RESEARCH PROTOCOL

PROJECT TITLE	EVALUATION OF THE DOSE-RESPONSE OF CELLAVITA HD INVESTIGATIONAL PRODUCT AFTER INTRAVENOUS APPLICATION IN PARTICIPANTS WITH HUNTINGTON'S DISEASE
PROTOCOL CODE	ADORE-DH (*in Portuguese: **A**valiação da **Do**se-**Re**sposta do Produto Investigacional CELLAVITA HD em Participantes com **D**oença de **Huntington*** – Evaluation of the dose-response of Cellavita HD investigational product after intravenous application in participants with Huntington's disease)
DOCUMENT VERSION	Amendment 6 dated February 23, 2021
STUDY PHASE	Phase II
INVESTIGATIONAL PRODUCT	Cellavita HD
SPONSOR	**CELLAVITA PESQUISA CIENTÍFICA LTDA** Rua Martinho Leardine, 296 Chácara Silvania, Valinhos/ SP - ZIP Code: 13271-650
ISSUANCE OF THE PROTOCOL	**AZIDUS BRASIL PESQUISA CIENTÍFICA E DESENVOLVIMENTO LTDA.** Rua General Osório, 507 Valinhos/SP - ZIP Code: 13271-130 Phone/Fax: (19) 3829-6160

I. Sponsor's Signature

My signature, together with the signature of the Investigator, confirms the treaty between both parties that the clinical study will be conducted in accordance with the protocol and all applicable laws and regulations, including, among others, the Guidelines of the International Conference on Harmonization (ICH) for Good Clinical Practice (GCP), the Code of Federal Standards (CFR), Resolution 466 of December 12, 2012, the FDA document *Considerations for the Design of Early-Phase Clinical Trials of Cellular and Gene Therapy Products - Guidance for Industry* (2015) applicable privacy laws.

This protocol was issued according to the international protocol preparation guide called 2013 SPIRIT Statement - *Standard Protocol Items: Recommendations for Interventional Trials*.

Signature - Sponsor	Signature Date (dd/mm/yyyy)
Name and Position of the Responsible - Sponsor (printed)	

II. Statement of the Principal Investigator

My signature, in conjunction with Sponsor's signature, confirms the treaty between both parties that the clinical study will be conducted in accordance with the protocol and all applicable laws and regulations, including, but not limited to, the Guidelines of the International Harmonization Conference (ICH) for Good Clinical Practice (GCP), the Code of Federal Standards (CFR), ethical principles originated through Resolution 466 of December 12, 2012 and applicable privacy laws.

Nothing contained in this document is intended to limit a physician's authority to provide emergency medical care in accordance with current regulations. I will provide copies of the protocol and all relevant information to all professionals involved in this study, which are under my responsibility. I will discuss this material with these professionals in order to ensure that they are fully informed regarding the research product and the conduct of the study.

I will use the Informed Consent Form (ICF) approved by the Research Ethics Committee and fulfill all responsibilities to present pertinent information to the Ethics Committee responsible for the ethical issues of this study.

I also agree to report all information or data in accordance with the protocol, and in particular, I agree to report any serious or non-serious adverse events related or not to the study medication.

Signature - Principal Investigator	Signature Date (dd/mm/yyyy)
Name and position – Principal Investigator (printed)	

III. Description of the professionals and institutions involved in the research

A. Research Center

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E. Contact in case of Adverse Events

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F. Persons authorized to sign the protocol by Sponsor

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Position	Administrative Coordinator
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IV.REVISION/VERSION CONTROL

Status	No.	Date	Reason for revision/version
Version	1.0	21/12/2015	Version after Sponsor's approval.
Version	1.0	25/05/2016	After the pending opinion (No. 1,521,528) issued by CONEP on May 17, 2016, the pending was answered and subsequently approved on 06/08/2016.
Amendment	1.0	06/09/2017	Changes to prevent the research participant from being exposed to unnecessary scans. Insertion of the 6-month uniformization period before randomization / initiation of treatment and reduction of total treatment time with the possibility of continuing treatment in an extension study. There was the exchange of the principal investigator of the study due to the resignation of the former PI of the company.
Amendment	2.0	29/09/2017	Elucidate the activities that will be performed in each visit of the study as well as change the primary parameter, which instead of evaluating only the motor scale, will evaluate all domains of the UHDRS scale.
Amendment	3.0	09/04/2018	Restructuring of study visits and laboratory tests.
Amendment	4.0	10/09/2018	Correction some information existing in the previous version and improve the textual understanding of the activity to be performed during the study.
Amendment	5.0	26/08/2019	Clarify questions related to inclusion visits, uniformization and initiation of treatment that could generate doubt, in addition to textual review of motor and cognitive evaluation.
Amendment	6.0	23/02/2021	Exclusion of dosing of the prognostic biomarker NF-L (neurofilament protein) as an exploratory objective and alterations pertinent to the protocol procedures.

V.SYNOPSIS OF THE PROTOCOL

Clinical Study Code	ADORE-DH
Clinical Study Title	EVALUATION OF THE DOSE-RESPONSE OF CELLAVITA HD INVESTIGATIONAL PRODUCT AFTER INTRAVENOUS APPLICATION IN PARTICIPANTS WITH HUNTINGTON'S DISEASE
***Status* of the document**	Amendment 6 dated February 23, 2021.
Population of the Clinical Study	Participants of both genders, aged between 21 and 65 years, with laboratory diagnosis (genetic) of Huntington's disease and presenting the eligibility criteria determined for the study.
Objective(s) for assessment of efficacy	• **Primary efficacy objective** The primary objective of this clinical trial is to identify the dose of Cellavita-HD product that has the best clinical response. ○ **Primary variable** Verified through the *Score* of the UHDRS (Unified Huntington's Disease Rating Scale) of the baseline in relation to the end of treatment (domains that comprise the assessments: motor, cognitive, behavioral, functional capacity and independence). • **Secondary efficacy objectives** The secondary objectives of the study are: a) Clinical neurological evaluation throughout the study; b) Overall assessment of Huntington's disease severity (CIBIS scale); c) BMI assessment; d) Assess the incidence of suicide attempts during treatment; e) Neurological evaluation by imaging. ○ **Secondary variables** a) Score of the UHDRS scale throughout the study (at each visit); b) Score of the CIBIS scale throughout treatment; c) Weight of the participant throughout the study; d) Score obtained in the suicide domain of the HAM-D scale during treatment; e) It will be assessed through the profiles obtained during the treatment of the following measures: • Cortical thickness; • Volume of the various brain structures, especially the core of the base, with special attention to the caudate; • Identifiable metabolic changes in proton spectroscopy.

Objective for safety assessment	The safety of the drug will be evaluated through the incidence and classification of adverse events occurring between treatments regarding type, frequency and intensity.
Inclusion criteria	a) Providing informed consent in writing, signed and dated; b) Participant of both genders aged ≥ 21 and ≤ 65 years*; c) Present confirmatory diagnostic report (PCR) of Huntington's disease with a number of CAG repeats on chromosome 4, greater than or equal to 40 and less than or equal to 50 (if the participant has not undergone the examination and/or if he/she does not have the report of the examination accessible, a new examination should be performed); d) Obtaining 5 points or more in the motor evaluation of the UHDRS (Unified Huntington's Disease Rating Scale) at the time of inclusion; e) Obtaining 8 to 11 points in the functional capacity of the UHDRS scale at the time of inclusion. *Note: The age of the participant will be considered on the day of the inclusion visit (V-3).
Exclusion criteria	a) Participant of the research who has participated in protocols of clinical studies in the last 12 (twelve) months, unless the researcher considers that there may be direct benefit to him/her (Resolution CNS 251, of August 7, 1997, item III, subitem J); b) Diagnosis of juvenile Huntington's disease*; c) Diagnosis of epilepsy; d) Diagnosis of major cognitive disorder; e) Decompensated active psychiatric disease; f) Current or past history of neoplasia; g) Current history of severe and uncontrolled gastrointestinal, hepatic, renal, endocrine, pulmonary, hematological, immunological, metabolic, or cardiovascular disease; h) Diagnosis of any active infection, be it viral, bacterial, fungal or caused by another pathogen; i) Participant who has contraindication to the tests performed in this study, such as having a pacemaker or surgical clip; j) Alcohol and drug abuse (previous diagnosis according to the criteria of the Diagnostic and Statistical Manual of Mental Disorders - DSM V); k) Use of illicit drugs; m) Present positive results in one of the serological tests: HIV 1 and 2 (Anti-HIV-1.2), HTLV I and II, HBV (HBsAg, Anti-HBc), HCV (anti-HCV-Ab) and FTA-ABS (Treponema pallidum); n) History of drug allergy, including contrasts for imaging, or bovine products; o) In use or foresight of the use of immunosuppressive drugs or prohibited drugs (item 6.9) during the first three months after the first administration of the investigational product; p) Any clinical change that is interpreted by the Investigator physician as a risk to the inclusion of the participant in the study. **Note: The diagnosis of juvenile Huntington's will be defined by the presence of 60 or more CAG repeats in the confirmatory diagnostic report for the disease and the appearance of characteristic signs and symptoms before the age of 21 (Nance, 2001).**

Methodology	The Clinical trial will be prospective, phase II, single-center, randomized (2:2:1), triple-blind, with two test-doses, placebo-controlled. The study will include 35 participants. Initially, participants will be randomized in ratio 2 : 2 : 1 in groups G1: lower dose ($1x10^6$ cells/weight range), G2: higher dose ($2x\ 10^6$ cells/weight range) or G3: placebo; i.e. of the 35 participants, 14 will be randomized to the lowest dose group, 14 for the highest dose group or 7 for the placebo group. The group receiving placebo is numerically smaller to prevent more people from not receiving treatment.
Product Under Investigation	**Test 1:** Cellavita-HD **Dose:** 1 x 10^6 cells/weight range per administration **Pharmaceutical form:** Liquid suspension of allogeneic cells. **Test 2:** Cellavita-HD **Dose:** 2 x 10^6 cells/weight range per administration **Pharmaceutical form**: Liquid suspension of allogeneic cells.
Comparator Product	• **Placebo:** test product base without allogeneic cells.
Dosage	**Product dosage:** both placebo and tests will be administered IV monthly until 3 doses (one cycle) are completed. There will be 3 cycles throughout the study.
Number of participants	35 participants.
Duration of the Treatment	The study will last approximately 14 months. (420 days) per participant. There are 15 scheduled visits, including: • Inclusion visit V-3 • Uniformization visits (V-3, V-2 and V-1), • Visits to the administration of the investigational product (cycle 1 - Visits 0, 1 and 2; cycle 2 - Visits 4, 5 and 6; cycle 3 - Visits 8, 9 and 10) • Follow-up visits (Visit 3, 7 and 11).

VI.List of abbreviations and acronyms

°C: Degree Celsius
AEs: Adverse Events
ANVISA: National Health Surveillance Agency
ASC: Area Under the Curve
BMI: Body Mass Index
BP: Blood pressure
CEP: Research Ethics Committee
CFR: Code of Federal Standards
CI: Confidence Interval
CK: Creatine Kinase
CKmB: Creatine Kinase mB
Cmax: Maximum Concentration
CNS: Central Nervous System
CROR: Clinical Research Organization Representative
DECH: graft-versus-host disease
DMSO: Dimethyl Sulfoxide
DRPLA: Dentatorubro-Palidoluisian Atrophy
ECG: Cardiogram
e-CRF: Clinical Record or Case Report Form
EDC: Electronic Data Capture
ELISA: Enzyme-Linked Immunosorbent Assay
HD: Huntington's disease
HR: Heart Rate
FDA: Food and Drug Administration
FES: Fatty Embolism Syndrome
Gama-GT: Gamma glutamyl transferase
GCP: Good Clinical Practices
Hb: Hemoglobin
HBV: Hepatitis B Virus
HCV: Hepatitis C Virus
HDL: High Density Lipoprotein
HES: Hydroxyethyl Starches
HGF: Hepatocyte Growth Factor

hIDPSC: Human Deciduous Tooth Pulp Stem Cells

HIV: Human Immunodeficiency

HSC: Hematopoietic Stem Cells

Ht: Hematocrit

HTLV: Human T Cells of Lymphotropic Virus

HTT: Huntingtin Protein

ICF: Informed Consent Form

ICH: International Harmonization Conference

IGF-1 and 2: Insulin-like Growth Factor

ITT: Population of Analysis with Intent to Treat (Intention-to-Treat)

IL: Interleukins

LDL: Low Density Lipoprotein

MSC: Mesenchymal Stem Cell

Nanog: Transcription Factor and, Key Factor of Pluripotency

NHC: National Health Council

Oct-4: Octomer-4 Transcription Factor

PCR: Polymerase Chain Reaction

PP: Analysis Per Protocol

SOPs: Standard Operating Procedures™

SAEs: Serious Adverse Events (Serious)

SD: Standard deviation

SDF: Stromal Derived Factor-1

SSEA-3 and 4: Stage-Specific Embryonic Antigens

TG: Triglycerides

TGO: Oxalacetic Glutamic Transaminase

TGP: Pyruvic Glutamic Transaminase

TNF-α: Tumor Necrosis Factor alpha

TRA 1-60, TRA 1-81: Tumor Recognition Antigens

TRALI: Transfusion-Related Acute Lung Injury

UHDRS: Unified Huntington's Disease Rating Scale

USA: United States of America

VEGF: Vascular Endothelial Growth Factor

VLDL: Very Low-Density Lipoprotein

Vx: Visit Number x

WHO: World Health Organization

VII.Framework of study activities - Inclusion and Uniformization Period

Activities	Inclusion	Uniformization	
	V-3	V-2	V-1
	-37 to -1 days before V-2	Up to 30 + 7 days after V-3	30 + 7 days after V-2
Consent form (ICF)	X		
Evaluation of inclusion/exclusion criteria	X	X[a]	X[a]
Medical evaluation: medical history, and physical examination/vital data	X	X	X
Demographic data (date of birth, gender and race)	X		
Confirmation or performance of laboratory diagnosis of Huntington's disease	X		
Evaluation of laboratory diagnosis of Huntington's disease, if applicable		X[b]	
Blood and urine collection for laboratory inclusion tests	X		
Evaluation of laboratory blood and urine tests		X	
Pregnancy urinary test for women	X	X	X
Blood collection for serology examination	X		
Evaluation of the serology examination		X	
ECG performance	X		
Evaluation of ECG result		X	
Performance of CNS MRI	X		
Assessment of the CNS MRI result		X	
UHDRS Scale	X	X	X
CIBIS Scale	X	X	X
Hamilton Depression Assessment Scale (HAM-D)	X	X	X
Activities	**Inclusion/uniformization**	**Uniformization**	
	V-3	**V-2**	**V-1**

	-37 to -1 days before V-2	**Up to 30 + 7 days after V-3**	**30 + 7 days after V-2**
Concomitant/prior medication registration	X	X	X
Evaluation of adverse events	X	X	X
Assessment of pregnancy risk and dispensation of contraceptive method, if applicable.	X	X	X
General guidelines	X	X	X

[a] Items "d" and "e" of the inclusion criteria will be evaluated only at the inclusion visit (V-3) due to the natural fluctuation of the disease
[b] For participants who need to carry out the laboratory diagnosis of Huntington's disease, the test will be performed at V-3 and evaluated at V-2

VII. Framework of study activities - Treatment and Follow-up Period

Activities	V0 Start of treatment - Up to 30 days after V-1	V1 30+5 days after V0	V2 60+5 days after V0	V3 90+5 days after V0	V4 120+5 days after V0	V5 150+5 days after V0	V6 180+5 days after V0	V7 210+5 days after V0	V8 240+5 days after V0	V9 270+5 days after V0	V11 300+5 days after V0	V11[c] 330+5 days after V0
	1st adm. Cycle 1	2nd adm. Cycle 1	3rd adm. Cycle 1	F-up.	1st adm. Cycle 2	2nd adm. Cycle 2	3rd adm. Cycle 2	F-up.	1st adm. Cycle 3	2nd adm. Cycle 3	3rd adm. Cycle 3	Final
Evaluation of inclusion criteria/ Exclusion (except criteria "d" and "e")	X											
Evaluation of discontinuation criteria		X	X	X	X	X	X	X	X	X	X	X
Medical evaluation: medical history and physical examination/vital data	X	X	X	X	X	X	X	X	X	X	X	X
Randomization	X											
Intravenous administration of Cellavita-HD or placebo (hospitalization for a period prior to administration and for another 06 hours)	X[a]	X[a]	X[a]		X[a]	X[a]	X[a]		X[a]	X[a]	X[a]	
Blood and urine collection for laboratory tests				X				X				X
Evaluation of laboratory test results					X				X			X
Pregnancy urinary test for women	X	X	X	X	X	X	X	X	X	X	X	X
Blood collection for serology												X
Evaluation of serology result												X
ECG performance				X				X				X
Evaluation of ECG result					X				X			X
ECG monitoring	X	X	X		X	X	X		X	X	X	
UHDRS and HAM-D scale and CIBIS	X	X	X	X	X	X	X	X	X	X	X	X
MRI for CNS evaluation												X
Evaluation of CNS MRI result												X

Activities	V0 Start of treatment - Up to 30 days after V-1	V1 30+5 days after V0	V2 60+5 days after V0	V3 90+5 days after V0	V4 120+5 days after V0	V5 150+5 days after V0	V6 180+5 days after V0	V7 210+5 days after V0	V8 240+5 days after V0	V9 270+5 days after V0	V11 300+5 days after V0	V11[c] 330+5 days after V0
	1st adm. Cycle 1	2nd adm. Cycle 1	3rd adm. Cycle 1	F-up.	1st adm. Cycle 2	2nd adm. Cycle 2	3rd adm. Cycle 2	F-up.	1st adm. Cycle 3	2nd adm. Cycle 3	3rd adm. Cycle 3	Final
Concomitant medication registration	X	X	X	X	X	X	X	X	X	X	X	X
Evaluation of adverse events	X	X	X	X	X	X	X	X	X	X	X	X
Assessment of pregnancy risk and dispensation of contraceptive method, if applicable.	X	X	X	X	X	X	X	X	X	X	X	X[b]
General guidelines	X	X	X	X	X	X	X	X	X	X	X	X
Dispensation from the study												X

[a] Hospitalization for 06 hours: all procedures provided for in the protocol should be performed at the times determined, including evaluation of vital signs, clinical examination and ECG. The length of stay can be extended at the discretion of the principal investigator.
[b] In this visit, because it is the last visit of the study, there will be no dispensation of contraceptive method, only assessment of the risk of pregnancy.
[c] If any examination performed on this visit presents any clinically significant abnormality, the research participants will be invited to perform an extraordinary visit.

Summary

1. INTRODUCTION

According to ANVISA definition, cell therapy aims to restore the function of an organ or tissue of the human body, through the transplantation of cells that have differentiation capacity. Cell therapy with stem cells is considered an example of regenerative medicine and is currently in the world stage, as it may represent the improvement or maintenance of quality of life for patients affected by pathologies that are extremely debilitating and do not have a cure so far, such as degenerative diseases. In Brazil, the use of stem cells for therapeutic purposes has also been the target of research, however, compared to other countries, it is currently in the early stages (Pereira & Queiroz, 2012, ANVISA, 2015).

The term *"Stem cell"* was proposed by the Russian histologist Alexander Maksimov in 1908 for scientific use. By definition, stem cells are primitive and undifferentiated cells, which have the ability to replicate indefinitely and/or differentiate into several cell types (Kim & Vellis, 2009; de Souza et al., 2013; Zuttion et al., 2013). Among the tissues known to have stem cells, the bone marrow was the most studied, as a source of both hematopoietic stem cells (HSC), which classically give rise to all blood lineage, as well as mesenchymall stem cells (MSC), which give rise to various connective tissues and other non-mesodermal cell types such as neural cells and hepatocytes (Bydlowski et al., 2009; Kim & Vellis, 2009; de Souza et al., 2013; Zuttion et al., 2013).

1.1. Tooth pulp as a source of mesenchymal stem cells

Mesenchymal stem cells are found not only in the bone marrow, but also in the umbilical cord, tooth pulp and fat, and are responsible for tissue regeneration in cases of injury during life. Mesenchymal cells secrete a variety of biomolecules such as cytokines, which have trophic activity and are capable of promoting a regenerative microenvironment, in addition to other molecules that contribute to tissue reconstruction, such as immunomodulatory mediators. In general, mesenchymal cells can reduce chronic inflammation, inhibit apoptosis, stimulate mitosis of tissue-intrinsic progenitors, stimulate angiogenesis and neurogenesis, and reduce tissue destruction due to oxidative stress, i.e., they are capable of improving the injured microenvironment and promoting tissue remodeling (Aleynik et al., 2014; Kerkis et al., 2015; Kerkis & Caplan, 2012; Kim & Cho, 2013).

Several studies have shown that the pulp of deciduous teeth (milk tooth) is a tissue rich in mesenchymal stem cells and that these cells have high differentiation capacity in several cell lines, in addition to rapid proliferation, high clonogenic capacity and excellent expansion *in vitro*, and can be kept in culture for a long period of time. In addition, the removal of these cells does not present any ethical restriction, since deciduous teeth are discarded after a natural process of primary resorption of the tooth root (exfoliation) and, if necessary, the technique of excision of the tooth, is considered simple and minimally invasive (Kerkis et al., 2015; Kerkis & Caplan, 2012).

1.2. Characterization of the investigational product

The investigational product object of study in this protocol, called Cellavita-HD is a biotechnological product developed by a group of researchers in stem cells of the Butantan Institute led by Prof. Dr. Irina Kerkis. It has been researched and validated for years and today is characterized as a product originating from human deciduous tooth pulp stem cells (*Human Immature Dental Pulp Stem Cells* – or in abbreviated form, hIDPSC). The cells that are present in the Cellavita-HD product correspond to the population of somatic/adult stem cells similar to mesenchymal stem cells (MSC) (Kerkis et al., 2015; Kerkis & Caplan, 2012). This technology was patented by the research group (Prof. Dr. Irina Kerkis) and the Butantan Institute (*Application number* CT/IB2014/059850). In 2014, this patent was licensed by a biotechnology company, 100% Brazilian *Start-up*, called Cellavita Pesquisa Científica Ltda.

This biotechnology developed by the group allows the origin from a single tooth pulp a large amount of stem cells, which allows the clinical investigation of possible beneficial properties of these cells in patients with neurodegenerative diseases (considering the ability of these cells to differentiate into neurons).

In 2006, Dr. Irina Kerkis et al. isolated hIDPSC cells from dental pulp following the pulp tissue explant technique (procedure used for the isolation of neural crest stem cells), under cultivation conditions similar to those used for embryonic stem cells (Kerkis et al., 2006; Kerkis & Caplan, 2012; Kerkis et al., 2015). Due to the characteristics related to their embryogenesis, these cells present promising and diversified therapeutic possibilities resulting from their rapid proliferation, high differentiation capacity in several cell lines (multipotential properties), in addition to

the ability to acquire the phenotype of another cell of a different tissue or organ (plasticity) (Kerkis et al., 2015; Kerkis & Caplan, 2012).

Immunocytochemical studies have shown that these cells express some markers of human embryonic stem cells such as Oct-4 (Oct-4 transcription factor), Nanog (transcription factor and key factor of pluripotency), SSEA-3 and 4 (stage-specific embryonic antigens), TRA 1-60, TRA 1-81 (tumor recognition antigens), but also mesenchymal stem cell markers such as CD105 (SH2), CD73 (SH3 and SH4), CD13 and CD31, which were observed in approximately 97% of the cells. No hematopoietic markers were found (Kerkis et al., 2006; Kerkis & Caplan, 2012; Kerkis et al., 2015).

To date, the Cellavita-HD investigational product has not presented any clinical or experimental evidence that could compromise the safety of the use of this therapy, such as tumor formation or risk of immune rejection. A relevant characteristic of this type of cell is its ability to migrate to pathological sites, a mechanism called pathotropism, in addition to its property of crossing the blood brain barrier and reaching the central nervous system (CNS) (Aleynik, et. al., 2014; Kerkis et al., 2015; Kerkis & Caplan, 2012). In addition, the process of *in vitro* expansion and proliferation of these cells is standardized to be cryopreserved in a Cell Bank (*Master Cell Bank*) and used in future clinical applications of cell therapy (Almeida, et. al., 2011; Kerkis & Caplan, 2012).

First, Huntington's disease was established as a therapeutic target of the Cellavita-HD investigational product. Next, the main aspects of this pathology are described.

1.3. Therapeutic target: Huntington's disease (HD)

Huntington's disease (HD) is a fatal, genetic, highly debilitating and progressive neurodegenerative disorder characterized by the following clinical signs and symptoms: cognitive, motor and psychiatric dysfunction. Usually, the onset of symptoms of the disease occurs between 25 and 45 years of age (ranging from 3-70 years). Once started, cognitive-behavioral symptoms and engines progress inexorably until they result in a fatal outcome. The most striking sign of the disease is the presence of involuntary movements, called choreic movements, which are continuous, brief, random and result in contractions of the orofacial muscle and limbs (Ross et al., 2014; Nuzzo et al., 2014). Emotional changes and behavioral changes

that result in anxiety and depression, although frequent, do not occur uniformly among patients, as motor and cognitive alterations occur (Ross et al., 2014).

Several factors alter the overall prevalence of the disease, such as: different ethnicities, geography, migration patterns and location. The disease has a global prevalence of about 5.7 cases per 100,000 people in the Western world, and the incidence is more prevalent in descendants of Western Europe, North and South America and Australia, and rare in Africans and Asians (Olanow and Schapira, 2012; Gonçalves, 2013). HD is an autosomal dominant genetic disorder caused by a mutation in the huntingtin protein gene (*HTT*), located on the short arm of chromosome 4 (location 4p 16.3). This genetic mutation causes increased repetition of the CAG trinucleotide sequence (responsible for encoding the glutamine). This abnormal repetition expanded produces a protein with a long chain of glutamine, known as polyglutamine or PolyQ, in the N-terminal region of exon 1 of the gene, which constitutes the abnormal huntingtin protein (Figure 1) (Squitieri et al., 2006; Gonçalves, 2013; Ross et al., 2014; Nuzzo et al., 2014). HD is one of the 9 PoliQ disorders, which additionally include spinocerebellar ataxias (ACE type 1, 2, 3, 6, 7 and 17), bulbospinal muscular atrophy and dentorubro-palidoluisian atrophy (DRPLA), and among these, HD is the most prevalent (Schols et al, 2004).

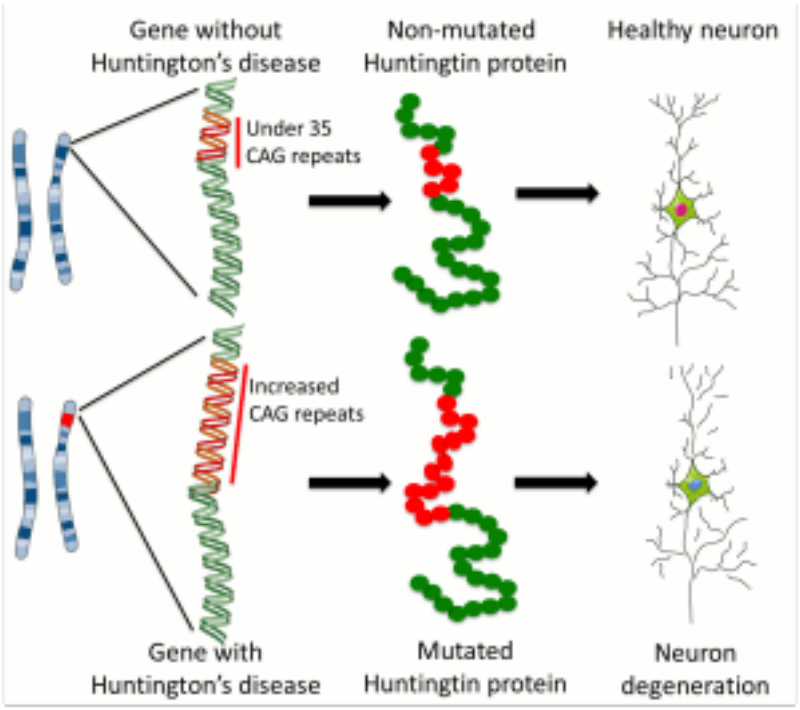

Figure 1. Illustration demonstrating how the Huntingtin gene mutation (*HTT*) produces increased repetitions of the CAG sequence, generating the abnormal huntingtin protein that causes neuronal degeneration.
Source:http://www.eurostemcell.org/factsheet/Huntington%E2%80%99s-disease-how-could-stem-cells-help

Although the first more detailed report of the disease occurred in 1872 by physician George Huntington, it was in the year of 1983 that the mutation of the gene *HTT* which causes the disease was localized and, in 1993, it was isolated by a group of researchers (*Huntington's Disease Collaborative Research Group*) who identified the mutation precisely on chromosome 4 in the IT15 part (Zuccato et al., 2010; Finkbeiner, 2011; Colle, 2012; Gonçalves, 2013). The gene *HTT* has a large DNA sequence (180 kb) consisting of 67 exons (Ambrose et al., 1994) and its sequence differs from any gene identified earlier (*The Huntington's Disease Collaborative Research Group*, 1993).

Individuals not affected by the disease who have normal huntingtin protein have on average a sequence of 19 repetitions of glutamine. On the other hand, HD patients have the number of CAG repeats in the *HTT* above 35 (Kremer et al., 1994; Hogarth, 2013). According to the number of repetitions that the mutation causes, different phenotypes of the disease may be observed, especially with regard to the age of onset of clinical manifestations. In this way, with CAG repeats of 36 to 39 (gray zone – incomplete penetrance) the patient may remain asymptomatic for many years and the onset of symptoms occur later, with slower progression (Rubinsztein et

al., 1996; Quarrell et al., 2007). However, higher repetitions of the PoliQ sequence (mainly above 60), may result in more severe conditions of the disease in which symptoms onset earlier (before 20 years of age) and progress faster than in patients with fewer repetitions (Telenius et al., 1993). These more serious conditions (called juvenile HD) represent about 7% of all cases of HD and, because having a rapid progression can lead these patients to death in about 11 years (Foroud et al., 1999; Gonçalves, 2013).

Despite being a hereditary and autosomal dominant disease, sporadic cases of HD have been reported in patients with asymptomatic parents who have 30-35 polyQ repeats (De Rooij et al., 1993; Goldberg et al., 1993; Hendricks et al., 2009). This fact corroborates the information found in the review by Gil-Mohapel & Rego (2011) and in Hogarth's article (2013), in which both discuss and describe the risk of HD transmission, according to the amount of CAG repetitions that the individual has. According to these authors, individuals who have 28-35 CAG repeats will not manifest the disease, however their descendants may have an increased risk of developing the disease if mutations occur in this gene (Gil-Mohapel and Rego, 2011; Hogarth, 2013). Figure 2 classifies the risk of transmission of the disease according to the number of CAG repetitions that the individual has.

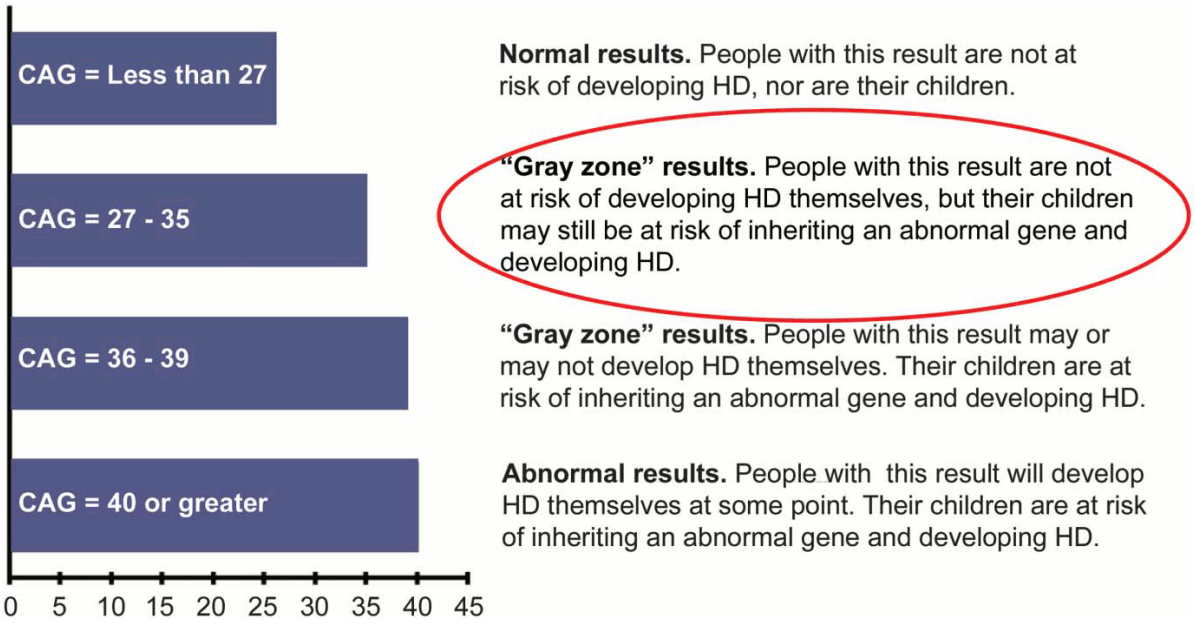

Figure 2. Classification of the risk of transmission of the disease according to the number of repetitions of the CAG sequence in individuals. CAG = less than 27 → Normal Result: there is no risk to develop the disease and transmit to descendants. CAG = 27 - 35 → the Gray Zone: no risk to develop the disease, but at risk of transmitting to the descendants. CAG = 36-39 → Gray zone: risk of manifesting the disease in adulthood and risk of transmission to descendants. CAG = greater than 40

→ Abnormal Outcome: manifestation of the disease at some point in life and transmission to descendants. Source: Hogarth, 2013.

The function of normal huntingtin protein (Htt) is not well established in humans, however it is suggested that this protein presents interaction with several proteins involved in transcription processes, cell signaling and intracellular transport. In addition, this protein has a vital function in embryonic development, a data collected from studies carried out with genetically manipulated animals to develop HD, in which they showed that the absence of Htt is directly related to the death of the embryo (Gonçalves, 2013).

Similarly, although the mechanism by which abnormal huntingtin protein acts in tissues is not fully known, it is known that it is potentially toxic to cells, affecting the middle spinous neurons in the CNS, specifically in the region of the *Striatum* at the onset of the disease and then in other areas of the brain such as thalamus and the cerebral cortex, as the disease progresses (Mink, 1996; Silverthorn, 2003; Vonsatteland Di Figlia, 1998; Gil-Mohapel and Rego, 2011).

The middle spinous neurons of the *Striatum* have inhibitory function over the other areas with which it communicates through gamma-aminobutyric acid (GABA) neurotransmitters and the co-transmitters dinorphine, Encephalin or substance P. Thus, the loss of the inhibitory effect caused by the death of the middle spinous neurons results in the loss of thalamus inhibition, which leads to increased activation of the direct circuit of the basal ganglia and consequent exacerbation of nerve impulses to the motor regions of the body, limbs and eyes, causing the undesirable choreic movements characteristic of HD (Mink, 1996; Wolf, 2009; Gil-Mohapel and Rego, 2011).

With the progression of the disease, there is an increase in cell death in other areas of the brain and as a result, patients with HD present emotional, cognitive and motivational difficulties, in addition to motor complications, behavioral and mood changes, loss weight, irritability, anxiety and depression (Foroud et al., 1999; Vonsattel and DiFiglia, 1998). Cognitively, there is a deficit on executive functions (Walker, 2007). With the progression of the disease, patients develop difficulties in coordination, including speech, swallowing and walking (Foroud et al., 1999). Disease-associated mortality is often related to complications such as pneumonia and heart disease (Sorensen and Fenger, 1992; Zuccato et al., 2010).

Huntington's disease has no cure and there are no medicines to date that can stop or reverse its progression. However, there are treatments with pharmacological and non-pharmacological approaches that only mitigate the symptoms presented by patients (symptomatic treatments).

1.3.1. Pharmacological approach

The medications most commonly prescribed for these patients include antipsychotics, anxiolytics, antidepressants, mood stabilizers and, in patients with juvenile HD, anticonvulsants. Dopamine-blocking agents, such as tetrabenazine, which has been approved by the United States of America (USA), can control the symptoms of chorea (Hayden et al., 2009; Wang et al., 2010). The same antipsychotic medications such as haloperidol, clozapine, chlorpromazine and olanzapine are prescribed to reduce chorea as they have dopamine antagonistic action and, additionally, the benefit of controlling the behavioral and/or psychotic characteristics that HD patients may present (Jankovic, 2009; Phillips et al., 2008).

To deal with symptoms of depression, suicidal thoughts and impulsiveness, Antidepressant medications (fluoxetine, escitalopram, sertraline, paroxetine and venlafaxine) are often prescribed for HD patients. In addition, anxiolytic medicines such as diazepam, benzodiazepines (Clonazepam, Alprazolam), paroxetine and venlafaxine are indicated to calm patients and mitigate anxiety symptoms associated with the disease (Phillips et al., 2008; Kaplan and Stockwell, 2012).

For mood stabilization, drugs such as lithium, valproate and carbamazepine can be prescribed to patients (Bonelli and Wenning, 2006). There are a lot of medications that can be used to control HD, however, many of these cause adverse effects that can worsen the patient's functionality and quality of life. To date, there is no adequate treatment for the inevitable cognitive and motor decline observed in HD progression (Olanow & Schapira, 2012).

1.3.2. Non-pharmacological approach

Physical and occupational therapies can promote improved motor coordination in the early stages of the disease and exercises can strengthen muscles and improve balance and posture. Psychiatric therapy can help reduce anxiety, depression, stress, as well as help cope with the changes associated with the

disease. Additionally, decreased stress and anxiety can help in the control of chorea. Speech therapists can work with HD patients assisting in communication and swallowing problems (Bilney et al., 2003).

1.3.3. Use of cell therapy in Huntington's disease

Cell therapy has been actively researched as a therapeutic alternative to modify the course of Huntington's disease, especially regarding neurodegeneration and selective neuronal loss of middle spinous neurons, which occurs in the striated body and deeper layers of the brain. Some studies have been published using striated brain tissue transplantation (*Striatum*) of a human fetus and an improvement in motor and cognitive performance in HD patients was observed, along with reduction of cerebral neuronal injury. However, the effects were considered temporary because the follow-up studies, who followed these participants 3 to 10 years after transplantation, showed that the therapy is safe, but its effects were not sustained (Bachoud-Lévi et al., 2000; Bachoud-Lévi et al., 2006; Barker et al., 2013; Fink et al., 2015). Additionally, animal studies have shown a positive effect on neuronal dysfunction when muscle cell transplantation of the striatum (Nakao & Itakura, 2000) was performed and demonstrated that the graft progressively integrated into the degenerate striated body in a transgenic model for HD. However, complete recovery has not been reported in any of these studies (Dunnett et al., 1998; Kim & Vellis, 2009).

An important limiting factor for the transplantation of fetal cells of the striatum is the difficulty in providing sufficient amount of cells and embryonic striated tissue, in addition to the ethical problems associated with the use of embryonic human tissues. In this sense, the stem cells grown *in vitro* and expanded neural or mesenchymal stem cell precursors are of great clinical interest (Kim & Vellis, 2009: Kerkis et al., 2015a). Recent studies also indicate that the regenerative effect caused by cell therapy actually occurs through secretory molecules in addition to cell replacement, such as paracrine communication. In this sense, mesenchymal stem cells stand out in relation to other cell types due to their ability to secretion of a variety of growth factors, cytokines and chemokines that regulate their biology in an autocrine/paracrine way and are able to interact with the microenvironment around them, creating a neuroprotective microenvironment. Growth factors such as VEGF (*Vascular endothelial growth factor*), HGF (*Hepatocyte growth factor*), IGF-1 and 2

(*Insulin-like Growth Factor*) and SDF (*Stromal derived factor-1*) secreted by mesenchymal stem cells are important for neuronal survival, neurogenesis, migration, differentiation and mitochondrial activation (Kerkis et al., 2015a*;* Aleynik, et. al., 2014; Kerkis et al., 2015; Kerkis & Caplan, 2012).

Thus, mesenchymal stem cells have two main therapeutic mechanisms, which are: (1) secretion of a variety of growth factors and signaling molecules that potentially increase the overall regenerative capacity of the tissue, as well as its immunomodulation, by creating a microenvironment capable of regulating intrinsic cell proliferation and promoting neuroprotection and/or, (2) replacement or repair of neurons. These positive effects combined with the ability of mesenchymal cells to migrate to the injured site could potentially function as a neuroprotective therapy and inhibit disease progression or even, in the best case, be able to recover the injured area, even partially, reducing disease-related symptoms (Kerkis et al.,2015a*;* Aleynik, et. al., 2014; Kerkis et al., 2015; Kerkis & Caplan, 2012.; Fink et al., 2015; Kim & Cho, 2013).

Based on this, it is assumed that, after crossing the blood brain barrier, the Cellavita-HD investigational product would be able to secrete neurotropic and immunomodulatory factors and act in places where there is tissue damage caused by Huntington's disease, in order to alleviate neurological damage and restore even partially degenerated tissue. Therefore, cell therapy with Cellavita-HD in Huntington's disease has as its ultimate goal the reduction of neuronal loss and stimulation of neurogenesis, both endogenous and newly transplanted cells, activating a neuroprotective environment that is able to integrate new cells and replace dead neural cells or in the process of apoptosis.

Some authors believe that although cell therapy with stem cells, embryonic or neural, shows a beneficial effect both experimental and clinical, they do not represent the effective and definitive cure for the natural progression of Huntington's disease, due to the genetic nature of it and the fact that the mutant huntingtin protein can cross the cell membranes and propagate in the new transplanted cells. Therefore, cell therapy would only be able to delay the onset or course of the pathology, which, given the devastating nature of the disease, would already represent a major advance for these patients (Fink et al., 2015).

2. JUSTIFICATION FOR CARRYING OUT THE STUDY

Huntington's disease (HD) is a genetic neurodegenerative disease characterized by choreic movements, cognitive impairment, and psychiatric disorders. Several pharmacological treatments are used to improve or mitigate the symptoms of the disease, however, these do not promote the cure of the condition and do not alter its natural evolution, so that, to date, there is no adequate treatment for the inevitable cognitive-behavioral decline and motor observed in HD. Disease-modifying or neuroprotective therapy that decreases disease progression represents the largest and most important clinical need not yet met in HD (Olanow & Schapira, 2012; Kim & Vellis, 2009).

Stem cell therapy is currently prominent on the world stage and represents a new possibility of treatment, mainly for neurological diseases and CNS injuries, partly due to its inherent ability to repair. Mesenchymal stem cells are of particular clinical interest, due to their ability to migrate to sites with injury and to release beneficial neurotrophic factors, with consequent promotion of a restorative microenvironment. While embryonic stem cells presented some technical and ethical problems, as well as limited clinical benefits, mesenchymal stem cell therapies proved clinically beneficial, safe and without ethical problems involved, as they are derived from adult non-embryogenic tissue (Fink et al., 2015: Kim & Cho, 2013). According to the recent review conducted by Fink et al. (published in August 2015), the historical profile of mesenchymal stem cell use of various sources is considered safe, both experimentally and clinically.

Cell and genetic therapies are considered innovative therapies for which there are no fully defined guides and guidelines in Brazil and on the world stage. However, in June 2015, the U.S. regulatory agency FDA (*Food and Drug Administration*) published a document entitled *Considerations for the Design of Early-Phase Clinical Trials of Cellular and Gene Therapy Products - Guidance for Industry* representing the FDA's current recommendations on the first clinical studies with cell and gene therapy. The present study called ADORE-DH (EVALUATION OF THE DOSE-RESPONSE OF CELLAVITA HD INVESTIGATIONAL PRODUCT AFTER INTRAVENOUS APPLICATION IN PARTICIPANTS WITH HUNTINGTON'S DISEASE) has as main objective to identify the dose of the Cellavita HD product that presents the best or most appropriate dose-response relationship, that is, that provides maximum efficacy and is considered safe. The definition of the most appropriate dose in the early stages of

the clinical study is decisive for a more optimized clinical development (FDA, 2015; FDA, 2010; Chow & Chang, 2008).

The target population of the ADORE-DH study will consist of participants of both genders, with laboratory diagnosis of Huntington's disease, with mild to moderate symptoms and that justifies the risks inherent to a new product, but at the same time, is able to provide results that can be interpreted for dose-response evaluation of the same.

The methodology to be used for the evaluation of the primary outcome (identification of the best dose) will be the score obtained in the evaluation in the motor part of the UHDRS scale (motor test), since motor degradation is considered one of the main aspects of Huntington's disease (ADF, 2010; Paulsen et al., 2014). As secondary objectives, other components of the UHDRS scale will be evaluated, as well as BMI and morphological aspects of the central nervous system by Magnetic Resonance. The safety of the investigational product will also be evaluated through the incidence and classification of adverse events presented by the participants during the study.

The intravenous route of administration will be used because it is minimally invasive and currently used in clinical studies using mesenchymal stem cell therapy for the treatment of various diseases, including those affecting the central nervous system such as multiple sclerosis, cerebral palsy and spinal cord injury (Kerkis et al., 2015a; Boncoraglio et al., 2010; Lee et al., 2008; 2012; Koç et al., 2002; Ra et al., 2011; Weiss et al., 2013; Horwitz et al., 1999; 2002; Karussis et al., 2010; Hare et al., 2009; Yamout et al., 2010; Liang et al., 2010a, 2010b; Sun et al., 2007; Carrion et al., 2010; Mohamadnejad et al., 2007; Kharaziha et al., 2009; Wilson et al., 2015). The chosen doses represent the clinical experience with other products for cell therapy (Hare et al., 2009; Koç et al., 2002; Lazarus et al., 2005; Prasad et al., 2011).

In relation to the proposed dosage, the basis of previous studies developed by the research group of Dr. Irina Kerkis in São Paulo, carried out in dogs with neurological sequelae derived from the distemper virus (human model of multiple sclerosis), in a genetic model of canine muscular dystrophy (Duchene muscular dystrophy) (Kerkis et al., 2008) and bone marrow aplasia (aplastic anemia in humans), using a regimen of 3 intravenous mesenchymal stem cell applications, with an interval of 30 days between each administration. The studies demonstrated

the safety of multiple administrations and, considering the treatment of neurological sequelae in dogs, the result was efficient in motor response, and 70% of treated dogs regained the ability to perform movements (unpublished data provided by Sponsor). Furthermore, previous studies in humans with graft-versus-host disease (DECH) have shown that multiple applications of mesenchymal stem cells have shown satisfactory clinical response in patients who were not responsive to a single dose (Ball et al., 2015; Chen et al., 2015, Erbey 2015). These data suggest that stem cell therapy can be performed through multiple applications, according to the patient's response to treatment.

Thus, with the identification of the dose of the Cellavita-HD product that presents the best response, measured from the parameters established in this protocol, the next planned studies can be initiated. The present study coded as ADORE-DH, corresponds to the second study of the development plan for the process of registration of the Cellavita-HD investigational product with the competent agencies.

3. OBJECTIVES

3.1. Primary objective of effectiveness

The primary objective of this clinical trial is to identify the dose of Cellavita-HD product that has the best clinical response.

3.1.1. Primary variable

Verified through the Score of the Unified Huntington's Disease Rating Scale (UHDRS) scale of the baseline in relation to the end of treatment (domain comprising the assessments: motor, cognitive, behavioral, functional capacity and independence.

3.2. Secondary objectives of effectiveness

The secondary objectives of the study are:

a) Clinical neurological evaluation throughout the study;
b) Overall assessment of Huntington's disease severity (CIBIS scale);
c) BMI assessment;
d) Assess the incidence of suicide attempts during treatment;
e) Neurological evaluation by imaging.

3.2.1 Secondary variables

The variables are as follows:

a) Score of the UHDRS scale throughout the study (at each visit);
b) Score of the CIBIS scale throughout treatment;
c) Weight of the participant throughout the study;
d) Score obtained in the suicide domain of the HAM-D scale during treatment;
e) It will be assessed through the profiles obtained during the treatment of the following measures:
    - Cortical thickness;
    - Volume of the various brain structures, especially the core of the base, with special attention to the caudate;
    - Identifiable metabolic changes in proton spectroscopy.

3.3. Safety objective

The safety of medicines will be evaluated through the incidence and classification of adverse events occurring between treatments regarding type, frequency and intensity.

3.4. Study hypothesis

It is expected that it is possible to identify the dose that presents the best therapeutic response compared to the doses administered when compared to the placebo group.

4. STUDY OVERVIEW

4.1. Study design

This clinical trial will be prospective, phase II, single-centric, randomized, triple-blind, with two-test doses, placebo-controlled.

4.2. Phase

Phase II

4.3. Possible treatment groups

The study will include 35 participants. Initially, participants will be randomized in ratio 2: 2: 1 in groups G1: lower dose ($1x10^6$ cells/by weight range), G2: higher dose ($2x\ 10^6$ cells/by weight range) or G3: placebo, i.e. of the 35 participants, 14 will be randomized to the lowest dose group, 14 for the highest dose group or 7 for the placebo group. Thus, the groups listed below represent the groups in which participants can be included in the course of the study.

- **G1: Lower dose:** dose of $1x10^6$ cells/by weight range per administration.
- **G2: Higher dose:** dose of $2x10^6$ cells/by weight range per administration.
- **G3: Placebo:** constituted by the vehicle of the investigational product.

4.4. Duration of the study

A phase of inclusion and uniformization lasting a maximum of 90 days will be part of the study; after this period, the participant will be included in the study and will begin the treatment and follow-up phase that will last 330 days.

The total period of participation in the study will be up to 420 days from the period of uniformization of the study; that is, about 14 months approximately.

4.5. Visit schedules

15 scheduled visits are planned, including: inclusion and uniformity visits (V-3 to V-1), management visits of the investigational product (cycle 1- Visits 0, 1 and 2; cycle 2 - Visits 4, 5 and 6; cycle 3- Visits 8, 9 and 10) and follow-up visits (Visit 3, 7 and 11).

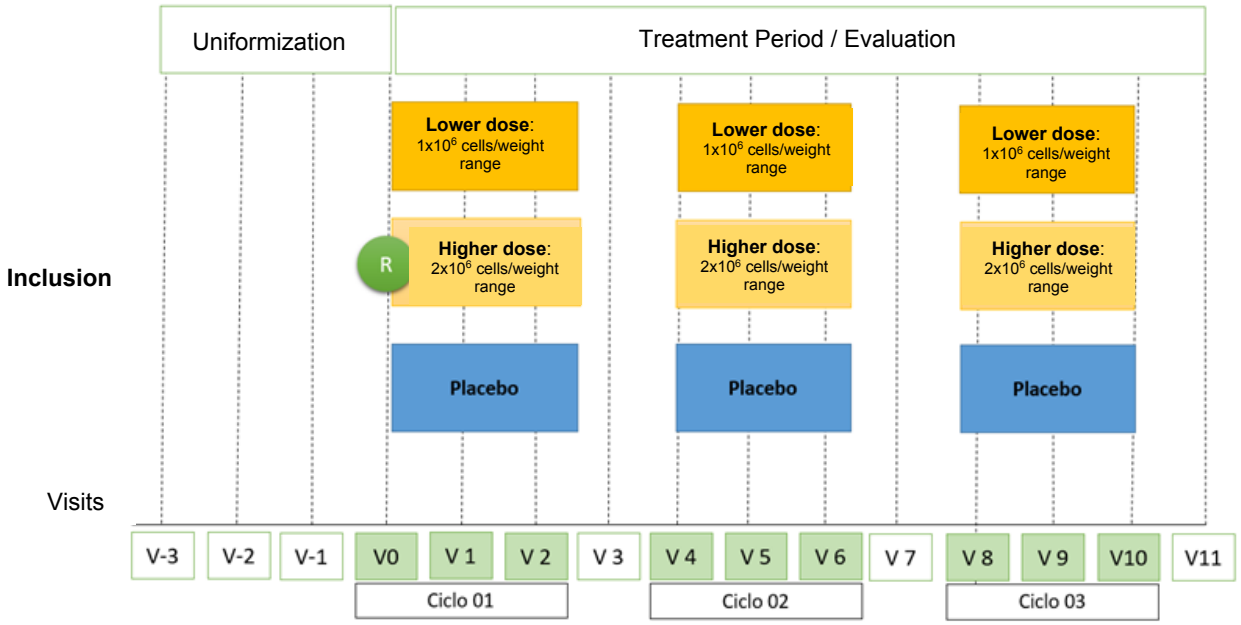

Figure 3. Study design.

The visits foreseen in the study are specified in a summarized form below:

- **Visit -3 inclusion (V-3):** -30 to -1 days before V-2;
- **Visit -2 (V-2):** up to 30 + 7 days after V-3
- **Visit -1 (V-1):** up to 30 + 7 days after V-2
- **Visit 0 (V0):** first administration of cycle 1; up to 30 days after V-1, and may occur even on the same day;
- **Visit 1 (V1):** second administration of cycle 1 (30 days after Visit 0 – window of + 5 days);
- **Visit 2 (V2):** third administration of cycle 1 (60 days after Visit 0 – window of + 5 days);
- **Visit 3 (V3):** follow-up visit (90 days after Visit 0 – window of + 5 days);
- **Visit 4 (V4):** first administration of cycle 2 (120 days after Visit 0 – window of + 5 days);
- **Visit 5 (V5):** second administration of cycle 2 (150 days after Visit 0 – window of + 5 days);
- **Visit 6 (V6):** third administration of cycle 2 (180 days after Visit 0 – window of + 5 days);
- **Visit 7 (V7):** follow-up visit (210 days after Visit 0 – window of + 5 days);

- **Visit 8 (V8):** first administration of cycle 3 (240 days after Visit 0 – window of + 5 days);
- **Visit 9 (V9):** second administration of cycle 3 (270 days after Visit 0 – window of + 5 days);
- **Visit 10 (V10):** third administration of cycle 3 (300 days after Visit 0 – window of + 5 days);
- **Visit 11 (V11):** follow-up visit/final visit (330 days after Visit 0 – window of + 5 days);

After the 11th visit, participants will enter an extension protocol.

4.6. Panoramic description of the study - Study plan

Participants should be informed about the objectives, methodologies, risks, benefits and their rights in the study, as well as the confidentiality and secrecy with which the collected data will be treated. If they agree to participate in the study, they must provide their consent by signing the two copies of the Informed Consent Form (ICF).

- **Inclusion (V-3) and beginning of uniformization:** after signing the Informed Consent, the participant will undergo a medical evaluation, which will include obtaining his medical history and performing a physical examination. At this time, the demographic data (birth date, gender and race) will be recorded, as well as the record of the use of prior/concomitant medication by the participant and adverse events not related to the medication. Huntington's disease will be confirmed through the existing diagnostic report (PCR), however, if the participant has not performed the test or if he/she does not have the accessible test report, a blood sample should be collected for this laboratory test. Subsequently, the following procedures will be performed: blood and urine collection for laboratory tests (including pregnancy testing for women) and serology, ECG and magnetic resonance imaging for CNS evaluation. In addition, UHDRS (*Unified Huntington's Disease Rating Scale*), HAM-D and CIBIS scales will be applied. All V-3 procedures must be performed within a maximum period of 37 days. If the participant is considered eligible, the participant will be invited for the next visits (V-2 to V-1). During the visit V-3 it will also be given an identification card (participation card in clinical trial), in which data such as address(s)

and contact telephone number(s) of the Principal Investigator and staff professionals for possible emergencies will be contained (Annex 7).

- **Uniformization:** V-2 (up to 30+7 days after V-3) the results of the inclusion tests will be evaluated. If the participant is considered eligible, the participant will start a period of uniformization, which will consist of 3 scheduled visits, including the inclusion visit (Visit -3), Visit -2 and Visit -1, held monthly, to monitor the clinical evolution and application of the UHDRS Scale, HAM-D and CIBIS. In all these visits will be performed urinary pregnancy test in female participants.

- **Randomization and initiation of treatment:** in V0 (may occur on the same day or up to 30 days after V-1): eligible participants will undergo a new medical evaluation, which will include updating medical history and collecting urine (including pregnancy testing for women), as well as performing physical examination and verification of inclusion/exclusion criteria. The applications of the UHDRS (*Unified Huntington's Disease Rating Scale*), HAM-D and CIBIS scales will also be carried out. If able to continue in the study, participants will be randomized to 2:2:1 in the lower Dose ($1x10^6$ cells/by weight range), Higher dose ($2x10^6$ cells/by weight range) or Placebo group and will perform the first administration of Cycle 1.

In this visit, participants will be admitted to the Intensive Care Unit (ICU) of the Research Center for a period prior to the administration of the investigational product and will remain for 06 hours after administration (the length of hospitalization may be extended at the discretion of the Principal Investigator) for evaluation of vital signs and clinical examination. Cardiac monitoring will be performed during hospitalization and urine will be collected for β-hCG testing in women. In this visit, as in all other scheduled visits, there should be the registration of concomitant/previous medications and adverse events.

- **Treatment**: V1 (30 days after V0 - window of + 5 days) and V2 (60 days after V0 - window of + 5 days): participants will return to the Research Center for further medical evaluation (including urinary pregnancy test for women) and verification of discontinuation criteria. In these visits, the second and third administration of cycle 1 of Cellavita-HD or placebo products will occur, respectively. Similarly, participants will be hospitalized before the administration of the investigational product and will remain for 06 hours after the end of it for clinical evaluation (the length of hospitalization may be extended at the discretion of the

Principal Investigator), and the same procedures performed in Visit 0 will be performed in V1 and V2.

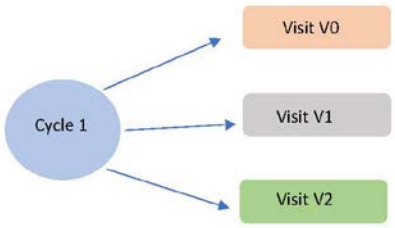

Figure 4. Cycle 1 visits.

- **Follow-up**: in V3 (90 days after V0 - window of + 5 days), participants will return to the Research Center for medical evaluation and verification of discontinuation criteria, in addition to collecting blood and urine laboratory tests (including urinary pregnancy test for women), as well as performing ECG. UHDRS (*Unified Huntington's Disease Rating Scale*), HAM-D and CIBIS scales will also be applied. There will be the registration of concomitant/prior medications and adverse events.

- **Treatment:** Visits V4 (120 days after V0 - window of + 5 days), V5 (150 days after V0 – window of + 5 days) and V6 (180 days after V0 – window of + 5 days) represent, respectively, the first, second and third administration of cycle 2, and in each visit the participants will undergo an update of the medical history and verification of the discontinuation criteria and ECG performed during the evaluation visit. Participants considered fit will be admitted to the Intensive Care Unit (ICU) of the Research Center for a period prior to the administration of the investigational product and will remain for 06 hours after the end of administration (the length of hospitalization may be extended at the discretion of the Principal Investigator) for evaluation of vital signs and clinical examination. In these visits, there should be the registration of concomitant/previous medications and adverse events. Applications of the UHDRS (*Unified Huntington's Disease Rating Scale*), HAM-D and CIBIS scales will also occur. Furthermore, in Visit 4, the laboratory and urine tests (including urinary pregnancy test for women) of the previous visit will be evaluated.

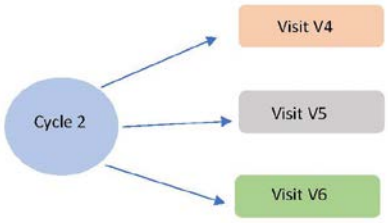

Figure 5. Cycle 2 visits.

- **Follow-up:** the Visit V7 (210 days after V0 with a window of + 5 days) represents a follow-up visit, and the same procedures and examinations performed in the V3 follow-up visit will be performed again in V7.
- **Treatment:** the visits V8 (240 days after V0 - window of + 5 days), V9 (270 days after V0 - window of + 5 days) and V10 (300 days after V0 - window of + 5 days) represent, respectively, the first, second and third administration of the last cycle (cycle 3) of administration, which will occur similarly to previous cycles, the same procedures mentioned during the hospitalization period.

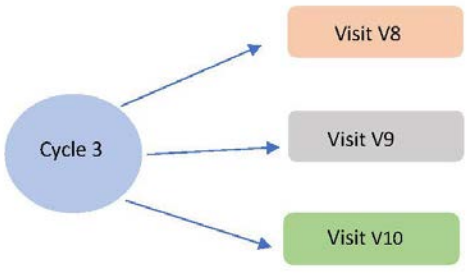

Figure 6. Cycle 3 visits.

Follow-up/Final Visit: V11 (330 days after V0 – window of ± 5 days): corresponds to the follow-up visit of the last cycle, as well as to the last visit of the study. On this visit, participants will return to the Research Center for medical evaluation and verification of discontinuation criteria, in addition to evaluating urine test results

(including pregnancy test for women). Blood and urine will be collected for further laboratory tests, magnetic resonance imaging of the CNS, as well as ECG. The UHDRS (*Unified Huntington's Disease Rating Scale*) HAM-D and CIBIS scales will be applied to participants. On the same day there will be the registration of concomitant/previous medications and adverse events.

If any test result is altered, the participant will be contacted for an extraordinary visit to take the relevant procedures to the case.

5. STUDY POPULATION

5.1. Population description

Thirty-five participants of both genders, aged between 21 and 65 years, with laboratory (genetic) diagnosis of Huntington's disease and presenting the eligibility criteria determined for the study will be included.

5.2. Inclusion and exclusion criteria

The inclusion and exclusion criteria of the study are:

5.2.1. Inclusion criteria

a) Providing informed consent in writing, signed and dated;
b) Participant of both genders aged ≥ 21 and ≤ 65 years*;
c) Present confirmatory diagnostic report (PCR) of Huntington's disease with a number of CAG repeats on chromosome 4, greater than or equal to 40 and less than or equal to 50 (if the participant has not taken the exam and/or if he does not have the exam report accessible, a new examination should be performed);
d) Getting 5 points or more in the motor evaluation of the UHDRS scale (*Unified Huntington's Disease Rating Scale*) at the time of inclusion;
e) Obtaining 8 to 11 points in the functional capacity of the UHDRS scale at the time of inclusion.

*Note: The age of the participant will be considered on the day of the inclusion visit (V-3).

5.2.2. Exclusion criterion

a) Participant of the research who has participated in protocols of clinical studies in the last 12 (twelve) months, unless the researcher considers that there may be direct benefit to it (Resolution CNS 251, of August 7, 1997, item III, subitem J);
b) Diagnosis of juvenile Huntington's disease*;
c) Diagnosis of epilepsy;
d) Diagnosis of major cognitive disorder;
e) Decompensated active psychiatric disease;
f) Current or past history of neoplasia;

g) Current history of severe and uncontrolled gastrointestinal, hepatic, renal, endocrine, pulmonary, hematological, immunological, metabolic, or cardiovascular disease;

h) Diagnosis of any active infection, be it viral, bacterial, fungal or caused by another pathogen;

i) Participant who has contraindication to the tests performed in this study, such as having a pacemaker or surgical clip;

j) Alcohol and drug abuse (previous diagnosis according to the criteria of the Diagnostic and Statistical Manual of Mental Disorders - DSM V);

k) Use of illicit drugs;

l) Present positive results in one of the serological tests: HIV 1 and 2 (Anti-HIV-1.2), HTLV I and II, HBV (HBsAg, Anti-HBc), HCV (anti-HCV-Ab) and FTA-ABS (Treponema pallidum);

m) History of drug allergy, including contrasts for imaging, or bovine products;

n) In use or foresight of the use of immunosuppressive drugs or prohibited drugs (item 6.9) during the first three months after the first administration of the investigational product;

o) Any clinical change that is interpreted by the Investigator physician as a risk to the inclusion of the participant in the study.

*Note: The diagnosis of juvenile Huntington's will be defined by the presence of 60 or more CAG repeats in the confirmatory diagnostic report for the disease and the appearance of characteristic signs and symptoms before the age of 21 (Nance, 2001).

5.2.3. Discontinuation criteria (interruption) of treatment with the investigational product:

Study participants are free to withdraw their consent and/or discontinue their participation in the study at any time, without prejudice to further treatment, as well as penalty or loss of benefits.

In addition to withdrawing consent, study participants may be discontinued for any of the reasons described below:

a) Study abandonment or loss of follow-up[a] ;

b) Adverse events at the investigator's discretion[b] ;

c) Fulfilling any of the exclusion criteria of the study (newly developed or not previously identified criteria), which at the investigator's discretion prevents him from remaining in the study (risk x benefit assessment);
d) Violation of the protocol, at the discretion of the Investigator and/or Sponsor;
e) Discontinuation of the study by Sponsor;
f) Death;
g) Need for the use of prohibited concomitant medications that at the investigator's discretion may interfere with the results of the investigational product of the study (item 6.9);
h) Any other clinical, laboratory or psychological condition, which in the opinion of the Investigator, is likely to discontinue treatment.

[a] In cases where there is loss of follow-up of the research participant, defined as "no return of the participant on the scheduled date", the Research Center should make telephone contact within a maximum period of one (1) business day after the scheduled visit. The procedure must be registered in a source document, and the new scheduled visit, respecting the maximum period of two (2) days. In situations where the telephone contact is not effective, a telegram should be sent to the research participant requesting the contact urgently. After two (2) telegrams with an interval of 5 working days, the participant of the research should be classified as abandonment. Telephone contact attempts as well as confirmations of receipt of telegrams must be recorded/attached to the source document of the research participant.

[b] Participants whose permanence in this study is interrupted by adverse events should be followed up until the resolution of the event. Their data will be included in the adverse event notification forms and considered for the efficacy and safety assessment of the investigational product used in this study.

5.3. Selection failure

The occurrence of participants who sign the informed consent and are not eligible before randomization will be considered a selection failure. All clinical records for participants representing selection failures will be collected in the source document. All participants considered selection failures will be replaced.

The rescreening of participants who failed to meet the inclusion and/or exclusion criteria will be allowed, if the criteria are reversible and may re-fit the eligibility criteria.

5.4. Replacement of study participants

Only participants considered selection failures will be replaced. There will be no replacement for those who are discontinued after randomization.

5.5. Follow-up of research participants whose continuation in this study is interrupted

Research participants excluded due to the emergence of adverse events (AEs) of any extension, related to the use of the product or not, will be monitored by the Research Center and monitored by Sponsor until its outcome. All necessary support will be provided for their clinical condition to be restored, i.e. stabilized at an acceptable level in the Investigator's assessment, and the costs are paid by Sponsor.

Research participants excluded for other particular reasons or for non-compliance with one of the study criteria will not be followed up once the non-occurrence of AEs has been established.

5.6. Recruitment strategies

Participants will be recruited for a natural demand from people diagnosed with Huntington's disease after the study is released under the regulatory rule of disclosure of clinical studies.

This recruitment strategy is considered specific for the target population and therefore presents a high eligibility rate for participants with diseases considered rare.

6. STUDY TREATMENT

6.1. Identification of the investigational product

The Cellavita-HD product contains viable human mesenchymal stem cells of allogeneic deciduous tooth pulp (of the same species but genetically different), in the form of a liquid suspension, as described in Table 01. The cells are not genetically modified.

Table 01. Description of Cellavita-HD investigational product

Product	Cellavita-HD
Characteristics and physical status	Liquid suspension of allogeneic cells
Concentration	1x10^6 Cells
Excipients	0.9% sodium chloride containing 10% HSA
Storage conditions	Cryopreservation -196º C
Pharmaceutical form	Suspension for infusion
Administration route	Intravenous use

Data provided by Sponsor

6.2. Treatment groups

The treatment groups will be arranged as follows:

- **G1: Lower dose:** 1x10 dose^6 cells/by weight range per administration.
- **G2: Higher dose:** 2x10 dose^6 cells/by weight range per administration.
- **G3: Placebo:** constituted by the vehicle of the investigational product.

6.3. Dosage

In this study, two different doses of the investigational product will be evaluated (1x10^6 cells/by weight range and 2x10^6 cells/weight range) compared to placebo. Therefore, each participant will receive according to randomization, one of the fractional doses of Cellavita-HD product in 0.9% saline or placebo.

Each participant will receive three cycles of three consecutive administrations (one every 30 days) with the drug according to randomization. The interval between cycles will be 30 days. The following table also summarizes the distribution of participants among the treatment groups, as well as the dosage and the route to be used.

Table 02. Possible treatment groups in the ADORE-DH study

Group	Dose	Dosage	Administration	Investigative Product	Way
G1	**Lower dose**	$1x10^6$ cells/weight range	3 administrations (one every 30 days) per cycle	Cellavita-HD	Intravenous
G2	**Higher dose**	$2x10^6$ cells/weight range	3 administrations (one every 30 days) per cycle		
G3	**Placebo**	Investigational product vehicle	3 administrations (one every 30 days) per cycle	Placebo	Intravenous

6.4. Treatment allocation / Concealment of allocation

The study will be randomized, including a ratio of 2:2:1 (14:14:7 participants, in the lower dose, higher dose and placebo groups, respectively) and the allocation of treatment will be performed through a randomization list generated by the person responsible for the statistical analysis of the study.

- G1 (lowest dose) = 14 participants;
- G2 (highest dose) = 14 participants;
- G3 (placebo) = 7 participants.

The Research Center will receive the identification code of the packaging containing the investigational or placebo products to be dispensed according to the order of inclusion of the participants.

6.5. Study blinding / Procedure for blind breakage

The study will be triple-blind, blind to the participant, the Researcher and those responsible for evaluating the parameters of efficacy and safety. The responsible for the Pharmacy of the Research Center, a component of the non-blind team, will dispense the investigational product in the center for the use of participants. The study's medications will be supplied in identical-looking packages to maintain the masking of the study. Neither the Investigator nor his study team will know which medication the participant is using. In addition, those responsible for evaluating the outcome will receive the results in a coded (hidden) way.

When necessary, for the safety and proper treatment of the participant, the Researcher may suspend the masking of the investigational product assigned to the participant, in order to define the appropriate treatment for clinical follow-up. The Investigator shall inform Sponsor of the breach of the blind code if it has not been notified previously.

Thus, if necessary, there will be the breaking of the blind code by opening the number of the medication kit from the randomization list. The reason for breaking the code must be recorded in the attendee's source document.

The professionals responsible for the analysis of the safety and efficacy parameters defined in the study will also be blind elements, as well as the *data manager* and the statistician involved.

For the accounting of the drugs used in the study, there will be a non-blind monitor designated for such function.

The pharmacist or delegated professional will also be non-blind and will administer the drugs to the research participants.

6.6. Packaging and labelling

The statistician of the study will forward to the Sponsor and, only to the Sponsor, the sequential randomization list. Sponsor shall perform sample masking and proper product labeling, including the sequential number featured on the packaging, so that the investigational product is randomized by the Research Center which will include participants in the appropriate sequence. The head of pharmacy at the Research Center, a component of the non-blind team, will dispense the investigational product in the center for the use of the participants. Therefore, physicians and participants will not have access to the packaging of medicines.

The product under investigation will be supplied on the packaging and will be labelled with the information necessary to identify the product intended for clinical research (Figure 7).

A

Sponsor:
Research Protocol:
Responsible Investigator:
Responsible Pharmacist:
Dosage form:
Batch:
Manufacturing date (month/year):
Expire date (month/year):
Weight:
Storage conditions: Keep at room temperature (15°C – 30°C). Protect from light and keep in a dry place.
Use instructions:
Route of administration:

⚠ **ATTENTION!**
USE EXCLUSIVE IN CLINICAL TRIALS.
ALL MEDICINES MUST BE KEPT AWAY FROM CHILDREN.
This product must not be marketed.
This product must not be used by another person. Its use is exclusive by the person for whom it was destined.
This package must not be discarded and must be returned in the next visit together with the product, even if it was not used completely.
This product must be used according to medical orientation.
In case of doubt, please contact the Center and talk to:

B

CELLAVITA-HD – **Syringe 1**
hIDPSC in 0,9% saline solution

Dose: n. of cells/kg of body weight
Batch: Syringe volume:
Total number of cells:
Total number of viable cells: Viability:
Storage conditions: 2 – 8°C
Responsible for preparation:
Date and time of preparation: Expiration:
Waiting time for the application of the next syringe:

Figure 7. Label template for products intended for research (A) Source: ANVISA; (B) Label model used for the study

6.7. Storage and stability

The investigational product will be stored in an appropriate location on the premises of Cellavita Pesquisa Científica Ltda (Sponsor), specifically in cryopreservation (liquid nitrogen -196ºC) and/or ultra-freezer (-80ºC) applications with temperature and access control, until the dates on which the administrations to the research participants will occur. Since Sponsor will have the product in custody most of the time, inventories as well as storage temperature control will be monitored by Sponsor, and any deviations from requirements, including the conducts employed, shall be recorded. After identifying any deviation, Sponsor shall judge whether it was significant and/or impactful on the quality and safety of the product, keeping it under quarantine or not, until cellular feasibility tests are performed to allow its use.

On the scheduled date for the participant's hospitalization, the investigational product (ready for use according to the "Manual of presentation and instructions for administration of the Cellavita-HD product") will be forwarded to the Research Center in containers with thermal insulation transported according to the GCP, so that there is maintenance of its temperature.

6.8. Chain of custody

The investigational product will be provided by Sponsor in sufficient quantity (depending on the participant's weight and treatment group) on the scheduled date for administration. The receipt and conference of the product by the Pharmacy of the research center will be carried out by a delegated professional of the Research Center, which will include the verification of the lots, visual physical integrity and the transport temperature. After the samples have been checked and the documentation regarding entry into the company have been completed, the products will be stored properly, according to the specifications of the "Manual of presentation and instructions for administration of the Cellavita-HD product" until the moment of infusion.

All manipulation of the investigational product will be carried out in a standardized manner, in accordance with the specifications present in the "Manual of presentation and instructions for administration of the Cellavita-HD product" and in accordance with the Standard Operating Procedures (SOPs) of the research center. Handling, dispensing, disposal and/or loss (if applicable) will be recorded by the

responsible professional. The labels of the packaging used will be kept in the management record of the investigational product of each participant.

The accounting of unused products will be carried out and they will be forwarded to Sponsor for disposal procedures. The chain of custody is summarized in Figure 8.

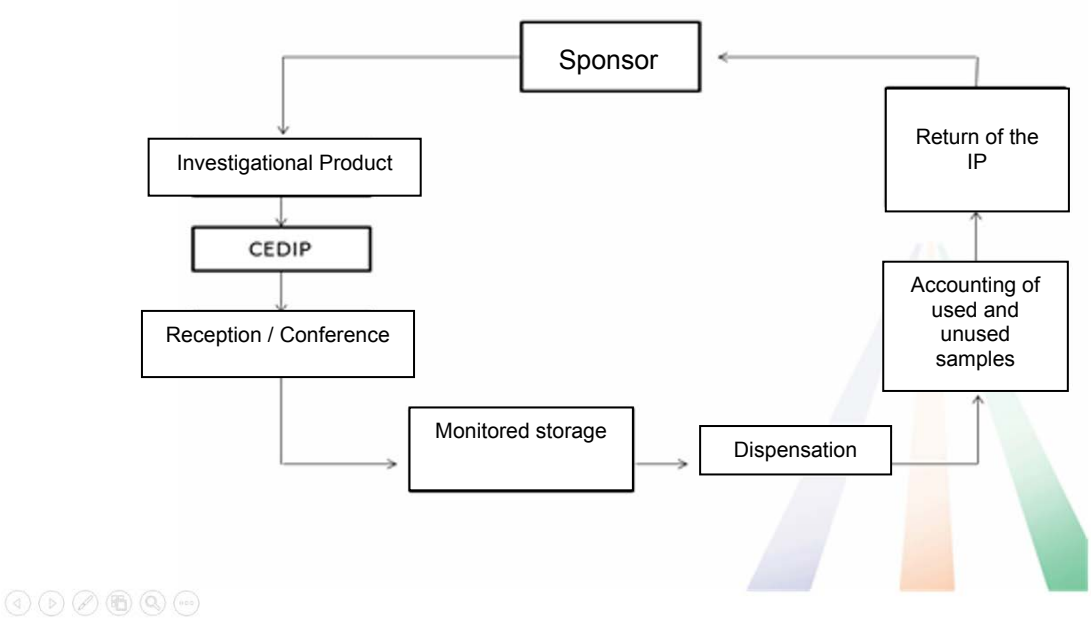

Figure 8. Chain of custody flowchart

6.9. Concomitant medicinal products prohibited during the study

Any medicinal product administered during each administration cycle with cytotoxic/cytostatic or immunosuppressive action, such as:

- **Cytotoxic/cytostatic drugs:** medicinal products belonging to the groups of nitrogen mustards, alkyl sulfonates, ethylenimines, methyl melamine, nitrosoureas and triazenes, as well as folic acid analogues, pyrimidine analogues and purine analogues.
- **Immunosuppressive drugs:** azathioprine, tacrolimus, cyclosporine, prednisone, sodium mycophenolate, mycophenolate mofethyl, everolimus, sirolimus. In the specific case of immunosuppressive drugs, the withdrawal of the participant will be performed at the discretion of the Principal Investigator, considering the administered dose and the frequency of administration.

Other drugs that may interfere with cell viability should be evaluated on a case-by-case basis.

7. SCHEDULE OF VISITS AND PROCEDURES OF THE STUDY

7.1. Procedure for entry of Participants

7.1.1. Informed Consent Form (ICF)

The benefits and risks related to participation in the study will be explained to the participant before performing any procedures related to the study. If the participant agrees to participate in the study, the participant must sign, date and rubric in his own hand, two original copies of the ICF, as well as the Researcher and/or the person responsible for the application of the document. One of the signed copies will remain with the Investigator and the other must be delivered to the participant.

If the research participant and/or his/her legal representative are unable to read and understand, an impartial witness must be present during the discussion of consent. After reading and explaining the Informed Consent, the research participant must orally provide his/her consent, and then his/her fingerprint must be collected in the document and the witness must sign and date the Informed Consent Form at the appropriate place. Due to the motor involvement characteristic of the studied condition, some participants may have difficulty writing their signature, despite being able from a cognitive point of view. For these participants, only the fingerprint should be collected in the document.

The ICF must have been previously approved by the CEP responsible for the institution where the study will be conducted.

7.1.2. Preserving the identity of research participants

Participants will be selected according to the inclusion and exclusion criteria, consecutively (order of inclusion). After the research participant agrees to participate in the study and sign the Informed Consent, he/she will receive a code, which will identify him/her during the study. This code is composed of the initials of the first name, the middle name (if it does not exist, a dash "-") and the last name must be inserted (disregard names that refer to kinship, for example, Junior, Filho, Neto, etc.), followed by the date of birth in the format "DDMMYYYY". So, we will have the following code as the example:

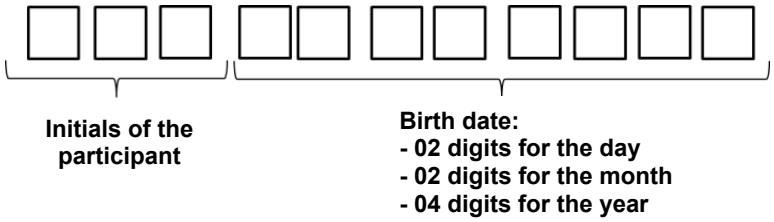

Figure 9. Participant code.

The participant's code must be included in all documentation involving the participant and must accompany him/her from the beginning to the end of the study, in order to preserve his/her identity.

In case of need for breach of confidentiality of the code, this will be done by the Principal Investigator, who will have access to the confidential coding data of the participants. Code breaking, which may occur in cases of health inspection, at Sponsor's request or at the discretion of the Investigator, must be carried out with the consent of the participant and the Investigator. The breaking of the codification without the participant's consent will only be performed in case of emergency.

7.1.3. Acceptable contraceptive methods

In this study, the following contraceptive methods are generally considered reliable when used correctly: oral contraceptives, contraceptive patches, contraceptive injection, male condom, diaphragm or cervical hood with spermicide, contraceptive vaginal ring, intrauterine device, surgical sterilization (bilateral tubal connection), vasectomized partner or sexual abstinence.

The Researcher and the participant himself will decide the appropriate method of contraception that will be used during the study, which, when necessary, will be provided by Sponsor if he/she is not using any method of contraception.

7.1.4. Exams performed for inclusion of study participants

In the Visit V-3 (inclusion) the procedures described below will be performed, that corresponds to the selection exams and/or tests necessary to obtain Baseline measurements. Additional information about each procedure performed and its methodological specifications can be found in item 7.2.

A. Laboratory diagnosis for Huntington's disease (PCR), if necessary;

B. Clinical evaluation: anthropometric data, medical history and physical examination/vital signs (with BMI value);

C. Laboratory tests: blood count, serum sodium, serum potassium, amylase, total cholesterol and fractions (LDL, HDL and VLDL), triglycerides, coagulogram (RNI, TAP and TTPA), alkaline phosphatase, glycated hemoglobin (HbA1c), urea, uric acid, TGO (oxalacetic glutamic transaminase), TGP (pyruvic glutamic transaminase), gamma-GT (gamma glutamyl transferase), total bilirubin and fractions (direct and indirect), serum creatinine, CK (creatine kinase) and CKmB (creatine kinase mB), β-hCG urinary for women and urine I;

D. Serology: HIV 1 and 2 (Anti-HIV-1.2), HTLV I and II, HBV (HBsAg, Anti-HBc), HCV (anti-HCV-Ab) and FTA-ABS (*Treponema pallidum)*;

E. Physician-reported electrocardiogram (ECG);

F. Application of the UHDRS (*Unified Huntington's Disease Rating Scale*), CIBIS and HAM-D Scales;

G. Evaluation of the CNS by magnetic resonance imaging of the brain.

7.2. Study-specific evaluations and procedures

7.2.1. Laboratory diagnosis for Huntington's disease (PCR)

Where applicable, laboratory diagnosis of Huntington's disease will be carried out using the PCR methodology (*polymerase chain reaction*) during the visit (V-3), as published by Warner and collaborators (1993). The strategy of the genetic analysis used will be the determination of the number of repetitions of the CAG trinucleotide, contained in the 5' region of the IT15 gene, in 4p16 by PCR (sequencing).

7.2.2. Clinical evaluation: demographic data, medical history and physical examination

Participants will be clinically evaluated by the study physician, and the following items should be evaluated and recorded in the source document:

a. Demographic data (birth date, gender and race);

b. Medical history:

- History of current pathology - Huntington's disease:
  - ✓ Start date;

✓ Evolution of signs/symptoms (includes motor, oculomotor, cognitive and psychiatric changes;

- Background:

✓ Personal history (other diagnoses/pathologies, dates and clinical evolution);

✓ Family history;

c. Physical examination and vital data:

- Physical examination assessment:

✓ General State: the participant may be in good general condition (GGC), regular general condition (REG) or bad general condition (BGC);

✓ The following should be evaluated: the degree of mucosal staining, hydration, perfusion (e.g., but not restricted to: clinical signs of cyaniasis) and respiratory pattern.

✓ Overall assessment: includes cardiovascular, pulmonary, digestive, osteomuscular and peripheral systems, with emphasis on neurological and other evaluation, if necessary.

- Evaluation of vital signs data:

✓ Weight: preferably measured on a calibrated digital scale. At the time of measurement, the participant must be wearing light clothing (without wearing shoes and empty pockets). Body weight will be recorded in kilograms (Kg);

✓ Height: a statometer (retractable train) attached to the digital scale will be used. During the procedure, the participant should be barefoot (or wearing socks) and position himself in the center of the equipment in an upright posture looking at a fixed point at eye height, with arms extended to the side of the body and palms facing back. The reading recorded after the measurement will be given in meters (m);

✓ BMI: will be determined by the BMI equation = weight/height^2 (kg/m^2). According to the value found, the participant is categorized as thinness, eutrophy, overweight and degrees of obesity;

✓ Blood pressure (BP): should be performed after the participant has remained at rest (without any physical effort) for at least 5 minutes. After this period, it will be checked 3 times at intervals of 1 to 2 minutes using a calibrated digital device. The measurement of the first measurement will

be discarded and the average of the last 2 measurements performed (ABP) will be recorded in a source document using the unit of measurement of millimeters of mercury (mmHg);

✓ Heart rate (HR): includes checking the frequency (number of palpated or ascultated pulses), rhythm (regularity of heartbeat) and amplitude (quality of pulses felt). This parameter will be measured by palpation of the radial artery. The result will be reported using the bpm unit of measurement (beats per minute);

✓ Body temperature: the measurement of the parameter will be performed using calibrated digital thermometer. The unit of measure used will be the centigrade scale, commonly called degrees Celsius (ºC).

7.2.3. Laboratory tests and serology

The tests to be performed in the study are described below. In visits in which there is the administration of the product, samples for laboratory tests should be collected before the beginning and after 06h 00min of the end of product administration.

Table 03. Laboratory tests to be performed on V-3 and in all post-cycle follow-up visits (V3, V7, V11)

Category	Test	Normality values
Hematological Analysis	Hemogram (erytrogram, leukogram and platelet count)	According to the reference values established by the laboratory.
Biochemical Analysis	Serum sodium	
	Serum potassium	
	Amylase	
	Total cholesterol and fractions (LDL, HDL and VLDL)	
	Triglycerides	
	Coagulogram (RNI, TAP and TTPA)	
	Alkaline phosphatase	
	Glycated hemoglobin (HbA1c)	
	Urea	
	Uric acid	
	TGO	
	TGP	
	Gamma-GT	

Category	Test	Normality values
	Total bilirubin and fractions	
	Serum creatinine	
	CK	
	CKmB	
Urological Analysis	Urine summary (urine I)	
Other	β-hCG urinary (women-only)	

Table 04. Serological tests to be performed in the study at visits V-3 and V11.

Serological Analysis	HIV (1 + 2) (Anti-HIV-1.2)	Negative
	HTLV I and II	
	HBV (HbsAg and Anti-HBc)	
	HCV (anti-HCV-Ab)	
	FTA-ABS (syphilis)	

7.2.4. Cardiogram

For the monitoring of cardiac function, an electrocardiogram (ECG) of 12 leads will be performed. The interpretation of the tracing should be performed by a physician and recorded both in the source document and in the individual clinical form (CRF) of each participant. Clinically significant abnormalities (and/or those related to current health condition) should be recorded as relevant medical history and/or current conditions during the Inclusion Visit (V-3), which should be judged by the Investigator Physician as a risk or not to inclusion in the study. Other clinically significant abnormalities found after product administration (compared to visit V-3) should be reported and evaluated by the Investigator physician as an adverse event. All ECG reports should be kept as part of the study documentation.

The electrocardiogram will be performed during visit V-3, in the scheduled post-cycle visits (V3, V7 and V11), and on the days when there is administration of the investigational product, the evaluation will be carried out during the 6 hours of hospitalization after the end of the administration (before the dismissal of the participant).

7.2.5. Application of the UHDRS Scale (*Unified Huntington's Disease Rating Scale*)

The UHDRS scale (*Unified Huntington's Disease Rating Scale)* is a clinical evaluation that presents 15 domains that assess motor, cognitive, behavioral and functional capacity functions. Each domain has from 0 to 4 categories, so that the higher the absolute value of the scale, the more severe the clinical situation of the participant. The version translated to Portuguese of this scale is found in Annex 1, being considered valid and reliable for the Brazilian population according to the article by Tumas et al. (2004) (Barreto, 2009; Januário, 2011; Huntington Study Group, 1996).

The motor evaluation (M) is obtained by the sum of the score of 31 items that evaluate different motor signs, which include oculomotor function (vertical and horizontal eye pursuit; saccadic movement – initiation and vertical and horizontal speed), orolingual movements (dysarthria and protrusion of the tongue), fine motor tasks (beats of fingers - right and left; pronation and supination - right and left), chorea (face, mouth, trunk and extremities), dystonia (trunk and extremities), parkinsonism and functions associated with gait. The score on this scale ranges from 0 to 124 and the highest scores indicate greater motor impairment (Barreto, 2009; Januário, 2011; Huntington Study Group, 1996).

This motor assessment may be filmed if the participant authorizes it by signing the Image Authorization Term.

The cognitive assessment (P) is done by applying three tests that integrate the cognitive component of UHDRS:

1. Verbal fluency test, which reflects the number of correct words produced per minute (series of three) (Benton & Hamsher, 1978);
2. Test Stroop Color-Word (Stroop, 1935), which reflects the number of correct items in the Stroop interference battery in a 45-second period;
3. Digit-digit test (Smith, 1973), which reflects the number of items produced in 90 seconds;

The Literal Verbal Fluency test is a test that assesses phonemic verbal fluency and the ability to establish verbal associations. The participant will be instructed to say the largest number of words started by a specific letter for 60 seconds, as long as the words are not morphologically similar or proper names. This

test is repeated 3 times using 3 different letters, and the commonly used letters are 'P', 'M' and 'R'. The total score is the sum of the number of words that the individual was able to say for the 3 letters (Barreto, 2009; Januário, 2011; Huntington Study Group, 1996).

The Stroop test is composed by means of three elements: color naming, word reading and interference. The participant will be guided correctly before the start of the test, and the participant will start only after the test has been understood. The Researcher or professional delegated by him must ensure that the participant does not have color blindness and should align the nomenclature of colors in order to avoid double naming of them due to regionalism. The first element consists of a blade containing only circles and/or squares with specific colors, in which the participant should read as soon as possible and without the assistance of indication (fingers or instruments such as ruler or marker), the color he is seeing. The second element consists of a blade containing colored words that repeat themselves by changing the order and color of the words. The third element consists of a blade containing color names, where the color nomenclature is one, and the color in which it was printed is another (ex: red; the word "red" is written in black). For each evaluated element, the response time will be timed. The test score is performed according to the number of errors, as well as the time required to perform the test, according to the standardized table of normality (Appendix 2).

The Digit-Symbol test (Digit span) is a test in which the participant must repeat the numerical sequence that the Investigator or professional delegated by him speak. This test consists of eight groups with two numerical sequences each, which must be reproduced in direct order and another with seven groups with two numerical sequences each, which must be reproduced in the reverse order in which it is spoken (E.g. 7 – 5 → 5 – 7). The test score is performed according to the number of correct answers; if the participant misses two consecutive numbers in the same sequence, the participant will lose points. The number of numbers stored by the participant (*Span*) will also be evaluated by the test. The score table with the normality values according to age as well as the Symbol-Digit test are shown in Annex 3.

For all three cognitive tests, higher scores translate better cognitive ability (Barreto, 2009; Januário, 2011).

The component of <u>behavioral and psychiatric evaluation</u> (B) of UHDRS aims to quantify the severity and frequency of neuropsychiatric symptoms

experienced by the participant. The evaluation score is obtained by the sum of different items (questions), each being defined by the product of gravity (0 = absent; 4 = severe) and frequency (0 = absent; 4 = almost always) of psychiatric symptoms (sadness/mood, apathy, low self-esteem, anxiety, suicidal thoughts, aggressive behavior, intolerant behavior, obsessions, compulsions, delusions and hallucinations). The evaluation is done along the lines of semi-structured interviews, and the clinician has the flexibility to introduce additional questions that help identify psychiatric symptoms. The score ranges from 0 to 176, and the highest scores indicate greater behavioral impairment. The frequency of psychiatric symptoms is graded from 0 to 4, zero signifying the absence of symptom and four their existence and permanence. The severity of the symptom is also scored between zero, without any severity, and four, indicating greater severity of this symptom interfering in the activity of the individual. In accordance with the quantification proposal referred to by Marder et al. (2000), the frequency of a symptom is multiplied by the severity of it, in order to obtain a *Score* (Barreto, 2009; Januário, 2011; Huntington Study Group, 1996).

The functional evaluation (F) includes an independence checklist scale and an assessment of total functional capacity (TFC - Total Functional Capacity). The functional Checklist is included in a questionnaire of 25 questions that include various tasks of daily living, which are filled out through the information provided by the patient or his companion, in an affirmative or negative way. Each affirmative answer corresponds to one point, and the maximum score of 25 points represents greater functional capacity. A "Total Functional Capacity" (TFC), assesses the participant's ability to perform elementary, basic and instrumental daily life tasks such as maintaining profession and employment, taking care of oneself, managing finances, doing personal hygiene and food. This classification is made by the Investigator and/or person delegated by him, integrating both the information of the patient and his/her family members. The TFC score may vary between scores 0 and 13, and the higher the score, the higher the functionality. Finally, the evaluation is complemented by an independence scale which classifies as a percentage the degree of dependence of the participant, and 100% corresponds to total independence, that is, greater functional capacity (Barreto, 2009; Januário, 2011; Huntington Study Group, 1996).

This scale will be performed at all study visits.

7.2.6. Application of the Hamilton Depression Assessment Scale (HAM-D)

The Hamilton depression scale is multidimensional, hetero-evaluative of the observer, developed for application to patients previously diagnosed with mood disorder. At first, it was created to evaluate hospitalized patients and, therefore, has emphasis on melancholic and physical symptoms of depressive syndrome. Considered a "gold standard" by psychiatry, it allows the quantification of depressive symptoms, being useful for application in clinical practice and in clinical trials, particularly in the psychopharmacology of antidepressants.

HAM-D originally has 17 items, which include the categories of mood, cognitive, somatic, motor, social and also anxiety. Thus, cognitive and somatic aspects represent 50% of the total score of the scale, while 16% refer to anxious symptoms and 8% to the mood category. The application of the scale should be performed by the Investigator or physician delegated by him, based on the presence/absence of the symptoms experienced by the participant in recent weeks, being ideal for application a time of up to 30 minutes. The score "zero" (0) should be given only when the symptom is absent, reduced due to symptomatic or dubious treatment. When there are doubts about the degree of intensity, one should always score to the most intense degree.

For adequate scoring, it is essential that the applicator points immediately after the patient's response. To transform the points into scores, it is up to the evaluator to select one of the categories already published previously to obtain the cut-off point of the scale, which often compromises the comparison of the results in a more standardized way. In current practice, however, it is accepted that scores with more than 25 points characterize deeply depressed patients; scores between 18 and 24 points, moderately depressed patients; and scores between 7 and 17 points, patients with mild depressive conditions.

The intention of using the HAM-D scale in this trial is to evaluate, especially, the "suicide" domain, since one of the most frequent causes of death in patients with Huntington's disease is suicide.

The full English HAM-D scale is available in Annex 4. This scale will be performed at all study visits.

7.2.7. CIBIS Scale Application – Clinician Interview Based Impression

The CIBIS scale (*Clinician Interview Based Impression of Severity*) is an instrument of global assessment of the severity of the disease that associates the impression of the interviewer physician with the opinion provided by both the participant himself and the responsible caregiver. The application of the scale is simple and fast and has been widely used in clinical trials as a basis for evaluating the overall clinical change during treatment, especially in patients with neurodegenerative diseases (dementia, Alzheimer's, schizophrenia, among others). Initially, the baseline status of the patient is determined from a first interview (before the start of treatment), in which this information will serve as a reference to determine in later evaluations whether or not there were significant clinical changes throughout treatment.

This evaluation is based on the observation of 15 domains related to the four categories that are part of the scale: cognition, behavior, social functioning and activities of daily living. It is not necessary for domains to be evaluated in a specific order. However, it is essential that each of them be examined and that observations are recorded in sufficient detail to facilitate subsequent evaluations.

Each question is graded on a 7-point scale, 1 = Normal, not sick and 7 = Patient extremely ill. Therefore, the Interviewer should indicate based on the information provided by the participant/caregiver associated with their clinical experience, which score best classifies the severity of the disease. The version in English of this scale is found in Annex 5. This scale will be performed at all study visits.

7.2.8. BMI Assessment (Body Mass Index)

Body mass index (BMI) will be calculated from the relationship between body mass (in kg) and squared height (in m^2) of the research participant. The BMI assessment will be carried out in all visits.

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

7.2.9. Evaluation of the CNS by magnetic resonance imaging of the brain

The test will be performed on an MRI machine. Conventional sequences will be obtained, used for the screening obtained before and after the use of paramagnetic contrast (gadolinium).

The sequences obtained will be submitted to a post-processing using the software FreeSurfer. This software is intended for the study of in vivo cortical and subcortical brain anatomy processed individually (evaluation of gray matter). The possibilities of FS are cortical surface reconstruction, cortical thickness calculation, cortical and subcortical structures segmentation, volumetric analysis, tractography analysis. Spreadsheets will be used to group individual data and generate a database.

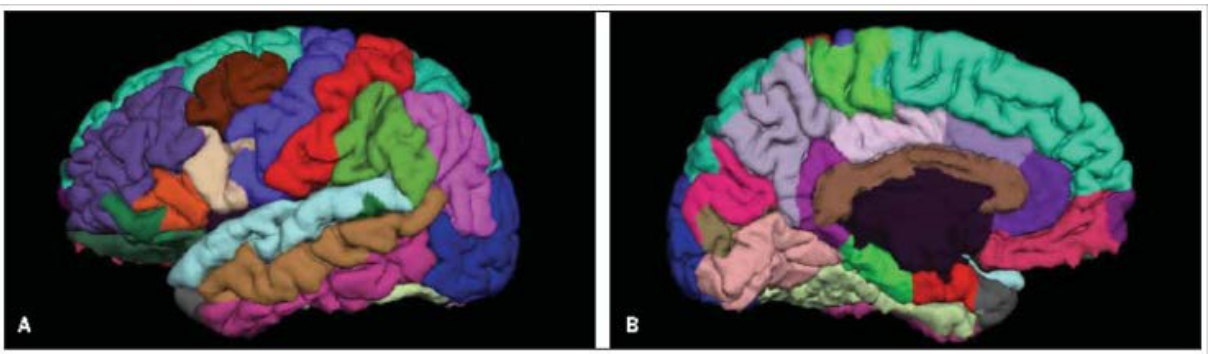

Figure 10. Example of areas evaluated for average cortical thickness in the following regions of both hemispheres: prefrontal, lateral orbitofrontal, superior frontal and caudal anterior cingulate.

The sequence by DTI (*Diffusion Tensor Imaging*) will also be evaluated for the purpose of studying the pathways of the brain white matter. The magnetic resonance tractography technique evaluates nerve fibers through the traces of the diffusion of water represented by a tensor, with the union of points and forming the image of a nerve pathway; this technique of RM tractography does not demonstrate fiber to fiber, but rather a set of them, indicating possible nerve paths by the white substance. The clinical applicability of the tractography is to measure the diameter of brain nerve fibers and their density, the state of myelinization in neurogenesis, evaluation of the degree of (de)myelinization throughout age and in cases of disease (Figure 11 and 12).

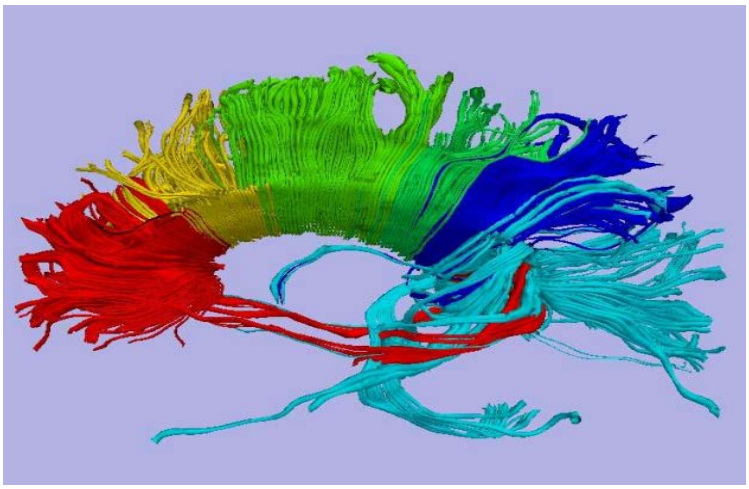

Figure 11. Figure of brain MRI using the DTI (tractography) sequence.

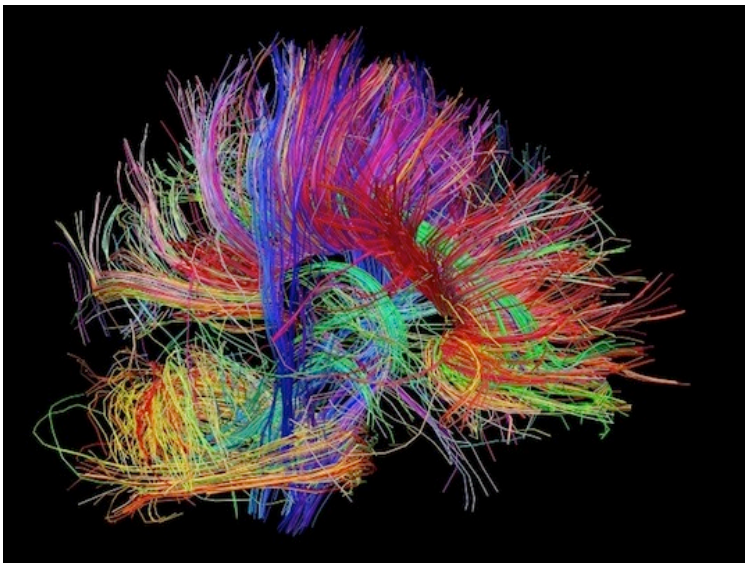

Figure 12. Figure of MRI using DTI (tractography) sequence of the cerebellum, bulb, bridge, midbrain and brain.

For the execution of this study, it may be necessary, depending on the clinical condition of the participant and the clinical evaluation performed by the physician, the use of sedative, in which it will be defined at the time of the examination which will be used. This evaluation will be carried out in visits V-3 and V11.

7.3. Plan of visits and activities to be carried out at each visit

7.3.1. Visit of inclusion and beginning of uniformization

A) Visit V-3: Inclusion (-37 to -1 days before V-2)

- Signature of the ICF (Item 7.1.1);
- Evaluation of inclusion and exclusion criteria (Item 5.2.1 and 5.2.2);
- Recording of demographic data (date of birth, gender and race);
- Medical evaluation: medical history and physical examination / vital signs data (Item 7.2.2 and 7.2.8) with BMI assessment - Body Mass Index;
- Laboratory diagnosis for Huntington's disease, if applicable (Item 7.2.2);
- Collection of blood and urine samples for testing:
    - Laboratory tests (Item 7.2.3 - Chart 3);
    - Serology (Item 7.2.3 - Chart 4);
- ECG 12 leads performance (Item 7.2.4);
- Application of the UHDRS, CIBIS and HAM-D scales (Item 7.2.5 to 7.2.7);
- Performance of magnetic resonance imaging of the CNS (Item 7.2.9);
- Assessment of pregnancy risk and dispensation of contraceptive method, if applicable;
- Registration of prior/concomitant medication;
- Record of adverse events not related to the drug;
- Performance of β-hCG in women;
- General guidelines.

7.3.2. Uniformization period

A) Visit V-2 (up to 30 + 7 days after visit V-3);

- Evaluation of inclusion - except items "d" and "e" - and exclusion criteria (Item 5.2.1 and 5.2.2);
- Medical evaluation: updating of medical history and personal history, physical examination/ vital signs (Item 7.2.2 and 7.2.8) with BMI assessment;
- Evaluation of the results of laboratory tests performed in the previous visit;
- Evaluation of the results of serology tests performed in the previous visit;
- Evaluation of ECG performed in the previous visit;
- Evaluation of the CNS magnetic resonance imaging result performed during the previous visit;
- Application of the UHDRS, CIBIS and HAM-D scales (Item 7.2.5 to 7.2.7);

- Record of adverse events/concomitant medication;
- Assessment of pregnancy risk and dispensation of contraceptive method, if applicable;
- Performance of β-hCG in women;
- Evaluation of laboratory examination for Huntington's disease, if applicable;
- General guidelines.

B) Visit V-1 (30 + 7 days after visit V-2);

- Evaluation of inclusion - except items "d" and "e" - and exclusion criteria (Item 5.2.1 and 5.2.2);
- Medical evaluation: updating of medical history and personal history, physical examination/ vital signs (Item 7.2.2 and 7.2.8) with BMI assessment;
- Application of the UHDRS, CIBIS and HAM-D scales (Item 7.5.5 to 7.5.7);
- Record of adverse events/concomitant medication;
- Assessment of pregnancy risk and dispensation of contraceptive method, if applicable;
- Performance of β-hCG in women;
- General guidelines.

7.3.3. Treatment and Follow-up Visits

A) Visit 0 (V0): 1st administration of cycle 1 (up to 30 days after V-1, which may occur on the same day)

- Verification of inclusion - except items "d" and "e" - and exclusion criteria (Item 5.2.1 and 5.2.2);
- Medical evaluation: updating of medical history and personal history and physical examination/ vital signs (Item 7.2.2 and 7.2.8) with BMI assessment;
- Randomization
- Intravenous administration of the investigational product or placebo;
- Hospitalization for a period prior to administration and for another 06 hours after the end of the same (the length of stay may be extended at the discretion of the Principal Investigator);

- ECG monitoring during hospitalization time (during 06 hours after IP administration) (Item 7.2.4);
- Application of the UHDRS, HAM-D and CIBIS scales (Item 7.2.5 to 7.2.7);
- Record of adverse events (AEs) and concomitant medications;
- Assessment of pregnancy risk and dispensation of contraceptive method, if applicable;
- Performance of β-hCG in women;
- General guidelines.

B) Visit 1 (V1): 2nd administration of cycle 1 (30 ± 5 days after V0)

- Verification of discontinuation criteria (Item 5.2.3);
- Medical evaluation: updating of medical history and personal history and physical examination / vital signs (Item 7.2.2 and 7.2.8) with BMI assessment;
- Intravenous administration of the investigational product or placebo;
- Hospitalization for a period prior to administration and for 06 more hours after the end of the administration (the length of hospitalization may be extended at the discretion of the Principal Investigator):
- ECG monitoring during hospitalization time (during 06 hours after IP administration) (Item 7.2.4);
- Application of the UHDRS, HAM-D and CIBIS scales (Item 7.2.5 to 7.2.7);
- Record of adverse events (AEs) and concomitant medications;
- Assessment of pregnancy risk and dispensation of contraceptive method, if applicable;
- Performance of β-hCG in women;
- General guidelines

C) Visit 2 (V2): 3rd administration of cycle 1 (60 + 5 days after V0)

- Verification of discontinuation criteria (Item 5.2.3);
- Medical evaluation: updating of medical history and personal history and general physical examination/vital signs (Item 7.2.2 and 7.2.8) with BMI assessment;
- Intravenous administration of the investigational product or placebo;

- Hospitalization for a period prior to administration and for 06 more hours after the end of the same (the length of hospitalization may be extended at the discretion of the Principal Investigator);
- ECG monitoring during hospitalization time (during 06 hours after IP administration) (Item 7.2.4);
- Application of the UHDRS, HAM-D and CIBIS scales (Item 7.2.5 to 7.2.7);
- Record of adverse events (AEs) and concomitant medications;
- Assessment of pregnancy risk and dispensation of contraceptive method, if applicable;
- Performance of β-hCG in women;
- General guidelines

D) Visit 3 (V3): follow-up visit (90 + 5 days after V0)

- Verification of discontinuation criteria (Item 5.2.3);
- Medical evaluation: updating of medical history and personal history and general physical examination/vital signs (Item 7.2.2 and 7.2.8) with BMI assessment;
- Collection of blood and urine samples for testing:
    - Laboratory tests (Item 7.2.3 - Chart 3)
- ECG 12 leads performance (Item 7.2.4);
- Application of the UHDRS, HAM-D and CIBIS scales (Item 7.2.5 to 7.2.7);
- Record of adverse events (AEs) and concomitant medications;
- Assessment of pregnancy risk and dispensation of contraceptive method, if applicable;
- Performance of β-hCG in women;
- General guidelines.

E) Visit 4 (V4): 1st administration of cycle 2 (120 + 5 days after V0)

- Verification of discontinuation criteria (Item 5.2.3);
- Medical evaluation: updating of medical history and personal history and general physical examination/vital signs (Item 7.2.2 and 7.2.8) with BMI assessment;
- Evaluation of the results of laboratory tests performed at the previous visit;
- Evaluation of the ECG result performed in the previous visit;

- Intravenous administration of the investigational product at the given dose or placebo;
- Hospitalization for a period prior to administration and for another 06 hours after the end of the administration (the length of hospitalization may be extended at the discretion of the Principal Investigator);
- ECG monitoring during hospitalization time (during 06 hours after IP administration) (Item 7.2.4);
- Application of the UHDRS, HAM-D and CIBIS scales (Item 7.2.5 to 7.2.7);
- Record of adverse events (AEs) and concomitant medications;
- Assessment of pregnancy risk and dispensation of contraceptive method, if applicable;
- Performance of β-hCG in women;
- General guidelines.

F) Visit 5 (V5): 2nd administration of cycle 2 (150 + 5 days after V0)

- Verification of discontinuation criteria (item 5.2.3);
- Medical evaluation: updating of medical history and personal history and general physical examination/vital signs (Item 7.2.2 and 7.2.8) with BMI assessment;
- Intravenous administration of the investigational product at the given dose or placebo;
- Hospitalization for a period prior to administration and for another 06 hours after the end of the administration (the length of hospitalization may be extended at the discretion of the Principal Investigator);
- ECG monitoring during hospitalization time (during 06 hours after IP administration) (Item 7.2.4);
- Application of the UHDRS, HAM-D and CIBIS scales (Item 7.2.5 to 7.2.7);
- Record of adverse events (AEs) and concomitant medications;
- Assessment of pregnancy risk and dispensation of contraceptive method, if applicable;
- Performance of β-hCG in women;
- General guidelines.

G) Visit 6 (V6): 3rd administration of cycle 2 (180 + 5 days after V0)

- Verification of discontinuation criteria (Item 5.2.3);
- Medical evaluation: updating of medical history and personal history and physical examination/vital signs (Item 7.2.2 and 7.2.8) with BMI assessment;
- Evaluation of the results of laboratory tests performed at the previous visit;
- Intravenous administration of the investigational product at the given dose or placebo;
- Hospitalization for a period prior to administration and for another 06 hours after the end of the administration (the length of hospitalization may be extended at the discretion of the Principal Investigator);
- ECG monitoring during hospitalization time (during 06 hours after IP administration) (Item 7.2.4);
- Application of the UHDRS, HAM-D and CIBIS scales (Item 7.2.5 to 7.2.7);
- Record of adverse events (AEs) and concomitant medications;
- Assessment of pregnancy risk and dispensation of contraceptive method, if applicable;
- Performance of β-hCG in women;
- General guidelines.

H) Visit 7 (V7): follow-up visit (210 + 5 days after V0)

- Verification of discontinuation criteria (Item 5.2.3);
- Medical evaluation: updating of medical history and personal history and physical examination/vital signs (Item 7.2.2 and 7.2.8) with BMI assessment;
- Collection of blood and urine samples for testing:
  - Laboratory tests (Item 7.2.3 - Chart 3);
- ECG 12 leads performance (Item 7.2.4);
- Application of the UHDRS, HAM-D and CIBIS scales (7.2.5 to 7.2.7);
- Record of adverse events (AEs) and concomitant medications;
- Assessment of pregnancy risk and dispensation of contraceptive method, if applicable;
- Performance of β-hCG in women;
- General guidelines.

I) Visit 8 (V8): 1st administration of cycle 3 (240 + 5 days after V0)

- Verification of discontinuation criteria (Item 5.2.3);
- Medical evaluation: updating of medical history and personal history and physical examination / vital signs (Item 7.2.2 and 7.2.8) with BMI assessment;
- Evaluation of the results of laboratory tests performed at the previous visit;
- Evaluation of the ECG result performed in the previous visit;
- Intravenous administration of the investigational product at the given dose or placebo;
- Hospitalization for a period prior to administration and for another 06 hours after the end of the administration (the length of hospitalization may be extended at the discretion of the Principal Investigator);
- ECG monitoring during hospitalization time (during 06 hours after IP administration) (Item 7.2.4);
- Application of the UHDRS, HAM-D and CIBIS scales (7.2.5 to 7.2.7);
- Record of adverse events (AEs) and concomitant medications;
- Assessment of pregnancy risk and dispensation of contraceptive method, if applicable;
- Performance of β-hCG in women;
- General guidelines.

J) Visit 9 (V9): 2nd administration of cycle 3 (270 + 5 days after V0)

- Verification of discontinuation criteria (Item 5.2.3);
- Medical evaluation: updating of medical history and personal history and physical examination/ vital data (Item 7.2.2 and 7.2.8) with BMI assessment;
- Intravenous administration of the investigational product at the given dose or placebo;
- Hospitalization for a period prior to administration and for another 06 hours after the end of the administration (the length of hospitalization may be extended at the discretion of the Principal Investigator);
- ECG monitoring during hospitalization time (during 06 hours after IP administration) (Item 7.2.4);
- Application of the UHDRS, HAM-D and CIBIS scales (7.2.5 and 7.2.7);
- Record of adverse events (AEs) and concomitant medications;

- Assessment of pregnancy risk and dispensation of contraceptive method, if applicable;
- Performance of β-hCG in women;
- General guidelines.

K) Visit 10: 3rd administration of cycle 3 (300 + 5 days after V0)

- Verification of discontinuation criteria (Item 5.2.3);
- Medical evaluation: updating of medical history and personal history and physical examination/vital signs (Item 7.2.2 and 7.2.8) with BMI assessment;
- Intravenous administration of the investigational product at the given dose or placebo;
- Hospitalization for a period prior to administration and for another 06 hours after the end of the administration (the length of hospitalization may be extended at the discretion of the Principal Investigator);
- ECG monitoring during hospitalization time (during 06 hours after IP administration) (Item 7.2.4);
- Application of the UHDRS, HAM-D and CIBIS scales (Item 7.2.5 to 7.2.7);
- Record of adverse events (AEs) and concomitant medications;
- Assessment of pregnancy risk and dispensation of contraceptive method, if applicable;
- Performance of β-hCG in women;
- General guidelines.

L) Visit 11 (V11): final follow-up visit (330 + 5 days after V0)

- Verification of discontinuation criteria (Item 5.2.3);
- Medical evaluation: updating of medical history and personal history and physical examination/ vital signs (Item 7.2.2 and 7.2.8) with BMI assessment;
- Evaluation of the ECG result performed in the previous visit;
- Collection of blood and urine samples for testing:
    - Laboratory tests (Item 7.2.3 - Chart 3);
    - Serology (Item 7.2.3 - Chart 4);
- MRI of the CNS (Item 7.2.9);
- ECG 12 leads performance (Item 7.2.4);

- Application of the UHDRS, HAM-D and CIBIS scales (Item 7.2.5 to 7.2.7);
- Record of adverse events (AEs) and concomitant medications;
- Assessment of pregnancy risk;
- Performance of β-hCG in women;
- General guidelines and exemption from the study.

Note.: If any participant presents a clinically significant result in any of the tests performed on V11, the participant will be contacted and an extraordinary visit will be made.

7.4. Extraordinary Visits

If the participant presents any adverse event or relevant signs and symptoms during the study period; or if the participant needs to be evaluated before regular visits, the participant should contact the Researcher or the Researcher himself can contact the participant if necessary. In such specific cases, the Investigator's assessment will be considered as an extraordinary visit.

If the extraordinary consultation results in the withdrawal of the participant of the study, it will be considered as a closing visit and all activities related to the Final Follow-up Visit (V11) should be carried out.

7.5. Adherence

Since, for safety reasons, the experimental therapy used in the study will have the total dose divided into 3 administrations, one every 30 days, the participant who, for some eventuality, does not attend the scheduled administration dates will not be discontinued from the study, since that the disease in question is considered serious and has drug treatments only for the management of symptoms. Safety and efficacy data obtained from these participants will only be considered in the ITT population analysis.

To increase the participant's adherence to the protocol, it will be discussed with the same in what the trial consists before his/her consenting, appointments will be scheduled at convenient times, expenses such as transportation and food will be reimbursed, and the participant will be encouraged to remain in the study.

8. STATISTICAL PROCEDURES

The study data will be recorded in the medical records of the research participant and will later be transcribed to the company's electronic CRF. The data inherent to the study will be tabled and sent to the statistician responsible for the study. Statistical analyses will be performed after the resolution of all inconsistencies and performance of data quality control.

The disposition of screened participants, included in the study, who received medication, who prematurely discontinued or completed the study will be summarized by treatment dose.

Demographic characteristics, pre-existing clinical conditions, medical history, previous treatments, reported at baseline will be summarized by treatment group, considering participants who received at least one dose of the drug under test.

Graphs, tables and descriptive analyses will be carried out using an appropriate statistical system.

8.1. Sample size determination

The estimated number of participants was calculated considering clinical/regulatory and non-statistical assumptions. In this way, the document *Considerations for the Design of Early-Phase Clinical Trials of Cellular and Gene Therapy Products - Guidance for Industry* (FDA, 2015) predicts that a smaller sample size is considered appropriate for diseases considered fatal, where a major potential benefit may warrant an unknown risk. Additionally, considering conditions provided by the FDA document such as the still limited capacity of the investigational product manufacturing and the relatively rare prevalence of the target disease, Sponsor suggests the sample size of 35 participants, which is considered a feasible sample size and at the same time adequate to meet the proposed objective.

8.2. Analysis Populations

The analysis of the study data will be based on the following populations as described below:

8.2.1. Population Analysis of Intention to Treat (ITT)

All participants who make use of the first administration of the investigational product of the study will constitute the population of intent to treat (ITT – *intention-to-treat*).

8.2.2. Population Analysis of Intention to Treat Efficacy (ITTe)

All ITT participants who have made at least one efficacy assessment.

8.2.3. Population Analysis Per Protocol (PP)

All participants who, according to the protocol guidelines, do not present major deviations, will constitute the population per protocol (PP - per protocol).

Major protocol violations will be reviewed before data analysis begins.

8.2.4. Safety Analysis Population

All participants who receive at least one administration of the investigational product of the study shall be included in the safety analysis population; identical to the ITT population.

8.3. Measurement of the primary variable

For the evaluation of the best dose-response, the score in the evaluation of the UHDRS scale (*Unified Huntington's Disease Rating Scale*) was defined as the primary outcome.

The evaluation of the primary variable will be performed considering the total result of the UHDRS scale. There will also be the evaluation of the composite UHDRS scale (cUHDRS).

The reports will include analyses of the values at the time of inclusion (V-3) in relation to the end of treatment (V11), as well as the analysis of the values of the uniformization period (mean values of visits V-3, V-2 and V-1) in relation to the end of treatment (V11).

The CAP score refers to the term in English ***C****AG - **A**ge **P**roduct* (*CAP*), which is an index calculated in relation to age and length of CAG repetition. This score will be used in order to complement the measurement of the primary variable, so that the participant's age and CAG repetition are taken into account in the analysis of the results over time.

The CAP score formula is: CAP= {100 x Age (CAG -L)}/ S

CAG being the number of repetitions on chromosome 4 (consider the allele with the highest numbering), L and S are constant (L=30 and S=627). In which "L" is a scale constant that anchors the length of the CAG approximately at the lower end of the distribution relevant to the pathology of HD. And "S" is a normalization constant chosen so that the CAP score is approximately 100 at the expected onset age of the patient, as estimated by Langbehn et al. 2004. For the calculation in this study, the Value of S used will be S = 627, which are estimates obtained by a reanalysis of the data of Langbehn et al presented by Warner and Hayden 2012.

8.4. Measurement of secondary variables

Secondly, the safety as well as preliminary evidence of efficacy of the Cellavita-HD investigational product will be evaluated through of the following variables

a) Check for clinical neurological improvement throughout the study:

The UHDRS scale will be evaluated at each visit to measure neurological improvement.

b) Check the overall improvement in disease severity by the CIBIS scale throughout treatment:

The profile of the CIBIS scale will be considered for the evaluation of the overall clinical improvement of the participant through the treatments of the study.

c) BMI assessment:

BMI (Body Mass Index) will be evaluated through BMI profiles obtained during treatment.

d) Evaluate the suicide domain of the HAM-D scale throughout treatment:

It will be evaluated especially the domain "Suicide" by means of classification scores, in which scores of 8 – 13 correspond to mild depression, 14 – 18 to moderate, from 19 - 22 to severe and above 23 points to very severe depression. The "suicide" domain of the HAM-D scale will be evaluated through the profiles obtained throughout the treatment.

e) Neurological evaluation by magnetic resonance imaging of the brain:

It will be assessed through the profiles obtained during the treatment of the following measures:

- Cortical thickness measurements;

- Volumes of the various brain structures, especially the core of the base, with special attention to the caudate;

- Identifiable metabolic changes in proton spectroscopy.

8.5. Measurement of the Safety Variable

During the study, the safety of the investigational product will be thoroughly assessed based on periodic evaluations including clinical, laboratory and imaging tests, so that any alteration is properly recorded. The safety data described below will be collected and recorded in the clinical file:

- Adverse events: all adverse events will be monitored and reported in the clinical form of the study from the signature of the Informed Consent, including type, frequency, intensity, seriousness, severity, action taken and relationship with the investigational product of the study. The adverse events may be reported by the research participant or observed by the Researcher. Adverse events will be standardized by the Investigator.
- Changes in vital signs, clinical examination and medical evaluations;
- Changes in laboratory or serology tests:
- Change in electrocardiogram (ECG);
- Incidences and classification of benign and malignant neoplasms.

The results obtained will be presented through the proportion of adverse events that occur, segmented by dose of the investigational product. Any clinically significant abnormalities that persist until the end of the study will be monitored by the Principal Investigator until resolution or until a clinically stable outcome.

8.6. Study hypotheses

8.6.1. Primary Hypothesis

Identify the dose of the Cellavita-HD investigational product that presents the best response.

8.6.2. Secondary hypotheses

Cellavita-HD is safe after intravenous administration in participants with Huntington's disease and provides evidence of efficacy in relation to the parameters evaluated.

8.7. Statistical analysis

The analyses will be carried out using an appropriate statistical system.

8.7.1.1. Analysis of demographic and baseline data

A description of the distribution of participants (number and percentage of randomized participants in the ITT and PP populations) will be provided by treatment.

The analyses of the qualitative variables of the ITT population will be described by frequency analysis. The quantitative variables will be described by mean, standard deviation (SD), minimum, maximum and median.

8.7.1.1.1. Hypothesis analysis 1

The treatments will be compared using a linear model in which the response is the motor evaluation of the UHDRS scale at the end of the three treatment cycles; where the treatment will be the factor and the basal measurement will be the variable.

Corrections will be made to multiple comparations (ANOVA).

8.7.1.1.2. Analysis of secondary hypotheses

The continuous (numerical) variables will be performed using a model of repeated measurements, including the factors treatment, time, interaction between time-treatment using the GEE method.

Analysis of residues will be carried out to evaluate the suitability of the model used. The multiple-way comparation method will take into account the adjustment of the significance level.

The analyses of categorical responses observed over time will take into account the factors treatment, time, time-treatment interaction. The significance level will be preserved with the preserved multiple-like comparation method.

8.7.1.1.3. Analysis of the safety hypothesis

The safety assessment will be performed at the end of each cycle and will be verified through the incidence of reports that adverse events occurred during the study, in relation to causality with the treatment under study.

8.7.1.1.4. Exploratory analyses

Additional exploratory analyses will be carried out if necessary to better understand the results. All methods used will be detailed described in the final study report.

8.8. Data management plan summary

The data of the study will be reviewed by the Data Management team and, if they present discrepancies, questions will be generated, which will be sent to those responsible for collecting them. A report will be issued with the questions raised and their answers / resolutions, which will be stored together with the other study documents.

The data management of the clinical studies conducted complies with current regulations and in compliance with the processes described below.

8.8.1. Deviations from the Original Statistical Plan

Any deviation from the original statistical plan shall be described and justified in the protocol and/or final statistical report, where appropriate.

8.8.2. Record of analyzed parameters

All study data will be recorded in the research participant's medical records and will later be transcribed into the company's CRF. The data inherent to the study will be tabled and sent to the statistician responsible for the study.

8.8.3. Data Capture - Clinical Record (CRF)

The clinical form of the clinical study will be used to record the data collected in the source documents, being considered an integral part of the clinical study. The process of evaluation, development and maintenance of the system is in accordance with the guidelines established by Article 21 part 11 of the CFR (*Code of Federal Regulations*) of the FDA. The tool will be validated prior to the beginning of data collection in accordance with the requirements established by Sponsor, in order to ensure its quality and performance.

The clinical data will be imputed by the Investigator or by the delegate(s) responsible.

The clinical form should be updated in order to express each phase during the clinical study, with the registration of all research participants who are screened, in order also to ensure the traceability of the records of the research participants considered as selection failures.

8.8.4. Data validation – Plan of Queries

The plan of queries describes the verification of data to be performed by data management, based on the crossing between different fields of the clinical record. Its development considers each field of the clinical record and the crossing of fields, within the same visit and between visits, allowing the verification of all fields considered primordial for statistical analysis and obtaining the results of the clinical study.

Manual checks contained in the queries will always be performed by Data Management, in order to ensure the quality of the data collected.

8.8.5. Database cleanup and freezing

Based on the queries, the database's data cleansing is carried out in order to ensure the quality of the collected data. The cleaning of the bank is carried out periodically by generating batch of queries submitted to the Research Center with requests for clarification. At the end of data collection, the last batch of queries is forwarded and after the final cleaning of the data, freezing is performed. The freeze will be carried out according to the procedures of the company responsible for monitoring the database.

8.9. Handling Missing or Invalid Data

Safety will be analyzed using the observed data set and no techniques will be used for imputation of missing or invalid data.

The analyses of the preliminary efficacy assessment will be performed using the LOCF principle, the last observation carried forward, for the absent post-baseline data. In the analysis of repeated measures, the imputation of data will be performed using the GEE method.

8.10. Interim analyses

There will be no interim analysis of the data.

9. ADVERSE EVENTS

9.1. Adverse events

An adverse event is defined as any unfavorable medical occurrence, which may occur during treatment with an investigational drug/product, but which does not necessarily have a causal relationship with this treatment.

All adverse events observed or reported spontaneously, regardless of the possible causal relationship with the investigational drug/product of the study, should be recorded on the Adverse Events page(s) of the clinical study form. Serious adverse events of any nature or unexpected events should be notified to Sponsor via specific Pharmacovigilance form.

Alterations (abnormalities) in the physical examination, considered as clinically significant by the Investigator, prior to the use of the investigational product should be reported in physical and/or historical clinical evaluation (if applicable) and if, after use of the drug, relative worsening, worsening of conditions or appearance of changes (abnormalities) during regular visits, these should be recorded as adverse events.

Changes (abnormalities) in the laboratory testing, considered as clinically significant by the Investigator and present in the period in which the participant does not use the medicine/ investigational product should also be recorded as adverse events. All laboratory alterations (abnormalities) should always be recorded in the participant's medical records.

For all adverse events, the Investigator should seek appropriate information, both to determine the consequence of the adverse event and to assess whether it is within the classification criteria as a serious adverse event, meaning that it requires immediate notification. The follow-up of the adverse event, even after the date of interruption of the study, will be necessary in case of persistence of the event or its sequelae. Such follow-up is necessary until the adverse event or its sequelae disappear or stabilizes to an acceptable level in the Investigator's assessment.

9.1.1. Serious adverse events

Serious adverse events include any undesirable medical occurrence that, at any dose:

- Results in death;

- Threatens life;
- Results in permanent or significant disability;
- Requires hospitalization or prolongation of hospitalization;
- Results in congenital anomaly;
- Constitutes other clinically important events (an event that may result in one of the consequences described above if not properly treated).

A person's substantial loss of competence in performing his or her routine duties is defined as incapacity.

It should be emphasized that, regardless of the above criteria, any other events that, in the Investigator's opinion, are considered serious, should be immediately reported.

All serious adverse events, regardless of the possible causal relationship with the study drug, should be immediately (within 24 hours) reported in the form of a severe adverse event reporting form filled out with all event data in the most detailed way possible and forwarded back to Sponsor.

The research ethics committee (CEP) should also be informed of serious adverse events.

Any serious adverse events occurring during the study shall be reported within 24 hours to Sponsor from the knowledge of the fact by the Center, regardless of the circumstances or probable cause, whatever the subsequent consequences.

9.1.2. Classification of adverse events according to expectation

- **Unexpected adverse event:** it is defined as any harmful experience that is not described in the research product leaflet, including events that may be symptomatic and physiologically related to an event described in the leaflet, but which differ from this event by the degree of severity and specificity. In addition, the adverse event whose nature, severity or outcome is inconsistent with the information contained in the leaflet is considered unexpected.
- **Expected adverse event:** it is defined as any adverse event whose nature or intensity is part of the information contained in the leaflet (for marketed medicines / investigational products) or in the Investigator's Brochure (for medicines / investigational products) in clinical research phase.

9.1.3. Classification of adverse events in terms of intensity

The classification of the intensity of adverse events will be performed according to the degree of qualifying intensity for health conditions (WHO) described below:

- **Mild:** a problem is present less than 25% of the time, with an intensity that a person can tolerate and that rarely happens in the last 30 days.
- **Moderate:** it is a problem that is present less than 50% of the time, with an intensity, which is interfering in the day-to-day of people and that happens occasionally in the last 30 days.
- **Severe:** it is that a problem that is present in more than 50% of the time, with an intensity that partially alters the day-to-day of people and that happens frequently in the last 30 days.
- **Complete commitment:** it is a problem that is present in more than 95% of the time, with an intensity that completely alters the day-to-day of the person and that occur every day for the last 30 days.
- **Unspecified:** It means there's not enough information to specify the intensity.
- **Not applicable:** it is inappropriate to use a gradation (e.g. menstrual functions).

9.1.4. Causality categories: WHO-UMC System

a) Certain / Defined

- Event or alteration (abnormality) in laboratory examination with plausible temporal relationship in relation to the administration of the intervention. It cannot be explained by disease or other intervention or medicine;
- Response to discontinuation or plausible withdrawal (pharmacologically, pathologically);
- Event defined pharmacologically or phenomenologically (e.g., an objective and specific disorder or a pharmacologically recognized phenomenon);
- Satisfactory re-exposure if necessary.

b) Probable

- Event or alteration (abnormality) in laboratory examination with a reasonable temporal relationship in relation to the administration of the intervention;
- Unlikely to be attributed to a disease or other intervention, medicinal product;
- Response to clinically reasonable discontinuation or withdrawal;
- Re-exposure not required.

c) Possible

- Event or alteration (abnormality) in laboratory examination with a reasonable temporal relationship in relation to the administration of the intervention;
- It can be explained by disease or other interventions, medicines;
- Information about withdrawal or discontinuation of treatment may be missing or obscure.

d) Improbable

- Event or alteration (abnormality) in laboratory examination that, in relation to the time of administration of the intervention, makes an unlikely (but not impossible) relationship;
- Disease or other treatments provide plausible explanations.

e) Conditional / Unclassified

- Event or alteration (abnormality) on laboratory examination;
- More data is needed for an appropriate assessment, or;
- Additional data under investigation.

Source: *The Uppsala Monitoring Centre* – World Health Organization

9.2. Contact with the Sponsor

The names and contact phones of the Principal Investigator, among others, are listed at the beginning of this protocol and will be made available to research participants together with the ICF for any consultations.

9.3. Follow-up of reports of adverse events

For all reports of adverse events regardless of their severity, in case there is any inconsistency in the registration and/or evaluation of the adverse event(s), it is the responsibility of the Investigator to obtain and provide the information to Sponsor.

In the final follow-up of the monitoring, the monitor should check the Clinical Form(s) if there are adverse events with a "not recovered" clinical outcome. For these reports, the monitor should ask the Investigator to follow-up and collect outcome information.

After the end of the study, the Investigator shall be responsible for informing Sponsor of any adverse event occurring within thirty (30) days, reported to them spontaneously, after the clinical study is completed or discontinued, if there is a possibility of a causal relationship between the adverse event and the product under investigation.

9.4. Reports of pregnancy occurrence with direct or indirect exposure to the investigational product of the study

In cases of pregnancy during clinical studies, we aim to assess toxicity in women and fetus directly through the passage of the product under investigation through the placental barrier (pregnancy with direct exposure to the medication under study) or due to the transfer of the product under investigation to the woman through the semen of the research participant (pregnancy with indirect exposure to the medication under study), therefore, both direct and indirect pregnancy should be analyzed with appropriate follow-up.

9.4.1. Gestational follow-up

The Investigator should follow-up on the evolution of pregnancy (with direct or indirect exposure to the medication under study) until the end of pregnancy and birth of the child, even after the exclusion of the research participant from the clinical study, considering that such information is important for the evaluation of the safety profile of the product under investigation.

9.4.2. Pregnancy with direct or indirect exposure to the investigational product under study

For research participants who may become pregnant after signing the Informed Consent, regardless of exposure to the product under investigation, gestational follow-up will be required, as described below:

- At the time of pregnancy knowledge;
- End of the 1st trimester: preferably between the 12th and 16th weeks, with ultrasound presentation;
- End of the 2nd trimester: preferably between the 24th and 28th weeks, with ultrasound presentation;
- End of the 3rd trimester: preferably between 36th and 40th weeks, with ultrasound presentation;
- After the birth of the child until the first year of life.

9.4.3. Notification to the Research Ethics Committee (CEP)

Although pregnancy is not a serious adverse event from a medical point of view, regulatory treatment is the same as a serious adverse event; therefore, the Research Ethics Committee (CEP) should be informed of cases of pregnancy occurring within a maximum of 24 hours, after knowledge by the Research Center, through its responsible investigators.

9.4.4. Consistency of information notified by Research Center to the Sponsor

In case of possible inconsistencies in the registration of the serious adverse event and/or pregnancy in the forms, the monitor will request clarification from the investigator. This communication will take place through monitoring reports and/or clarification letters.

9.5. Expected adverse events with cell therapy

Few adverse events have been reported after administration of mesenchymal stem cells in terms of immediate toxicity, infusion and late effects. Thus, as the number of patients on therapy with this type of cell is still relatively small, adverse events and safety related to it have not yet been fully elucidated. However, in some specific pathological cases, the use of mesenchymal stem cells

was related to the appearance of neoplasms (history of neoplasms) and worsening of arthritis (history of arthritis) (Wang, Qu & Zhao, 2012).

In order to gather information on adverse events related to mesenchymal stem cell therapies and to support their use in clinical studies, Lalu et al. (2012) conducted a meta-analysis in which no significant associations were verified between treatment with mesenchymal stem cells (of several lines) and the development of acute infusion toxicity, systemic complications, infections, neoplasms or deaths. However, the authors found that although there is a significant correlation between cell therapy and the onset of transient fever, it is possible to observe good tolerability and safety (Lalu et al., 2012).

Although the appearance of neoplasms is a risk to the use of cell therapies, the analysis performed by the authors did not find an association between both. The emergence of this correlation started from non-clinical studies that demonstrated neoplastic increase in animals treated with therapy, however, in current clinical studies the occurrence of neoplasia presented was low and present only in patients with a history of neoplasms (Lalu et al., 2012).

Thus, the authors concluded that despite the need for broader and more controlled clinical studies involving the use of mesenchymal stem cells for long periods, so far there is no evidence that this therapy represents a safety risk to humans (Lalu et al., 2012).

This way, due to the variety of theoretical risk factors involving this therapy, a thorough clinical evaluation should be performed, taking into account the risks and benefits that treatment may present for the patient. This assessment at the beginning and during follow-up of cell therapy can help determine the extent and focus of safety plans for such treatment (Herberts, Kwa & Hermsen, 2011; Wang, Qu & Zhao, 2012).

The table below summarizes the possible theoretical risk factors involving the use of cell therapy with mesenchymal stem cells of several lines.

Table 05. Theoretical risk factors involved in mesenchymal stem cell therapy.

Factors	Possible risks
Intrinsic	• Rejection of administered cells • Susceptibility to opportunistic diseases • Grafting cells in an unwanted location • Unwanted biological effect (differentiation *in vivo* in unwanted cell types) • Toxicity (infusion or systemic) (Symptoms: transient fever, bronchospasm and/or laryngospasm, chills, diarrhea, dyspnea, headache, facial edema, glottis and/or larynx, fever, hemoglobinuria, hypotension, nausea, itching, facial flushing, abnormal bleeding, sweating, tachycardia, dizziness, hives, and vomiting). • DMSO toxicity (Symptoms: cough, flushing, rash, chest tightening and wheezing, nausea and vomiting, cardiovascular instability). • Formation of neoplasms (malignant or benign)
Extrinsic	• Transmission of diseases • Reactivation of latent viruses • Unwanted immune response • Cell contamination (with unwanted cells, culture media components or chemical compounds); • Mixture of autologous patient material • Cardiac, vascular, renal, neurological, pulmonary, hematological or hepatic dysfunctions • Formation of neoplasms (malignant or benign)

Source: Circulation of Information for use of cellular therapy products – Revised November 2013. Helberts, Kwa & Hermsen, 2011; Lalu et al., 2012 - Adapted.

9.6. Record of safety parameters

Every safety data will be recorded in the participant's medical records and will later be transcribed to the company's CRF. The database will be converted to the format of tables and sent to statistical advisory.

9.6.1. Suspension of administration of the investigational product

If after the first administration there is a suspicion of the occurrence of an adverse event that may possibly threaten the participant's life, the Medical Investigator of the ADORE-DH project should judge whether the treatment should be discontinued or suspended indefinitely. Among such events, it may be considered (not restricted to):

- Systemic Inflammatory Response Syndrome (SIRS);
- Anaphylactic shock or allergic reaction;
- Significant reduction in total platelet count and granulocytic cells (neutrophils, eosinophils and basophils);

- Prolongation of prothrombin (TP) time and activated partial thromboplastin (TTPa);

The suspension of the administration to a participant due to the occurrence of one of the events mentioned above must be informed to Sponsor within a maximum period of 24 hours and notified to the Research Ethics Committee.

The safety assessment should be carried out at each visit in which the drug of the study takes place.

9.6.2. Study Interruption/Suspension

Due to the unknown risk related to the frequency and severity of adverse reactions involving cellular products, the first clinical studies conducted with these products included standards/rules aimed at temporary interruption of the inclusion of participants until any condition that put the participant's safety at risk was fully evaluated.

According to the document *Considerations for the Design of Early-Phase Clinical Trials of Cellular and Gene Therapy Products - Guidance for Industry* (FDA, 2015), these rules called "*stopping rules"*, shall specify a number of adverse events or deaths that will lead to the temporary interruption of the study in order to have a systematized review of some study criteria such as dose, number of administrations, eligibility criteria, mode of administration or manufacture of the investigational product. These rules aim to increase the safety of the investigational product in relation to the unpredictable risks that will be evaluated, identified and resolved during the clinical follow-up of the study.

For the ADORE-DH study, the following rules (*stopping rules)* are considered decisive in determining the temporary interruption of the study:

- Death of one of the research participants related to therapy with investigational product (necropsy to define the cause of death);
- Occurrence of one or more serious adverse events, such as SIRS, pulmonary thromboembolism, vascular thrombosis, acute myocardial infarction, hemorrhage, stroke, among others, in more than one participant with a proven relationship with the investigational product and that at the investigator's discretion may jeopardize the safety of the other participants.

Any occurrences related to the events reported above, shall be informed to Sponsor within a maximum of 24 hours and reported to the Research Ethics Committee (CEP).

9.7. Providing data and tracking of participants

For all adverse events, the Investigator is obliged to obtain and provide information, in addition to reporting it in the medical records and CRF, in accordance with what is requested by the clinical monitor and pharmacovigilance. In general, this includes a sufficiently detailed description of the adverse event to allow a thorough medical evaluation of the case and determination of possible causality.

In the event of the death of a participant, the *causa mortis* (cause of death) should be reported in the initial notification, when known, or later with the *follow-up of the causa mortis.* A copy of the death certificate should be forwarded to Pharmacovigilance.

The Investigator shall ensure that information about such cases, reported by telephone or by any other means, and the information recorded in the clinical study form is accurate and consistent.

In case of possible damage to the participant's health, proven by the investigational products used in the study, the participant will be indemnified in accordance with current legislation. In addition, in case of need for medical treatment with or without the need for hospitalization, it will be provided by the institution that assists it. Pre-planned surgical treatments or procedures should be recorded in the initial documentation during the protocol period and for each individual in particular.

9.7.1. Contact with the Sponsor

The names and contact telephone numbers of representatives of Cellavita Pesquisa Científica LTDA, the Principal Investigator, among others, are listed in item III of this protocol and will be made available to participants.

10. ETHICAL AND REGULATORY CONSIDERATIONS

This Protocol will be conducted in accordance with the guidelines of the International Conference on Harmonization - ICH for Good Clinical Practices and Document of the Americas, as well as national and international legislation.

The Research Center with its Investigators, sub-investigators, study coordinators and internal monitors declare that the protocol and the Informed Consent Form will be conducted in accordance with current ICH GCP and in accordance with applicable local regulations (Resolution No. 466 of December 12, 2012, Resolution No. 251 of August 7, 1997) of the CNS-MS.

10.1. Declaration of the Americas and Regulation

The study will be conducted in accordance with resolutions 466/12 and 251/97 of the National Health Council/MS, respecting mainly the participant and the Good Clinical Practices/Document of the Americas.

10.2. Belmonte Communication

The study will comply with the guidelines of ethical conduct of the Belmonte Communication, which has 3 basic principles:

a) Respect;

b) Beneficence;

c) Justice.

10.3. Informed Consent Form (ICF) and Image Authorization Term

The Investigator will protect the integrity of the participants by following all applicable regulations. These regulations are made available upon Sponsor's request. The ICF used during the consent-obtainment process must be reviewed by Sponsor, approved by CEP and made available for inspection.

Before any procedure required by the protocol is performed, the participant must:

- Be informed of all relevant aspects of the study and the elements of informed consent.
- Have enough time to resolve doubts and think about the decision to participate.

- Voluntarily agree to participate in the study.
- Sign and date an informed consent form approved by the CEP.

The Image Authorization Term must be signed for participants who allow them to be filmed for scientific purposes. The same standards of Good Clinical Practice so followed for obtaining the ICF will apply to this term as well.

10.4. Risks and benefits

The risks involving the drugs in the study are: rejection of the administered cells, susceptibility to opportunistic diseases, grafting of cells in an unwanted place, unwanted biological effect (in vivo differentiation in unwanted cell types), toxicity (infusion or systemic) (symptoms: transient fever, bronchospasm and/or laryngospasm, chills, diarrhea, dyspnea, headache, facial edema, glottis and/or larynx, fever, hemoglobin, hypotension, nausea, itching, facial flushing, abnormal bleeding, sweating, tachycardia, dizziness, hives and vomiting), toxicity to DMSO (symptoms: cough, flushing, rash, chest tightening and wheezing, nausea and vomiting, cardiovascular instability), formation of neoplasms (malignant or benign), transmission of diseases, reactivation of latent viruses, unwanted immune response, cellular contamination (with unwanted cells, components of the culture media or chemical compounds), mixture of autologous material of the patient, cardiac, vascular, renal, neurological, pulmonary, hematological or hepatic dysfunctions (malignant or benign).

When applicable, adverse events will be minimized and if they evolve it will be the responsibility of the Sponsor of the study to follow-up the participant until the solution thereof.

The potential benefits of the investigational product are the reduction of neuronal loss and the stimulation of neurogenesis of both endogenous and newly transplanted cells, activating a neuroprotective environment that is able to integrate new cells and replace dead neural cells or in the process of apoptosis. Therefore, cell therapy could be able to delay the onset or course of pathology, which would represent a major advance for these patients (Fink et al., 2015).

Based on these data and due to the devastating and fatal nature of the disease, it is possible to observe that the benefits presented in this study outweigh the possible risks involved.

10.5. Preserving the identity of research participants

In the process of maintaining the right of the participants' privacy, each participant will be codified at the time of inclusion and this code should be used in the study documentation.

10.6. Post-study drug supply

Sponsor understands and is aware of the foregoing in item III.3.d of CNS Resolution N. 466/2012 that all participants are assured, at the end of the study, to get free and indefinite access to the best therapeutic methods that prove effective. Sponsor is also aware of sub-item d1 that access will be guaranteed in the interval between the end of individual participation and the end of the study, and in this case, this warranty can be given through an extension study, according to the duly justified analysis of the participant's attending physician.

The benefit weighting of the investigational product is done in two distinct ways: collectively or individually. The individual benefit analysis is performed when the participant terminates his/her participation in the study, and not when the research is completed. If the investigational product has proved beneficial to the individual, the product must be supplied for as long as necessary (guarantee of continuity). The definition of the individual benefit is not the exclusive prerogative of the study physician and can also be performed by the participant's personal physician (assistant). The collective benefit is that defined in interim or final analyses, when it is possible to conclude whether the investigational product proved beneficial, or not, to the experimental group. In this case, the supply of the investigational product should also extend to the control group.

Thus, in the event of individual benefit, Sponsor shall ensure the provision of the investigational product free of charge, if efficacy is demonstrated, and this is a weighting of the study physician or personal physician. Furthermore, in the case of collective benefit, Sponsor shall ensure, free of charge, the investigational product to all participants of the research if collective benefit is observed, identified in interim analysis or at the end of the study. Therefore, Sponsor undertakes, before the Brazilian regulatory authorities, to ensure access to the investigational product of the study to all participants who participate in this study (for both discontinued or non-discontinued participants), according to the commitment established in the Informed

Consent Form, and according to the judgment/prescription of the Investigator and/or clinical team.

Access to the study medication will be ensured for as long as necessary at the discretion of the physician, or in case the treatment is replaced or presents any safety-related problems.

10.7. Declaration of interest

The Research Center with its Investigators and sub-investigators declare absence of any conflict of interest.

10.8. Ethics and publication policy

Sponsor's policy is to publish or otherwise communicate the results of its clinical case-checking and exploratory studies, whatever the evolution, for marketed products, compound(s) or research product(s) subsequently approved for commercialization.

Clinical hypothesis test studies are those that aim to provide significant results through the analysis of pre-specified questions, using statistically valid predefined plans for data analysis, thus providing strong evidence of safety and/or efficacy to corroborate their claims about the products.

On the other hand, exploratory studies serve to determine the direction of possible future studies. They have significant statistical limitations, provide only preliminary information about a disease, condition, or product, and are not designed to provide final conclusions about product claims.

Therefore, in accordance with Resolution 466/12, Sponsor declares that the search results will be made public, whether favorable or not.

11. PROTOCOL CHANGES

11.1. Amendments

Any change in the study plan requires an amendment to the protocol. The Investigator shall not make any changes to the study without the approval of the CEP and Sponsor, except where necessary to eliminate immediate obvious risks to participants. An amendment to the protocol to eliminate an immediate obvious risk to participants can be promptly implemented, but the change must then be documented in an amendment, informed to the CEP within 5 working days, and forwarded to the regulatory agency within the required time frame. All amendments to the protocol must be analyzed and approved, following the same process as the original protocol.

11.2. Amendment submission

The amendments, in the context of RDC 09/2015, are defined as any changes made to the clinical protocol, whether substantial or not, and it is at Sponsor's discretion to evaluate in which of these categories it will fit and its impact on clinical development.

Substantial amendments are considered those that interfere with the safety, physical or mental integrity of research participants, as well as significant changes in the scientific value of the clinical trial protocol, such as the change from a placebo comparator to an active comparator, insertion of additional experimental arms, or changes in the statistical analysis plan. On the other hand, non-substantial amendments are those that do not entail alterations relevant to the scientific value of the protocol, such as small clarifications, spelling correction, among others.

All amendments (substantial or non-substantial) must be submitted to the country CEP at the same time. However, substantial amendments must wait for both ethical assessment and manifestation of CONEP to be implemented to the protocol already presented, while non-substantial ones can be promptly implemented, being only described as part of the annual report of the clinical trial.

11.3. Deviations and protocol violations

Deviation from protocol: any lack of compliance with the procedures or requirements defined in the version of the protocol approved by the Research Ethics

Committee (CEP) without major implications for the integrity of the study, the quality of the data or the rights and safety of the participants.

Violation of protocol: deviation from the protocol that may affect the quality of the data, that compromises the integrity of the study or that may affect the safety or rights of participants.

In general, a significant deviation from the protocol is considered as a violation of the protocol, while a minor deviation is considered as a deviation from the protocol.

Any deviation from the original statistical plan shall be described and justified in the protocol and/or final report, if appropriate. Table 06 exemplifies the significant deviations from the protocol and the necessary actions and documentation.

Table 06. Model of protocol deviations and corrective actions.

Significant Deviations from the Protocol	Action/Documentation Required
Some Baseline procedure was performed after the administration of the medication under study	The deviation must be notified by the monitor via the Protocol Deviations form. In addition, the reason for the deviation should be documented in the participant's medical records.
Deviations from inclusion and exclusion criteria	When this type of deviation is found after randomization, the study team should be notified to determine whether the participant should remain in the study. The reason for the deviation should be documented in the participant's medical records.
Variance in The Statistical Test to Be Used	Any deviation from the Statistical Test to be used must be thoroughly justified in the final report.

12. SUSPENSION, CLOSURE AND COMPLETION OF THE STUDY

Sponsor may suspend or terminate a study, or part of it, at any time for any reason. After the decision of suspension or cancellation, Sponsor shall notify the CEP within a maximum of 15 calendar days, except in cases of temporary suspension as a security measure, when the period is 7 calendar days from the date of suspension. It is emphasized that cancellations under RDC 09/2015 are definitive and apply only to clinical trial protocols already initiated, with no possibility of subsequent reactivation.

If the Investigator suspends or terminates the study, he/she shall inform Sponsor and CEP immediately and provide them with detailed written information. The Investigator will also return to Sponsor all products under investigation, their containers and other study materials. At the time of completion of the study, the Researcher will provide Sponsor and CEP the final reports and abstracts, as required by current regulations.

13. QUALITY CONTROL

Sponsor performs quality control and warranty checks on all clinical studies that it sponsors. Before including any participant in this study, the Sponsor team and the Investigator review the protocol, the Investigator's Brochure, the CRF's and the instructions for its completion, the procedure for obtaining informed consent, and the procedure for reporting AEs and SAEs. A qualified Sponsor representative will monitor the conduct of the study. During these visits to the centers, the information recorded in the CRF's will be compared with the source documents.

14. DATA CONTROL AND RECORD MAINTENANCE

14.1. Investigator

The Investigator will allow monitoring related to the study, audits, CEP analysis and regulatory inspections, authorizing direct access to data and source documents.

All information will be recorded in the source documents. CRF's must be fully populated and include all required data. All CRF data must be submitted to Sponsor during the study and at the end of the study. Remote data capture (*electronic data capture* – EDC - e-CRF) will be used to register and transmit data electronically to Sponsor.

If any Investigator ceases to conduct the study for any reason, he/she shall notify Sponsor to discuss an acceptable storage solution. Regulatory agencies will be notified with proper documentation.

An updated statement on the Investigator's form will be on file with the Sponsor for any study staff changes reported in the current statement on the Investigator's form.

Investigators must notify the CEP of protocol violations in accordance with local and CEP regulatory requirements.

14.2. Sponsor

E-CRF data is stored in a database and processed electronically. The data are reviewed for completeness and logical coherence. Automated validation programs identify missing data, discrepant data, and other data inconsistencies. Requests for data clarification are sent to the Research Center for resolution.

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