

1 OVERALL INFORMATION AND BACKGROUND

Agalsidase beta is a recombinant form of human α-galactosidase A and is obtained by recombinant DNA technology from a cell line culture from Chinese hamster ovary (CHO). The amino acid sequence of the recombinant form, as well as the nucleotide sequence that encoded it, are identical to those of the natural form of α-galactosidase A. ATC Code: A16ABO4.

1.1.1 Fabry Disease and Enzyme Replacement Therapy

Fabry disease is a rare lysosomal disease, associated with the X chromosome and caused by mutations in the gene encoding the α-galactosidase A enzyme. Decreased enzyme activity results in a progressive lysosomal accumulation of glycosphingolipids, mainly globotriaosylceramide (GL-3) in several tissues, including vascular endothelial cells, neurons, myocytes, and renal cells. This accumulation leads to cellular dysfunction, tissue remodeling, fibrosis, and ultimately, organ failure such as heart failure or stroke.

The severity of manifestations varies depending on the remaining enzyme activity of α-galactosidase A.

Fabry disease is diagnosed by decreased α-galactosidase A activity in plasma leukocytes or dermal fibroblast culture. Urine or plasma GL-3 concentrations are used as a biomarker for diagnosis and monitoring.

Plasma globotriaosylsphingosine (lyso-GL-3) concentrations have also been shown to be used as a diagnostic marker. This is because lyso-GL-3 level has shown to be increased in patients who have not shown increased GL-3 level.

Treatment with agalsidase beta is indicated as long-term enzyme replacement therapy in patients with a confirmed diagnosis of Fabry disease and may be used in adults, adolescents, and children $\geq$ 8 years of age.

Currently, three therapies are approved worldwide: agalsidase α (Replagal by Shire and Agalsidase Beta BS by Amicus) and agalsidase β (Fabrazyme® by Sanofi-Genzyme or JR-051 by JCR Pharmaceuticals). Treatment with these drugs decreases GL-3 accumulation in the kidneys, heart, and epithelium. It also reduces pain and maintains renal function.

1.1.2 Mechanism of Action

The rationale for the enzyme replacement therapy is to restore enzyme activity to a level sufficient to remove accumulated substrate from organ tissues, thereby preventing, stabilizing, or reversing the progressive decline in organ function before irreversible damage occurs.

Following intravenous infusion, agalsidase beta is rapidly cleared from bloodstream and is taken up by the lysosomes of parenchymal and endothelial cells, which they probably enter via mannose-6 phosphate, mannose, and asialoglycoprotein receptors.

1.1.3 Pharmacokinetics

Following intravenous administration of agalsidase beta to adults dosed at 0.3 mg, 1 mg, and 3 mg/kg of body weight, AUC values increased more than dose-proportionally due to decreased clearance, which indicated saturated clearance. The elimination half-life was not dose-dependent and ranged from 45 to 100 minutes.

Following intravenous administration of agalsidase beta to adults with an infusion time of approximately 300 minutes and dosed at 1 mg/kg/body weight, every two weeks, mean plasma C_{max} ranged from 2,000 to 3,500 ng/mL, while that the AUC_{0}-∞ ranged from 370 to 780 mcg.min/mL. The mean volume of distribution at steady state ranged from 8.3-40.8 L, plasma clearance from 119-345 mL/min. and the mean elimination half-life from 80 to 120 minutes.

Beta agalsidase is a protein and is predicted to be metabolically degraded by peptic hydrolysis. Therefore, hepatic impairment is not expected to impact on the pharmacokinetics of agalsidase beta in a clinically significant manner. Renal clearance of agalsidase beta is considered a secondary clearance pathway.

1.1.4 Dosing Regimen and Administration

The dose to be used in this study on agalsidase beta is 1 mg/kg/body weight, administered as an intravenous infusion.

The rationale is as follows:

- The recommended dose of agalsidase beta comparator is 1 mg/kg of body weight, administered once every 2 weeks by intravenous infusion.
- It is the dose that did not cause adverse events in preclinical studies in animals, conducted on the same Biosidus S.A. molecule, which defines this dose as the NOAEL.
- It is the dose used in the biosimilar study conducted in Japan[15].

The initial infusion rate should not exceed 0.25 mg/min (15 mg/h) to minimize the potential for infusion-related reactions. Once the volunteer's tolerance has been established, the infusion rate can be gradually increased for subsequent infusions.

1.1.5 Contraindications

Life-threatening hypersensitivity (anaphylactic reaction) to the drug substance or to any of the inactive ingredients.

1.1.6 Warnings

Immunogenicity:

As agalsidase beta (recombinant human α-galactosidase A or r-hαGAL) is a recombinant protein, development of IgG antibodies is expected to occur in patients with little or no residual enzyme activity. In studies that resulted in the approval of the reference medicine, most patients developed IgG antibodies to r-hαGAL, mainly within 3 months of the first drug infusion. Over time, most seropositive patients in clinical trials showed a decreasing trend in titers (based on a ≥ 4-fold reduction in titers from peak measurement to the last measurement) (40% patients) or they showed tolerance (no detectable antibody was confirmed by 2 consecutive radioimmune precipitation assays (RIPA)) (14% patients) or showed a plateau (35% patients). Healthy volunteers participating in this study, whose enzyme activity is normal, are expected to have little or no antibody response to agalsidase beta administration.

Infusion-Related Reactions:

Patients with antibodies to recombinant agalsidase beta (recombinant human α-galactosidase A or r-hαGAL) are more likely to experience infusion-related reactions (IRRs) defined as any related adverse effect occurring on the day of the infusion. These patients should be treated with caution when re-administering agalsidase beta. Antibody status in patients with Fabry disease and on treatment should be monitored regularly.

Hypersensitivity

Allergic hypersensitivity reactions may occur, as with any other protein medication for intravenous administration.

If serious allergic or anaphylactic reactions occur, agalsidase beta administration should be discontinued immediately and appropriate treatment should be initiated. Current standards for emergency medical treatment of this situation will be followed.

Patients with Advanced Kidney Disease

The renal effect of agalsidase beta treatment may be limited in patients with advanced kidney disease.

1.1.7 Interactions

Biosidus has not conducted any *in vitro* metabolism or drug interaction studies. A literature search did not provide data on this type of study either. Given its metabolism, cytochrome P450-mediated drug interactions are unlikely to occur with agalsidase beta. It should not be administered with chloroquine, hydroxychloroquine, amiodarone, or aminoglycosides (amikacin, streptomycin, gentamicin, or tobramycin), due to the theoretical risk of inhibition of α-galactosidase A activity.

1.1.8 Adverse Reactions

Safety Profile Summary

As agalsidase beta (r-hαGAL) is a recombinant protein, development of IgG antibodies is expected to occur in patients with little or no residual enzyme activity.

Patients with antibodies to r-hαGAL are more likely to experience infusion-related reactions. Cases of reactions suggestive of immediate hypersensitivity (type I) have been reported in a small number of patients.

Chills, pyrexia, feeling cold, nausea, vomiting, headache and paraesthesia are included as very common adverse reactions. 67% of patients experienced at least one infusion-related reaction. In some cases anaphylactoid reactions have been reported. Treatment considered for anaphylactic reactions is adrenaline, corticosteroids (dexamethasone/hydrocortisone) and antihistamine (diphenhydramine).

1.1.9 Pregnancy and Breastfeeding

There are no sufficient data from the use of agalsidase beta in pregnant women.

Studies in animals do not indicate direct or indirect harmful effects on embryonic/fetal development.

Agalsidase beta should not be used during pregnancy unless clearly necessary.

Agalsidase beta may be excreted in breast milk. As there are no data on the effects on newborns exposed to the drug through breast milk, breastfeeding discontinuation is recommended when initiating treatment.

1.1.10 Overdose

Clinical studies with agalsidase beta have used up to 3 mg/kg of body weight.

1.1.11 Storage Conditions

Store in the refrigerator at a temperature between 2 °C and 8 °C in its original container.

Reconstituted solution should not be stored and should be diluted quickly. Diluted solution may be kept for up to 24 hours at a temperature between 2 °C and 8 °C.

2 STUDY OBJECTIVES

Primary Objective

The objective of this trial is to compare the pharmacokinetic behavior after administration by intravenous infusion over a period of 5 hours of a single administration of 1 mg/kg

Biosidus Agalsidase beta (Test Formulation, “T”) versus an intravenous infusion of Sanofi-Genzyme Fabrazyme® at the same strength (Reference Formulation, “R”).

Secondary Objectives

Evaluate and compare the plasma enzyme activity of agalsidase beta, the eventual induction of anti-agalsidase beta antibodies and the occurrence and frequency of adverse effects and tolerance to the formulations studied.

3 SITE

Volunteer recruitment, evaluation, control, clinical follow-up, hospitalization and processing of the samples to obtain the serum and plasma aliquots applicable to each time point will be conducted at Sanatorio Nuestra Señora del Pilar Research Center (SNSDP). It was authorized by ANMAT to conduct Phase I studies. The authorizing provision is attached in Annex 6 (DI-2019-5344-APN-ANMAT#MSYDS).

Samples will be sent with a numerical code (see section 6.2 MATERIAL PREPARATION AND LABELING) to be able to later identify the patient and the formulation received, with the submission of results. In this way, not only is the anonymity of the volunteer ensured, but also the work done in a blinded manner.

Volunteers will be hospitalized at SNSDP and the serum and plasma samples will be sent to Biosidus S.A. Code described in section 6.2 using a dry ice transport system for biological substances.

All members in the research team agree to adhere to the ethical, legal and administrative standards that govern research on human beings.

4 STUDY DESIGN

Pharmacokinetics, biological activity and immunogenicity will be studied in 20 healthy volunteers starting with a single dose of 1 mg/kg/body weight after administration via intravenous infusion of one (1) dose of:

- Sanofi-Genzyme Fabrazyme®. (Reference Formulation, “R”), or
- Biosidus SA Agalsidase beta (Test Formulation, “T”)

This study was conducted under an open, randomized, parallel-arm, balanced, and sequential design. As it is a sequential design, if the statistical analysis of the first 20 volunteers does not show bioequivalence, additional volunteers will be included until the similarity criterion is verified with adequate power.

For volunteers and clinical staff, the study is open. Laboratory determinations will be completed in a blinded fashion, with coded samples.

Below (Figure 1), a study visit scheme is presented:

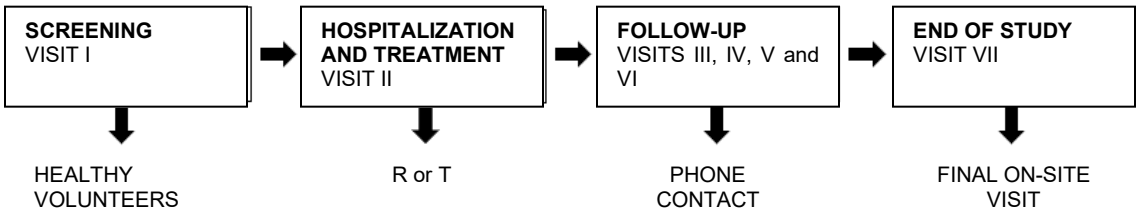

Figure 1 Study Visit Scheme

4.1 RANDOMIZATION PROCEDURE

Randomization to the study will be performed by chance, using a table of random numbers, using the RANDOM software of Randomness and Integrity Services Ltd. Premier Business Centres, 8 Dawson Street, Dublin 2, D02 N767, Ireland. It will be carried out in a balanced way in the anthropometric variables between both arms.

Annex 4 contains the randomization table, which indicates the treatment (R or T) assigned to each patient.

4.2 CODE UNBLINDING IN BLINDED STUDIES

This study is open to clinical staff, and is only blind for measurements to be made in the laboratory, which will be further carried out, when all the study samples are collected. Therefore, both the sponsor and the principal investigator will know the treatment received by each volunteer. The pharmacokinetic, enzyme activity and immunogenicity samples will be blind during dosing, and will be sent to the relevant laboratories presenting only the identification code of each subject.

4.3 STUDY DURATION AND DISCONTINUATION SCHEDULE

The study duration is approximately 6 months since the approval by the ANMAT, for the recruitment of volunteers, study conduction, analysis of results and final report preparation. Times for completion are subject to the COVID-19 Pandemic progress in Argentina, especially in the Province of Buenos Aires, and the availability of beds at SNSP.

The sponsor and the investigators independently may discontinue the study at any time at the possibility or occurrence of a serious adverse event or situation that could impact on the volunteer´s health.

4.4 STUDY COURSE AND PROCEDURES

The study will be conducted by staff trained in the Good Clinical Practice (GCP) Standards by the International Council for Harmonization (formerly International Conference on Harmonization), in compliance with ANMAT Regulation 6677/10.

Principal Investigator, Dr. Eduardo Pirotzky will be responsible for conducting this study.

4.4.1 Procedures to be made at each Visit

VISIT I (Screening)

After signing the informed consent, each volunteer will undergo a physical examination that will include: medical history, neurological semiology, cardiovascular, (ECG, blood pressure, chest x-ray). Volunteers weight will be measured, taking this body weight to adjust the dose should they enter the study. Lab tests will also be done within 30 days, including:

- CBC
- Erythrocyte sedimentation
- Uremia
- Blood glucose
- Uricemia
- Gamma GT
- LFT
- Quick time
- KPTT
- Platelet count
- Hepatitis B and C and HIV screening tests
- Urinalysis for screening drugs of abuse (cannabinoids and cocaine)
- Serum electrolyte panel
- Complete urinalysis
- PCR for COVID-19
- Serum creatinine
- Glomerular filtration rate (GFR)
- Urine protein

In the event that outliers are detected, volunteer will be contacted by phone, email or by mail to arrange a new visit where he/she will be informed about the reasons why he/she cannot participate and will also be instructed to see an applicable medical specialist.

VISIT II (Hospitalization)

During this visit, volunteer will receive the formulation randomly assigned. Volunteers must come to the clinic the day before, at 8:00 p.m. and the infusion will begin at 8:00 a.m. the following day, with the volunteer in fasting state.

In this instance, the Principal Investigator will ensure whether volunteers meet the conditions to proceed with the drug infusion (interrogation and physical examination).

There will be a staggered hospitalization of two volunteers at a time.

After the hospitalization of the first two volunteers (1 Reference volunteer and 1 Test volunteer), a week will be allowed to admit the next two volunteers. At the end of each hospitalization, volunteers will be evaluated for AE occurrence. If no AEs that jeopardize the participant´s safety occur, the next volunteers will be hospitalized according to the randomization table at the time intervals deemed appropriate by the Principal Investigator.

To draw blood samples and avoid multiple punctures, an abbocath-type intravenous line with a three-way stopcock will be placed in the arm contralateral to the one used for agalsidase infusion. Through it, all blood samples will be drawn during hospitalization.

At each time point, 30 mL of blood will be drawn, which will be placed in adequate tubes to obtain serum or plasma, as appropriate (for example, up to 500 mL of blood is drawn from a volunteer blood donor at a time and without consequences for him/her).

Samples to be obtained from each volunteer will be drawn from the contralateral arm to which the infusion of the experimental drug is being made.

After verifying its correct operation, the pre-dose sample (hour 0) will be drawn. Next, the intravenous administration of the corresponding infusion will begin according to the order of randomization.

Sampling times during Visit II will be the following:

1. For pharmacokinetic analysis: predose (0 hour), 1 h, 3 h, 5 h, 5,5 h, 6 h, 7 h, 8 h, 10 h, 12 h from the beginning of infusion.
2. For enzyme activity analysis: predose (0 hour), 1 h, 3 h, 5 h, 5,5 h, 6 h, 7 h, 8 h, 10 h, 12 h from the beginning of infusion.
3. For immunogenicity analysis: predose (0 h), 12 h and day 35.

On the study day, volunteers will be allowed to drink fluids freely.

Alcoholic drinks or xanthines will not be allowed (since 48 hours before the test).

No medication (neither allopathic nor homeopathic) will be allowed during the two weeks prior to the study.

Snacks during Visit II:

During hospitalization, volunteers may consume only standardized food, provided by the clinic. No other type of food will be allowed other than that which complies with the guidelines that are developed below:

Meal	Time Schedule	Menu
Lunch	4 hrs after the beginning of the infusion (approximately between 12 and 12:30 p.m.)	A piece of chicken (breast or leg and thigh), with no skin A portion of white rice 500 mL of mineral water 1 sachet of salt 1 small package of crackers 1 apple.
Afternoon snack	8 hrs after the beginning of infusion (approx. 4:00 p.m.)	1 cup of coffee or tea (decaffeinated with 100 mL of whole milk), 2 butter croissants each with 2 cooked ham slices and 2 cheese slices.
Dinner	12 hrs after the beginning of the infusion (approx. 8:00 p.m.)	A piece of chicken (breast or leg and thigh), with no skin A portion of white rice 500 mL of mineral water 1 sachet of salt 1 small package of crackers 1 apple.

VISIT III, IV, V y VI (phone contact):

The principal investigator or the delegated medical staff will contact the volunteer by telephone after visit II to ask about tolerance to medication and the occurrence of adverse events on days 3, 7, 14 and 21 after visit II respectively.

VISIT VII (on-site)

Volunteers will come to SNSDP after 35 days, to complete the end-of-study visit on an outpatient basis in order to assess the patient overall clinical status. The same studies carried out at the initial visit will be repeated.

For the immunogenicity analysis, a serum sample will be collected.

4.4.2 Blood sampling

Sterile, disposable tubes with a stopper will be used for the collection of blood samples for complete blood count. A 30-mL sample of venous blood should be drawn before the beginning of the infusion (time point 0, pre-dose).

Once the infusion begins, venous blood collection will continue at the following time points:

- 1 h, 3 h, 5 h, 5.5 h, 6 h, 7 h, 8 h, 10 h, 12 h from the beginning of the infusion for the pharmacokinetic study.

- Day 35 from the beginning of infusion for immunogenicity study.
- 1 h, 3 h, 5 h, 5.5 h, 6 h, 7 h, 8 h, 10 h, 12 h from the beginning of the infusion for the enzymatic activity study

Once the infusion finishes (visit II), volunteers will be observed until 20:00 p.m, the same day. Then, they will be discharged to return to their homes.

For immunogenicity sample collection: At visit II, racks with 2 2-mL polypropylene tubes with stoppers will be prepared to separate serum from each volunteer in two samples (sample and counter sample) from each collection time point for anti-agalsidase beta antibody measurement, immunogenicity measurement.

After collecting the blood sample, tubes will allowed to reach room temperature for 30 minutes to separate the clot and serum. Then they will be centrifuged at 2000g for 15 minutes to separate the serum.

To receive the serum, polypropylene tubes will be used. Each serum sample will be separated into 2 aliquots (sample and counter sample) to measure immunogenicity.

For pharmacokinetic sample collection: At visit II, racks with 10 2-mL polypropylene tubes with stoppers (heparinized tubes) will be prepared for each volunteer for the separation of plasma into two samples (sample and counter sample) applicable to each collection timepoint for the quantification of agalsidase beta, pharmacokinetics measurement. After collecting the blood sample, tubes will be kept cold and then centrifuged at 4 °C at 8000 rpm for 10 min for plasma separation.

Sample quantification will be carried out in an independent laboratory. The mass of agalsidase present in each sample will be quantified.

For sample collection for enzyme activity: At visit II, the same tubes used for PK measurement will be used for each volunteer. An aliquot of the plasma separated at each time point will be used for measuring enzyme activity.

After collecting the blood sample, tubes will be kept cold and then centrifuged at 4 °C at 8000 rpm for 10 min to separate the plasma.

Biological activity will be measured by the fluorimetry method in plasma samples, obtained in heparin tubes.

To receive the serum, polypropylene tubes will be used. Each serum sample will be separated into 2 aliquots (sample and counter sample) for agalsidase beta, enzyme activity or immunogenicity measurements. Tubes will be labeled with the study code, volunteer number and collection time points.

The complete process from the blood sample collection to obtaining the 2 (two) aliquots of serum will take 60 minutes. Tubes will be frozen at -20 °C at the Site's laboratory and will be taken after each hospitalization by Biosidus for their storage until measurements are made.

Criteria for rejecting a sample: pronounced hemolysis, accidental thawing.

All tubes for blood collections, and polypropylene tubes for serum collection will be labeled with a self-adhesive label where the study code, volunteer number and collection timepoint will be indicated.

Storage of Plasma and Serum Samples

Serum or plasma obtained at each time point will be placed in duplicate in polypropylene tubes properly labeled and stored at -20 ºC in a secured freezer with temperature control, at the Site laboratory, with restricted access to the staff until its transfer to Biosidus.

4.4.3 Analytical Method

Samples

All serum or plasma samples will be stored in duplicate at -20°C. Before the assay, the samples should be allowed to reach room temperature (15 °C - 28 °C) and mixed by gentle stirring. Aliquots from the samples will be made to prevent freezing - repeated thawing. The duplicate shall be kept at -20 °C.

Quantification Method

Pharmacokinetic Quantification:

Samples will be shipped at -20°C to Shin Nippon Biomedical Laboratories (SNBL), Ltd.

The agalsidase content will be quantified by the electrochemiluminescence method. The SNBL laboratory will validate the quantification method before the study is initiated.

Enzyme Activity Quantification

For the measurement of the biological enzyme activity in the plasma samples, the fluorimetric method of hydrolysis of the synthetic substrate 4-MU-Gal will be used.

Immunogenicity Measurement

Serum samples will be incubated with 1 ng beta agalsidase for 15 minutes at room temperature. Then, the sample enzymatic activity will be measured using the 4-MU-Gal substrate.

The inhibition percentage of the samples will be determined by comparing the activity value of 1 ng agalsidase beta with the treated samples. Those samples with percentage inhibition > 50% will be designated as positive.

4.5 VOLUNTEER FOLLOW-UP

The study will be conducted under medical supervision and all the information will be transcribed to the Medical Record and the CRF.

During the hospitalization period, the volunteer will undergo a permanent clinical evaluation by the principal investigator and his group of collaborators.

Before each blood draw, blood pressure, heart rate and respiratory rate will be measured. In the pre-dose and final draw, hematocrit will also be recorded. These data together with the theoretical and real times of collection will be recorded in the Medical Record and will be transcribed to the Case Report Form (CRF).

During the study, signs and symptoms of intolerance to the medication must be observed, which will be documented in the Medical Record and the CRF.

4.6 VOLUNTEER IDENTIFICATION

The system for assigning numbers to volunteers (after signing the ICF) is based on the obligation to keep anonymity. Volunteers will be assigned a number according to their order of inclusion in the study (according to the table in Annex 4 - Randomization Table). They will be identified by the number assigned from 01 to 20 consecutively (in case new volunteers need to be added because it is a sequential study, they will be assigned numbers from 21), a two-letter code (first letter of the last name and first letter of the first name) and their date of birth (day-month-year):

- Assignment Number: based on the randomization table and according to the order of inclusion: NN
- First letter of the last name and first letter of the first name. XX
- Date of birth (day-month-year): xxxxxx

  NNXXxxxxxx

EXAMPLE: Juan Perez, Assigned number 004, Date of birth May 27, 1960 Patient ID: 04PJ270560

4.7 SAMPLE IDENTIFICATION

Collection tubes:

Tubes must be identified with the study protocol code (RD-BIO-AGA-01/03/21) at the top, then the patient identification code (NNXXxxxxx) must be placed, then two letters that will identify the type of quantification to be made on the sample (pharmacokinetics: PK, enzyme activity: AE or Immunogenicity: IM), then a two-digit number that corresponds to the sample number.

EXAMPLE:

RD-BIO-AGA-01/03/21

04PJ270560: PK 10

In this case, this sample applies to a pharmacokinetic quantification, to the tenth sample from volunteer number 04.

Aliquot tubes:

The aliquot number will be added to the above information (sample: A1 or counter sample: A2) with a digit followed by the letter A.

EXAMPLE:

RD-BIO-AGA-01/03/21

04PJ270560: PC 10 A1

In this case, this sample is to perform the pharmacokinetic quantification of Aliquot 1 of the tenth sample from volunteer number 04.

The types of sample obtained in this study are detailed below:

Table 1 PK Samples (PK)

Timepoint (h)	**0**	**1**	**3**	**5**	**5.5**	**6**	**7**	**8**	**10**	**12**
Sample	1	2	3	4	5	6	7	8	9	10
Aliquot 1	A1	A1	A1	A1	A1	A1	A1	A1	A1	A1
Aliquot 2	A2	A2	A2	A2	A2	A2	A2	A2	A2	A2

Table 2 Samples for Enzymatic Activity (EA)

Timepoint (h)	**0**	**1**	**3**	**5**	**5.5**	**6**	**7**	**8**	**10**	**12**
Sample	1	2	3	4	5	6	7	8	9	10
Aliquot 1	A1	A1	A1	A1	A1	A1	A1	A1	A1	A1
Aliquot 2	A2	A2	A2	A2	A2	A2	A2	A2	A2	A2

Table 3 Immunogenicity Samples (IM)

Timepoint (h or Days)	**0 h**	**12 h**	**Day 30**
Sample	1	10	3
Aliquot 1	A1	A1	A1
Aliquot 2	A2	A2	A2

5 CRITERIA FOR SCREENING VOLUNTEERS

5.1 POPULATION

In order to be selected and included in the study, volunteers must meet all the screening and inclusion criteria and none of the exclusion criteria.

The number of subjects to be included in the study will be a total of 20 male volunteers. As a sequential study, additional volunteers may be included.

Those volunteers who meet all the inclusion criteria and none of the exclusion criteria will be incorporated into the study, after having explained the purpose of the clinical trial and having signed the informed consent for participation.

Presence of the following inclusion criteria and absence of exclusion criteria must be verified by the Principal Investigator in the medical record and in the applicable form in the CRF book of each subject.

5.2 INCLUSION CRITERIA

1) Male subjects aged between 18 and 40 years.
2) Body mass index (BMI) between 19 and 25 kg/m²
3) Volunteers whose investigations (ECG, chest X-ray, blood and urine, PCR for COVID-19), performed prior to their inclusion and are within normal limits and/or without clinical significance at the investigator's discretion.
4) Subjects with systolic blood pressure higher than 110 mm Hg and lower than 139 mm Hg, a diastolic pressure higher than 70 mm Hg and lower than 89 mm Hg with a heart rate higher than 50 and lower than 90 beats per minute after being 5 minutes in a sitting position and then in a standing position (outliers included).
5) Volunteer being willing to participate in the study and who has signed the informed consent approved before the study initiation.

5.3 EXCLUSION CRITERIA

1) History of clinically significant allergies (except asymptomatic untreated seasonal allergies),
2) ≥ 20 mm Hg drop in systolic pressure or ≥ 10 mm Hg in diastolic pressure in the first 3 minutes of postural change.
3) Volunteers who are or have been taking other drugs (prescription or over-the-counter) within two weeks prior to the study.
4) Volunteers with a history of autoimmune diseases.
5) Organic CNS mental and/or psychiatric conditions: bipolar disorder, severe depression, insomnia, changes in personality.
6) Active or chronic infections
7) Reception of live or dead virus, bacteria vaccines within fifteen days prior to visit 1 or 2.
8) In the case of COVID vaccines, a window period longer than 15 days must be observed before visits 1 or 2. This period is contemplated based on the recommendations by the National Ministry of Health published on 5-29-2021. The volunteer may receive the vaccine 35 days after receiving the investigational product.
9) Known allergy to any of the components of the formulations.
10) Active smoker of more than 10 cigarettes/day.
11) Current clinical evidence of severe digestive disorders, digestive tract surgeries (except appendectomy)
12) Current clinical evidence of kidney disease.
13) Current evidence of liver disorders
14) Current clinical evidence of respiratory and cardiac diseases.
15) Presence of diabetes mellitus, thyroid dysfunction or other endocrine disorder.
16) Evidence of active gastroduodenal disease.
17) History of peripheral thrombotic phenomena
18) Underlying neurological disease
19) Current presence of any progressive chronic disease
20) History of drug or alcohol abuse or addiction during the last three years.
21) Participation in a clinical study within the last three months.
22) Use of any drug within fourteen days prior to the study initiation that, at the principal investigator´s discretion, may interfere with the drug biodistribution.

23) Subject who donated or experienced blood loss within the last twelve weeks prior to the study initiation, or willing to donate blood within three months after the end of the study.

24) Excessive drinker of tea, cocoa, mate, coffee and/or beverages containing caffeine (>5 cups/day) or wine (>0.5 L/day) or alcoholic drinks (>50 ml/day).

25) Significant abnormalities in the electrocardiogram

26) PCR positive for COVID-19

27) Serology positive for HIV, hepatitis B or hepatitis C.

28) Abnormal clinical laboratory results (which, at the principal investigator´s discretion, are considered clinically significant)

29) Non-cooperative volunteer

5.4 STUDY WITHDRAWAL CRITERIA

Subjects may freely withdraw from the study at any time, and without giving reasons. For safety, behavior or administrative reasons, they may also be withdrawn from the study, at the Principal Investigator´s discretion. If a subject does not come for the next scheduled visit, every effort should be made to contact the subject, document the reasons for his withdrawal, and ask for any adverse events.

If the subject decides to withdraw during the study, the safety telephone follow-up will be made on days 3, 7, 14 and 21. The investigator may retain and continue using any data collected prior to the withdrawal of consent.

A patient will be withdrawn from the study due to any of the following causes:

1. If you have study drug related- adverse events considered unacceptable.
2. Free decision by the volunteer who no longer wishes to participate in the study without any type of reason
3. Serious adverse event
4. Decision by ANMAT
5. Decision by the Sponsor, or Ethics Committee at DIM CLINICA PRIVADA Site.
6. Risk to the volunteer
7. Decision by the principal investigator, who considers the volunteer is at undue risk
8. Participant who has the possibility of receiving the first or second dose of the COVID vaccine during the study.
9. If the study doctor thinks that continuing in the study may not be at patient's health best interest.
10. If the Sponsor, the Argentine Public Health authority (A.N.M.A.T.) or other applicable regulatory authorities decide to discontinue the study.

11. Non-compliance by the volunteer
12. Not meeting the inclusion criteria or meeting some exclusion or withdrawal criteria at the time of hospitalization.

6 STUDY DRUG

6.1 DRUG NAME, FORMULATION AND STORAGE

6.1.1 Study Formulations

Test Formulation (T)

Biosidus beta agalsidase. Powder for concentrate for solution for infusion. It is supplied as both 35 mg and 5 mg per vial to adjust for the indicated dose

The following data will be specified on the labels of the infusion vials: Lot, Study and expiration date.

Qualitative-quantitative formula:

Biosidus beta agalsidase 35 mg: Each vial contains lyophilized powder to reconstitute:

Drug Substance	Agalsidase beta	35 mg
Inactive ingredients	Sodium phosphate monobasic anhydrous	42 mg
	Sodium Hydroxide	q.s. pH 6.8
	Mannitol	210 mg

Once reconstituted with 7.2 mL WFI, each vial contains 5 mg/mL (35 mg/7 mL) agalsidase beta. Reconstituted solution must then be diluted.

Biousidus agalsidase beta 5 mg: each vial contains lyophilized powder to reconstitute:

Drug Substance	Agalsidase beta	5.5 mg
Inactive ingredients	Sodium phosphate monobasic anhydrous	6 mg
	Sodium Hydroxide	q.s. pH 6.8

	Mannitol	30 mg

Once reconstituted with 1.1 mL WFI, each vial contains 5 mg/mL agalsidase beta. Reconstituted solution must then be diluted.

Reference Formulation (R)

Sanofi-Genzyme Fabrazyme®. Powder for concentrate for solution for infusion. It is supplied as both 35 mg and 5 mg per vial to adjust for the indicated dose.

The following information shall be specified on the labels of infusion bottles: Lot, Study and expiration date.

Qualitative-Quantitative Formula:

Fabrazyme® 35 mg: each vial contains lyophilized powder to reconstitute:

Drug Substance	Agalsidase beta	37 mg
Inactive ingredients	Sodium phosphate monobasic monohydrate	20.4 mg
	sodium phosphate dibasic heptahydrate	59.2 mg
	Mannitol	222 mg

Once reconstituted with 7.2 mL WFI, each vial contains 5 mg/mL (35 mg/7 mL) Fabrazyme® agalsidase beta. Reconstituted solution must then be diluted.

Fabrazyme® 5 mg: each vial contains lyophilized powder to reconstitute:

Drug Substance	Agalsidase beta	5.5 mg
Inactive ingredients	sodium phosphate monobasic monohydrate	3 mg
	sodium phosphate dibasic heptahydrate	8.8 mg
	Mannitol	30 mg

Once reconstituted with 1.1 ml WFI, each vial contains 5 mg/mL Fabrazyme agalsidase beta. Reconstituted solution must then be diluted.

Justification for the difference in drug substance Agalsidase Biosidus and Frabrazyme® strengths is found in Annex 7.

6.1.2 Content and Potency Uniformity

Prior to the clinical phase of this study, the Biosidus Development Department will evaluate the comparative enzyme activity of all batches of the Test and Reference products to be used in the clinical study. Content uniformity of the formulations will also be determined by reverse phase HPLC.

Justification for the difference in drug substance Agalsidase Biosidus and Frabrazyme® strengths is found in Annex 7.

6.1.3 Storage

All the medication for this study will be stored in the refrigerator at 2 °C – 8 °C, not in the freezer, under adequate conditions, indicated by the sponsor in writing, with restricted access to the staff in charge.

6.2 MATERIAL PREPARATION AND LABELING

Medication used in the study will be provided by Biosidus S.A., in accordance with current regulations. Preparation for the medication used for each volunteer will be made by the principal investigator, or his designee, as follows.

Each volunteer will receive one (1) intravenous infusion in the arm. Vials will be labeled with a self-adhesive label where the study code and other information necessary for the label of the investigational product will be mentioned, in accordance with ANMAT Provision 6677/2010, as indicated in Annex 3. Reconstitution and dilution for each volunteer will be made based on his body weight.

6.3 DOSE AND ROUTE OF ADMINISTRATION

Each volunteer will receive a single 1 mg/kg agalsidase beta dose.

To adjust the required dose based on the volunteer´s weight on the hospitalization day, the following formulations will be available:

1. Fabrazyme® 35 mg lyophilized powder to be reconstituted for infusion
2. Fabrazyme® 5 mg lyophilized powder to be reconstituted for infusion
3. Biosidus Agalsidase 35 mg lyophilized powder to be reconstituted for infusion
4. Biosidus Agalsidase 5 mg lyophilized powder to be reconstituted for infusion

6.4 TREATMENT DURATION

Study duration will be approximately 56 days for each volunteer, assuming: 21 days for the screening period (Visit 1), 1 day for hospitalization and treatment (Visit 2), 4 telephone contacts (days 3, 7, 14 and 21 - Visit 3, 4, 5 and 6) and 1 day for the Final Visit (day 35 – End of study).

Each volunteer will receive only one study formulation (T or R) at visit II (Hospitalization).

7 ENZYME ACTIVITY ANALYSIS

For measurement of the biological enzyme activity in plasma samples, the fluorimetric method of hydrolysis of the synthetic substrate 4MU-Gal will be used.

7.1 PHARMACOKINETIC ANALYSIS OF RESULTS

From the results of plasma concentration of agalsidase beta at the different sampling times, the following variables will be calculated:

$ABC_{0-t:}$ Area under the plasma concentration curve between time 0 and time t (in this case 12 hours).

$ABC_{0-\infty}$: Area under the plasma concentration curve, result of extrapolation between time 0 and time ∞.

C_{max}: Peak plasma concentration of agalsidase beta.

T_{max}: Time to reach the peak plasma concentration of agalsidase beta.

C_{max} / $ABC_{0-\infty}$: ratio between the peak plasma concentration and the area under the plasma concentration curve between time 0 to ∞, as an absorption rate index of less variability than the T_{max}.

The drug elimination constant and elimination half-life in the body will be calculated. The summary measures (arithmetic and geometric means, standard deviation, CV% and range) for each infusion will be presented, as well as the individual results of each volunteer.

7.1.1 Comparative pharmacokinetic analysis between formulations

ABC_T/ABC_R: Ratio between the area under the curve of each test formulation and the area under the curve of the reference formulation.

For logarithmic transformation, the 90% confidence interval of the AUC ratio (for $ABC_{0-\ 120\ h}$ and $ABC_{0-\infty}$) should be within the range of 0.80-1.25.

$C_{max\ T}/C_{max\ R}$: Ratio between the C_{max} of each Test formulation and C_{max} of the Reference formulation. For logarithmic transformation, the 90% confidence interval of the C_{max} ratio should be first within the range 0.80-1.25 (80% to 125%). Considering the high intra-individual variability of this pharmacokinetic parameter, if a variability > 30% is confirmed, this interval for the C_{max} may be extended to 0.75-1.33 (75-133%).

The FDA assumes that the test product is considered bioequivalent to the reference product if the 90% confidence interval (CI) of the geometric mean ratio of AUC and C_{max} between the test product and the reference product fall within the range 80-125%, even though the application of such criterion to drugs with high variability is controversial and regulatory agencies as strict as the FDA use different criteria. In Argentina, renowned specialists such as R. Bolaños, MD and S. Giarcovich, MD have analyzed the current status of the bioequivalence in the world proposing the most appropriate study designs.

Globally, drugs with high intra-individual variability are considered to be those with a coefficient of variation (CV) greater than 30% in pharmacokinetic parameters such as C_{max} and AUC. Different strategies have been tested given the high variability issue and in order to meet the conventional bioequivalence principles (90% CI 80-125). One has been to significantly increase the number of volunteers, sometimes to an unfeasible number, although this procedure does not guarantee that the results will fall within that equivalence interval. In this regard, there are reference products with high variability that are not capable of demonstrating bioequivalence with themselves. Additionally, the increase in volunteers to minimize variability poses an ethical problem, since we expose a greater number of healthy individuals to drugs that involve a risk in order to meet the bioequivalence criteria, which have been arbitrarily established. Other approaches have additional problems.

Given this difficulty, most regulatory agencies consider expanding the classical limits of bioequivalence (80-125%) in bioequivalence studies with drugs with high intra-individual variability. These considerations are made on the C_{max} and not on the AUC, where there is a consensus to keep the range 80 to 125% in all products. The reason for the greatest C_{max} variability compared to the AUC is the fact that this parameter arises from the measurement of an individual point, which makes it highly dependent on time, sampling frequency, and drug elimination rate.

Several proposals are available in the literature to modify the bioequivalence criteria for drugs with high variability. In general, the proposals are based on reducing the level of the confidence interval, on widening the equivalence limits, or on both.

A reduction in the level of the confidence interval, for example, from 90 to 85%, would imply a possible increase in the risk of patients, which is usually not acceptable. In contrast, the extension of the equivalence limits, which is the generally accepted criterion and the one used here for C_{max}, represents what limits are admissible and tolerable for the ratios (or differences) of the means between the compared products.

Any adjustment of these limits should be based on considerations of the statistical data properties, as well as the clinical characteristics of the individual drug. Statistically, the extension of the limits can be done by directly modifying the tolerated value of the bioequivalence limits, which is what some agencies such as the EMEA, Japan and South Africa have adopted. In countries like Canada, confidence limits for C_{max} are not required, but rather that the geometric mean ratios for T/R be within 80 -125%.

Using simulation techniques, Tothfalusi L et al.[8] compared the gradual limit escalation for high variability drugs and the direct expansion of the limits around C_{max}. Escalation involves extending the limits of the confidence interval based on the variability of the reference product. Authors conclude that escalation would significantly reduce the sample size needed for bioequivalence studies of high variability drugs. They also conclude that the same results are obtained simply by extending the regulatory limits to 75-133% or even 70-143% for C_{max}.

To calculate the pharmacokinetic/pharmacodynamic parameters, specific pharmacokinetic software will be used (EquivTest version 2.0 (Equivalence Testing Software) from Statistical Solutions Ltd.)

Outliers: for measuring potential outliers, a selective statistical method will be applied. K&H Consulting S.A. will be responsible for statistical processing.

7.2 PHARMACODYNAMIC ANALYSIS OF RESULTS

As an estimator of agalsidase beta biological activity, blood samples will be drawn to determine the variation in the agalsidase beta enzyme activity of the end-of-infusion sample (5 h) compared to baseline activity (pre-infusion).

Ratio of activity variation in both groups to be close to the unit and that the 90% CI is within the range 0.8 to 1.25 will be taken as bioequivalence criterion.

8 IMMUNOGENICITY ANALYSIS

The incidence of anti-agalsidase beta antibodies will be determined both at the start of treatment, at the end of visit II and 31 days after the end of the infusion, for both treatment groups.

Serum samples will be incubated with 1 ng agalsidase beta for 15 minutes at room temperature. Then, the enzyme activity for the samples will be determined using the 4-MU-Gal substrate.

The inhibition percentage of the samples will be determined by comparing the activity value of 1 ng agalsidase beta with the treated samples. Those samples with percentage inhibition > 50% will be reported as positive.

9 SAFETY ANALYSIS

9.1 ADVERSE EVENTS

All adverse events that occurred in the subjects during their participation in the study will be recorded. The record will be documented in the subject's Medical Record and on the applicable pages of the Case Report Form.

All solicited and unsolicited reported adverse events, regardless of treatment group or presumed causal relationship to the investigational product(s), will be reported as described in the following sections.

For all adverse events, the investigator must search and obtain adequate information, both to determine the outcome of the adverse event, and to assess whether or not it meets the criteria for its classification as a serious adverse event, which require immediate reporting to the ANMAT. For all adverse events, the investigator must obtain sufficient information to determine the adverse event causality. The investigator is required to assess such causality. For adverse events with a causal relationship to the investigational product, follow-up is required by the investigator until the event or its sequelae resolve or stabilize at a level acceptable to the investigator.

9.2 ADVERSE EVENT REPORTING

All adverse events detected by the investigator or his collaborators, or reported spontaneously by the subjects at each visit, must be reported in a special section in the Case Report Form. Adverse events will be described preferably using WHO terminology.

The study monitor will check the completion in the CRF.

The following information must be recorded for each adverse event, whether it is related to the study drug or not:

- description of the adverse event
- start date / end date
- duration of the event
- characteristics of the event (severity, expectedness, seriousness)
- action taken (report any required treatment or dose adjustments in the CRF)
- progress
- relationship to study drug (causality)

All adverse events must be documented and followed until their resolution or adequate justification, even when the subject has completed the study. Any change in the dose of the drug administered will be reported on the Case Report Form.

All non-serious adverse events (expected and unexpected) will be described in the final study report.

The Serious Adverse Event report will be prepared in compliance with ANMAT 6677/2010 provision (Section B, item 7).

The investigators will report Serious Adverse Events (SAE) immediately, within 24 hours, to the sponsor by phone 49098049/ fax no. 49243601 (Biosidus Development Department) and/or email farmacovigilancia@biosidus.com.ar, except those events that the protocol identifies as not requiring immediate reporting.

According to provision 6677/2010, item 7.1, the Sponsor must inform ANMAT of all Serious and Unexpected Adverse Drug Reactions (*RAMSI* – by its Spanish acronym) related to the product under study within 10 administrative business days of becoming aware of it. The RAMSI caused by a comparator product already registered in ANMAT for being marketed in the country or those related to placebo must be reported only to the ANMAT Pharmacovigilance System. The Investigator must report Serious and Unexpected Adverse Events to the Teaching and Research and Ethics Committee(s) in accordance with their requirements, in a timely manner.

Based on provision 6677/2010, item 7.3, RAMSI reporting must include the following information:

- name of the adverse reaction;
- investigational product: code, INN name and/or trade name;
- type of report: initial, update (update number) or final;
- sponsor name;
- investigational site and investigator name;
- number of ANMAT Provision authorizing the study;
- study name or title;
- participant code, age and sex;
- dates of RAMSI occurrence, investigator's report and date it is received by the sponsor;
- RAMSI seriousness criterion;
- brief description for the RAMSI;
- investigational product information: daily dose, route of administration, start and stop dates, duration of therapy, and indication;
- specify if the reaction went away when treatment was discontinued;
- specify if the reaction reoccurred with treatment rechallange;
- concomitant medications: dosage and start and end dates;
- Participant history relevant to the RAMSI.

A single RAMSI Report per drug will be accepted, referring to all files and approval provisions by A.N.M.A.T. of studies that are being conducted in the country with that drug.

The Sponsor must immediately notify the Investigator(s) and the Ethics Committee at DIM CLINICA PRIVADA site that approved the study of all Adverse Drug Reactions that are both Serious and Unexpected that occur in other national investigational sites.

The Sponsor must inform the Investigator(s) and the Ethics Committee at DIM CLINICA PRIVADA that approved the study of all other relevant information regarding the research

product within a period not exceeding 10 business days from receiving initial information by the Sponsor.

Adverse Reactions attributable to placebo or to the comparator will not be subject to this individualized reporting system. Reports submitted to ANMAT will comply with the item 7.1, provision 6677/2010.

According to section E: Glossary, provision 6677/2010 establishes the following definitions of Adverse Drug Reaction (ADR) and Serious and Unexpected Adverse Drug Reaction (RAMSI).

Adverse Drug Reaction (ADR): noxious and unintentional response to a medicinal product related to any dose. In clinical experience prior to the approval of a new medicinal product or its new uses, particularly when the therapeutic dose cannot be established, an adverse drug reaction should be considered any reaction that involves a causal relationship between a medicinal product and an adverse event such as a reasonable possibility, that means, that the relationship cannot be ruled out.

Serious and Unexpected Adverse Drug Reaction (RAMSI): is the adverse reaction that results in death, is life threatening, requires hospitalization or prolongs an existing hospitalization or produces persistent or significant disability or incapacity, and their nature or severity is not consistent with the product information described in the IB or other documentation.

9.3 ADVERSE EVENT DEFINITION

An adverse event is any adverse medical event, occurring in a clinical research subject who has been administered a product or who uses a medical device. The event does not necessarily have to have a causal relationship with the treatment or use.

Examples of adverse events include, but are not limited to, the following:

- Abnormal findings in investigations.
- Clinically significant symptoms and signs;
- Changes in physical examination findings;
- Hypersensitivity;
- Progression/worsening of an underlying disease;

Additionally, they may include signs and symptoms resulting from:

- Drug overdose;
- Drug withdrawal;
- Drug abuse;
- Inappropriate drug use;

- Drug interactions;
- Drug dependency;
- Extravasation

9.4 ABNORMAL FINDINGS IN THE INVESTIGATIONS

Criteria for determining whether an abnormal finding on investigations should be reported as an adverse event are as follows:

- The result of the investigations is associated with accompanying symptoms, and/or
- The test result requires additional diagnostic testing and/or medical/surgical intervention, and/or
- The investigator considers the test result as an adverse event.
- A repeated abnormal investigation, in the absence of any of the conditions mentioned above, does not constitute an adverse event. Any result of an abnormal investigation determined as an error does not require being reported as an adverse event.

9.5 SERIOUS ADVERSE EVENT

A serious adverse event is any unfavorable medical event that at any dose:

- results in death;
- is life-threatening; (immediate risk of death)
- requires subject hospitalization or prolongation of existing hospitalization;
- results in a persistent or significant disability/incapacity;
- results in a congenital abnormality/birth defect.
- Important / significant medical events.

In determining whether an event is a significant medical event, medical and scientific judgment must be exercised. An important medical event may not be immediately life-threatening and/or result in death or hospitalization. However, if it is determined that the event may jeopardize the subject and require intervention to prevent one of the other outcomes listed in the definition above, the important medical event should be reported as serious.

Examples of such events are: intensive treatments in an emergency room or at home for allergic bronchospasms; blood dyscrasias or seizures that do not result in hospitalization; or the development of drug dependence or abuse

9.6 HOSPITALIZATION

Reported adverse events from clinical trials associated with hospitalizations, or prolongation of hospitalizations, are considered serious. Any initial admission (even for less than 24 hours) to a health care institution meets such criteria. Admission also includes transfer within the hospital to an acute/intensive care unit (e.g., from a psychiatric ward to a clinical ward, clinical ward to coronary unit, neurology ward, etc.).

Hospitalizations do not include the following:

- rehabilitation institutions;
- hospices;
- qualified nursing facilities;
- nursing sites;
- routine admission to an emergency room;
- same-day surgeries (outpatient/same day procedures).
- any hospitalization or prolongation of an existing hospitalization, in the absence of a clinical adverse event that precipitates it, does not constitute, in itself, a Serious Adverse Event. Examples include the following:
- admission for treatment of a pre-existing condition, not associated with the development of a new adverse event, or with the worsening of a pre-existing condition (for example, to treat a persistent laboratory abnormality prior to treatment);
- admission for social reasons (for example, the subject has nowhere to sleep);
- administrative admission (for example, for an annual physical examination);
- protocol-specified admission during a clinical trial (eg, for a procedure required by the trial protocol);
- optional admission not associated with a precipitating adverse event (for example, for elective cosmetic surgery);
- previously planned surgical treatments or procedures must be recorded in the baseline documentation for the entire protocol and/or for the individual subject.
- non-invasive and invasive diagnostic and therapeutic procedures, such as surgery, should not be reported as adverse events. However, the medical condition for which the procedure is performed must be reported, if it meets the definition of an adverse event. For example, acute appendicitis beginning during the adverse event reporting period should be reported as an adverse event, and the resulting appendectomy recorded as treatment of the adverse event.

9.7 SEVERITY ASSESSMENT

If required by the adverse event case report forms, the investigator will use the adjectives MILD, MODERATE, or SEVERE to describe the maximum intensity of the adverse event. For purposes of consistency of information, these degrees of intensity are defined as follows:

MILD	Does not interfere with the subject's usual activities
MODERATE	Somehow interferes with the subject's usual activities
SEVERITY	Significantly interferes with the subject's usual activities

Please note the differentiation between severity and seriousness of an adverse event. A severe event is not necessarily a serious event. For example, a headache may be severe (significantly interferes with the performance of the subject's usual functions), but would not be classified as serious, unless it meets one of the criteria considered for serious adverse events, detailed above.

9.8 CAUSALITY ASSESSMENT (RELATIONSHIP TO MEDICATION, RELATEDNESS)

The investigator's causality assessment must be provided for all Adverse Events (serious and non-serious). The investigator's causality assessment consists of determining whether or not there is a reasonable possibility that the product under investigation caused or contributed to an adverse event.

Causality may be assigned with one of the following two possibilities:

- No: Definitely not drug related.
- Yes: remotely, possibly, probably, or definitely related to the drug

If the investigator's final determination of causality is unknown, and the investigator does not know whether the event was caused by the investigational product, then the event will be considered “related to the investigational product” for the purposes of event reporting. If the investigator's assessment of causality is “unknown, but clearly not related to the investigational product,” that fact must be clearly documented in the study records. In addition, if the investigator determines that a serious adverse event is associated with the trial procedures, the investigator must record such causal relationship in the source documents and Case Report Forms, as applicable, and report such assessment in accordance with the requirements for reporting serious adverse events, if applicable.

All Adverse Events judged by the investigator or sponsor to be "drug related" qualify as an Adverse Drug Reaction (ADR).

10 STATISTICAL CONSIDERATIONS AND ANALYTICAL PLAN. SAMPLE SIZE CALCULATION

A recent study with JR-051, the first agalsidase beta biosimilar to be approved in Japan, was conducted in 20 volunteers (10 per treatment group), based on the fact that previous studies with this molecule assumed the geometric mean of C_{max} and $ABC_{0–24}$ with a value of 1.0 and a coefficient of variation of 0.15.

Therefore, with a power = 0.80, significance level α = 0.05, and a limit for accepting bioequivalence where the 90% confidence interval of the AUC ratio (for $AUC_{0\text{-}120h}$ and $AUC0_{-\infty}$) should be within the range of 0.80-1.25 9 volunteers per group are required for the study, estimating the possibility of 1 volunteer lost to follow-up per group, the sample size was calculated in 20 volunteers.

11 ETHICAL ASPECTS

11.1 LOCAL REGULATIONS/ DECLARATION OF HELSINKI

The Principal Investigator, the Co-Investigators and the members of the Research team will ensure that this study is conducted in full compliance with the principles of the "Declaration of Helsinki 1964" (latest version 64th General Assembly, Fortaleza, Brazil de October 2013) and with the Argentine laws and regulations, in accordance with the ones that most protects the individual. The study must strictly adhere to the principles enunciated in the "Guidelines for Good Clinical Practice" (ICH Tripartite Guideline, January 1997) or local laws, if they provide greater protection for the patient.

11.2 INFORMATION FOR THE SUBJECT AND INFORMED CONSENT

The informed consent form must be approved by DIM CLINICA PRIVADA Ethics Committee and local regulatory agencies (ANMAT) and must be in accordance with the the International Conference on the Harmonization Good Clinical Practice guidelines and local legal and regulatory requirements.

The investigator or the investigator's designee (if permitted by local regulations) is responsible for obtaining the written informed consent from each individual participating in this study or undergoing any examination to assess inclusion in the study, after the objectives, methods, and potential risks of the study have been adequately explained. It is also necessary to explain to the volunteers that they are completely free to refuse to participate in the study or to withdraw from it at any time and for any reason and they will be provided with the corresponding forms for the written informed consent.

11.3 INDEPENDENT ETHICS COMMITTEE (IEC)

It is the investigator's responsibility to obtain advance approval of the Study protocol, protocol amendments, Informed Consent forms and other relevant documents, notices, if applicable, by the DIM CLINICA PRIVADA Ethics Committee

The Principal Investigator will submit to DIM CLINICA PRIVADA Ethics Committee, this protocol, any protocol amendment, as well as all accompanying material. Before starting the study, approval by this Committee must be obtained, which must be documented in a letter addressed to the investigator, specifying the date on which the Committee met and granted its approval.

All correspondence and emails with the DIM CLINICA PRIVADA Ethics Committee must be kept at the Investigator's file.

12 CONDITIONS FOR AMENDING THE PROTOCOL

All amendments to the protocol must be submitted to DIM CLINICA PRIVADA Ethics Committee and ANMAT. No changes may be implemented until such approval is obtained, except if they are necessary to eliminate an immediate risk to trial patients or if the change(s) involve(s) logistical or administrative aspects of the trial (e.g., change of monitor(s), change of telephone number(s)).

13 CONDITIONS FOR COMPLETING THE STUDY

Study early termination may occur upon Principal Investigator, Sponsor, ANMAT Regulatory Authorities, the Ethics Committee decision or due to drug safety problems.

The investigator has the right to terminate the study at any time. When terminating a study, the investigator should ensure that special consideration has been given to patient protection.

Following notification, the investigator must contact all participating subjects within one month of becoming aware of trial termination or discontinuation.

14 STUDY DOCUMENTATION, CRF and RECORDS

14.1 INVESTIGATOR FILES / DOCUMENT RETENTION

The investigator, the clinical test laboratory and the plasma measurement laboratory must keep accurate records so that the study is adequately documented and its data can be verified. These documents should be classified into two different categories: (1) Investigator file and (2) patient source clinical documents.

Patient source clinical documents will include hospital/clinic generated patient records, physician and nursing notes, visit schedule, original laboratory reports, ECG, pathology and special assessment reports, signed informed consent forms, mailing and patient screening and recruitment logs.

Principal Investigator will file copies of all the information and documentation submitted to the ANMAT regulatory authorities, treatment sequence randomization, monitoring reports

conducted at the hospitalization site, the original copies of receipt and return of materials and leftover drugs that is carried out in each hospitalization cycle.

Medical records, clinical laboratory reports, anteroposterior chest X-ray and admission electrocardiogram, Case Report Forms, copies of receipt and return of materials, will be available at the hospitalization sit.

The data and all information from the agalsidase beta enzyme activity method and its results will be available at Biosidus Analytical Department - Constitución 4234 - C1254ABX Buenos Aires, Argentina – Phone 4909 8000 - Fax 4909 8055.

The investigator must retain these documents in his file for at least 10 years from the study termination or discontinuation. In case of not being able to retain the documentation during that period, it will be kept by the sponsor. After that period, documents may be destroyed, in accordance with local regulations. (GCP - Version July 1991).

14.2 AUDITS AND INSPECTIONS

Principal Investigator and his team will accept that the source documents for this trial are available to the study monitors, previously agreed sponsor audits, members of DIM CLINICA PRIVADA Ethics Committee and inspections by the ANMAT or other regulatory authorities with prior notification and agreement of inspection date and time. Verification of the data contained in the CRFs will be carried out by direct inspection of the source documents. In no case may the inspectors remove original documents, photocopies or data from the study volunteers from Sanatorio Nuestra Señora del Pilar site.

14.3 CASE REPORT FORMS (CRF)

Principal investigator or authorized study staff delegated must complete and sign a CRF for each enrolled patient. This will also apply to the records of those patients who do not complete the study (even when this occurs during the screening period prior to randomization, if a CRF has already been initiated). If a patient withdraws from the study, the reason should be recorded in the CRF. If a patient is withdrawn from the study due to an adverse treatment effect, every effort should be made to clearly document the course.

Forms must be completed in printing or with indelible black ink and must be legible. Errors must be crossed out with a transversal line —never erased—, the correction must be inserted, and the change must be signed and dated by the investigator or its authorized designee. Investigator must ensure that data reported to the sponsor in the CRFs and in all requested reports are complete, accurate, legible, and timely.

14.4 CONTRACT RESEARCH ORGANIZATIONS

The study protocol has been prepared by Dr. Eduardo Pirotzky, who assumes the duties of Principal Investigator for this study, as agreed with the sponsor in the agreement signed between both parties.

15 STUDY MONITORING

The monitor will contact and meet regularly with the investigator and upon request, will be allowed to inspect the various trial records (CRF and other relevant data) as long as patient confidentiality is kept, in accordance with the local requirements.

The monitor will be responsible for inspection of CRFs at regular intervals during the study to verify the degree of compliance with the protocol and that the data entered are complete, consistent and accurate.

The monitor will be able to access laboratory test reports and other patient records needed to verify CRF entries.

Investigator (or his representative) will agree to cooperate with the monitor to ensure the any problems detected during these visits are resolved.

Monitors should review source documents to confirm that the data recorded on the CRFs is accurate.

Investigator and the Hospitalization Center must allow monitors, regulatory authorities and members of the DIM Clínica Privada Ethics Committee who require it, direct access to the source documents to comply with this verification.

In no case will it be authorized to withdraw from the hospitalization center: originals, photocopies or written data of the volunteers of the source documents.

It is important that both the respondent and relevant personnel are available during monitoring visits, and possible audits or inspections, and that they are able to devote sufficient time to this process.

16 CONFIDENTIALITY OF THE STUDY DOCUMENTS AND PATIENT RECORDS

The Principal Investigator and his team, Sanatorio Nuestra Señora del Pilar site must ensure the anonymity of the participating individuals and it will not be authorized to pick up the original, photocopy or annotation that violates the data confidentiality, violating the confidentiality and anonymity of the volunteer.

In the CRF or other documents, patients will not be identified by their names but by their identification code.

The investigator must keep a patient recruitment log in which the codes, names and addresses appear.

All information resulting from this study may be seen by monitors, auditors, members of the DIM CLINICA PRIVADA Ethics Committee and ANMAT regulatory authorities, after arranging the date and time of the visit with the investigator, without violating confidentiality and secrecy about the identity of the volunteer to the extent permitted by applicable laws and regulations.

If the results of the study are published, the volunteer identity will be kept confidential.

According to Law 25,326 on Personal Data Protection: the information collected "...cannot be used for purposes other than or incompatible with those for which it was obtained...", "...must be destroyed when it is no longer necessary or pertinent to the purposes for which it was collected..." and "...the processing of personal data is unlawful when the owner has not given his free, express and informed consent, which must be in writing, or by another equal means, according to the circumstances."

According to the same law, the personal data bearer is allowed to exercise the right of access to them free of charge at intervals no shorter than six months, unless a legitimate interest is accredited to that effect, in accordance with the provisions of section 14, paragraph 3 of Law No. 25326.

The National Directorate for the Personal Data Protection, controlling Body of Law No. 25326, has the power to deal with complaints and claims that are filed in relation to non-compliance with the rules on personal data protection.

All data generated during the study (Registration Forms, patient identification list, laboratory findings) will be kept for 10 years. In the event that the principal investigator could not retain the documentation during this time, the sponsor will be informed, who will be in charge of safeguarding it.

If the results of the study are published, the volunteer identity will be kept confidential. Data related to this study and its results are the property of the sponsor and may not be made public, including presentations at congresses or scientific publications, without the sponsor written permission.

17 QUALITY ASSURANCE

Good Clinical Practice

The study will be conducted adhering to the GCPs, following the international recommendations (ICH) or, otherwise, the European (EMA) and national (ANMAT) Guidelines.

The study quality control and the GCP audits may be carried out by the Sponsor and its quality assurance service or by an inspector from ANMAT. In particular, this protocol will be submitted for the Independent Ethics Committee (IEC) approval. Consistency between study development and the protocol will be controlled. No amendment to the protocol will be made without approval by the CEI that has agreed to the study conduct, and without documented authorization from the Sponsor. Sponsor will be notified of any minor changes to the protocol. In case of important and written modification (modification that could compromise the integrity of the people participating in the study and modifications that could change the scientific validity of the study) the protocol will be submitted again for IEC approval.

Each patient will receive detailed written information, in the information letter, including active name under study, a summary of its properties and potential side effects, doses administered, administration times, number of visits, nature and number of previous exams, previously sent to the IEC.

The patient will be informed that she can withdraw from the study at any time without justification.

The system for assigning numbers to patients is based on the obligation to maintain anonymity. Patients will be numbered in the order of inclusion in the study. They will be identified by the Sponsor by the assigned number, a two-letter code (first letter of the last name and first letter of the first name), their date of birth (day-month-year).

Compliance with the study development will be controlled from the patient screening and throughout the trial. To control the study development, the Principal Investigator will make available to the monitor or to any other person designated, the necessary documents for the control of the data reported in the observation book (medical records, investigations results, etc.).

The Principal Investigator will be available by phone for each patient he has included. The CRFs will be completed legibly with a black pen without deletions or overwriting. Deletions and overwriting, modifications, will be corrected by the Principal Investigator by signing and dating them. The CRF and any other document considered source document that may be filed (biological tests, written informed consent, etc.).

Quality Control

Control actions will be taken within the frame of the Quality Assurance system to verify that the quality requirements of the trial are met.

The original documents, produced during the study, will be controlled by both the Sponsor representative of the Sponsor, the person responsible for the study, and the Principal Investigator at each stage of the study in order to guarantee the reliability of the data analyzed.

Internal audits may be carried out during the course of the study by the representative of the Sponsor or an independent body. These would make it possible to verify that the study is conducted in accordance with the protocol and the regulations and recommendations in force.

18 FINAL REPORT AND PUBLICATION

The Principal Investigator or the Person responsible for conducting the Study agrees to:

- Keep the Sponsor informed in writing of the results of the study.
- Write a study results report for the Sponsor.

19 CALENDAR PROVIDED

The clinical study will begin immediately after the approval of the protocol by the DIM CLINICA PRIVADA Ethics Committee and obtaining the authorizing provision by ANMAT. The duration of the study for a patient will be 56 days.

20 FUNDING AND INSURANCE

The Sponsor will regulate all expenses related to the study. A commercial agreement has been signed between the Sponsor and Research & Development RA. S.A. (CRO Contracted) and with Dr. Eduardo Pirotzky and/or all the doctors participating in the study who are under his control.

The Sponsor assures full and total responsibility for any damage that may result from the experimentation described in this protocol, subject to the fact that the terms and conditions of the study have been thoroughly respected by the Principal Investigator.

21 SIGNATURE PAGE

I have carefully read and reviewed the protocol. Having read and understood the requirements and conditions of the protocol, I agree to conduct this study following international guidelines for good clinical practice and local regulatory requirements, for verification of source documents, study audits and inspections.

I agree to use the study material, including medication, only as stated in this protocol.

I understand that any change to the protocol must be made in the form of an amendment that must be previously approved by the sponsor, DIM CLINICA PRIVADA Ethics Committee and the ANMAT.

I understand that any protocol violation may lead to the study early termination. I agree with the following study schedule. It will begin after the ANMAT approval and will end in a period not exceeding five months later.

I agree to report to Biosidus S.A. within 24 hours of any serious adverse event, whether considered related or not to the drug administered.

I understand that any protocol violation may lead to early study termination.

Principal Investigator

_____	_____	_____
Date	Signature	Name

Biosidus S.A.

_____	_____	_____
Date	Signature	Name

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Annex 1: SCHEDULE OF ACTIVITIES

Protocol activity	**Visit 1**	**Visit 2**	**Visit 3 to 6**	**Visit 7**
	Screening	**Hospitalization**	**Phone Follow-up**	**Final Visit**
	21 days	**1**	**3, 7, 14 and 21**	**35**
Informed Consent	**X**			
Preparation of Medical Record/Progress Form	X	X		
Physical Examination	X			
ECG	X			
Laboratory tests	X			
Evaluation of inclusion/exclusion criteria	X			
Investigational Product Administration		X		
BP, HR and RR measurement prior to blood sampling for evaluation of pharmacokinetic parameters and immunogenicity		X		
Sampling for the evaluation of pharmacokinetic, pharmacodynamic and immunogenicity parameters		X		
* Adverse event evaluation *		X	X	X
Clinical status evaluation		X	X	X
PCR COVID-19	X			
COVID-19 Rapid Test		X		

BP: blood pressure, HR: heart rate and RR: Respiratory Rate

*****.- Please note that the Serious Adverse Event reporting must be made from the time the volunteer signs the Informed Consent and in the time and manner indicated in the protocol.

Annex 2: Declaration of Helsinki of the World Medical Association Ethical Principles for Medical Research Involving Human Subjects

Adopted by the

18th General Assembly, Helsinki, Finland, June 1964 and amended by the:

29th General Assembly, Tokyo, Japan, October 1975

35th General Assembly, Venice, Italy, October 1983

41st General Assembly, Hong Kong, September 1989

48th General Assembly, Somerset West, South Africa, October 1996

52nd General Assembly, Edinburgh, Scotland, October 2000

Note of Clarification added by the WMA General Assembly, Washington 2002

Note of Clarification added by the WMA General Assembly, Tokyo 2004

59th General Assembly, Seoul, Korea, October 2008

64th General Assembly, Fortaleza, Brazil, October 2013

- Introduction
- Basic Principles for all Medical Research
- Principles Applicable When Medical Research Is Combined with Healthcare

DECLARATION OF HELSINKI - Introduction

1. The World Medical Association (WMA) has developed the Declaration of Helsinki as a statement of ethical principles for medical research involving human subjects, including research on identifiable human material and data.

The Declaration is intended to be read as a whole and each of its constituent paragraphs should be applied with consideration of all other relevant paragraphs.

2. Consistent with the mandate of the WMA, the Declaration is addressed primarily to physicians. The WMA encourages others who are involved in medical research involving human subjects to adopt these principles.

General Principles

3. The Declaration of Geneva of the WMA binds the physician with the words, “The health of my patient will be my first consideration,” and the International Code of Medical Ethics declares that, “A physician shall act in the patient’s best interest when providing medical care.”

4. It is the duty of the physician to promote and safeguard the health, well-being and rights of patients, including those who are involved in medical research. The physician’s knowledge and conscience are dedicated to the fulfilment of this duty.

5. Medical progress is based on research that ultimately must include studies involving human subjects.

6. The primary purpose of medical research involving human subjects is to understand the causes, development and effects of diseases and improve preventive, diagnostic and therapeutic interventions (methods, procedures and treatments). Even the best proven interventions must be evaluated continually through research for their safety, effectiveness, efficiency, accessibility and quality.

7. Medical research is subject to ethical standards that promote and ensure respect for all human subjects and protect their health and rights.

8. While the primary purpose of medical research is to generate new knowledge, this goal can never take precedence over the rights and interests of individual research subjects.

9. It is the duty of physicians who are involved in medical research to protect the life, health, dignity, integrity, right to self-determination, privacy, and confidentiality of personal information of research subjects. The responsibility for the protection of research subjects must always rest with the physician or other health care professionals and never with the research subjects, even though they have given consent.

10. Physicians must consider the ethical, legal and regulatory norms and standards for research involving human subjects in their own countries as well as applicable international norms and standards. No national or international ethical, legal or regulatory requirement should reduce or eliminate any of the protections for research subjects set forth in this Declaration.

11. Medical research should be conducted in a manner that minimises possible harm to the environment.

12. Medical research involving human subjects must be conducted only by individuals with the appropriate ethics and scientific education, training and qualifications. Research on patients or healthy volunteers requires the supervision of a competent and appropriately qualified physician or other health care professional.

13. Groups that are underrepresented in medical research should be provided appropriate access to participation in research.

14. Physicians who combine medical research with medical care should involve their patients in research only to the extent that this is justified by its potential preventive, diagnostic or therapeutic value and if the physician has good reason to believe that participation in the research study will not adversely affect the health of the patients who serve as research subjects.

15. Appropriate compensation and treatment for subjects who are harmed as a result of participating in research must be ensured.

Risks, Burdens and Benefits

16. In medical practice and in medical research, most interventions involve risks and burdens.

Medical research involving human subjects may only be conducted if the importance of the objective outweighs the risks and burdens to the research subjects.

17. All medical research involving human subjects must be preceded by careful assessment of predictable risks and burdens to the individuals and groups involved in the research in comparison with foreseeable benefits to them and to other individuals or groups affected by the condition under investigation.

Measures to minimize the risks must be implemented. The risks must be continuously monitored, assessed and documented by the researcher.

18. Physicians may not be involved in a research study involving human subjects unless they are confident that the risks have been adequately assessed and can be satisfactorily managed.

When the risks are found to outweigh the potential benefits or when there is conclusive proof of definitive outcomes, physicians must assess whether to continue, modify or immediately stop the study.

Vulnerable Groups and Individuals

19. Some groups and individuals are particularly vulnerable and may have an increased likelihood of being wronged or of incurring additional harm.

All vulnerable groups and individuals should receive specifically considered protection.

20. Medical research with a vulnerable group is only justified if the research is responsive to the health needs or priorities of this group and the research cannot be carried out in a non-vulnerable group. In addition, this group should stand to benefit from the knowledge, practices or interventions that result from the research.

Scientific Requirements and Research Protocols

21. Medical research involving human subjects must conform to generally accepted scientific principles, be based on a thorough knowledge of the scientific literature, other relevant sources of information, and adequate laboratory and, as appropriate, animal experimentation. The welfare of animals used for research must be respected.

22. The design and performance of each research study involving human subjects must be clearly described and justified in a research protocol.

The protocol should contain a statement of the ethical considerations involved and should indicate how the principles in this Declaration have been addressed. The protocol should include information regarding funding, sponsors, institutional affiliations, potential conflicts

of interest, incentives for subjects and information regarding provisions for treating and/or compensating subjects who are harmed as a consequence of participation in the research study.

In clinical trials, the protocol must also describe appropriate arrangements for post-trial provisions.

Research Ethics Committees

23. The research protocol must be submitted for consideration, comment, guidance and approval to the concerned research ethics committee before the study begins. This committee must be transparent in its functioning, must be independent of the researcher, the sponsor and any other undue influence and must be duly qualified. It must take into consideration the laws and regulations of the country or countries in which the research is to be performed as well as applicable international norms and standards but these must not be allowed to reduce or eliminate any of the protections for research subjects set forth in this Declaration.

The committee must have the right to monitor ongoing studies. The researcher must provide monitoring information to the committee, especially information about any serious adverse events. No amendment to the protocol may be made without consideration and approval by the committee. After the end of the study, the researchers must submit a final report to the committee containing a summary of the study's findings and conclusions.

Privacy and Confidentiality

24. Every precaution must be taken to protect the privacy of research subjects and the confidentiality of their personal information.

Informed Consent

25. Participation by individuals capable of giving informed consent as subjects in medical research must be voluntary. Although it may be appropriate to consult family members or community leaders, no individual capable of giving informed consent may be enrolled in a research study unless he or she freely agrees.

26. In medical research involving human subjects capable of giving informed consent, each potential subject must be adequately informed of the aims, methods, sources of funding, any possible conflicts of interest, institutional affiliations of the researcher, the anticipated benefits and potential risks of the study and the discomfort it may entail, post-study provisions and any other relevant aspects of the study. The potential subject must be informed of the right to refuse to participate in the study or to withdraw consent to participate at any time without reprisal. Special attention should be given to the specific information needs of individual potential subjects as well as to the methods used to deliver the information.

After ensuring that the potential subject has understood the information, the physician or another appropriately qualified individual must then seek the potential subject's freely-given informed consent, preferably in writing. If the consent cannot be expressed in writing, the non-written consent must be formally documented and witnessed.

All medical research subjects should be given the option of being informed about the general outcome and results of the study.

27. When seeking informed consent for participation in a research study the physician must be particularly cautious if the potential subject is in a dependent relationship with the physician or may consent under duress. In such situations the informed consent must be sought by an appropriately qualified individual who is completely independent of this relationship.

28. For a potential research subject who is incapable of giving informed consent, the physician must seek informed consent from the legally authorised representative. These individuals must not be included in a research study that has no likelihood of benefit for them unless it is intended to promote the health of the group represented by the potential subject, the research cannot instead be performed with persons capable of providing informed consent, and the research entails only minimal risk and minimal burden.

29. When a potential research subject who is deemed incapable of giving informed consent is able to give assent to decisions about participation in research, the physician must seek that assent in addition to the consent of the legally authorised representative. The potential subject's dissent should be respected.

30. Research involving subjects who are physically or mentally incapable of giving consent, for example, unconscious patients, may be done only if the physical or mental condition that prevents giving informed consent is a necessary characteristic of the research group. In such circumstances the physician must seek informed consent from the legally authorised representative. If no such representative is available and if the research cannot be delayed, the study may proceed without informed consent provided that the specific reasons for involving subjects with a condition that renders them unable to give informed consent have been stated in the research protocol and the study has been approved by a research ethics committee. Consent to remain in the research must be obtained as soon as possible from the subject or a legally authorised representative.

31. The physician must fully inform the patient which aspects of their care are related to the research. The refusal of a patient to participate in a study or the patient's decision to withdraw from the study must never adversely affect the patient-physician relationship.

32. For medical research using identifiable human material or data, such as research on material or data contained in biobanks or similar repositories, physicians must seek informed consent for its collection, storage and/or reuse. There may be exceptional situations where consent would be impossible or impracticable to obtain

for such research. In such situations the research may be done only after consideration and approval of a research ethics committee.

Use of placebo

33. The benefits, risks, burdens and effectiveness of a new intervention must be tested against those of the best proven intervention(s), except in the following circumstances:

Where no proven intervention exists, the use of placebo, or no intervention, is acceptable; or where for compelling and scientifically sound methodological reasons the use of any intervention less effective than the best proven one, the use of placebo, or no intervention is necessary to determine the efficacy or safety of an intervention.

The patients who receive any intervention less effective than the best proven one, placebo, or no intervention will not be subject to additional risks of serious or irreversible harm as a result of not receiving the best proven intervention.

Extreme care must be taken to avoid abuse of this option.

Post-Trial Provisions

34. In advance of a clinical trial, sponsors, researchers and host country governments should make provisions for post-trial access for all participants who still need an intervention identified as beneficial in the trial. This information must also be disclosed to participants during the informed consent process.

Research Registration and Publication and Disemination of Results

35. Every research study involving human subjects must be registered in a publicly accessible database before recruitment of the first subject.

36. Researchers, authors, sponsors, editors and publishers all have ethical obligations with regard to the publication and dissemination of the results of research. Researchers have a duty to make publicly available the results of their research on human subjects and are accountable for the completeness and accuracy of their reports. All parties should adhere to accepted guidelines for ethical reporting. Negative and inconclusive as well as positive results must be published or otherwise made publicly available. Sources of funding, institutional affiliations and conflicts of interest must be declared in the publication. Reports of research not in accordance with the principles of this Declaration should not be accepted for publication.

Unproven Interventions in Clinical Practice

37. In the treatment of an individual patient, where proven interventions do not exist or other known interventions have been ineffective, the physician, after seeking expert advice, with informed consent from the patient or a legally authorised representative, may use an unproven intervention if in the physician's judgement it offers hope of saving life, re-establishing health or alleviating suffering. This intervention should subsequently be made the object of research, designed to evaluate its safety and efficacy. In all cases, new information must be recorded and, where appropriate, made publicly available.

Annex 3: Storage and labeling

Each treatment unit corresponds to one bottle for intravenous administration of Biosidus Agalsidase or Sanofi Genzyme Fabrazyme® for a period of 5 hours.

Bottle label for each patient contains the following information: Protocol No: RD-BIO—AGA-01/03/21

Bottle N°:

Patient Initials: _____

Pacient N°: __

Investigator´s Name: Eduardo Pirotzky, MD

Date of provision: ___

Lot N°:

Date of Expiration: November 30, 2021

Store between 2°C to 8°C, protect from heat, light and excessive moisture.

For exclusive use in clinical research. Keep out of reach of children.

Biosidus Cazadores de Coquimbo 2841 - Building 4 – 1st Floor (B1605AZE) Munro - Buenos Aires, Argentina

Note: Bottle N° 1 with 35 mg or Bottle N° 2 with 5 mg.

Annex 4: Randomization Table

Patient number	Treatment assigned
1	R
2	T
3	T
4	R
5	T
6	R
7	R
8	T
9	T
10	R
11	R
12	R
13	R
14	T
15	T
16	R
17	T
18	R
19	T
20	T

Table generated using RANDOM software by Randomness and Integrity Services Ltd. Premier Business Centres, 8 Dawson Street, Dublin 2, D02 N767, Ireland.

Annex 5: Adverse reactions organized by system and frequency – Reference Product

Classification System	Very Common	Common	Uncommon	Unknown frequency
infections and infestations		Nasopharyngitis	Rhinitis	
Immune system				Anaphylactoid reaction
Nervous system	Headache, paraesthesia	Dizziness, drowsiness, hypoesthesia, burning, lethargy, syncope	Hyperesthesia, tremor	
Eye		Increased lacrimation	pruritus, ocular hyperaemia	
Ear and labyrinth		Tinnitus, vertigo	Auricular inflammation earache	
Cardiac		Tachycardia, bradycardia, palpitations	Sinus bradycardia	
Vascular		Flushing, hypertension, paleness, hypotension, hot flushes	Limb coldness	
Respiratory, thoracic and mediastinal		Dyspnoea, nasal congestion, throat tightness, wheezing, cough, exacerbated dyspnea	Bronchospasm, pharyngolaryngeal pain, rhinorrhea, tachypnoea, upper respiratory tract congestion	hypoxia
Gastro-intestinal	Nausea, vomiting	Abdominal pain, abdominal discomfort, upset stomach, oral hypoaesthesia, diarrhea	Dyspepsia, dysphagia	
Skin and subcutaneous tissue		Pruritus, hives, rash, erythema, generalized pruritus, angioneurotic edema, facial swelling,	Livedo reticularis, erythematous, rash, pruritic rash, skin dispigmentation,	Classical leukocytic vasculitis

		maculopapular rash	skin discomfort	
Musculoskeletal and connective tissue		Limb pain, myalgia, back pain, muscle spasms, arthralgia, muscle tightness, musculoskeletal stiffness	Musculoskeletal pain	
Administration site and general disorders	Chills, pyrexia, feeling cold	Fatigue, chest discomfort, feeling hot, peripheral edema, pain, asthenia, chest pain, face edema, hyperthermia	Feeling hot and cold, flu-like syndrome, pain at the infusion administration site, reaction at infusion site, thrombosis at injection site, general discomfort, edema	
Investigations				Decreased oxygen saturation

Annex 6: Authorization to conduct Phase I Clinical Pharmacology Studies

Let the company Sanatorio Nuestra Señora del Pilar S.A. be authorized to conduct PHASE I CLINICAL PHARMACOLOGY STUDIES IN THE ADULT POPULATION and BIOEQUIVALENCE STUDIES, at Sanatorio Nuestra Señora del Pilar.

[Argentina's coat of arms]

Argentine Republic – National Executive Branch

2019 – Export Year

Provision

Number: DI-2019-5344-APN-ANMAT#MSYDS

CITY OF BUENOS AIRES

Wednesday, July 3, 2019

Reference: EX-2019-00632005-APN-DGA#ANMAT

IN VIEW OF ANMAT Provisions N° 6677/10, 4008/17 and 4009/17 and File N° EX-2019-00632005-APN-DGA#ANMAT of the "Registro de la Administración Nacional de Medicamentos, Alimentos y Tecnología Médica" *(Registry of the National Administration of Drugs, Food and Medical Technology)* and,

WHEREAS,

By means of these proceedings the company Sanatorio Nuestra Señora del Pilar S.A., requests authorization to conduct Phase I Clinical Pharmacology Studies and Bioequivalence Studies, under the terms of ANMAT Provisions N° 4008/17 and 4009/17 at Sanatorio Nuestra Señora del Pilar.

ANMAT Provision No. 4008/17 in section 11 states the Specific Considerations for Phase I Clinical Pharmacology Studies, indicating in its sections 11.2 and 11.4 the specifications for the investigational site and for the approval and authorizations, respectively.

ANMAT Provision No. 4009/17 approves the requirements and conditions that Healthcare Sites must meet in order to be authorized to conduct Phase I Clinical Pharmacology Studies and/or Bioequivalence studies.

Sanatorio Nuestra Señora del Pilar is duly authorized and registered in Registro Federal de Establecimientos de Salud (REFES) (Federal Registry of Healthcare Sites) of the National Ministry of Health.

Dirección de Evaluación y Registro de Medicamentos (DERM) (Directorate of Drug Evaluation and Registration), through Inspection Order 2019/838-INAME-116, carried out the verification procedure provided for in ANMAT Provision No. 4009/17.

Final technical report by DERM is added, considering site is acceptable for conducting Phase I Clinical Pharmacology Studies in the adult population and Bioequivalence studies.

Directorate of Drug Evaluation and Registration of the National Drug Institute and the General Directorate of Legal Affairs have intervened accordingly.

Action is taken under the powers conferred by Decree No. 1490/92 and its amendments.

Therefore,

THE NATIONAL ADMINISTRATOR OF THE ADMINISTRACION NACIONAL DE MEDICAMENTOS, ALIMENTOS Y TECNOLOGÍA MÉDICA

STATES THAT:

1° SECTION: Let the company Sanatorio Nuestra Señora del Pilar S.A. be authorized to conduct PHASE I CLINICAL PHARMACOLOGY STUDIES IN THE ADULT POPULATION and BIOEQUIVALENCE STUDIES, at Sanatorio Nuestra Señora del Pilar, located at Av. Gaona 3979 PB, Ciudadela, Province of Buenos Aires – CP1702, in accordance with data and conditions described in Annex I herein.

2° SECTION: Let it be stated that the authorization granted by virtue of 1° section will be valid for 5 (five) years, being the bearer obliged to notify this National Administration of any modification in the conditions of health authorization or the conditions of approval in accordance with this provision and ANMAT Provision No. 4009/17.

3° SECTION: - Let it be stated that the authorization granted in accordance with 1° section will be without effect, by operation of law, in the event the Site does not conduct any Phase I Clinical Pharmacology or Bioequivalence research study during the 2 (two) years following the date of granting such authorization.

4° SECTION.- Let it be registered. Let the Admission Desk Department notify the interested party, delivering this provision. Let the Directorate of Drug Evaluation and Registration be notified. Let it be filed once completed these proceedings.

File: EX-2019-00632005-APN-DGA#ANMAT

ANNEX I

DATA AND CONDITIONS FOR THE HEALTHCARE SITE AUTHORIZATION TO CONDUCT PHASE I CLINICAL PHARMACOLOGY STUDIES IN THE ADULT POPULATION AND BIOEQUIVALENCE STUDIES

Information on Investigational Site for Phase I Clinical Pharmacology studies in the adult population and Bioequivalence studies	
Site Name	Sanatorio Nuestra Señora del Pilar
Site address	Av. Gaona 3979 PB, Ciudadela, Province of Buenos Aires-CP1702
Name or Corporate Name of the bearer of the Site Authorization	Sanatorio Nuestra Señora del Pilar
Name of Site Director	Juan Domingo Pugliese, MD (Medical Chief Officer)
Authorized activity	INVESTIGATIONAL SITE FOR PHASE I CLINICAL PHARMACOLOGY STUDIES IN ADULT POPULATION AND BIOEQUIVALENCE STUDIES
Location of area authorized destined to studies	Ground Floor - Recovery room: 8 beds for bioequivalence. When phase 1 studies are conducted in the area, the use of up to 2 simultaneous beds is authorized. - ICU - Rehabilitation Site - Kitchen - Pharmacy - Doctor offices on Ground Floor, 1° and 2° Floors 3° Floor - Meeting room, Filing room, administrative office
Registration in Registro Federal de Establecimientos de Salud (REFES) Disposition 1070/09	Code: 10068402300461 Healthcare Facility with general hospitalization. Category: High risk with intensive therapy
Phone/Fax	4469-6700/6767
Contact	Medical Manager: Néstor Zungri, MD nestor@sanatoriodelpilar.com.ar Principal Investigator: Eduardo Pirotzky MD

	epirotzky@yahoo.com 15 6554-7584 Eduardo Pirotzky MD

File: EX-2019-00632005-APN-DGA#ANMAT

[There follows a digital logo and a statement that reads:] Carlos Alberto Chiale – Administrator – National Administration of Drugs, Food and Medical Technology

Annex 7: Justification for the difference in drug substance strengths of both Agalsidase Biosidus and Frabrazyme®.

Fabrazyme and AGA Biosidus content

To determine the beta agalsidase content in the 35 mg and 5 mg presentations according to our methodology, volume and mass measurements were performed on Fabrazyme.

To that end, several vial batches of each of the Fabrazyme® presentations were reconstituted according to the instructions on the package insert (7.2 mL for the 35 mg presentation and 1.1 mL for the 5 mg presentation). The extractable volume was measured according to RTD-AGA-091 and the agalsidase strength by reverse phase HPLC (RTD-AGA-022).

The RF-HPLC method uses a standard with a quantification value that is previously assigned by optical density (OD). The extinction coefficient to determine the agalsidase beta content in the standard was 2.55 based on the experimental results carried out in Protagen (Germany) using time resolved analysis of said standard (RTD AGA117).

The Fabrazyme results were:

Fabrazyme® 35 mg	Date	cc (mg/mL)	Volume (mL)	Content (mg)
Fabrazyme® 8W0716	February 21, 2019	5.4	7.2	39
Fabrazyme® 8W1890	February 21, 2019	5.4	7.2	39

Fabrazyme® 5 mg	Date	cc (mg/mL)	Volume (mL)	Content (mg)
Fabrazyme® 7HV1176	November 18, 2018	5.4	1.0	5.4
Fabrazyme® 8HV1569	February 21, 2019	5.3	1.0	5.3
Fabrazyme®	November	5.3	1.0	5.3

9W1763	15, 2019			

In other words, although Fabrazyme declares a total of 37 mg (in 7.2 ml), with our methodology the content was 39 mg.

In order to have a target concentration in our product and to be biosimilar to Fabrazyme (not to differ more than 3% in content), the value of 5.3 mg/mL was taken as the target concentration of agalsidase beta in the reconstituted product. Therefore, our product is formulated with approximately 6% overage compared to the declared theoretical concentration.

In other words, each vial of Biosidus Agalsidase labeled 35 mg will contain 5.3 mg/mL when reconstituted in 7.2 mL, total: 38 mg.

With these values, we ensure biosimilarity in content with respect to the reference Fabrazyme, which according to our methodology has a total of 38-39 mg.