CLINICAL TRIAL PROTOCOL

Endometrial regeneration in Asherman's Syndrome through autologous bone marrow stem cell transplantation ("ENdometrial TIssular REnovation, ENTIRE)

*IGX1-ENT-XS-16-01**

** Code assigned by Study Sponsor*

Version 1.2 dated February 19, 2020

Clinical trial full title:

A phase I/II, prospective, open-label, non-randomized, uncontrolled (pre- / post-study), explanatory, multicenter, national, interventional clinical trial using advanced cell therapy on a single allocation group (one treatment group only) of patients of child-bearing potential seeking pregnancy and diagnosed with Asherman's Syndrome grade II, III or IV treated with screened autologous non-expanded bone marrow stem/progenitor cells (IGX1).

Brief title:

Endometrial regeneration in Asherman's Syndrome through autologous bone marrow stem cell transplantation. ("ENdometrial TIssular REnovation", ENTIRE)

EudraCT number: 2016-003975-23

Protocol code: IGX1-ENT-XS-16-01

Version: 1.2

Type of study: Clinical Trial

Phase: I-II

Investigational medicinal product: IGX1 is a product based on non-expanded bone marrow stem/progenitor cells (CD133+ cells) screened following Peripheral Blood Progenitor Cell (PBPC) mobilization and collection and posterior apheresis and transplantation of these cells in the patient. The aim being the de novo regeneration of the endometrium of patients with physiopathologies, which are associated with diminished endometrial stem cells.

Release date: 9/23/2016

Key words:

Bone marrow stem cells, Assisted Reproductive Technology, endometrial regeneration, endometrial physiopathology, CD133+, endometrial atrophy, Asherman's Syndrome, endometrial thickness.

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Responsibilities and signatures

By signing this protocol titled **"Endometrial regeneration in Asherman's Syndrome through autologous bone marrow stem cell transplantation" ("ENdometrial TIssular REnovation", ENTIRE).**

The undersigned state the following:

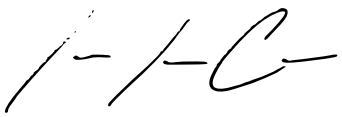
- The clinical trial respects the ethical and legal guidelines applicable to this type of studies, they and will follow the Guidelines for Good Clinical Practice when conducting the study.
- They have all material and human resources necessary to conduct the clinical trial, without interfering in the performance of any other type of studies or other currently entrusted tasks.
- They ensure every subject will be treated and monitored according to the protocol with the favorable opinion of the Ethics Committee and authorized by the Spanish Agency of Medicines and Medical Devices.
- The collaborating investigators included in the study have received the appropriate training in the conduct of studies, they will be actively involved and consent to this study.

Sponsor

Dr. Carlos Simon Vallés
Administrator

Date 02/19/2020

Principal Investigator



Dr. Xavier Santamaría
Gynecologist
Scientific Director of Asherman Therapy
Asherman Therapy

Date 02/19/2020

List of abbreviations

3D	Three dimensions
AE	Adverse Event
AF	Attributable Fraction
AFC	Antral follicular count
AFC	Antral Follicle Count
AIS	Autoimmunity Study
alloHPCT	Allogenic hematopoietic progenitor cell transplantation
ALT	Alanine aminotransferase
AMH	Anti-Müllerian hormone
AR	Attributable Risk
AR	Adverse reaction
ART	Assisted Reproductive Treatment
ASRM	American Society of Reproductive Medicine
AST	Aspartate aminotransferase
BMI	Body mass index
BrdU	Bromodeoxyuridine
BST	Banc de Sang i Teixits
CD133+	133+ stem cell
CL	Corpus Luteum
CRA	Clinical Research Associate
DET	Double Embryo Transfer
DRN	Deoxyribonucleic Acid
E_2	Estradiol
EB	Endometrial biopsy
EC	Ethics Committee
ECO	External Cervical Os
ECOG	Eastern Cooperative Oncology Group
eCRF	Electronic Case Report Form
eGFR	Estimated glomerular filtration rate
ERA	Endometrial Receptivity Analysis
ESGE	European Society for Gynaecological Endoscopy
ESH	European Society of Hysteroscopy
EU	European Union
FISH	Fluorescence in situ hybridization
FSH	Follicle-stimulating hormone

FV	Study Final Visit
FVP	Final Visit Post-treatment
GCP	Good Clinical Practice
G-CSF	Granulocyte-colony stimulating factor
GnRH	Gonadotropin-releasing hormone
HBcAg	Hepatitis B core antigen
HBsAg	Hepatitis B surface antigen
hCG	Human chorionic gonadotropin
HCV	Hepatitis C virus
HIV	Human immunodeficiency virus
HLA	Human leukocyte antigen
HRT	Hormone replacement therapy
HSC	Hysteroscopy
HUVH	Hospital Universitario Vall d´Hebron
ICH	International Conference on Harmonization
ICH-GCP	International Conference on Harmonization/Good Clinical Practice
ICSI	Intracytoplasmic sperm injection
IF	Implantation failure
IMP	Investigational medicinal product
IR	Implantation rate
IVF	In Vitro Fecundation
LB	Live Born
LBR	Live Birth Rate
LH	Luteinizing hormone
MII	Metaphase II Oocytes
OC	Oral contraceptives
OPR	Ongoing pregnancy rate
OVODON	Oocyte Donation
P4	Progesterone
PBPC	Peripheral Blood Progenitor Cells
PBS/EDTA	Phosphate-buffered saline/Ethylenediaminetetraacetic acid
PGD	Pre-implantation genetic diagnosis
PIS/IC	Patient information sheet / Informed consent
PMN	Polymorphonuclear cells
PR	Pregnancy rate
QoL	Quality of Life
qPCR	Quantitative polymerase chain reaction
RNA	Ribonucleic Acid
RR	Relative risk

SAE	Serious Adverse Event
SET	Single Embryo Transfer
SETH	*Sociedad Española de Trombosis y Hemostasia* (Spanish Society of Thrombosis and Hemostasis)
SIVIS	IVI Digital information management platform
SNS	Spanish National Health System
SOP	Standard Operating Procedures
SUSAR	Suspected Unexpected Serious Adverse Reaction
ULN	Upper Limit of Normal
US	Ultrasound
WOI	Window of Implantation
β-hCG	Beta-Human Chorionic Gonadotropin

1. Table of contents

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2. Synopsis

Protocol title: A phase I/II, prospective, open-label, non-randomized, uncontrolled (pre- / post-study), explanatory, multicenter, national, interventional clinical trial using advanced cell therapy on a single allocation group (one treatment group only) of patients of child-bearing potential seeking pregnancy and diagnosed with Asherman's Syndrome grade II, III or IV treated with screened autologous non-expanded bone marrow stem/progenitor cells (IgX1).
Brief title: Endometrial regeneration in Asherman's Syndrome through autologous bone marrow stem cell transplantation. ("ENdometrial TIssular REnovation" – ENTIRE).
Protocol number: IGX1-ENT-XS-16-01
EudraCT number: 2016-003975-23
Protocol version: 1.2
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Study phase: I-II
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Person and/or company responsible for monitoring the study: Carlos Gómez De la Cruz IGENOMIX S.L. Director of the Clinical Study Department / Clinical Research Associate Parque Tecnológico de Paterna. Calle Narcís Monturiol Estarrio, 11. Parcela B, Edificio Europark. 46980. Paterna (Valencia) España Tel. +34 96 390 53 10 Ext. 18543 / Fax +34 96 390 34 98 carlos.gomez@igenomix.com

Name of the investigational medicinal product(s): IGX1

Description of investigational medicinal product(s):

IGX1 is a product based on non-expanded bone marrow stem/progenitor cells (CD133+ cells) screened following Peripheral Blood Progenitor Cell (PBPC) mobilization and collection and posterior apheresis and transplantation of these cells in the patient. The aim being the de novo regeneration of the endometrium of patients with physiopathologies such as Asherman's Syndrome, which are associated with diminished endometrial stem cells.

Study design: A phase I/II, prospective, open-label, non-randomized, uncontrolled (pre- / post-study), explanatory, multicenter, national, interventional clinical trial using advanced cell therapy on a single allocation group (one treatment group only).

Hypotheses: The endometrium will undergo de novo regeneration through the use of autologous bone marrow stem cells obtained from Peripheral Blood Progenitor Cell (PBPC) mobilization and collection, involving apheresis and selection of CD133+ cells and subsequent transplantation of those cells in patients with Asherman's Syndrome (grade II, III or IV) planning to undergo Assisted Reproductive Treatment (ART).

Primary objective:

The primary objective of this study is to assess safety and tolerability of the IGX1 investigational medicinal product (CD133+ cells screened after mobilization and collection of peripheral blood progenitor cells (PBPCs) from the same patient) in improving post-treatment reproductive prognosis, as measured by:

1. Identification of incidence, prevalence and frequency of different adverse events that may occur in the patient throughout the study up until Month 15 post-treatment.
2. In the event of a term pregnancy, identification of incidence, prevalence and frequency of different adverse events that may occur during birth.
3. In case of a Live Birth (LB), identification of incidence, prevalence and frequency of the different adverse events that may be associated with the investigational medicinal product (IMP) until the first month of age.

Secondary objectives:

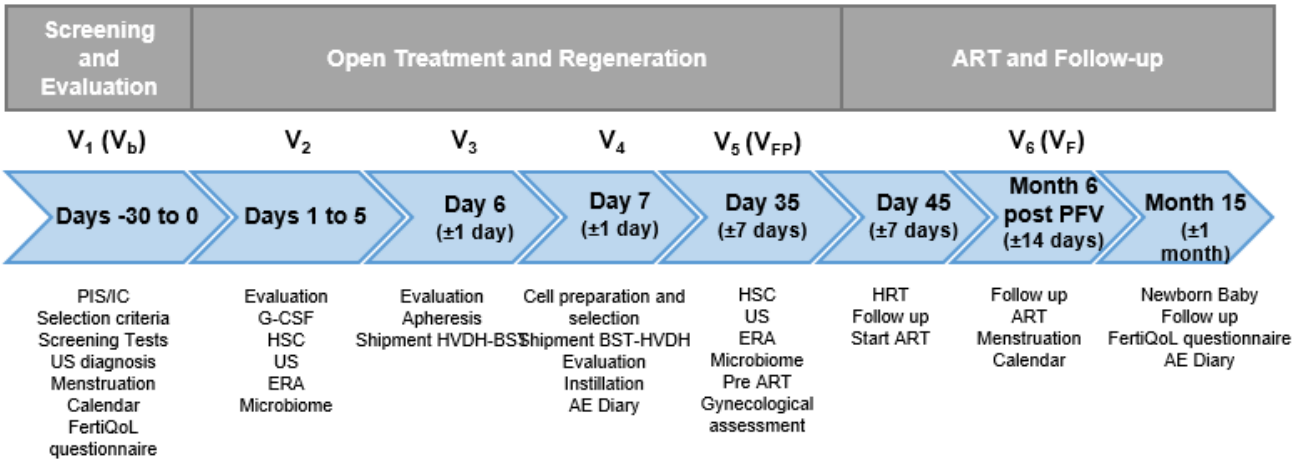
- Efficacy of the investigational medicinal product IGX1 in improving post-treatment reproductive prognosis, as measured by:
  1. Implantation rate (IR) per transferred embryo, pregnancy rate (PR) and ongoing pregnancy (OPR) per transfer performed from embryo transfer to birth.
  2. Improved hysteroscopic score in accordance with the European Society of Hysteroscopy (ESH) classification and the European Society for Gynaecological Endoscopy (ESGE) 28 days after stem cell treatment.
  3. Improvement in endometrial thickness and pattern as determined by ultrasound 28 days after stem cell treatment.
- Pregnancy follow-up, evaluated by live birth rate (LBR), rate of (clinical and biochemical) miscarriage and rate of ectopic pregnancy from embryo transfer until birth.
- Recovery of endometrial volume as measured by 3D ultrasound 28 days after stem cell treatment.
- Analysis of endometrial vascularization via pre- and post-treatment endometrial biopsy 28 days after stem cell treatment.
- To diagnose any alterations in the window of implantation (WOI) as measured by the ERA test before and after stem cell treatment.
- To study the endometrial microbiome in order to set up its possible impact in the Asherman´s Synrome and thus, its relation in the assisted reproduction treatments results by using the new throughput sequencing platforms (Next Generation Sequencing – NGS) comparing the microbial populations found in endometrial fluid versus endometrial tissue.
- To evaluate reappearance of menstrual episodes (if they did not exist prior to treatment) or compare differences in duration and quantity of episodes after embryonic stem cell treatment until embryo transfer, with respect to menstrual episodes occurring prior to treatment.
- To determine the cell dose, cut-off point of CD133+ cells that maximizes sensitivity and specificity of test needed to discriminate between pregnancy/non-pregnancy (response variable) in keeping with minimum

quality requirements established (sterility, cell viability greater than or equal to 50% and purity greater than or equal to 70%).

- To evaluate and describe quality of life (QoL) of participating patients, using the 2008 FertiQoL International tool, in order to estimate the influence and impact of infertility-related psychological problems on the various spheres of their daily lives, before and after the treatment.

Methodology: Patients aged between 18 and 45 years diagnosed with Asherman's Syndrome grade II, III or IV will be screened following the criteria established by the European Society of Hysteroscopy (ESH) and the European Society for Gynaecological Endoscopy. Adhesiolysis or evaluation of endometrial cavity will be conducted after Hormone Replacement Therapy (HRT) via hysteroscopy and study of endometrial receptivity using Endometrial Receptivity Analysis (ERA) after mobilization of bone marrow stem cells in peripheral blood with G-CSF (Granulocyte Colony-Stimulating factors). Next, polymorphonuclear cells will be obtained through apheresis and CD133+ cells will be screened and then re-instilled in the uterine artery via catheterization. Finally, the endometrial cavity will be re-evaluated in the following month via hysteroscopy and ERA; after this, patient monitoring and Assisted Reproductive Treatment (ART) will begin. If after the first 6 months the patient does not undergo ART as planned upon enrollment in the study, she will join the safety population and follow-up visits will be performed at 9, 12 and 15 months (End of Study Visit). If the patient proceeds with their ART according to plan and no ongoing pregnancy ensues, follow-up visits will still be performed at 9, 12 and 15 months (End of Study Visit). During the first 6 months, the patient can receive one or more ART (in the event of negative β-hCG, Ectopic Pregnancy, or Biochemical or Clinical Miscarriage occurring after treatment and recovery), always proceeding in accordance with standard clinical practice. Nevertheless, should ongoing pregnancy ensue, pregnancy follow-up visits will be performed at 12-14, 22-24, 34-36 (these coinciding with gestational ultrasounds part of standard clinical practice) and a follow-up visit at Month 15. In addition, patients will have a postpartum visit within a month from LB coinciding with EOS visit. If the patient gives birth before Month 15, follow-up will continue until Month 15.

Overall study schedule:



Endpoints:

Primary variables

For primary objective:

- Adverse events (list) at each checkup/medical visit
- Period of time between apheresis and appearance of adverse event (in days)
- Hospitalization for adverse event (YES/NO)
- Treatment required for adverse event (list)
- Each blood test performed at visits, quantitative variables
- Hysteroscopic score according to ESH/ESGE (numerical variable)
- Endometrial thickness in mm at each visit (numerical variable)
- Endometrial pattern (trilaminar/diffuse), categorical variable
- Pregnancy YES/NO, categorical variable
- Implantation YES/NO, categorical variable
- Pregnancy in progress YES/NO, categorical variable

- Apgar score, categorical variable

For secondary objectives:

- Live birth (LB) YES/NO, categorical variable
- Pregnancy outcome (categorical list: Pregnancy in progress, clinical or biochemical miscarriage, ectopic pregnancy)
- Endometrial vascularization analysis measured using immunohistochemistry with presence of α-ASMA expression.
- Endometrial Receptivity diagnosed using Receptive/Non-Receptive ERA (categorical variable)
- Frequency of menstrual episodes in days (numerical variable) during 6 consecutive months following treatment if any (if patient becomes pregnant prior to this, then they will not be performed)
- Duration of menstrual episodes (numerical variable) during 6 consecutive months following cell instillation
- Number of sanitary napkins/day following treatment (numerical variable) during 6 consecutive months
- Number of CD133+ cells, explanatory variable (quantitative).
- Pregnancy/No-Pregnancy, response variable (qualitative).
- One variable per each microorganism identified in the endometrial biopsy
- Microbiome found in endometrial biopsy (LD/NLD)

Presence of bacterial microbiome; Identified bacteria are classified into the following groups:

- Group 1 (LD): *Lactobacillus* dominated microbiome (≥90% *Lactobacillus spp.*), composed of different species of the genus *Lactobacillus*: *L. crispatus*, *L. gasseri*, *L. iners*, *L.jensenii*, among others.
- Group 2 (NLD): non-*Lactobacillus* dominated microbiome (<90% *Lactobacillus spp. with at least 10% of dysbiotic bacteria*), formed by species that modify the physiological endometrial conditions, therefore decreasing the prevalence of *Lactobacillus*

- One variable per each microorganism identified in EF
- Microbiome results in EF (LD/NLD)
- Microbiome results LD/NLD
- Microbiome results in EB (LD/NLD)

Control Variables: These will be used as controls for the study primary variables

- Number of CD133+ cells injected (numerical variable) greater than 30 million cells
- Percentage of cell viability (numerical variable) greater than or equal to 50%
- Percentage of cell purity (numerical variable) greater than or equal to 70%
- Each baseline blood test (before treatment), quantitative variables
- Baseline endometrial thickness in mm (numerical variable)
- Frequency of baseline menstrual cycles in days (numerical variable)
- Duration of baseline menstrual cycles in days (numerical variable)
- Number of sanitary napkins/day before treatment (numerical variable)
- Baseline endometrial receptivity diagnosed using Receptive/Non-Receptive ERA (categorical variable)
- Information from HRT for biopsy at baseline ERA: Days of estradiol, progesterone (P4) concentration, P4 supplementation start day, P4 start time, time biopsy is performed (numerical variables)
- Day samples are taken (baseline biopsy), categorical variable with respect to day Progesterone is administered (P+4, P+5, P+6, P+7).

Descriptive Variables: They are used for evaluating homogeneity of population and avoiding possible biases considering their distribution among each of the study groups or identifying factors that have an effect on them:

- Age (numerical variable from 18 to 45)
- Body Mass Index (BMI), numerical variable from 18.5 to 30
- Obstetric formula: Numerical variables from obstetric history (pregnancies, births, cesarean sections, prior miscarriages, curettage).
- ART indication (list), categorical variable.
- Number of prior implantation failures (IF), numerical variable.

- Baseline FSH, numerical variable.
- Baseline AMH, numerical variable.
- Sperm concentration, numerical variable.
- Treatment type (IVF/ICSI, DONOR EGG), categorical variable.
- Cycle type (vitrified eggs/embryos), categorical variable.
- Information from HRT for ERA biopsy: Days of estradiol, progesterone (P4) concentration, P4 supplementation start day, P4 start time, time biopsy is performed, numerical variables
- Day samples are taken (biopsy), categorical variable with respect to day Progesterone (P+4, P+5, P+6, P+7) is administered.
- Information on ovarian stimulation (stimulation type, number of stimulation days, Estradiol (E_2) levels on hCG test day, numerical variables.
- AFC (antral follicle count), numerical variables.
- Number of days between ovarian puncture and embryo transfer, numerical variable.
- No. oocytes in metaphase II (MII) stage, numerical variable.
- No. oocytes fertilized (to know fertilization rate), numerical variable.
- Information on HRT for embryo transfer: Days of estradiol, progesterone (P4) concentration, P4 supplementation start day, P4 start time, time embryo transfer is performed, numerical variables.
- Transfer day (5/6), categorical variable.
- No. embryos transferred, numerical variable.
- Embryo quality through morphology of transferred embryos, categorical variable (A, B, C, D).
- Pre-implantation Genetic Diagnosis (PGD) YES/NO, categorical variable.
- No. vitrified embryos, numerical variable.
- No. implanted sacs, numerical variable.

Outcome variables:

The implantation rate will be defined as the proportion of gestational sacs observed via vaginal ultrasound at Week 6, over the number of transferred embryos. The clinical gestation rate per cycle will be defined as the percentage of pregnant patients with sac with respect to the total number of cycles performed. The clinical gestation rate per transfer will be defined as the percentage of pregnant patients with respect to the total number of transfers performed. All pregnancies will be considered evolutive if they reach >20 weeks of pregnancy.

Statistical considerations:

Population: Patients diagnosed with Asherman's Syndrome grade II, III or IV who intend to undergo ART with own or donated oocytes and transfer of frozen embryos on Day 5/6.

Population sets: In this study, four population sets have been defined.

- Intention-to-treat set: All of the patients enrolled in the study, including screening failures with/without apheresis performed.
- Safety set: All of the patients enrolled in the study for whom the product IGX1 has been instilled, regardless of whether they have received ART or not.
- Population for whom procedure (or cycle) has been started: All of the patients enrolled in the study, who have the IGX1 product instilled and begin ART, regardless of whether embryo transfer is performed or not.
- Per Protocol set: All of the patients enrolled in the study who have completed all study phases through to completion including embryo transfer. No mayor deviations from protocol can occur during the study period (see section X).

Total number of subjects: A total of 22 patients will be included.

Our hypothesis is that stem cell transplantation into the uterine cavity improves the live birth rate (LBR) as the primary dependent variable and endometrial thickness as the secondary dependent variable.

The study will use a historical control group, which may include either the same patient undergoing ART before treatment or historical patients with similar clinical characteristics (a matched cohort "before-and-after" study).

We assume that patients will undergo single embryo transfer (SET) on day 5/6 of development.

A. IMPROVEMENT IN LIVE BIRTH RATE (LBR)

Our hypothesis is that the treatment will increase the live birth rate from 0% in the control group to 28% in the treatment group (the average LBR reported in ART according to the European IVF-Monitoring Consortium (EIM) et al., *Human Reproduction*, 2016). This rate is calculated based on the total number of completed cycles (embryo transfers).

B. Calculation of the LBR as a binary variable

(Sample size refers to the number of embryos transferred):

- **Proportion (%) of events in:**
  - Control Group (Group 0) = 0.00%
  - Treatment Group (Group 1) = 28.00%
- **Minimum expected effect size:** Difference = 28.00%
- **Alpha risk = 5%**
- **Dropout rate = 20%**
- **Endpoint significance level:** 0.05
- **Test:** One-sided
- **Power (%):** 80
- **Sample size per group:** 18
- **Considering dropouts (20%):** 22 per group

C. Patient Calculations

To determine the number of patients equivalent to 22 embryo transfers (ET):

- We estimate that **50% of patients will need only one ET**, equivalent to **11 patients**.
- Of the remaining 11 patients who require a second ET, we expect **70% to become pregnant**, which equals **7 patients**.
- These calculations assume that all patients will have at least 2 embryos and undergo SET.

Therefore, the **protocol-required number of patients (with ET completed)** is **18 in total** (expected to conceive after 2 ETs).

D. Total Sample Size for Intention-to-Treat

To calculate the total sample size for intention-to-treat (i.e., total patients starting the study, including those who do not reach the ET phase), we account for potential losses due to various reasons before reaching the ART phase (e.g., dropout, loss of information, complications with Neupogen® treatment, apheresis, or IGX1 instillation).

Given that this is a small, highly controlled population with high treatment expectations, we estimate a **20% dropout rate**. Thus, the total number of patients for intention-to-treat is:
18 + (18 x 0.2) = **22 patients in total**.

Statistical analysis:

Interim analysis and guidelines for safety and tolerability assessment: The data obtained in this study will be reviewed by an Independent Data Monitoring Committee (IDMC). This committee will review cumulative data

after 10 patients have entered the study and have been evaluated X days after receiving treatment with IGX1. During this review, the IDMC will monitor all study data obtained, paying special attention to all data pertaining to safety and tolerability of the IGX1 investigational medicinal product. This committee may make some recommendations for the sponsor regarding continuation, early suspension, modification or interruption of this study. Similarly, any recommendations made by this committee will be taken into consideration for future related studies.

Inclusion criteria:

1. Patients whose written informed consent approved by the Ethics Committee (EC) has been obtained, after having been duly informed of the nature of their illness and voluntarily accepted treatment program, while being fully aware of the potential risks, benefits and any discomfort involved.
2. Patients diagnosed with Asherman's Syndrome grade II, III or IV, in accordance with the criteria set forth by the European Society of Hysteroscopy (ESH) and the European Society for Gynaecological Endoscopy (ESGE), who intend to undergo Assisted Reproductive Treatment (ART) with Double Embryo Transfer (DET) of blastocysts (Day 5/6 of development) once cell therapy for endometrial regeneration has been completed.

   Cases involving a single embryo transfer (SET) in blastocyst stage will be accepted if, for medically determined reasons, this was deemed necessary in order to safeguard the patient's health and well-being.
3. Patients who, prior to study start, plan to undergo ART in a *Hormonal Replecement Therapy* (HRT) with donated oocytes or own vitrified embryos in blastocyst stage (Day 5/6 of development).

   We will accept cases where, in accordance with standard clinical practice, Pre-implantation Genetic Diagnosis (PGD) is indicated, in compliance with current legislation on human assisted reproductive techniques (Law 14/2006 of May 26). The most common indications for PGD are as follows: Advanced maternal age (age ≥ 38 years), recurring implantation failure, repeated miscarriages or alterations in karyotype of one or both parents as well as any detected in the FISH of sperm cells.
4. Women of child-bearing potential between 18 and 44 years (both included).
5. IMC: 18 – 30 Kg/m^2 (both included).
6. Adequate liver and kidney function, defined as follows:

   Total bilirubin < 1.5xULN

   AST and ALT< 2.5x ULN and

   Serum creatinine < 1.0 mg/dL; if serum creatinine is > 1.0 mg/dL, then estimated glomerular filtration rate (eGFR) should be > 60 mL/min/1.73 m^2.
7. Absence of severe heart disease.
8. Negative blood pregnancy test.
9. ECOG= 0-1.
10. Negative HIV, HCV, HbsAg, HBcAg and Syphilis tests (recent, at least 30 days).
11. Normal coagulation study.
12. Adequate peripheral venous access. Otherwise, the investigator will assess whether central venous catheter should be implanted.
13. Absence of severe psychiatric illnesses.
14. Patient can adhere to and follow study procedures and checkups, that is, patients who are able to understand and comply with parameters as indicated in the protocol.

Exclusion criteria:

1. Patient refuses to receive central venous catheter as proposed by the investigator in cases where there is no peripheral venous access.
2. Patients who are allergic to iodine contrast.
3. Patients for whom an optimal investigational medicinal product cannot be obtained or infused after performing apheresis and selection. The product is unusable if any of the following criteria are identified:

   Dose to infuse having less than 30 million CD133+ cells.

Cell viability lower than 50%.

Less than 70% purity.

Non-sterile.

4. Patients who have participated in another clinical trial or who have received an investigational treatment in the 30 days prior to the study, unless expressly approved by the sponsor.
5. Existence of severe or uncontrolled bacterial, fungal or viral infections, which in the opinion of the Principal Investigator may interfere with patient's participation in the study or with the evaluation of the study results.
6. Any illness or medical condition that is unstable or which may put at risk the patient's safety and her compliance in the study.

Investigational medicinal product, dose and administration route:

IGX1 is composed of CD133+ cells screened after mobilization and collection of peripheral blood progenitor cells (PBPCs) from the same patient by way of apheresis.

This is performed using a sterile pre-filled single-use 50 mL syringe, which will be administered intra-arterially at Visit 4 within 24 hours after it has been obtained via apheresis from each patient.

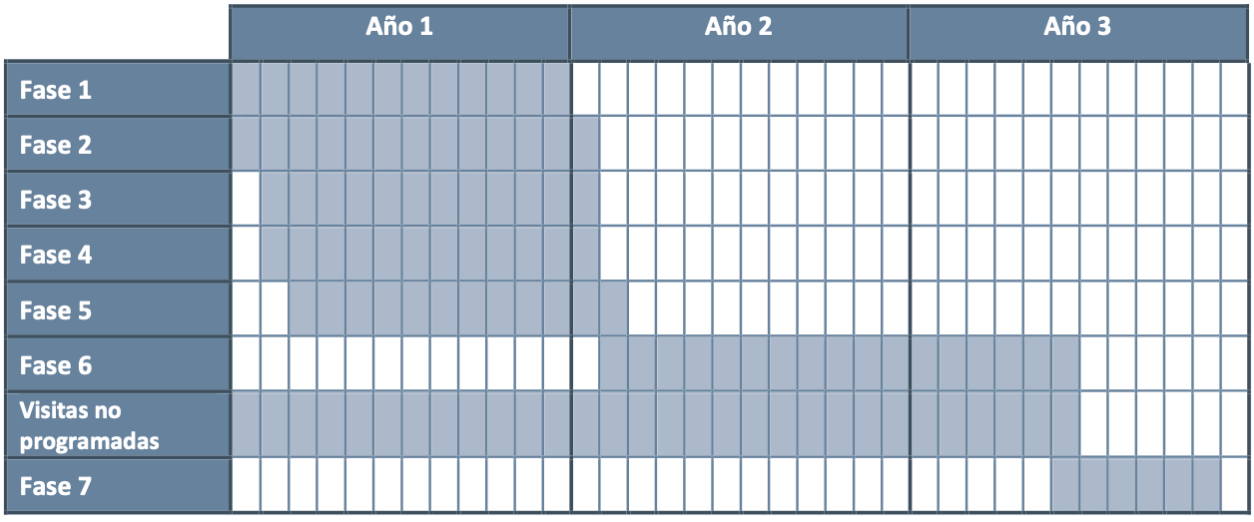
Concomitant treatment, dose and administration route:

Prior to infusion of Investigational Medicinal Product (IMP) and apheresis process, patients will have received for cell mobilization ***Neupogen***® (Filgrastim or G-CSF) 30 mU 0.6 mg/mL in pre-filled sterile, disposable 30-50 mL syringe, administered subcutaneously with a dose of 10 µg/Kg every 24 hours for 5 consecutive days (Visit 2).

Study duration:

- Total approximate duration of study per patient: 15 months
- Planned start date: May 2017
- Planned completion date: April 2019
- Recruitment period: 8 months

Study schedule:



EudraCT No.: 2016-003975-23

3. General information

3.1 Trial identification

Protocol number: IGX1-ENT-XS-16-01

EudraCT No.: 2016-003975-23

Protocol title: "Endometrial regeneration in Asherman's Syndrome through autologous bone marrow stem cell transplantation". (ENTIRE)

Brief title: “ENdometrial TIssular REnovation” – ENTIRE

3.2 Type of clinical trial

A phase I/II, prospective, open-label, non-randomized, uncontrolled (pre- / post-study), explanatory, multicenter, national, interventional clinical trial using advanced cell therapy on a single allocation group (one treatment group only).

3.3 Name of the investigational medicinal product(s)

IGX1

3.4 Description of the investigational medicinal product(s)

IGX1 is a product based on non-expanded bone marrow stem/progenitor cells (CD133+ cells) screened following Peripheral Blood Progenitor Cell (PBPC) mobilization and collection and posterior apheresis and transplantation of these cells in the patient. The aim being the de novo regeneration of the endometrium of patients with physiopathologies such as Asherman's Syndrome, which are associated with diminished endometrial stem cells.

Presentation format, pharmaceutical administration and labeling: This is performed using a sterile pre-filled single-use 50 mL syringe, which will be administered intra-arterially at Visit 4 within 24 hours after it has been obtained via apheresis from each patient.

Each syringe will be labeled at a processing site (BST) in accordance with EU standards and the ICH E6 Guideline for GCP under the responsibility of the study Sponsor. This label will feature a text in any of the official languages at the sites in the participating countries (preferably Spanish or Catalan) and will include a description of the study drug, study code and title, warnings, storage conditions, usage instructions reference, batch, expiry date, site code and patient code, visit number, name and contact details of the Principal Investigator and Sponsor.

Other concomitant treatments:

Prior to infusion of IMP and apheresis process, patients will have received for cell mobilization **Neupogen®** (Filgrastim or G-CSF) 30 mU 0.6 mg/mL in pre-filled sterile, disposable 30-50 mL syringe (based on the concentrate volume obtained after apheresis for each patient), administered subcutaneously with a dose of 10 µg/Kg every 24 hours for 5 consecutive days (Visit 2).

It is a drug that is already being marketed, and therefore already labeled, which will be purchased and supplied to the sites by the Sponsor to be subsequently supplied to the participants in the study. It will be re-

labeled with a text in any of the official languages at the sites in the participating country (preferably Spanish or Catalan). This will include a description of the study drug, study code and title, warnings, storage conditions, usage instructions reference, batch, expiry date, site code and patient code, visit number, contact name and details of the Principal Investigator and Sponsor.

3.5 Sponsor

ASHERMAN THERAPY SL S.L.
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46980. Paterna (Valencia) España
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3.6 Person and/or company responsible for monitoring the study:

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IGENOMIX S.L.
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3.7 Study Coordinator and Principal Investigator:

Xavier Santamaría
Investigator. Asherman project manager. Asherman Therapy SL (Spain)
Specialist Obstetrician and Gynecologist, Department of Reproductive Medicine at the Instituto Valenciano de Infertilidad (IVI). IVI Barcelona
Biomedical researcher specializing in Gynecology. Hospital Vall D´Hebron, Barcelona.
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3.8 Participating site(s) and Principal Investigator(s) for each site

Site	Investigator	Function

IVI Barcelona	Xavier Santamaría	Principal Investigator and Study Coordinator
IVI Valencia	Carlos Simón Vallés	Principal Investigator
Hospital Universitario Vall d´Hebron	Julio Herrero	Principal Investigator
Banc de Sang i Teixits	Sergi Querol	Principal Investigator

3.9 Expected participating countries

This is a national, multicenter study, and is therefore expected to be performed in one country: Spain.

3.10 Study duration

Estimated total duration of the study: 3 years.
Planned start date: March 2020.
Planned end date: March 2023.
Approximate total study duration per patient: 18 months (approximately 1–2 months corresponding to the Selection, Evaluation, Open Treatment, and Regeneration Period + a maximum of 16 months [6+9+1] corresponding to the TRA period and follow-up if a viable pregnancy and live birth occur).
Recruitment period: 12 months.
(See Figure 3, "Study Duration and Timeline," Section 10).

4. Introduction

4.1 Background

The endometrium is the tissue that lines the inside of the uterine cavity whose function is to permit implanting an embryo at the right time. If it is not implanted, the endometrium is partially destroyed and menstruation takes place, 2/3 of this tissue is generated de novo in the next menstrual cycle. It is therefore a tissue with a high self-renewal capacity under hormonal control (Chan RWS et al., 2004).

From a histological point of view the endometrium is divided into two layers: the base layer and the functional layer. The functional layer responds to progesterone and estradiol, and is shed during menstruation. The base layer does not respond to steroid hormones and is not shed, renewing the mucous from it. In general. the endometrium consists of different cell types, including luminal and glandular epithelium, stroma, endothelial cells and smooth muscle, and temporarily immune system cells such as polymorphonuclear (PMN) cells, macrophages and T lymphocytes. The tissue regeneration that takes place in the endometrium is not similar to the entire organism. Under the influence of ovarian steroid hormones - estrogen and progesterone - the endometrium undergoes dramatic changes every 28 days between 400 and 500 times in the reproductive life of women.

Three phases can be identified in the menstrual cycle: The proliferative phase, the secretory phase and menstruation.

The proliferative phase is characterized by rapid regeneration of the endometrium, mostly directed by estradiol. Glandular mitosis is very frequent during this phase and the functional layer of the endometrium grows; the tubular glands become more winding, acquiring a longitudinal morphology.

Ovulation then takes place, during which the mitosis ratio reaches its maximum as a result of the peak of the Luteinizing Hormone (LH) and subsequent formation of the Corpus Luteum (CL). Formation of the CL gives rise to an increase in progesterone plasma concentrations leading to the start of the luteum phase. During the first five post-ovulation days the number of vacuoles in the glandular cells containing glycoproteins and mucopolysaccharides increases dramatically. After the sixth day, the glands actively secrete this material in the luminal space. At this point, the glands account for 25% of the endometrium.

During the late luteum phase the glands start their involution process and the leukocytes begin infiltrating the luminal epithelium.

Finally, menstruation takes place as a result of the decrease in circulating estradiol and progesterone levels. First small lesions appear on the luminal epithelium and finally rapid, incomplete degeneration of all the functional layers takes place.

All these changes take place in multiple cell types, including the epithelium, underlying stroma and the vascular tissue. These cellular and endocrine changes lead to a functional receptiveness window during which the embryo can be implanted in the endometrium.

The gestation rates per cycle in healthy, fertile couples is around 25%. The embryo quality, and development of the receptive endometrium are determining factors for gestation to develop. If, on the other hand, implantation does not take place, other types of tissue changes take place so that menstruation occurs several days later. The re-epithelialization process starts at the same time as endometrium tissue is destroyed and menstruation begins. The stroma components regenerate and a new proliferative stage begins. The multipotent and unipotent cells in the bone marrow take part in regenerating different cell types at endometrium level which could open the gate to clinical use thereof for this purpose.

Regarding tissue source, work has been conducted with women who have received allogenic hematopoietic progenitor cell transplantation (alloHSCT) from male donors where the existence of cells from the donor marked XY in the endometrium of the recipient has been proved (Taylor HS., 2004). This data suggests that adult hematopoietic stem cells are capable of establishing in the endometrium and taking part in its renewal.

All adult tissues consist of a percentage of stem cells that account for a minority of the total tissue. These cells are in charge of renewing and regenerating the mature tissue, always in accordance with the pre-established capacity for each organ. The tissue localization of these stem cells represents the niche. The niche has a specific micro-environment that permits maintaining these stem cells in an indifferent status and on standby in terms of cell cycles. The micro-environment of this niche regulates access to messengers and the concentration of oxygen, temperature, chemical gradients and mechanical forces in order to protect the stem cell. This niche is comprised of stem cells and differentiated neighbor cells that communicate via

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positive and negative signals. Nevertheless, the niche is not only able to protect the resident stem cells, but can also recruit new stem cells from other organs such as bone marrow (Fuchs E et al., 2004). This process, called "homing" is known to take place after tissue damage. The molecular mechanisms that mediate this process are still not widely known, but the phenomena were proven by Kai and Spradig in Drosophila germ cells. In their study, they observed that when germ stem cell niches were reduced, they produced a number of signals causing mesenchymal stem cells to migrate and repopulate the niche (Kai T., 2003).

On the other hand, the bone marrow has a well-characterized population of stem cells (Jiang, 2004). These were the first somatic stem cells to be identified. The hematopoietic stem cells have the ability to renew themselves and produce progenitors through asymmetric differentiation which are later differentiated in all manner of mature, circulating blood cells such as granulocytes, erythrocytes, lymphocytes and megakaryocytes. Moreover, the mesenchymal stem cells also have the ability to migrate to the endometrium during certain periods of the menstrual cycle (Taylor, 2004; Cervello 2011).

The mesenchymal stem cells in bone marrow were the first to be studied and described in 1966 by Friedenstein in rats. Mesenchymal stem cells have been isolated in multiple different tissues such as the liver, umbilical cord blood, placenta, kidneys, uterus and the central nervous system (Mezey, 2003, Poulson, 2001, Jiang, 2002). These cells are multi-potential cells with differentiation to different tissues in the three germ layers having been proved in vitro such as myocardium (mesoderm), β-pancreatic cells (endoderm) and neurons (ectoderm) (Wolff EF et al., 2007; Santamaria X et al., 2010.).

In recent years, several groups have suggested that a group of stem cells with a niche such as the one previously described can be found in the deepest base layer of the endometrium.
Chan et al. (Chan RWS et al., 2004.). They proved that there are putative stem cells, both stromal and epithelial in the human endometrium. Schwab et al. subsequently proved that these cells have clonogenic potential (Schwab et al., 2007). Finally, our group proved that there are cells that retain BrdU (bromodeoxyuridine) that co-express stem cell markers (Cervello et al., 2007).

Despite the presence of endometrium stem cells not being a surprising fact given the characteristics of this tissue, proving donor cells in the endometrium of bone marrow transplantation patients in the endometrium is still interesting. Hence, Taylor et al. studied endometrium biopsies in patients who had previously received bone marrow transplants from donors other than the HLA group. They observed that whilst some glands were practically entirely composed of donor cells, other were not, which means that not all the glands were of a clonal origin. The predominance of donor stromal cells in relation to the epithelial cells proves different percentages of trans-differentiation depending on the cell type. Some cells also reported the presence of functional cilia in immunohistochemistry and expression of calcitonin indicating complete differentiation.
Several studies have determined that between the stem cells existing in bone marrow, the cells that express the surface antigen AC133 or CD133+ represent an enriched stem cell population in progenitors known as hemangioblastoma (cells with the ability to differentiate both hematopoietic cells and endothelial cells (Wynter et al., 1998; Gehling et al., 2000). These CD133+ cells cannot only be isolated from bone marrow,

but it has also been proved that they are also in peripheral blood mobilized with G-CSF and although most AC133+ cells co-express CD34+, there is a small percentage that are CD34 negative (Menendez et al., 2001). These cells apparently not only have a higher long-term engraftment capacity in tissue (Lang et al., 2004) but they also have the ability to differentiate in other non-hematopoietic linages. Finally, several studies have determined that therapy with this kind of cells has an excellent safety profile (Rafii et al., 2003). That is why all these results mean we are able to look at common diseases of the uterus and endometrium with different outlooks and which show the following physiopathology: Symptoms associated with the absence, reduction or dysfunction of stem cells in the endometrium. In this sense, there are two published cases suggesting the therapeutic use of stem cells from bone marrow to treat certain endometrial diseases (Chaitanya et al., 2011; Gargett CE, 2011).

4.2 Rationale

At the Plenary Session of the National Congress held by the Spanish Thrombosis and Hemostasis Society (SETH), Arrieta R. et al., presented a study assessing the beneficial effects of therapy with intra-myocardial CD133+ cells in candidate patients for revascularization and to determine the associated risks and benefits. In the aforementioned study, along with others (Goussettis E et al., 2006) they proved that intra-myocardial cell therapy can account for a safe procedure with improvements in cardiac function and in other aspects such as renal function in patients with ischemic heart disease. Moreover, other studies have also proved safe, effective use of this type of cell therapy in other pathologies (Karussis D, et al., 2010).
In view of the aforementioned knowledge (Reyes M et al., 2001), this study aims to use autologous Peripheral Blood Progenitor Cells (PBPC) obtained through prior mobilization and collection via apheresis for subsequent transplantation in order to regenerate the endometrium in patients receiving ART. The results of this study will allow a new therapeutic approach for treating infertility in patients who have a reduction or dysfunction of stem cells in the endometrium, since today there is no specific treatment to tackle this kind of endometrial physiopathology.

Scientific / technical impact

This study aims to present a new approach to a certain type of physiopathology in reproductive medicine for which there is currently no treatment (reduction or dysfunction of stem cells in the endometrium). In order to achieve this, the aim is to regenerate endometrial tissue by instilling stem cells from bone marrow which are known to participate in the homeostasis of this tissue.

The expected results consist of obtaining gestation in patients who are unable to conceive owing to endometrial cavity problems, characteristic of this type of physiopathology. After intra-arterial instillation of bone marrow stem cells (use of CD133+ cells) an improvement in the uterine cavity caused by endometrial regeneration is expected.

This is obviously a very novel study and could provide a new tool to treat a subgroup of patients who today have a fairly negative prognosis in terms of reproduction.

In fact, our group already performed an independent prospective, non-randomized, uncontrolled clinical study in which we treated a total of 18 patients (5 with Endometrial Atrophy and 13 with severe or moderate Asherman's Syndrome) through instillation of CD133+ after adhesiolysis. In that study, we proved a significant clinical improvement in terms of duration and severity of periods, and a hysteroscopic improvement of the cavity after treatment. At reproduction level, we obtained a significant improvement in the thickness of the endometrium and an improvement in reproductive results compared to surgery with 5 healthy Live Births in 4 patients (one with twins), 1 ectopic pregnancy and 2 clinical and 3 biochemical abortions. Moreover, no side effects related to the treatment were observed in the study population.

Social and economic impact

The prevalence of patients reporting this physiopathology is between 0.5% and 1% in women receiving ART (in Spain around 55,000 cycles per year are performed and in Europe this figure is almost 10 times higher, 400,000 cycles per year) and between 1% and 2% of patients underwent hysteroscopy. The data are still rather ambiguous because there are no figures for the number of hysteroscopies that are performed annually either in Spain or in Europe. Whichever the case, we are talking about a significant number of patients per year in Spain who want to be parents but for whom there is no kind of treatment.

In our field, the cost of IVF is around €6000 - €8000. Bearing in mind that practically none of these patients manage to conceive, in the National Health System (NHS) a major investment is being made without obtaining any results. Increasing the possibility of patients with this physiopathology conceiving would reduce health care costs for the NHS, and would also entail a saving for society in terms of indirect costs (loss or reduction of ability to work or enjoy leisure by hospitalized patients or those convalescing at home...) and an increase in the quality of life of patients and their partners. A more detailed cost / benefit analysis would obviously be necessary to assess the effectiveness of the proposed intervention in this investigation protocol.

We once again place emphasis on the fact that we are able to provide a response, not only for the Spanish population, but for the international community at large, since in accordance with our knowledge, no other group in the world has published such a wide number of documented, protocolized cases as our group, and therefore the application of this new therapy for patients with this kind of endometrial dysfunctions could entail a significant development in techniques to permit gestation in patients for whom to date it is impossible.

5. Study hypotheses and objectives

5.1 Hypotheses

The endometrium will undergo de novo regeneration through the use of autologous bone marrow stem cells obtained from Peripheral Blood Progenitor Cell (PBPC) mobilization and collection, involving apheresis and selection of CD133+ cells and subsequent transplantation of those cells in patients with physiopathologies associated with endometrial stem cell decrease or dysfunction (such as Asherman's Syndrome or Endometrial Atrophy) planning to undergo Assisted Reproductive Treatment (ART).

5.2 Primary objective

The primary objective of this study is to assess safety and tolerability of the IGX1 investigational medicinal product (CD133+ cells screened after mobilization and collection of peripheral blood progenitor cells (PBPCs) from the same patient) in improving post-treatment reproductive prognosis, as measured by:

1. Identification of incidence, prevalence and frequency of different adverse events that may occur in the patient throughout the study up until Month 15 post-treatment.
2. In the event of a term pregnancy, identification of incidence, prevalence and frequency of different adverse events that may occur during birth.
3. In case of a Live Birth, identification of incidence, prevalence and frequency of the different adverse events that may be associated with the investigational medicinal product (IMP) until the first month of age.

5.3 Secondary objectives

- Efficacy of the investigational medicinal product IGX1 in improving post-treatment reproductive prognosis, as measured by:
    1. Implantation rate (IR) per transferred embryo, pregnancy rate (PR) and ongoing pregnancy (OPR) per transfer performed from embryo transfer to birth.
    2. Improved hysteroscopic score in accordance with the European Society of Hysteroscopy (ESH) classification and the European Society for Gynaecological Endoscopy (ESGE) 28 days after stem cell treatment.
    3. Improvement in endometrial thickness and pattern as determined by ultrasound 28 days after stem cell treatment.
- Pregnancy follow-up, evaluated by live birth rate (LBR), rate of (clinical and biochemical) miscarriage and rate of ectopic pregnancy from embryo transfer until birth.
- Recovery of endometrial volume as measured by 3D ultrasound 28 days after stem cell treatment.
- Analysis of endometrial vascularization via pre- and post-treatment endometrial biopsy 28 days after stem cell treatment.
- To evaluate reappearance of menstrual episodes (if they did not exist prior to treatment) or compare differences in duration and quantity of episodes after embryonic stem cell treatment until embryo transfer, with respect to menstrual episodes occurring prior to treatment.
- To determine the cell dose, cut-off point of CD133+ cells that maximizes sensitivity and specificity of test needed to discriminate between pregnancy/non-pregnancy (response variable) in keeping with minimum quality requirements established (sterility, cell viability greater than or equal to 50% and purity greater than or equal to 70%).
- To evaluate and describe quality of life (QoL) of participating patients, using the 2008 FertiQoL International tool, in order to estimate the influence and impact of infertility-related psychological problems on the various spheres of their daily lives, before and after the treatment.

5.4 Exploratory objectives

- Study of the endometrial receptivity profile to detect potential alterations in the window of implantation (WOI) measured by i.e the ERA test (Endometrial Receptivity Analysis) before and after stem cell treatment. Additionally, the study aims to investigate factors or potential predisposition causes that could link Asherman's Syndrome to endometrial receptivity genes.

- Study of the endometrial microbiome to establish its possible impact on Asherman's Syndrome and its relationship to the outcomes of assisted reproduction treatments, using next-generation sequencing (NGS) technologies to compare microbial profiles from endometrial fluid and biopsies.

- Histological study of endometrial biopsies before and after stem cell treatment to compare differences in endometrial structure and tissue regeneration mechanisms, aiming to evaluate potential mechanisms of action.

- Identification and characterization of the CD133+ cell population using new cell markers to define therapeutic cell populations through the collection of a small aliquot.

- Execution of a follow-up substudy that includes macroscopic, microscopic (anatomopathological), and genetic evaluations of the placentas of newborns from pregnant women treated with IGX1, compared to a control group. The goal is to identify potential placental issues (such as placenta accreta or percreta).

6. Detailed study design

6.1 Trial design and scheme

This is a Phase I-II clinical trial of advanced cell therapy, prospective, open-label, non-randomized, non-controlled (before-and-after study), explanatory, multicenter, and interventional, with a single treatment group. It is aimed at patients of reproductive age diagnosed with moderate and/or severe Asherman's Syndrome (excluding mild cases) based on the criteria of various European Societies (ESH and ESGE; see Appendix 3). The trial involves patients planning to undergo assisted reproductive treatment with a single blastocyst embryo transfer (day 5 or 6 of development) following cell therapy for endometrial regeneration. Exceptionally, cases requiring double embryo transfer (DET) for medical reasons may also be included.

A total of 22 patients are expected to be enrolled across participating centers within the Quirónsalud Group (Hospital El Pilar and Quirónsalud Barcelona) and Vall d'Hebron Hospital. Additionally, the participation of the Banc de Sang i Teixits de Catalunya (BST) as the processing center for the investigational product and the Igenomix Laboratory for endometrial receptivity and microbiome analysis will be essential for the study's activities and tests. The Príncipe Felipe Research Center is also expected to participate by

conducting preclinical research on murine models in parallel with the study, using a small aliquot of IGX1 obtained at the processing center.

In general, Quirónsalud centers will handle the recruitment, evaluation, and selection of patients, as well as the pre- and post-treatment endometrial diagnosis following the use of stem cells derived from CPSP. They will also conduct patient follow-up throughout the study, including post-treatment monitoring after the infusion of selected stem cells and post-assisted reproduction treatment (ART) follow-up for up to 15 months after the post-cell infusion hysteroscopy (Day 37 ± 7/Visit 6).

The other two participating centers, HUVH and BST, will be involved in patient evaluation, hematological assessment, mobilization, collection, and processing of CPSP, as well as the instillation of the selected stem cell unit (CD133+ cells) and immediate postoperative monitoring. Patient monitoring and follow-up will be conducted collaboratively across all centers throughout the study.

The total estimated duration of the study is approximately 3 years:

- 12 months for patient recruitment.
- 18 months per patient (approximately 1–2 months for the Selection, Evaluation, Open Treatment, and Regeneration Period + a maximum of 16 months [6+9+1] for the ART period and follow-up in the case of a viable pregnancy and live birth).
- 6 months for data analysis, conclusions, and preparation of the final report.

Recruitment will take place through patients who contact the investigators at the participating centers (Vall d'Hebron University Hospital, Hospital El Pilar, and Quirónsalud Barcelona).

Alternatively, given that this is a rare disease, contact will be made with patient associations such as the Spanish Asherman Association, and a campaign will be conducted through websites frequented by patients to raise awareness about the study.

The study consists of seven visits divided into three periods:

1. Selection and Evaluation Period: This period includes the baseline visit (Visit 1), which lasts approximately 45 days. During this time, patient selection and evaluation will take place across the various departments of the participating centers (Gynecology and Reproductive Medicine Services at Hospital del Pilar and Quirónsalud Clinic, and Hematology, Radiology, and Gynecology Services at Vall d'Hebron University Hospital).
2. Open Treatment and Regeneration Period: This period includes five visits (Visits 2 to 6), spanning approximately from Day 1 to Day 37.
    - Day 1 (Baseline Visit/Visit 2): Preferably on a Tuesday, the Hematology Service will evaluate the patient before prescribing G-CSF. Once the hematologist approves, the patient will collect the medication from the Pharmacy Service.

- Day 4 (+1) (Friday or Saturday): The patient will begin at-home treatment with Neupogen® as instructed by Hematology and Pharmacy (10 µg/kg every 24 hours for five consecutive days via subcutaneous administration).
- Days 5 and 6 (+1) (Saturday and Sunday or Sunday and Monday): The patient continues the at-home treatment.
- Day 7 (Visit 3, Monday or Tuesday): The patient will undergo pre-treatment endometrial evaluation and diagnosis (control) through hysteroscopy, ultrasound, and an endometrial biopsy for the ERA test. Additionally, a sample of endometrial fluid (EF) will be collected for microbiome analysis via NGS, just before tissue collection for the ERA test.
- Day 8 (+1) (Visit 4, Tuesday or Wednesday): After completing five days of Neupogen® treatment, the patient will receive the last dose, followed by an evaluation and CPSP collection via apheresis. The collected CPSP will be sent to the processing center (BST central laboratory) for selection and preparation ahead of instillation on Day 10 (Visit 5).
- Day 9 (+1) (Wednesday or Thursday): The BST will process and isolate the cells to produce the investigational product, IGX1.
- Day 10 (+1) (Visit 5, Thursday or Friday): The IGX1 product will be sent early in the morning, and during Visit 5, the cell instillation will take place within 24 hours of completing the IGX1 processing. The patient will undergo a prior medical evaluation by the clinical team.

3. Post-Treatment Final Visit: Approximately four weeks after the cell instillation (Visit 6 or Final Post-Treatment Visit, Day 37 ± 7), equivalent to one menstrual cycle, the patient will undergo a post-treatment endometrial diagnosis to evaluate treatment outcomes and endometrial tissue regeneration. The same diagnostic tests as in Visit 3 (ultrasound, hysteroscopy, endometrial biopsy, ERA test, and EF sampling for microbiome analysis) will be performed.

After the gynecological evaluation, the results will be discussed with the patient, and a joint assessment will be made before initiating the Assisted Reproductive Treatment (ART) that the patient had planned. ART is expected to occur approximately 10 days after Visit 6 (Day 50 ± 7). During this visit, the patient will start Hormone Replacement Therapy (HRT) in preparation for ART, which can take place within the first six months following the Final Post-Treatment Visit.

Regardless of whether the patient and/or medical team decides to proceed with the initially planned ART, a follow-up period of up to 15 months from the day of the second hysteroscopy to assess the endometrial cavity (ART and Follow-up Visit or Final Visit, i.e., from Visit 6 to Month 15). This follow-up period (including both in-person and remote visits), regardless of whether the patients become pregnant following a possible embryo transfer, will include at least five scheduled contacts, aligned with standard clinical practice.

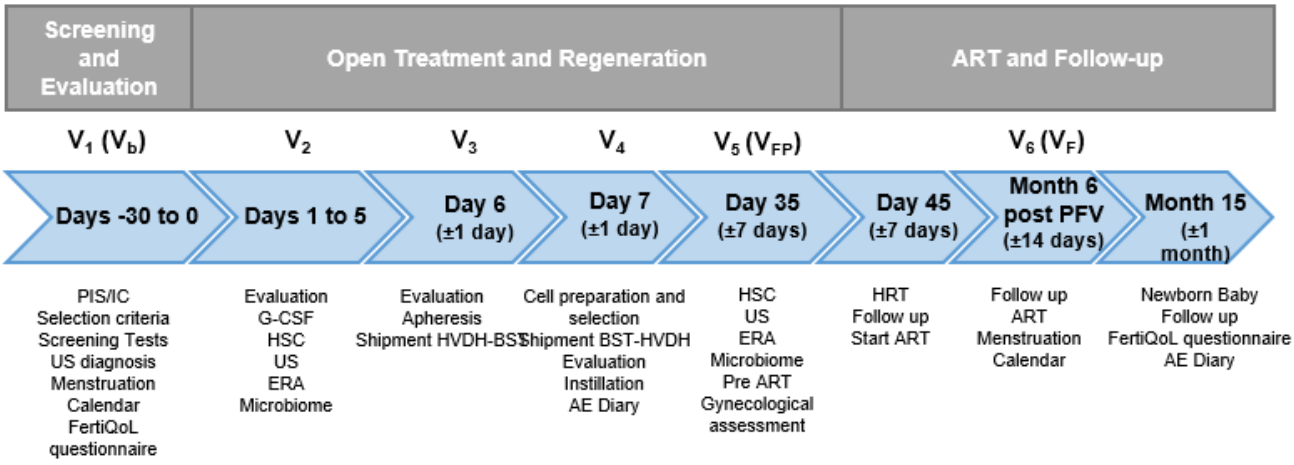
Once the follow-up period begins, both patients who complete all scheduled visits and those who withdraw before the end of this period (estimated at up to 15 months) will undergo a corresponding Final Visit (FV) of the study.

Additionally, in the case of a full-term pregnancy, a follow-up contact (in-person or remote) will be conducted 30 days after the Final Visit (approximately Month 15 or earlier). This follow-up visit will assess the condition of both the newborn (RNV) and the mother.

Throughout the study, strict monitoring will be conducted for all adverse events (AEs) as well as any medication the patient may receive during their participation. To facilitate this, a diary will be provided to the patient during Visit 5 (Day 10(+1)) to record all AEs and any concomitant medication taken. The diary will be reviewed by the medical team during each contact with the patient and documented in the source documents as well as in the electronic Case Report Form (eCRF). In the case of a newborn, any AEs occurring during delivery or the first month postpartum will also be recorded.

If the patient requests it or if the investigator deems it necessary, an unscheduled visit may be conducted at any time during the study. The date and reason for the visit will be recorded in the eCRF, along with any other relevant data, especially concerning concomitant medication, adverse events, or other procedures or tests.

Figure 2. Overall study schedule



SCREENING AND EVALUATION PERIOD

BASELINE VISIT (VISIT 1/BASELINE VISIT)

At **VISIT 1 (Day -30 to 0)**, patients of child-bearing potential, between 18 and 45 years old, diagnosed with Asherman's Syndrome grade II, III or IV who intended to undergo assisted reproductive treatment with double embryo transfer and who were candidates for the study will be contacted through any of the participating IVI sites and will be asked to participate in the study. These patients will be assessed at the participating IVI site and in the different involved HUVH departments in order to ensure they meet the different selection criteria to participate in the study.

During this visit and before any study activity or assessment is performed, that is not a part of the standard clinical practice at the site, the Principal Investigator at the site or another appointed doctor will inform the

patient of all the aspects of the study, including all scheduled activities and visits and must obtain the relevant signed and dated informed consent form in writing as approved by the Ethics Committee. Likewise, a signed and dated copy of the written informed consent must be given to the patient.
Any assessments prior to signing the informed consent that have been obtained through standard clinical practice within the periods established in the protocol will be considered valid.

A total of 20 patients will be recruited (transfer of 40 embryos since this is DET) during a recruitment period estimated to last 8 months.

After informed consent has been obtained, in order to protect the identity of the patients enrolled in the study, each patient will be coded through assignation of a code consisting of a permanent 3-digit identification number. The first digit will denote the site, followed by 2 digits in accordance with chronological enrollment in the study (the first identification number will be 01, the following 02 and so on). In this way, the complete patient identification number will consist of a site identification number followed by the patient identification number (01, 02, etc.). For example, the first patient at the first site will be assigned 101, the following patient 102, the first patient at site 2 will be 201 followed by 202 and so on.

Any patients who have been enrolled in the study, therefore receiving their identification number but who have not received treatment will stem cells, will be considered screening failures (for example because they do not meet the selection criteria, or because the patient withdraws prematurely from the study, for medical reasons...) and the same identification code will not be assigned to a new patient. This means that each recruited patient will be assigned a unique identification number that will remain the same through to the end of the study.

Since this is a non-randomized study, all patients will be assigned to the same treatment group, owing to which it will not be necessary to assign a randomized number or code.

During this visit patients will be given their clinical trial participation card which they must keep throughout the duration of the trial. (See Appendix 21.2).

The screening visit will be conducted no more than 30 days before the start of the G-CSF treatment (start of the endometrial diagnosis and cell mobilization phase). During this phase, the following procedures will be conducted at the sites indicated as follows:

<u>Site IVI</u>

1. Detailed explanation of the study, including activities and scheduled visits, obtaining written informed consent before any tests related to the study are performed.
2. Review of inclusion/exclusion criteria.
3. Review of medical history and demographics.
4. Review of medication records.

5. Physical examination of the patient and vital sign measurement.
6. Start/review of the estradiol and progesterone regimen.
7. Dispensing 2D/3D ultrasound diagnosis in the proliferative stage of all patients.
8. Dispensing the menstrual calendar and entering of previous menstrual cycle data.
9. Dispensing the quality of life questionnaire in assisted reproduction FertiQoL International 2008.

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1. Review of inclusion/exclusion criteria.
2. Review of medical history and demographics.
3. Review of medication records.
4. Physical examination of the patient and vital sign measurement.
5. Complete blood tests: Chemistry, hematology, hemostasis and serology.
6. Determination of blood group and autoimmunity study (AIS).
7. Pregnancy test in blood (β-hCG).
8. ECOG (Eastern Cooperative Oncology Group) assessment.
9. Electrocardiogram (ECG).
10. Assessment of arteries for catheterization.
11. Prescription and collection of Neupogen (G-CSF). Directions for use, conservation and subsequent return at the end of treatment.
12. Explanation about the procedures to perform and signing informed consent for each of the procedures in accordance with standard clinical practice.

OPEN-LABEL TREATMENT AND REGENERATION PERIOD

ENDOMETRIAL DIAGNOSIS AND CELL MOBILIZATION (VISIT 2)

During the first of these visits (Visit 2 / Days 1 to 5), pre-treatment endometrial diagnosis (control) will be conducted by means of ultrasound, hysteroscopy and ERA test. At **VISIT 2 (Days 1 to 5)**, patients will start subcutaneous treatment with G-CSF (Neupogen®), preferably on a Thursday or Friday at a dose of 10 µg/Kg, every 24 hours for a total of 5 days in order to mobilize the stem cell population (including CD133+ cells which will be selected) to peripheral blood for subsequent collection via apheresis. On the fourth day mobilization of the CD133+ cells will be checked via blood tests to rule out failure of the effects of Neupogen®.

Prior to starting the treatment, the investigator staff, including the doctor in charge of collection, will ensure that the patient is in optimum conditions to start treatment confirming that all the assessments and results conducted at Visit 1 are available and are suitable. Moreover, it will be confirmed that peripheral vein access assessment is adequate. If this is not the case, fitting a catheter will be considered for the procedure. The relevant request for PBPC collection will be completed and signed and the date and time for starting collection by the Apheresis Unit will be confirmed.

After treatment with estradiol and progesterone, in accordance with standard clinical practice, and after 5 days of treatment with G-CSF, prior to apheresis, a second 2D/3D ultrasound test in the luteum phase will be performed to assess endometrial pattern, thickness and volume and a hysteroscopy surgical diagnosis to release adherences along with an ERA test at the IVI site in Barcelona to act as control, permitting assessment of the status and receptivity of the endometrium prior to treatment with stem cells.
In addition, at the same time on the scheduled day of the ERA biopsy in day P+5 during the HRT cycle (after 5 days of progesterone treatment) according to the clinical standard practice, a sample of endometrial fluid (EF) will be aspirated immediately prior to EB for ERA. This EF sample, as well as a small portion of the endometrial tissue obtained for the endometrial receptivity diagnosis by ERA, will be used for the study of the endometrial microbiome. These samples will help us to set up its possible impact in the Asherman´s Synrome and thus, its relation in the assisted reproduction treatments results by using the new throughput sequencing platforms (Next Generation Sequencing – NGS) comparing the microbial populations found in endometrial fluid versus endometrial tissue.

Depending on the grade and case of Asherman's Syndrome, the doctor will assess instillation of 10cc of hyaluronic acid (HialuBarrier®) to prevent formation of new adherences in accordance with standard clinical practice. At the time of discharge, the hormone replacement therapy guidelines will be reviewed and provided in accordance with standard medical practice.

Any adverse events (AE) arising from the patient recruitment in the study and any concomitant medication the patient may have taken will be recorded.

CELL COLLECTION (VISIT 3)

At **VISIT 3 (Day 6 ±1)**, after treatment with Neupogen® to mobilize the progenitor cells to the peripheral blood, the patient will go to the site to start collection of those cells and the packages of G-CSF medication that were used over the 5 days prior to treatment will be returned to the Pharmacy Department.

The cell collection process will be carried out via apheresis by BST hematologists in the HUVH. This process will be carried out in accordance with standard conditions, via peripheral vein access (or central blood access if deemed necessary by the medical staff), with hospitalization and discharge on the same day, and will last approximately between 30 minutes and 2 hours.

During this apheresis process the plasma and leukocyte fractions of the blood will be separated out, returning the rest of the components to the patient's bloodstream. A total plasma volume of between 200 and 500 mL is expected to be obtained, although this could vary depending on the type of patient.
Before starting the collection process, in accordance with standard clinical practice for this kind of procedure, the BST staff will have performed a new blood test (independent of the test performed by the Hematology Department at HUVH). Likewise, a physical examination and follow-up of the patient's vital signs will be performed just before and after apheresis.

After the collection process, the bag with the plasma and white blood fraction (where the CD133+ cells that can be selected are found) will be labeled and sent to the central BST laboratory following the usual transport and storage conditions for this type of biological material (in refrigerator between 2 and 8ºC). This laboratory is located at: Banc de Sang i Teixits. Passeig Taulat, 116. Edificio Dr. Frederic Duran i Jordá. 08005. Barcelona. The person responsible for shipping and custody before and after cell selection will be Dr. Sergi Querol's team.

Shipping will be in an isothermal container (between 2 and 8ºC) with temperature log via TempTale of those normally used for transport of this kind of biological material and which permits ensuring the integrity and contents thereof throughout the process.

Once the unit is received at the processing site, verification will be performed (condition of the bag and temperature monitoring) by trained personnel at the site and will be stored in a refrigerator for this purposes between 2 and 8ºC until the following morning which is when the CD133+ cell selection process will be carried out. This selection process will be carried out the following morning, coinciding with the infusion day in order to ensure conservation and viability of the selected CD133+ cells.

In the event of there being any incidents during shipping, reception or custody of the unit at the processing site (central BST laboratory) which could affect the quality or viability of the contents, this must be immediately reported to the Sponsor and the investigator staff, who will ultimately decide whether to interrupt or continue with the process in accordance with criteria concerning the health and welfare of the patient. The

entire process will be monitored and any abnormalities will be recorded in the relevant source documents and in the eCRF.

Any adverse events that arise, as will any concomitant medication the patient may have taken during this period, will be recorded.

CELL SELECTION AND INSTILLATION (VISIT 4)

At **VISIT 4 (Day 7 ±1),** selection of the CD133+ cells, and manufacture of the drug at the processing site in accordance with standard operating procedures (SOP) at that site will begin.
The CliniMACS® System will be used for preparation. The CD133+ cell concentrate obtained will be diluted along with PBS/EDTA buffer to obtain a final volume of 30-50 mL which will be pre-loaded in a sterile, disposable syringe for direct intra-arterial use. This drug will be labeled in accordance with the requirements of current legislation (see Appendix 21.3) with the batch being the donation code (each patient equivalent to her own batch) and the selection and instillation day being the expiry date.

The pertinent quality, safety, pureness and viability tests on the CD133+ cell concentrate will be performed.

In accordance with the study criteria the minimum quantity of CD133+ cells to be instilled will be 30 million. In any cases where the minimum is not reached, the PI responsible for the study will agree to compassionate use of these isolated cells with the patient. If the patient does not accept, the patient will be excluded from the study. If the patient agrees to compassionate use, the cells will be instilled and the patient will continue being monitored until the end of the study, but her data will not be compiled or used for assessment of treatment efficacy. Nevertheless, any appearance of adverse events in accordance with the established protocol will be monitored. In other words, the patient will be considered to have been excluded from the study for the purposes of effectiveness and efficacy analyses, but safety and tolerability controls will be performed as with the rest of the patients enrolled in the study.

In cases where a quantity higher than 35 million CD133+ cells is isolated, an aliquot of 3-5 million cells will be prepared which will be used in an animal Asherman model (preclinical studies). The preparation of the aliquot must never compromise the patient's participation in the study, and therefore in cases with less than 35 million isolated CD133+ cells, all the cells will be used for the clinical trial and consequently no aliquot will be made.
The samples divided into aliquots will be prepared in a 2 to 15 mL tube, identified with a process code and other identification specifying that they are cells for preclinical study. The samples will be delivered with a document containing a description of the concentrate and the volume of the samples, as well as the sample type, the name of the recipient at HVH, location and contact method. The cells will be transported using a refrigerated system at 2-8ºC inside a second safety container. The person in charge of receiving the samples for preclinical studies is Julia Vallve, an employee at *Vall Hebron Institut de Recerca* (VHIR) who will be in charge of freezing and subsequent custody of the cells. These cells will be used for studies in animal models

that will be performed concomitantly to the clinical trial so that there are no frozen cells at the end of the clinical trial (ENTIRE).

The director or person responsible for the logistics at BST will issue a certificate with the cell quality, safety, viability, pureness and concentration results which will be sent to the site by e-mail or fax or along with the selected CD133+ cells to confirm "release of the product for shipment", guaranteeing the viability of the process prior to instillation.
If any of the required specifications are not met, the clinical team will assess whether or not to continue with the instillation process with the patient.

After product release, the sterile syringe with the duly labeled drug will be shipped in an isothermal container (between 2 and 8ºC) with temperature log via TempTale of those normally used for transport of this kind of biological material and which permits ensuring the integrity and contents thereof during shipping. On arrival at the hospital where instillation is to take place (HUVH), the medical staff at the Radiology Department will check the conditions of the medication and documentation, and it may not be infused without having been previously verified in order to guarantee correct status thereof.

The drug may not be radiated or exposed to any other source that could damage the contents.

The patient will be hospitalized at mid-morning on the instillation day, with the drug having previously been received at the site, and will thus be ready for instillation.

In accordance with standard clinical practice at the site for this kind of procedure, the patient will be examined immediately on arrival at the Obstetrics Department for observation, 2-4 hours after instillation of the drug and at discharge 5-7 hours after the procedure. During this physical examination and vital sign measurement, at least blood pressure, heart rate and pedal pulse must be measured. The involved personnel must agree with the requirements for registering the data for this study.

The medication will be infused manually, using intra-arterial catheters in accordance with standard clinical practice. In order to do so, after accessing the femoral artery using the Seldinger technique, a 4F introducer is fitted in the artery through which both hypogastric arteries are fitted with angiographic catheters with Cobra 2 curve and a Terumo 0.035" guide wire. A 2.5 F micro-catheter is fitted through the Cobra catheter with a 0.014" guide wire and the catheter is introduced in the uterine artery to the ascending curve or to the most distal level allowed by the micro-catheter. Once the catheter is stabilized and the position has been checked, instillation of the drug will take place. The inner diameter of the catheter is approximately 500-600 microns (the inner diameter is 0.014 inches). Approximately 15-20 mL will be injected in the most distal portion of each uterine artery in the direction of the arterioles. If there are any anatomical or technical variations that impede bilateral access, the cells will be instilled through the uterine artery on one side. The approximate time for this process is around 60 minutes, although this could vary depending on each patient.

The patient's assessment process, catheterization and instillation of the drug will always be carried out by the doctors and nurses at the HUVH Radiology Department.

Once the cell instillation process has ended, the patient will be transferred to the Obstetrics day hospital for observation and follow-up until discharge during the afternoon or evening on the same day.

At the end of this visit patients will be given and informed about a diary where they will be able to record any medication and adverse events that could arise throughout the entire follow-up period. This diary will be reviewed by the clinical team at the site at each contact with the patient (either in person or by telephone) until Month 15 or the study Final Visit when it will be returned.

Any adverse events or concomitant medication patients may take during this time will be recorded in the patient's source documents and the eCRF.

ENDOMETRIAL DIAGNOSIS POST-TREATMENT AND FINAL VISIT POST-TREATMENT (VISIT 5/FVPT)

At **VISIT 5 (Day 35 ±7),** approximately 4 weeks after the cell selection and instillation day, the patient will go to the IVI site where any possible regeneration of the new endometrium, the uterine pattern and its endometrial receptiveness will be assessed. The following tests will be performed to ascertain this:

1. Review of the estradiol and progesterone regimen.
2. 2D/3D ultrasound to assess thickness, pattern and volume of the endometrium in the luteum phase.
3. Hysteroscopy diagnosis.
4. ERA Test.
5. Endometrial microbiome analysis immediately prior to endometrial biopsy for ERA indication (a single EF aspiration for the NGS analysis together with a portion of the endometrial tissue for the ERA test).

Additionally, both in the eCRF and the patient's source documents, all concomitant medication and any adverse events that could have arisen since the previous visit will be recorded.

After carrying out the pertinent medical check-up about any possible evolution and endometrial regeneration, the site doctor will discuss the possibility of carrying on ART treatment or not with the patient (always in accordance with the indications of the selection criteria in the protocol).
Regardless of whether the clinical team or the patient decide not to continue with ART or decide to perform one or more ART (if not conceiving during that attempt or if suffering a biochemical or clinical abortion or an ectopic pregnancy), a follow-up period up to Month 15 from the end of treatment visit (Visit 5/FVPT) will be established, coinciding with the second hysteroscopy. (See following section "Follow-up Period").

Any adverse events or concomitant medication patients may take during this time will be recorded in the patient's source documents and the eCRF.

FOLLOW-UP PERIOD

FOLLOW-UP AND STUDY FINAL VISIT (FROM FVPT TO MONTH 15)

The **FOLLOW-UP VISIT** will start as soon as the final visit post-treatment has taken place (Visit 5 or Day 35 ±7) and will last for a total of 15 months (study Final Visit day).
Around 10 ±7 days after V5/FVPT, coinciding with the first menstruation, the patient will start hormone replacement therapy (HRT) in order to initiate her ART which will take place within the first 6 months (±14 days) following FVPT. Once the ART has been performed, a total follow-up period of 15 months (±1 month) will be established until the End of Study Visit, regardless of whether or not there is an ongoing pregnancy.
This follow-up phase will consist of the following periods ordered chronologically from FVPT to Month 15 or the study Final Visit:

1. **First 6-month follow-up (±14 days) / ART Period**

   During these first 6 months, the patient will undergo ART in accordance with the schedule when entering in the study. ART will be carried out in accordance with standard clinical practice with double embryo transfer of two blastocysts on day 5 or 6 of development. During the ART process, ultrasound and analytical controls will be performed by determining estradiol and progesterone levels in the blood in accordance with standard procedures at the site.

   Once embryo transfer has been performed, the β-hCG result will be measured between 10 and 14 days after transfer (in-person visit) and a review of any concomitant medication and adverse events recorded in the diary, and also after directly asking the patient herself.

If the patient or the investigator staff have decided not to continue with ART, a follow-up visit will be scheduled (in-person or otherwise) in Months 9, 12 and 15 (Final Visit) to assess the general condition of the patient.

The investigator will inform the Sponsor about the patient's decision to continue or withdraw from the scheduled ART. If no ART is performed in the first 6 months, the data obtained from these patients concerning their safety will be compiled (patients included in the Safety Population set).

If ART is carried out, the investigator must report the results of the ART to the Sponsor immediately within 24 hours once the results are known (pregnancy / non-pregnancy) and perform special follow-up of pregnant women and evolution through to the pregnancy outcome. This notification must also include pregnancies that lead to normal live births. During or after this 6-month period when patients complete their Assisted Reproduction Treatment (ART), two situations could arise:

a. **If there is an ongoing pregnancy** controls coinciding with standard gestation check-ups will be carried out at 12-14, 22-24, y 34-36-week gestation, and at Week 44 (postpartum) coinciding with the postpartum follow-up visit of the LB 1 month after birth (End of Study Visit). If this takes place before the 15 months post second hysteroscopy (V5 or FVPT), it will be completed at this point at the last follow-up visit (follow-up will always be up to Month 15). To do so, the normal scans will be made on each patient as per standard gestation controls in pregnant women after a positive β-hCG result until standard gestation discharge. These in-person visits will at least include a 2D/3D ultrasound scan.

   Pregnancy will not be considered an AE since it is one of the objectives of the study, and in turn the initial desire or objective of participants.

   If the pregnant participant experiences a SAE during pregnancy or the outcome of the pregnancy meets the criteria of a SAE, the investigator must follow the procedures to report SAEs, i.e. report the event to the Sponsor within 24 hours after becoming aware of the event.

b. **If there is no ongoing pregnancy**, additional safety follow-up will be performed until Month 15, with scheduled visits (in-person and/or otherwise) at Month 9, 12 and 15 (EOS) after V5/FVPT.

   During the first 6 months, the patient can receive one or more ART (in the event of negative β-hCG, Ectopic Pregnancy, or Biochemical or Clinical Miscarriage occurring after treatment and recovery), always proceeding in accordance with standard clinical practice. If, after subsequent transfer, there is an ongoing pregnancy after a second or third embryo transfer, the process will be as described in the previous section (see Section a).

2. Global follow-up 1 month after Live Birth (LB) after delivery

In the case of birth, an additional follow-up procedure will be established for the newborn immediately after birth, approximately at Week 44, i.e. approximately one month after birth. This visit can be in-person or otherwise. The parents will be contacted to ask about the baby's health and to ask about any possible relevant findings.
In certain cases, this visit may coincide with the EOS in Month 15. Nevertheless, if the baby is born before Month 15, follow-up will always be until Month 15.

All neonatal or infant deaths, congenital abnormalities or other significantly relevant findings (such as the presence/absence of defects at birth, complications with the newborn or the mother during pregnancy or immediately afterwards) and those that occur within the first month following birth (regardless of the cause) must be reported to the Sponsor as an AE or a SAE as applicable.

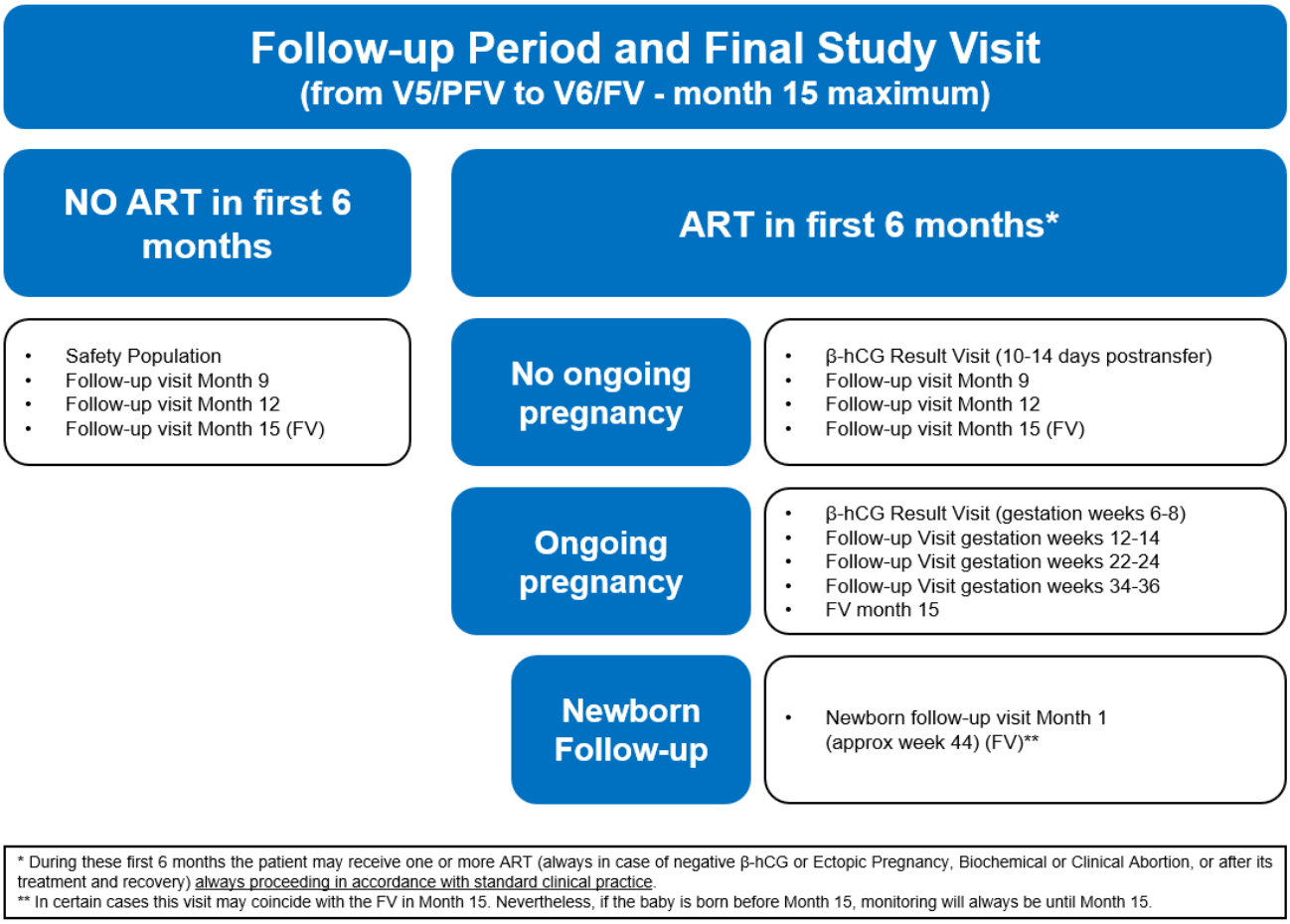
During the study Final Visit, the patient must deliver the adverse event diary that was given to them at Visit 4, duly completed. The diary will help the investigator staff to compile all the AEs that have occurred during

that period of time and all concomitant medication the patient may have taken in the CRF. This source document will be kept with the rest of the patient's source documents.
Moreover, a quality of life questionnaire for assisted reproduction FertiQoL International 2008 will be filled in again (the same as at the baseline visit) which will help to assess and describe the evolution of the patient's quality of life before and after treatment. This questionnaire will help us estimate the evolution on the influence and impact of psychological problems related to infertility in different aspects of everyday life, such as the impact on self-esteem, emotions, general health, family, partner, social relations, work, future life projects and the emotional impact of assisted reproduction treatment. This is an internationally validated questionnaire developed by Boivin, Takefman and Braverman at Cardiff University School of Psychology in Wales, which was born in 2002 in cooperation with the two main assisted reproduction medical societies, namely the European Society of Human Reproduction and Embryology (ESHRE) and the American Society for Reproductive Medicine (ASRM), in conjunction with Merck S.A to measure the quality of life of people with fertility problems. This instrument, made available to professionals free of charge around the world is a self-report, designed to be completed by the patients themselves lasting for around 10 to 15 minutes.

The data compiled after the specified follow-up period will not be included in the eCRF. Nevertheless, in the event of such data being collected, they will not be analyzed. Patients with ongoing AE or test results that could be relevant (according to the investigator's interpretation) will be controlled until resolution, stabilization or until the patient is referred for medical care or until it is determined that the condition is no longer related to any specific study treatment or procedure.

Patients who decide to withdraw from the study before the end or who are lost to follow-up without having specifically withdrawn their informed consent, will be considered lost to follow-up and all the available data until the last date of contact will be recorded.

Figure 2. Visit schedule during the Follow-up Period.



UNSCHEDULED VISITS

Unscheduled visits can be held at any time during the study if requested by the patient or if the investigator considers it necessary. The date and reason for unscheduled visits will be recorded in the CRF, as will any other data obtained. (For example, medication and concomitant treatment, procedure or test results, etc.).

Table 1. Study activity schedule

	SCREENING AND EVALUATION PERIOD	OPEN-LABEL TREATMENT AND REGENERATION PERIOD				FOLLOW-UP PERIOD	
	BASELINE VISIT	ENDOMETRIAL DIAGNOSIS AND CELL MOBILIZATION	CELL COLLECTION	CELL SELECTION AND INSTILLATION	ENDOMETRIAL DIAGNOSIS POST-TREATMENT / FINAL VISIT POST-TREATMENT	FOLLOW-UP/STUDY FINAL VISIT (from V5/FVP to Month 15) FV	UNSCHEDULED VISITS
Visit and assessment schedule	**V1 Day -30 to 0**	**V2 Day 1 to 5**	**V3 Day 6 (±1)**	**V4 Day 7 (±1)**	**V5 Day 35 (±7)**	**V6 Day 45 (±7) to Month 15 (±1 month)**	
Inclusion/ exclusion criteria [A,B,1]	X						
IC signature [A,2]	X						
Medical history and demographics [A,B]	X						
Prior medication [A,B,3]	X						
Physical examination and vital signs [A,B,4]	X	X	X[i]	X[ii]	X		X
Complete blood tests (chemistry, hematology, hemostasis and serology) [B,5]	X		X[iv]				X
Determination of blood group and AIS [B]	X						
Blood pregnancy test (β-hCG) [A,B,6]	X					X	
ECOG [B]	X						
ECG [B,7]	X						
Estradiol and progesterone regimen [A,8]	X					X[v]	X
Assessment of arteries for catheterization [B,9]	X						
2D/3D US [A,10]	X	X			X	X	X
Menstrual calendar [A,11]	X					X	
FertiQoL questionnaire [A,12]	X					X	
Prescription and collection of Neupogen (G-CSF) [B,13]	X						
HSC [A,14]		X			X		
EF sampling for the microbiome [A,15]		X			X		X
ERA test/Biopsy [A,16]		X			X		X
Patient assessment [A,B,C]		X	X	X	X		X
PBPC mobilization (Treatment with G-CSF) [17]		**X**					
Return of Neupogen (G-CSF) [B,18]			X				
Collection of PBPC/Apheresis [C,19]			X				

Shipment of the unit[B,C,20]		X	X			
Preparation and selection of CD133+ cells and instillation of stem cells (catheterization) [B,C,21]			X			
Patient diary for follow-up and recording of AEs and Concomitant Medication[22]			X	X	X	X
ART[23]					X	
Follow-up [24]					X	X
Review of AEs[A,B,C, 25]	X	X	X	X	X	X
Concomitant medication[A,B,C,25]	X	X	X	X	X	X

[A] Procedure performed at any of the participating IVI sites: IVI Valencia or IVI Barcelona.

[B] Procedure performed at Hospital Universitario Vall d´Hebrón (HUVH).

[C] Procedure performed at Processing Center: Banc de Sang i Teixits (BST).

[1] Patients must meet all the inclusion criteria and none of the exclusion criteria. The selection criteria for the study will be verified at the participating IVI site and at HUVH in accordance with Section 6.

[2] Consent must be signed before performing any protocol specific tests or procedures that are not a part of standard clinical practice at the site.

[3] Medication in the last 30 days prior to enrolling the patient in the study, and prior ongoing medication when the patient is enrolled in the study.

[4] Including temperature, blood pressure, pulse rate, respiratory rate, weight, height and BMI in accordance with the standard clinical practice at the site.

[i] Measured just before and after apheresis. Including at least blood pressure and pulse rate.

[ii] Measured just before and after instillation of stem cells (at hospitalization and 2h after instillation). Including at least blood pressure, heart rate and pedal pulse according to standard clinical practice at the HUVH Radiology Department.

[5] According to standard parameters examined at the site. At Visit 1, the chemistry analysis must at least include hepatic and renal functions (Total Bilirubin < 1.5xULN, AST and ALT< 2.5xULN and Serum Creatinine < 1.0 mg/dL; if the serum creatinine is not > 1.0 mg/dL, then estimated glomerular filtration rate (eGFR) should be >60 mL/min/1.73 m^2). Serology at Visit 1 must at least include the following tests: HIV 1 and 2 (Anti HIV-1,2 antibodies), Hepatitis B (HBsAg. Anti-HBc), Hepatitis C (Anti-HCV antibodies; in cases of hematopoietic parents, PCR will also be required) and Syphilis. Recent tests within the previous 30 days will be accepted.

[iv] Following on with the standard procedures at the site for this type of treatment, and regardless of those already performed at the Hematology Department at Visit 1, a blood count in the BST will be performed before and after cell collection.

[6] A blood pregnancy test will be performed (β-hCG) at HUVH at Visit 1 (mandatory in all cases) and at Visit 6 at the IVI site after ART (if ART is carried out within the first six months following V5) in accordance with standard clinical practice at the site (approximately 10-14 days after embryo transfer: β-hCG result visit).

[7] Tests performed within 6 weeks before screening are acceptable.

[8] Patients who have started estrogen and progesterone treatment in accordance with standard clinical practice at the site 2 weeks before entering the study will be accepted.

[v] Start of treatment (ART) prior to performing the ERA test in accordance with standard clinical practice in ART cycles, in V6 (approximately Day 45 ±7).

[9] Performed at the HUVH Radiology Department at Visit 1.

[10] 2D/3D Ultrasound diagnosis in Proliferative Phase at Visit 1, 2D/3D ultrasound at Visit 2 after starting G-CSF treatment and prior to apheresis, and 2D/3D ultrasound control at Visit 5 (FVPT) coinciding with the second HSC after ART treatment in accordance with standard clinical practice, around 4±1 weeks after instillation and after having deprived the patient of menstruation. If ART coincides with standard clinical practice, a 2D/3D ultrasound will be performed at Visit 6 just before embryo transfer and the relevant gestation ultrasound tests (Weeks 12-14, 22-24 and 34-36) if there is an ongoing pregnancy.

[11] When being enrolled in the study at VI/BV and at V6 at the end of the 6 months after ART.

[12] When being enrolled in the study at VI/BV and at V6 and at the Final Visit in Month 15.

[13] The collection of Neupogen (G-CSF) will take place at the Pharmacy Department at HUVH at Visit 1 after assessment and prior prescription by the hematologist at the HUVH Hematology Department at Visit 1.

[14] A diagnosis hysteroscopy at Visit 2 will be conducted, after treatment with estradiol and progesterone in accordance with standard clinical practice, prior to apheresis and at Visit 5 (post-treatment control), after ART in accordance with standard clinical practice, around 4±1 weeks after instillation and after depriving the patient of menstruation.

[15] One sample of EF immediately before the EB for the ERA test on day 5 in HRT cycle according to the clinical standard practice.

[16] An ERA test at Visit 2 will be conducted, after treatment with estradiol and progesterone in accordance with standard clinical practice, prior to apheresis and at Visit 5 (post-treatment control), after ART in accordance with standard clinical practice, around 4±1 weeks after instillation and after depriving the patient of menstruation.

[17] Treatment with G-CSF should start on a Thursday or Friday. Drug treatment with G-CSF 10 µg/Kg every 24 hours over 5 consecutive days will be started.

[18] Used drug may be returned directly at the HUVH Pharmacy Department or to the HUVH Hematology Department staff, before or after the start of apheresis, as indicated by the site investigator.

[19] Hospitalization of the patient in the morning for the cell instillation process. Once instillation has been completed, the patient will be discharged on the same day after spending 6-8 hours in observation.

[20] At Visit 3, shipment of the unit (variable volume of resulting plasma between 200-500 mL, depending on patient) from HUVH to BST. At Visit 4, shipment of the resulting unit after cell preparation and selection (drug ready for instillation) from BST to HUVH.

[21] Cell preparation and selection at BST. Instillation of stem cells (catheterization) at the HUVH Radiology Department, previously assessing the patient.

[22] At the end of Visit 4 the patient will be given a diary to facilitate recording of any AE and Concomitant Medication. This will be reviewed at Visit 5 and subsequent follow-up visits, whether in person or by telephone, and at any unscheduled visits through to the study Final Visit in Month 15 when it will be returned.

[23] ART in the first six months after FVPT in patients who will to undergo a double transfer (DET) with frozen embryos in substituted cycle (ART) in accordance with standard clinical practice. Any cases of single embryo transfer (SET) will be accepted if, owing to medical decisions, it is necessary to safeguard the welfare and health of the patient.
During the first 6 months, the patient can receive one or more ART (in the event of negative β-hCG, Ectopic Pregnancy, or Biochemical or Clinical Miscarriage occurring after treatment and recovery), always proceeding in accordance with standard clinical practice.

[24] From Visit 5/FVPT to Visit 6/EOS (Month 15 at latest). In the case of not having ART in the first 6 months, the patient will be part in the safety population with follow-up visits at Months 9, 12 and 15 (FV). In case of ART in the first 6 months, a β-hCG result visit will be scheduled in accordance with standard clinical practice (around 10-14 days post-transfer). If there is an ongoing pregnancy, gestation follow-up visits will be performed in accordance with standard clinical practice, i.e. Weeks 12-14, 22-24 and 34-26, In the event of LB, postpartum follow-up of the LB will be conducted 1 month after birth (FV), which may coincide with the EOS in Month 15 or if birth takes place before Month 15, a follow up visit will always take place until Month 15. If there is no ongoing pregnancy, follow-up visits will be held at Months 9, 12 and 15 (FV).
During the first 6 months, the patient can receive one or more ART (in the event of negative β-hCG, Ectopic Pregnancy, or Biochemical or Clinical Miscarriage occurring after treatment and recovery), always proceeding in accordance with standard clinical practice.

[25] From the day treatment with Neupogen (G-CSF) starts until the study Final Visit in Month 15.

6.2 Trial phase

Phase I/II.

6.3 Screening and randomization process Blinding techniques

This is an open-label study and therefore there is no need to blind treatment either for the investigator or the patient. No blinding / unblinding techniques are required.

This is a non-randomized study with a single allocation group, i.e. with just one treatment group in patients whose endometrial physiopathology is associated with a reduction of endometrial stem cells (patients diagnosed with Asherman's Syndrome grade II to IV). All patients will be given the same treatment. Autologous stem/progenitor cells from bone marrow selected (CD133+) after prior mobilization and collection of PBPC and subsequent apheresis and transplantation of those cells in the patient in order to renew the endometrium.

Moreover, it is an uncontrolled clinical trial (pre- / post- study) where the aim is to assess the efficacy, response, tolerability and safety of intervention in this group of patients before and after intervention, with each subject acting as her own control. We assume that any changes at endometrium level will be due to the treatment with stem cells the patient has been administered.

6.4 Unused investigational medicinal products

The disposal of unused study drug and all materials that have come into contact with it will be carried out in accordance with the local regulations of each participating center.

According to the study criteria, the minimum dose to be infused must be ≥30x10⁶ CD133+ cells, and under no circumstances may the maximum dose exceed 236x10⁶ CD133+ cells. In cases where the minimum dose is not reached, and provided that the Investigational Medicinal Product (IMP) meets the remaining quality requirements for infusion, the patient may receive the IGX1 treatment even if the dose is below the minimum indicated. The results of this patient will not be included in the Per Protocol Population but may be included in other population groups. If the patient does not consent, they will be excluded from the study.

Whenever possible, an aliquot of 3-7 million cells will be taken to proceed with preclinical trials. If the patient ultimately does not receive the infusion of the IMP, authorization will be requested for the use of their cells in the planned preclinical trials.

6.5 Collection of biological samples

A single EF aspiration followed by a single EB collection will be taken in an HRT cycle and it will be performed ERA biopsy day. The procedure will be performed as described (Ruiz-Alonso et al., 2013). Both, the aspiration of EF and the collection of EB, are common procedures in clinical standard practice.

Collection of endometrial biopsy

The endometrial biopsy, to be used for clinical indication of ERA test, will be immediately collected after the aspiration of endometrial fluid with the help of a Pipelle cannula of Cornier Devices (CCD Laboratories) or similar which has in the tip transverse holes and chamfers. Once introduced through the cervical orifices, the biopsy will be obtained by scraping the endometrium from the bottom of the uterus downwards, and rotating the catheter each time, so that tissue from the walls of the endometrial cavity is taken. Once the sample is obtained, half of the same (about 30 mg tissue) will be used for ERA analysis and will transfer to a cryotube containing 1.5 mL of RNALater (Qiagen), which should be shaken vigorously for a few seconds and stored at 4°C for 4 hours and subsequently sent to Igenomix (Parque Tecnológico de Valencia. C/ Narcís Monturiol Estarriol nº11 Parcela B, Edificio Europark. 46980 Paterna–Valencia.) at room temperature. The EB sample will be divided for the endometrial receptivity diagnosis with ERA and the study of the endometrial microbiota. The EB will be performed without anaesthesia. The risk and complications associated to this technique are rare, with the most frequent being a slight pain after the introduction of the cannula or a scanty spotting after the biopsy. Nonetheless, in several cases the following complications have been described: uterine or cervical perforation, genital infection or prolonged bleeding.

Aspiration of endometrial fluid

With the patient in the lithotomy position, the vagina and cervix will be cleaned and all the material that will be used should be disinfected. After the introduction of the disinfected speculum, a sterile and flexible catheter, the same used for embryo transfer, will also be introduced and endometrial fluid will be aspirated with an average volume of between 20 and 40 microliters with a syringe of 10ml.

The endometrial aspiration is often part of the usual procedures of clinical practice. This is a procedure that is usually painless and rarely generates mild discomfort to the patient because it is not a biopsy of endometrial tissue but a slight suction of endometrial fluid with painless cannulas that are used daily in performing embryo transfers. It should be noted that taking endometrial aspiration before embryo transfer does not affect implantation rates in the same cycle (van der Gaast et al., 2003).

Endometrial fluid samples will be collected in a sterile tube containing a preservation solution that will be provided by Igenomix to the participant centers. Those tubes will be vigorously shaked and store at 4ºC for at least 4h. Then, they will be sent at room temperature to Igenomix headquarters.

Samples will be stored at -80ºC in a safe and restricted-access area, accessible only to personnel who carry out this research for a limited and proportional period to the time required to carry out the established goals. The sample is completely consumed for the study indicated in this project so it will not be necessary to proceed to their final destruction. The person responsible for the custody of the samples will be Dr. Diana Valbuena. The patient must give her consent to the preservation and storage of the sample and must be informed about where, how long and for what purposes it is stored.

The endometrial biopsy for the ERA test will be stored and shipped following the usual procedure for this type of sample.

6.6 Trial duration

The trial is scheduled to start in May 2017 and to end in April 2019 with patients participating for approximately 15 months, including the follow-up period (1 month for screening and evaluation, 1 month for treatment and regeneration and 13 months for follow-up including the ART period).
Twenty patients are expected to be enrolled in the trial at 4 investigation sites, all of them national sites.

6.7 Termination and interruption criteria

The trial will be ended when all the patients have completed the follow-up period and the study Final Visit.
In general terms, the trial may be interrupted due to the following reasons:

1. Safety reasons that invalidate or place prior positive assessment of the benefit/risk at risk.
2. Decision by the Sponsor if the expected objectives or the scheduled recruitment deadlines cannot be met or if the trial is not being conducted in accordance with the Guidelines for Good Clinical Practice (GPC).

Likewise, the study may be terminated at a participating site at any time if:

1. The site is unable to meet the guidelines for GPC.
2. The site is not capable of meeting the requirements defined in the protocol.

In the event of early termination of the study, all study materials supplied by the Sponsor must be returned.

6.8 Source data

The entries made in the electronic Case Report Form (eCRF) must be verifiable with the source documents. All the patient details must match the original source documentation recorded in the medical histories or equivalent before being entered in the eCRF. The source data parameter to be verified and identification of the source document must be confirmed and recorded. The data cannot be directly recorded in the eCRF and considered source documents unless the site obtains written authorization from the Sponsor before starting the study, indicating which data can be directly recorded in the eCRF. The completeness and accuracy of all required documents in terms of regulation must also be checked. The data processed by any of the participating sites (for example, laboratory results, etc.) may be sent electronically for recording in the eCRF. The study file and all source documents must be kept until notification is issued by the Sponsor or its representative to destroy them. The data recorded in the eCRF will be filed at the end of the study in the TMF.

6.9 End of Trial

The trial will be ended when each recruited patient has concluded the follow-up period, having made the final scheduled follow-up visit (Final Visit scheduled for Month 15) or due to interruption as described in the section "Termination and Interruption Criteria".
Termination of the clinical trial will be notified in accordance with the procedures set forth in Royal Decree 1090/2015 of December 24, 2015 (Article 30).

7. Patient screening and withdrawal

7.1 Inclusion criteria

1. Patients whose written informed consent approved by the Ethics Committee (EC) has been obtained, after having been duly informed of the nature of their illness and voluntarily accepted treatment program, while being fully aware of the potential risks, benefits and any discomfort involved.
2. Patients diagnosed with Asherman's Syndrome grade II, III or IV, in accordance with the criteria set forth by the European Society of Hysteroscopy (ESH) and the European Society for Gynaecological Endoscopy (ESGE), who intend to undergo Assisted Reproductive Treatment (ART) with Double Embryo Transfer (DET) of blastocysts (Day 5/6 of development) once cell therapy for endometrial regeneration has been completed.
   Cases involving a single embryo transfer (SET) in blastocyst stage will be accepted if, for medically determined reasons, this was deemed necessary in order to safeguard the patient's health and well-being.
3. Patients who, prior to study start, plan to undergo ART in a *Hormonal Replecement Therapy* (HRT) with donated oocytes or own vitrified embryos in blastocyst stage (Day 5/6 of development).
   We will accept cases where, in accordance with standard clinical practice, Pre-implantation Genetic Diagnosis (PGD) is indicated, in compliance with current legislation on human assisted reproductive techniques (Law 14/2006 of May 26). The most common indications for PGD are as follows: Advanced maternal age (age ≥ 38 years), recurring implantation failure, repeated miscarriages or alterations in karyotype of one or both parents as well as any detected in the FISH of sperm cells.
4. Women of child-bearing potential between 18 and 44 years (both included).
5. BMI: 18 – 30 Kg/m^2 (both included).
6. Adequate liver and kidney function, defined as follows:
   Total bilirubin < 1.5xULN
   AST and ALT< 2.5x ULN and
   Serum creatinine < 1.0 mg/dL; if serum creatinine is > 1.0 mg/dL, then estimated glomerular filtration rate (eGFR) should be > 60 mL/min/1.73 m^2.
7. Absence of severe heart disease.
8. Negative blood pregnancy test.
9. ECOG= 0-1.
10. Negative HIV, HCV, HbsAg, HBcAg and Syphilis tests (recent, at least 30 days).
11. Normal coagulation study.
12. Adequate peripheral venous access. Otherwise, the investigator will assess whether central venous catheter should be implanted.
13. Absence of severe psychiatric illnesses.
14. Patient can adhere to and follow study procedures and checkups, that is, patients who are able to understand and comply with parameters as indicated in the protocol.

7.2 Exclusion criteria

1. Patient refuses to receive central venous catheter as proposed by the investigator in cases where there is no peripheral venous access.
2. Patients who are allergic to iodine contrast.
3. Patients for whom an optimal investigational medicinal product cannot be obtained or infused after performing apheresis and selection. The product is unusable if any of the following criteria are identified:
   Dose to infuse having less than 30 million CD133+ cells.
   Cell viability lower than 50%.
   Less than 70% purity.
   Non-sterile.
4. Patients who have participated in another clinical trial or who have received an investigational treatment in the 30 days prior to the study, unless expressly approved by the sponsor.
5. Existence of severe or uncontrolled bacterial, fungal or viral infections, which in the opinion of the Principal Investigator may interfere with patient's participation in the study or with the evaluation of the study results.
6. Any illness or medical condition that is unstable or which may put at risk the patient's safety and her compliance in the study.

7.3 Expected number of subjects

The inclusion of 22 evaluable patients is planned. If, for medical reasons, a patient is included but later found not to meet the established criteria for the per-protocol statistical data analysis group (see section 9), additional patients may be recruited to ensure sufficient statistical power to carry out the analysis.

7.4 Withdrawal criteria and analysis planned for withdrawals and discontinuations

In accordance with the Declaration of Helsinki, any patient may be withdrawn from the study at any time if, in the opinion of the investigator, the treatment is not in her best interest. Likewise, patients have the right to withdraw from the study at any time for any reason without having to give any explanations and without prejudice to any future medical care by the doctor or the site.

Withdrawal of consent for a study means that the patient no longer wishes to receive any further study treatment and does not wish to or cannot continue participating in the study. Any patient may completely withdraw consent to participate in the study at any time. The investigator will discuss the most suitable manner to withdraw in order to guarantee their health. Any patient may fully withdraw their consent to participate in the study and will be discontinued from the treatment and/or study observations immediately after the date when withdrawal is requested.

All efforts must be made to complete and notify observations as in much detail as possible until the withdrawal date. All information must be recorded in the relevant source documents and the eCRF.

Patients enrolled in the study who do not receive CD133+ selected stem cell treatment will be considered Screening Failures (SF). These patients must be recorded in the eCRF (the available data must be entered including the reason for screening failure up until the date this takes place) and will be included in the intention-to-treat statistical study, but will not be included in the statistical analysis per protocol and may be substituted by new patients.

If patients withdraw prematurely from the study, or if the investigator decides to withdraw the patient after treatment, the utmost must be done to ensure completion and notification of all observations until the time of withdrawal. A final assessment of the patient by the investigator must be performed at the time of withdrawal in accordance as indicated by the site and an explanation provided for the reason why the patient withdraws or is withdrawn from the study. All efforts must be made to complete the final visit post-treatment and the follow-up visit.

Whichever the case, the reason for and date of withdrawal from the study must be recorded in the source documents and the eCRF.

If a patient wishes to withdraw prematurely from the study after treatment, without specifically withdrawing her informed consent, this does not mean that she no longer wishes to provide the trial Sponsor with information about the clinical results (for example, supplying information about safety, adverse events that could be relevant, information about her gestation or the pregnancy outcome, etc.) during the follow-up period (until Month 15). This will allow us to continue compiling clinical information about patients who no longer actively participate in the study and do not withdraw their informed consent, and to include them in the analysis of all the clinical variables.

Patients may waive providing all or part of that information at any time, or definitively withdraw their specific informed consent at any time during the study.

All patients who, having received the study treatment, decide to end/interrupt their continuity in the study for the reasons described below, will remain in the follow-up phase, except for those patients who withdraw their informed consent, die or are lost to follow-up.

A patient may withdraw or be withdrawn from the study for the following reasons:

1. Withdrawal of informed consent by the patient or the wish to end treatment or if failing to cooperate.
2. Toxicity, adverse event or intercurrent illness which, in the investigator's opinion, justifies withdrawing the patient from the study.
3. Decision by the Principal Investigator, if interrupting the study is in the patient's interests.
4. Request by the Sponsor to withdraw the patient.
5. Death of the patient, disease progression or any other serious adverse event (SAE) that affects the patient's safety.

6. Appearance of any exclusion criteria that is clinically relevant and that affects the patient's safety.
7. Significant deviation or non-compliance with the protocol, in the opinion of the Principal Investigator, that justifies withdrawing the patient from the study.
8. Lost to follow up.

7.5 Duration of the recruitment period

The duration of the recruitment period will be 8 months from the start of the CT.

8. Treatment of subjects

8.1 Description, supply and manufacturing of the drug

IGX1 is a product based on non-expanded bone marrow stem/progenitor cells (CD133+ cells) screened following Peripheral Blood Progenitor Cell (PBPC) mobilization and collection and posterior apheresis and transplantation of these cells in the patient. The aim being the de novo regeneration of the endometrium of patients with physiopathologies such as Asherman's Syndrome, which are associated with diminished endometrial stem cells.

The autologous stem cells are firstly obtained from bone marrow through prior mobilization and collection of Peripheral Blood Progenitor Cells (PBPC) via apheresis. The obtained concentrate is then sent to the processing center where it is stored and kept until the start of the selection and purification process of the CD133+ cells for ulterior intrauterine transplantation in the patient within the first 24 hours in order to renew the patient's endometrial tissue.

IGX1 will be made at the processing center "Banc de Sang i Teixits" (BST), in a clean room in compliance with current regulations, instructions and procedures for production as provided by the Sponsor. The CliniMACS® System will be used for preparation. The CD133+ cell concentrate obtained will be diluted along with PBS/EDTA buffer to obtain a final volume of 30-50 mL which will be pre-loaded in a sterile, disposable syringe for direct intra-arterial use.

Quality control tests will be performed throughout the process and on the final product. Process samples will be taken which will permit safety tests and bioassays to be conducted in accordance with the manufacturer SOPs. Those results, both preliminary and final, will be appropriately documented and will serve as the criteria for release of the product IGX1.

IGX1 will be packed and labeled according to the manufacturer's procedures.

Shipment of the plasma bag and the white fraction of the blood, obtained after apheresis from the clinical site to the processing center, and the drug once the manufacturing process has taken place, from the processing center to the clinical site, will be carried out in accordance with the standard clinical practice between both centers, in accordance with the BST SOPs. Shipping will be in an isothermal container (between 2 and 8ºC) with temperature log via TempTale of those normally used for transport of this kind of biological material and which permits ensuring the integrity and contents thereof throughout the process.

The Sponsor will control the entire shipping and reception process in order to guarantee the aforementioned shipping conditions. These checks will be documented in a special form in accordance with the Sponsor SOPs. Acknowledgment of receipt of each delivery by the investigator staff will be issued.

The entire manufacturing, quality, custody, shipping and labeling process of the product will be carried out in accordance with the procedure described in the manufacturer SOPs (BST or processing center SOPs).

At the time the batch is released, some safety tests and bioassays may not be yet available. In this case, in accordance with the manufacturer SOPs, a preliminary and final analysis certificate will be issued once all the results are available, along with a batch release statement.

Table with quality tests (safety + bioassays)????

The product will be considered suitable for infusion provided that the following minimum quality criteria are met:

Dose to infuse higher or equal to 30 million CD133+ cells.

Cell viability higher or equal to 50%.

Pureness higher or equal to 70%.

Sterility.

If any of the results of one or more of these safety tests and/or bioassays do not meet the specifications, a deviation form will be completed and an investigation carried out to permit implementation of the relevant corrective actions in order to avoid incident recurring. A copy of this report will be filed with the batch records.

If any kind of incident related to product quality is detected, if the results are confirmed, this must be notified immediately to the clinical site, the CRA and the Sponsor. The Sponsor will inform the local regulatory authority if applicable.

In cases where a quantity higher than 35 million CD133+ cells is isolated, an aliquot of 3-5 million cells will be prepared which will be used in an animal Asherman model (preclinical studies). The preparation of the aliquot must never compromise the patient's participation in the study, and therefore in cases with less than 35 million isolated CD133+ cells, all the cells will be used for the clinical trial and consequently no aliquot will be made.

The samples divided into aliquots will be prepared in a 2 to 15 mL tube, identified with a process code and other identification specifying that they are cells for preclinical study. The samples will be delivered with a document containing a description of the concentrate and the volume of the samples, as well as the sample type, the name of the recipient at HVH, location and contact method. The cells will be transported using a refrigerated system at 2-8ºC inside a second safety container. The person in charge of receiving the samples for preclinical studies is Julia Vallve, an employee at *Vall Hebron Institut de Recerca* (VHIR) who will be in charge of freezing and subsequent custody of the cells. These cells will be used for studies in animal models that will be performed concomitantly to the clinical trial so that there are no frozen cells at the end of the clinical trial (ENTIRE).

8.2 Description of the administration dose, interval, route, and regimen

IGX1 will be supplied to the site in pre-filled, sterile syringes containing 30-50 mL. (depending on the concentrate volume obtained from each patient), for single use, with a single dose equal to or higher than 30 million CD133+ cells with cell viability equal to or higher than 50%, pureness equal to or higher than

70%, sterile and which must be administered intra-arterially through the intrauterine artery at Visit 4 within the first 24 hours after it is obtained via the apheresis process for each patient.

This drug will be labeled in accordance with the requirements of current legislation (see Appendix 21.3) with the batch being the donation code (each patient equivalent to her own batch) and the selection and instillation day being the expiry date.

8.3 Criteria for changing treatment regimen during the trial

If any of the aforementioned required specifications are not met for instillation of the drug, the production center, along with the site medical staff and the Sponsor will assess whether or not to continue with the instillation process in the patient.

If the investigator staff finally decides not to infuse the product, this must be discarded and the patient will be considered to be excluded from the study. All this information, along with the reasons behind the decision not to infuse must be recorded in the patient's source documents and in the CRF. The investigator staff must ensure the safety and well-being of the patient through to resolution of any adverse events that could still be ongoing.

If the investigator staff decides to infuse the product even if it does not meet the aforementioned criteria, the patient will be considered non-evaluable (excluded). Nevertheless, recording and follow-up of the patient will continue in the same way as for enrolled, evaluable patients. The investigator staff must ensure the safety and well-being of the patient throughout the entire study, and beyond if necessary until resolution of any uncontrolled relevant episodes that could still be ongoing.

8.4 Concomitant and supportive treatment

All prior or concomitant treatment taken or administered during the 30 days prior to patient screening until the end of the treatment period will be recorded in the patient's medical history and in the CRF. The generic or commercial name, indications and dose will be recorded. The Sponsor will be in charge of coding this in accordance with the World Health Organization medical dictionary and terminology.

At each visit the investigator or personnel assigned to this task will ask patients if they have taken any concomitant medication, other than the drugs that are not stipulated in the study (also including over-the-counter medicines, vitamins or other dietary supplements or herbal medicines).

All patients will receive the following concomitant medication during the study:

- Filgastrim or C-CSF (Neupogen®).
- Hormone Replacement Treatment with Estradiol Valerate (Progynova®, Evopad® or Estradot®) and Natural Micronized Progesterone (Progeffik® or Utrogestan®).
- Hyaluronic Acid (Hyalubarrier®).

Neupogen® will be administered subcutaneously at a dose of 10 µg/Kg, every day for a total of 5 days in order to mobilize the stem cell population (including CD133+ cells which will be selected) via peripheral blood for subsequent collection via apheresis. On the fourth day mobilization of the CD34+ cells will be checked via blood tests to rule out failure of the effects of Neupogen®.

Patients will have previously been administered **Hormone Replacement Therapy (HRT)** in order to prepare the endometrium for the ERA test and the hysteroscopy at the time of the assumed implantation window. The following will be administered for this purpose:

Estradiol Valerate 6 mg/day orally (Progynova®) or Estradiol Valerate transdermally at a dose of 150 mg/48h (Evopad 75 mg® or Estradot 75 mg®) or for a minimum of 10 days and a maximum of 4 weeks;

Natural Micronized Progesterone will be added (Progeffik® or Utrogestan®) vaginally at a dose of 400mg/12h for a total of 5 days or 10 doses before performing the hysteroscopy and biopsy for ERA.

Immediately after hysteroscopic adhesiolysis, 10 cc of **hyaluronic acid (Hyalubarrier®)** will be administered with the aim of preventing the formation of new adhesions as per standard clinical practice.

Then for the ART the same endometrial preparation as for the ERA biopsies will be carried out and the Progesterone days will be within the implantation window.

No medication is not permitted. Nevertheless, the use of non-steroid anti-inflammatory drugs (NSAIDs) is not recommended from the day of the diagnostic hysteroscopy (Visit 2) to the day of β–hCG after assisted reproduction treatment owing to the possible side effects. Likewise, if the β–hCG result is positive, administering NSAIDs is not recommended either unless, under medical criteria, it is absolutely necessary due to the contraindications thereof during pregnancy.

No other investigational drugs should be administered unless approved by the Sponsor.

9. Statistical Analysis Plan

9.1 Population

Patients between 18 and 45 years old diagnosed with Asherman's Syndrome grade II, III or IV who intend to undergo ART with their own or donated oocytes and transfer of two embryos (DET) frozen on Day 5/6 of development. These patients must have normal hepatic and renal functions, and there must be an absence of serious serological infections and heart, coagulation and psychiatric disorders.

In this study, four population sets have been defined.

- Intention-to-treat set: All of the patients enrolled in the study, including screening failures with/without apheresis performed.
- Safety set: All of the patients enrolled in the study for whom the product IGX1 has been instilled, regardless of whether they have received ART or not.
- Population per-procedure (or cycle) start: All of the patients enrolled in the study, who have the IGX1 product instilled and begin ART, regardless of whether embryo transfer is performed or not.
- Per Protocol set: This includes all patients enrolled in the study who meet all selection criteria and complete all study phases through to the end, including embryo transfer. During the study period, there must be no major protocol deviations, loss to follow-up, or discontinuation from the study.

In the ITT (intention-to-treat), safety, efficacy, and procedure-initiation populations, the live birth rate (LBR) objective will still be evaluated. However, since some patients may not proceed to embryo transfer, there is likely to be a loss of statistical power in these populations.

9.2 Sample size

A total of 22 patients will be included.
Our hypothesis is that stem cell transplantation into the uterine cavity improves the live birth rate (LBR) as the primary dependent variable and endometrial thickness as the secondary dependent variable.

The study will use a historical control group, which may consist of the same patients who underwent ART prior to treatment or historical patients with similar clinical characteristics (matched cohort "Before and After" study).

We assume that all patients will undergo single embryo transfer (SET) on day 5/6 of embryo development.

IMPROVEMENT IN LIVE BIRTH RATE
Our hypothesis is that the improvement will involve an increase from 0% in the control group to 28% in the treatment group (which corresponds to the average LBR published for ART according to the European IVF-Monitoring Consortium (EIM) et al., *Hum Reprod* 2016). It should be noted that this rate is calculated based on the total number of completed cycles (embryo transfers).

Calculations of the Live Birth Rate (LBR) as a binary variable (the sample size will refer to the number of embryos transferred):

Proportion (%) of events in:
Group 0 = 0.00%
Group 1 = 28.00%

Minimum expected effect size:
Difference = 28.00%
Alpha Risk = 5%
Dropouts: 20%

[Endpoint]	
Test significance level, a	0.05
1 or 2 sided test	1
Proportion difference, m_1 - m_2	0-28%
Power (%)	80
n per group	18
dropouts	20%
n per group considering dropouts	22

To determine how many patients the 22 embryo transfers (ET) correspond to, we calculate that 50% will need 1 ET = 11 patients. Of the remaining 11 (who need a second ET), we expect that 70% will become pregnant = 7. These calculations are made considering that all patients will have at least 2 embryos and assuming that SET will be performed.

Thus, we would need 18 patients in the study by protocol (with ET done), which we expect will become pregnant after 2 ET.
Therefore, the number of patients to treat by protocol would be 18.

To calculate the total sample size by intention to treat (the total number of patients who start the study) and who do not reach ET, we must consider possible losses for various reasons before reaching the ART phase (withdrawal, loss of information, complications in the treatment with Neupogen®, in apheresis, or in the IGX1 instillation, etc.). Since it is a small, highly controlled population and the expectation for treatment is high, we estimate a 20% dropout rate. Therefore, the number of patients by intention to treat is 18 + 3.6 = 22 patients in total.

9.3 Study and database variables

Primary variables

Main Variables

For the primary objective:

- Adverse events (list) during each medical review/visit from IGX1 instillation until 1 month post-live birth (if applicable).
- Time elapsed between IGX1 instillation and the occurrence of adverse event(s) (in days).
- Hospitalization due to adverse event: YES/NO.
- Treatment required for adverse event (list).
- Each of the control blood analyses performed during the visits (quantitative variables).
- Apgar score, categorical variable.
- Adverse events in the live newborn (list).

For secondary objectives:

- Pregnancy: YES/NO, categorical variable.
- Implantation: YES/NO, categorical variable.
- Ongoing pregnancy: YES/NO, categorical variable.
- Live Birth (LB): YES/NO, categorical variable.
- Hysteroscopic score according to ESH/ESGE (numerical variable) before and after treatment.

- Endometrial thickness in mm during each visit (numerical variable) before and after treatment and at each visit.
- Endometrial pattern (trilaminar/diffuse), categorical variable before and after treatment and at each visit.
- Pregnancy outcome (categorical list: ongoing pregnancy, clinical miscarriage, biochemical miscarriage, ectopic pregnancy).
- Measurement of endometrial volume via 3D ultrasound before and after treatment (numerical variable).
- Analysis of endometrial vascularization measured by immunohistochemistry through the presence of α-SMA expression before and after treatment and at each visit.
- Frequency of menstrual episodes in days (numerical variable) before and during 6 consecutive months after treatment, if applicable (if pregnancy occurs before this period, measurements will not be performed).
- Duration of menstrual episodes (numerical variable) before and during 6 consecutive months after cell instillation.
- Number of sanitary pads/day before and after treatment (numerical variable) before and during 6 consecutive months.
- Number of CD133+ cells, explanatory variable (quantitative).
- Evolution of quality of life before and after treatment (quantitative and qualitative variables as described in Appendix 20.4).

For exploratory objectives:

- Endometrial receptivity diagnosed by ERA: Receptive/Non-Receptive (categorical variable) before and after treatment. Within Non-Receptive patients, the following subcategories may be identified:
    - F: Proliferative phase.
    - PREd2: Pre-Receptive day 2 (48h to achieve receptivity).
    - PREd1: Pre-Receptive day 1 (24h to achieve receptivity).
    - PREt: Late Pre-Receptive (12h to achieve receptivity).
    - R: Receptive (moment of receptivity).
    - eT: Early Post-Receptive (12h after achieving receptivity).
    - T: Post-Receptive (more than 12h after achieving receptivity).
- One variable for each gene found in the ERA test in the endometrial biopsy and endometrial fluid before and after treatment.

- One variable for each microorganism found in the biopsy and endometrial fluid before and after treatment.
- Microbiome found in biopsy and endometrial fluid (LD/NLD) before and after treatment:

Presence of bacterial microbiome; identified bacteria will be classified into two groups:

  - Group 1 (LD): Lactobacillus-dominant microbiome (>90% Lactobacillus spp.), composed of various species within the Lactobacillus genus: *L. crispatus, L. gasseri, L. iners, L. jensenii*, among others.
  - Group 2 (NLD): Non-Lactobacillus-dominant microbiome (<90% Lactobacillus spp. with 10% dysbiotic bacteria), composed of species that alter physiological conditions in the endometrium, thereby reducing the prevalence of Lactobacillus.

- Histological findings of endometrial biopsies before and after treatment (categorical variable: normal/abnormal).
- Surface markers VEGFR (Vascular Endothelial Growth Factor Receptor), CD38+, and others.

Control Variables: will be used to adjust for the main variables in the study.

- Number of injected CD133+ cells (numerical variable), greater than 30 million cells.
- Percentage of cell viability (numerical variable), equal to or greater than 50%.
- Percentage of cell purity (numerical variable), equal to or greater than 70%.
- Baseline blood analyses (pre-treatment), quantitative variables.
- Details of HRT for biopsy collection for baseline ERA and post-treatment ERA: Days of estradiol administration, progesterone concentration (P4) on the day P4 supplementation begins, time of P4 initiation, and time of biopsy collection (numerical variables).
- Biopsy collection day (baseline and post-treatment), categorical variable relative to the day of progesterone administration (P+4, P+5, P+6, P+7).

Descriptive Variables: Used to assess population homogeneity and avoid potential bias by evaluating distribution across study groups or identifying factors influencing outcomes:

- Age (numerical variable: 18–44).
- Body Mass Index (BMI), numerical variable: 18.5–30.
- Ethnicity/Race (categorical variable).
- Obstetric history: numerical variables for obstetric background: pregnancies, deliveries, cesarean sections, miscarriages, curettages.
- ART indication (list), categorical variable.
- Number of previous implantation failures (IF), numerical variable.

- Baseline FSH, numerical variable.
- Baseline AMH, numerical variable.
- Sperm concentration, numerical variable.
- Treatment type (IVF/ICSI, egg donation), categorical variable.
- Cycle type (vitrified/fresh oocytes), categorical variable.
- Details of HRT for biopsy collection for ERA: Days of estradiol administration, progesterone concentration (P4) on the day P4 supplementation begins, time of P4 initiation, and time of biopsy collection (numerical variables).
- Biopsy collection day (baseline and post-treatment), categorical variable relative to the day of progesterone administration (P+4, P+5, P+6, P+7).
- Ovarian stimulation data: type of stimulation (long or short protocol), stimulation days, estradiol (E2) levels on the hCG day, numerical variables. Dosage of follicle-stimulating hormones (FSH, LH, HMG).
- Antral Follicle Count (AFC), numerical variable.
- Number of metaphase II (MII) oocytes, numerical variable.
- Number of fertilized oocytes (to calculate the fertilization rate), numerical variable.
- Details of HRT for embryo transfer: Days of estradiol administration, progesterone concentration (P4) on the day P4 supplementation begins, time of P4 initiation, and time of embryo transfer, numerical variables.
- Embryo transfer day (Day 5/6), categorical variable.
- Number of embryos transferred, numerical variable.
- Embryo quality based on the morphology of transferred embryos, categorical variable (A, B, C, D).
- Preimplantation Genetic Testing (PGT): YES/NO, categorical variable.
- Number of vitrified embryos, numerical variable.
- Number of implanted sacs, numerical variable.

Outcome Variables:

The implantation rate will be defined as the proportion of gestational sacs observed via vaginal ultrasound at week 6, relative to the number of embryos transferred. The clinical pregnancy rate per cycle will be defined as the percentage of patients with a gestational sac relative to the total number of cycles performed. The clinical pregnancy rate per transfer will be defined as the percentage of patients with a gestational sac relative to the total number of embryo transfers performed. Ongoing pregnancies will be considered those that exceed 20 weeks of gestation. The information for these variables will be stored on an electronic platform (CRDe) that can be easily converted later to R and/or SPSS files for statistical analysis.

9.4 Statistical analysis

As previously indicated, a database will be made covering all the aforementioned variables by patient. The data will be exported to the eCRF and statistical analysis will be performed using the SPSS and/or R software as required.

Descriptive statistics:

The means, proportions and corresponding confidence intervals of the descriptive variables of the patient population enrolled in the study will be calculated, such as patient age, body mass index, used stimulation protocol, vitrification protocol and other variables in order to check if it is a homogeneous population. If it is considered insufficient, a logistic regression analysis will be considered including all the significant confusion factors in order to assess the effects sought in the study.

Analytical statistics:

a) Intention-to-treat analysis: All patients recruited into the study will be included.
b) The safety analysis of the study will be determined by the number and severity of adverse events (AEs) recorded in the CRDe as specified in Section 12 for all patients undergoing IGX1 cell instillation.
c) The efficacy analysis will include morphological and functional changes in the endometrium after cell instillation, as described in Section 11 (efficacy evaluation). It will also include analyses by cycle or by procedure initiation (calculated for all patients undergoing ART, regardless of whether embryo transfer occurs) and protocol analysis (calculated only for patients who underwent embryo transfer).
d) For the primary and secondary objectives, means, proportions, and their corresponding confidence intervals for the main variables will be compared with baseline values (before treatment) using Student's T-test and Chi-square test or Fisher's exact test for quantitative and categorical variables, respectively.
e) The Relative Risk (RR), Attributable Risk (AR), and Attributable Fraction (AF) will be calculated for implantation, pregnancy, and ongoing pregnancy rates of patients after treatment compared to before treatment, to establish whether the observed differences are attributable to the study treatment.
f) The ROC (Receiver Operating Characteristic) curve will be calculated as a graphical representation of sensitivity versus specificity for a binary classification system, varying the cutoff point of the explanatory variable under study.

In general, the following AUC (Area Under the Curve) intervals will be used to determine the test's quality:
[0.5, 0.6): Poor test.

[0.6, 0.75): Fair test.

[0.75, 0.9): Good test.

[0.9, 0.97): Very good test.

[0.97, 1): Excellent test.

The statistical significance will be delimited at p-value <0.05.

All statistical analyses and study procedures will be documented in a detailed Statistical Analysis Plan (SAP) that will be developed throughout the study and finalized before data analysis begins.

9.5 Interim analysis and guidelines for safety and tolerability evaluation

The data obtained in this study will be reviewed by an Independent Data Monitoring Committee (IDMC). This committee will evaluate the accumulated data after 11 patients have entered the study and been assessed 30 days after treatment with IGX1. During this review, the IDMC will monitor all study data collected, paying special attention to safety and tolerability regarding the investigational product IGX1. The quality of the recorded data, all adverse events reported to date, and the study's progress in relation to the initially proposed objectives will be assessed. The committee may make recommendations to the sponsor regarding the continuation, premature suspension, modification, or termination of the current study. Additionally, the committee's recommendations will be considered for the design of future related studies.

10. Planning

Phase 1: Selection and evaluation

Phase 2: Hematological evaluation, collection, initiation, and treatment with Neupogen®

Phase 3: Endometrial diagnosis and cellular mobilization

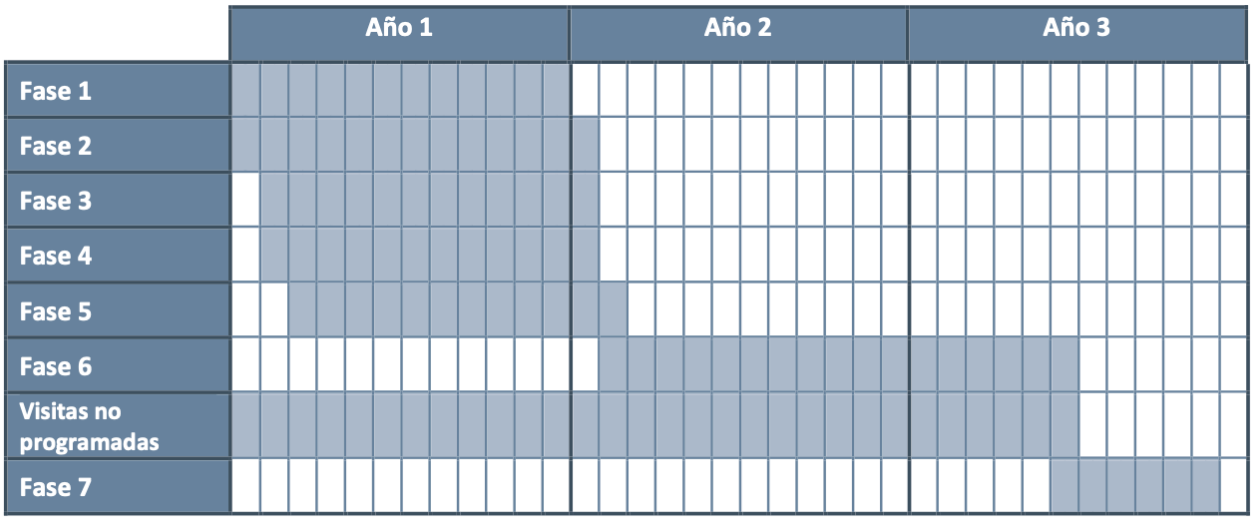
Phase 4: Collection, selection, and cellular instillation

Phase 5: Post-treatment endometrial diagnosis / Post-treatment final visit (PTFV)

Phase 6: Follow-up / ART / Final study visit (FSV)

Unscheduled visits

Phase 7: Results analysis. Conclusions. Final report



11. Efficacy evaluation

The efficacy of the intervention can be assessed based on the outcomes of the objectives we have set, as follows:

1. Morphological and functional changes in the endometrium, measured by:

- Improvement in the hysteroscopic score 28 days after treatment with stem cells. This evaluation will be conducted using the ESHRE/ESGE scale. Improvement in the hysteroscopic score according to these scales will be assessed.
- Improvement in endometrial thickness and pattern via ultrasound 28 days after treatment with stem cells. A minimum improvement of 20% in endometrial thickness is expected, along with an improvement in endometrial regularity and pattern (it should be regular and secretory).
- Recovery of endometrial volume measured by 3D ultrasound 28 days after treatment with stem cells. An increase of at least 25% in endometrial volume is expected.
- Analysis of endometrial vascularization through endometrial biopsy pre- and post-treatment, conducted 28 days after stem cell treatment. An increase in the number of vessels, measured via immunofluorescence using the Alpha-ASMA marker, is expected.
- Evaluation of the reappearance of menstrual episodes (if absent before treatment) or a comparison of differences in their frequency, duration (in days), and quantity (number of pads used) after treatment with embryonic stem cells, up until embryo transfer, in relation to the menstrual episodes prior to treatment.
- Diagnosis of alterations in the window of implantation (WOI) measured by the ERA test 28 days before and after treatment with stem cells.

2. ART outcomes, measured by:
    - Implantation rate (IR), calculated as the number of implanted embryos relative to the number of embryos transferred.
    - Pregnancy rate (PR).
    - Ongoing pregnancy rate (OPR) per transfer performed, from embryo transfer to delivery.
3. Gestational follow-up, evaluated by:
    - Live birth rate (LBR).
    - Miscarriage rate (clinical and biochemical).
    - Ectopic pregnancy rate, from embryo transfer to delivery.

As defined in the statistical analysis section, means, proportions, and their corresponding confidence intervals for the variables explained above will be compared with baseline values (prior to treatment) using Student's T-test and Chi-square test, or Fisher’s exact test for quantitative and categorical variables, respectively.

Note: As explained in the population and statistical analysis sections, all calculations will be based on the number of transfers performed to determine rates per transfer (protocol or transfer analysis); in patients who initiate ART, whether or not they reach embryo transfer (procedure initiation or cycle analysis); in patients undergoing cell instillation, regardless of whether or not they undergo ART (safety and efficacy analysis); and in all patients recruited into the study, whether or not they undergo cell instillation and apheresis.

12. Safety evaluation

12.1 Safety parameters and definitions

According to Good Clinical Practice (GCP) guidelines by the ICH, an adverse event (AE) is any unfavorable medical occurrence experienced by a patient or a person included in a clinical research study who has received a pharmaceutical product, regardless of the causal relationship. Therefore, an adverse event may include:

- Any unfavorable and unexpected sign (including, for example, abnormal laboratory test results), symptom, or disease temporally associated with the use of a drug, regardless of its causal relationship to the medicinal product.
- Any new disease or exacerbation of an existing disease (worsening in nature, frequency, or intensity of a known condition).
- Recurrence of an intermittent condition (e.g., headache) that was not present at baseline.
- Any deterioration in a laboratory value or other clinical test (e.g., ECG, X-ray) accompanied by symptoms or leading to an adjustment of the study treatment, administration of concomitant treatment, or discontinuation of the study drug.
- Adverse events related to an intervention required by the protocol, including those performed before treatment allocation in the study (e.g., invasive procedures during screening, such as biopsies).

Any adverse event that occurs will be recorded in the Case Report Form (CRF) for each patient as well as in their medical record.

Identification of adverse events

At all interim points of the assessment (study visits, telephone calls, etc.) a uniform methodology will be used to ask patients about AEs without suggesting any responses. The following are examples of open questions:

How have you been since your last visit?

Have you had any new health problems or noticed any changes since your last visit?

Assessment of severity and causality of adverse events

The investigators will request information about AEs and serious adverse events (SAEs) whenever they contact the patient. All AEs and SAEs reported by the patient or authorized study staff will be documented in the patient's medical history and on the relevant AE page of the CRF.

The investigator will evaluate the severity, intensity (initial and maximum), and causality of each AE and SAE recorded in the CRF. The terms "intense" and "severe" are not synonymous. **Intensity** refers to the magnitude of the AE (e.g., mild, moderate, or intense pain); the event may have relatively low medical significance (e.g., intense headache). **Severe** is a regulatory definition based on the outcome of the patient

or the event or on action criteria typically associated with events that pose a life-threatening risk or vital function impairment to the patient. **Severity** (not intensity) is the guiding criterion used to define reporting obligations to health authorities.

Intensity and severity should be evaluated independently when recording AEs and SAEs in the CRF.

Table 2 provides instructions for assessing AE intensity, while **Table 3** provides instructions for assessing causal relationships with investigational products.

Severity	Description
Mild	Presence of discomfort, although normal daily activity is not affected.
Moderate	Sufficient discomfort to reduce or interfere with normal daily activity.
Severe	Disabling (unable to work or perform normal daily activity).

Table 2. Intensity of Adverse Events (AEs)

To ensure consistency in causality assessments, investigators must apply the following general guidelines:

Is the AE/SAE suspected to be caused by the investigational product based on facts, evidence, scientific data, and clinical judgment?	
YES	The temporal relationship between the AE/SAE and the administration of the investigational product makes the existence of a causal relationship possible, AND other medications, therapeutic interventions, or underlying conditions do not sufficiently explain the AE/SAE.
NO	The temporal relationship between the AE/SAE and the administration of the investigational product makes the existence of a causal relationship unlikely, OR other medications, therapeutic interventions, or underlying conditions sufficiently explain the AE/SAE.

Table 3. Causality of AEs

Assessment of causality

The investigator must make every effort to explain each adverse event and assess its relationship, if any, with the investigational drug treatment. Causality should be assessed using the following categories:

- **Definitive:** The adverse event follows a reasonable temporal sequence from the time of drug administration, aligns with a known response pattern of the investigational drug, and cannot reasonably be explained by other factors such as the subject's clinical condition or other therapeutic interventions or concomitant medications administered to the subject. Additionally, one or more of the following apply:
  a) The event occurs immediately after drug administration.

b) The event improves upon discontinuation of the drug.
c) The event reappears upon reintroduction of the drug.

- **Probable:** The adverse event follows a reasonable temporal sequence from the time of drug administration, aligns with a known response pattern of the investigational drug, and cannot reasonably be explained by other factors such as the subject's clinical condition or other therapeutic interventions or concomitant medications administered to the subject.
- **Possible:** The adverse event follows a reasonable temporal sequence from the time of drug administration and/or aligns with a known response pattern of the investigational drug, although it could also be caused by other factors such as the subject's clinical condition, other therapeutic interventions, or concomitant medications administered to the subject.
- **Unlikely:** The adverse event is more likely caused by other factors, such as the subject's clinical condition, therapeutic interventions, or concomitant medications administered to the subject, and does not align with a known response pattern of the investigational drug.
- **Unrelated:** The adverse event is clearly related to other factors, such as the subject's clinical condition, therapeutic interventions, or concomitant medications administered to the subject.
- Unknown Relationship

All adverse events classified by the investigator outside the group of unrelated to the drug will be classified as adverse drug reactions.

Serious Adverse Event

An SAE is an adverse event that results in one of the following outcomes:

- It is fatal (i.e., the adverse event contributes to or directly causes death) or life-threatening (i.e., the investigator determines that the adverse event poses an immediate risk of death to the patient).
- Requires hospitalization or the prolongation of an existing hospitalization, unless the hospitalization is for:
  • Routine treatment or monitoring of the studied indication, not associated with any disease deterioration.
  • Elective or previously planned treatment for a pre-existing condition unrelated to the studied indication and that has not worsened since the start of the investigational drug.
  • Emergency outpatient treatment for an event that does not meet any of the SAE definitions described above and does not result in hospital admission.
  • Social reasons or residential care in the absence of any deterioration in the patient's overall condition.
- Causes persistent or significant disability/incapacity (i.e., the adverse event results in substantial impairment of the patient's ability to perform daily life functions).

- Results in a congenital anomaly or birth defect in a neonate/infant whose mother has been exposed to the investigational product.
- The investigator considers it to be a medically significant event (e.g., one that may endanger the patient or require medical or surgical intervention to prevent one of the outcomes listed above).

All adverse events that do not meet any of the criteria for severity will be considered non-serious adverse events.

It is the responsibility of the investigator to document all adverse events in the CRF, whether observed by them or spontaneously reported by the study participant, regardless of their relationship with the treatments.

The adverse event reporting period begins upon signing the informed consent form. Each subject will be asked about the presence of new adverse events or the progression of pre-existing ones.

The following information related to the adverse event will be collected:

- Description.
- Duration and resolution: start and end dates.
- Maximum intensity.
- Causal Relationship with Study Treatment(s)
- Maximum severity.
- Actions taken and outcome.

Adverse Reaction (AR)

Any harmful and unintended response to an investigational drug, regardless of the administered dose.

Unexpected Adverse Reaction or Unexpected Adverse Event

An unexpected adverse event is any adverse reaction to a drug whose nature or severity does not match those described in the current Investigator's Brochure (or in the drug's Summary of Product Characteristics, if already marketed). Additionally, reports that provide significant new information about the specificity or intensity of a known and documented adverse event constitute unexpected adverse events. For example, an event that is more specific or severe than what is described in the Investigator's Brochure should be classified as "unexpected."

12.2 Safety plan

Recording of adverse events

During the period following the acquisition of informed consent and before the initiation of study treatment, only serious adverse events (SAEs) resulting from a protocol-required intervention (e.g., SAEs related to invasive procedures such as biopsies) should be collected.

During the period from the administration of the first dose of the study treatment to the final follow-up visit, the investigator or medically qualified site staff must collect all adverse events, regardless of severity, causality, or actions taken. At each visit, the investigator or medically qualified site staff will ask the patient if they have experienced any adverse events since the last visit. The investigator is responsible for documenting all adverse events in the patient's original medical records and in the CRF.

Adverse Evento Reporting Period

After obtaining informed consent but before the administration of study drugs, only SAEs caused by a protocol-required intervention (e.g., SAEs related to invasive procedures such as biopsies, medication washout, or pre-inclusion without treatment) will be collected.

Once the administration of study drugs has begun, all adverse events (AEs) and serious adverse events (SAEs), regardless of their attributed cause, will be documented until 30 days after the last administration of the study treatment or until study termination/cancellation, whichever occurs later.

After this period, the investigators should only report SAEs if they are considered to be related to the study treatment.

Reporting of serious adverse events

Any SAE or laboratory abnormality that occurs during the study, regardless of the treatment received by the patient, must be reported to the sponsor immediately (within a maximum of 24 hours from awareness).

Additionally, the sponsor will be responsible for notifying Serious and Unexpected Suspected Adverse Reactions (SUSARs) to the Health Authorities, the Research Ethics Committee (CEIm), and investigators, in accordance with Article 51 of Chapter XII of Royal Decree 1090/2015, dated December 4, 2015.

The minimum information to include in the initial SAE notification is as follows: patient identification, AE identification, start date, reason for considering the event serious, and the name of the person initially reporting the event.

Any additional information about a previously reported SAE must also be submitted using the corresponding form, in compliance with the sponsor's Standard Operating Procedure (SOP) (or that of the subcontracted CRO, if applicable), as soon as possible.

Immediate reporting of the SAE using the form is supplementary to documenting these data in the CRF.

Management and Follow-Up of Adverse Events

Adverse events (AEs), particularly those whose relationship with the investigational drug cannot be classified as "unrelated," must be followed up until they return to baseline or stabilize. If a clear explanation is established, it should be documented in the CRF. Reports on the subject's progress must be submitted to the clinical trial monitor.

Laboratory Test Abnormalities

Laboratory test results will be recorded in the corresponding section of the CRF. Abnormal laboratory test values will not be documented as adverse events unless the abnormality is deemed clinically significant by the investigator or if:

- It is associated with a clinical condition in the patient requiring treatment.
- It leads to a modification of concomitant treatment.
- It results in the interruption or delay of study treatment or a change in the dosage of one or more study drugs.
- It qualifies as an SAE.
- The patient is permanently withdrawn from the investigational drug due to the abnormal test value.

Adverse events related to the study medication and/or procedures

The expected adverse events (side effects) in this study are listed as follows:

• **Expected Adverse Events for IGX1:**

These are detailed in the IMPD and the Investigator's Brochure.

• **Adverse Events Related to Hysteroscopy:**

Hysteroscopy is an endoscopic technique that allows visualization of the uterine cavity through a small-caliber optical instrument and enables the treatment of certain intracavitary uterine pathologies, such as Asherman's Syndrome, by releasing intrauterine adhesions.

This surgical procedure is typically performed in an operating room under local anesthesia or sedation.

The most common discomforts that may arise from this surgery include mild abdominal pain similar to menstrual cramps, shoulder pain due to phrenic nerve irritation, and mild vaginal discharge. Complications associated with this procedure are rare but may include, without being limited to, the following:

- Intraoperative complications: Uterine perforation, circulatory overload, and, in rare cases, acute pulmonary edema, electrical accidents, anesthetic accidents, and even the need for emergency laparoscopic or laparotomic surgery.
- Postoperative complications: Vaginal bleeding, gynecological infection, cervical stenosis, hematometra, and adenomyosis.

The specificities of this technique may necessitate suspending an intervention before completion or performing an emergency laparoscopy or laparotomy, requiring unscheduled surgery.

Absolute success cannot be guaranteed, and recurrence of the endometrial pathology cannot be ruled out.

• **Adverse Events Related to Angiography and Catheter Insertion (Catheterization):**
The angiography procedure involves inserting a small tube called a catheter into a blood vessel, in this case an artery, followed by the administration of contrast material that allows visualization of the uterine arteries via X-rays. Finally, previously isolated cells are instilled under fluoroscopic control.

In general, this is a safe procedure performed by qualified professionals in facilities equipped with the necessary infrastructure to ensure appropriate patient care at all times. However, certain complications may occur, such as:

- Blood accumulation (hematoma) at the catheter insertion site.
- The introduction of contrast material into the circulatory system may cause allergic reactions or worsen kidney function. For this reason, a “non-ionic” contrast agent will be used, as it has fewer side effects.
- On rare occasions, very severe reactions have been described, which can potentially lead to the patient's death. These events are extremely rare, with an approximate incidence of 1 in 100,000 procedures.

Adverse events related to the apheresis:

Apheresis of progenitor cells is a procedure that allows for the collection of blood cells necessary for transplantation, returning the rest of the blood components to the donor.

Before initiating apheresis, blood samples are collected to screen for certain transmissible diseases. The donor receives prior treatment to mobilize progenitor cells from the bone marrow into the peripheral blood. The ability to mobilize cells varies from person to person, and it may be necessary to repeat the apheresis procedure the following day.

The apheresis process involves connecting the donor or patient via venous access (two sites) or a central catheter to a cell separator machine using a sterile collection system of bags and tubes. The blood flows into the cell separator, where it is processed, and mononuclear cells, including progenitor cells for transplantation, are collected. The remaining blood components are returned to the donor. The procedure takes between 3 and 5 hours, depending on the number of progenitor cells mobilized.

Throughout the process, nursing staff remain with the patient to monitor pulse, blood pressure, and general well-being. If necessary, they will alert the responsible physician, who will assess and treat any side effects that may occur.

Main Side Effects:

- The most common side effect is muscle cramps, which can be easily resolved by administering calcium.

- Other less frequent side effects include hypotension due to extracorporeal circulation, general discomfort or syncope, hematomas, and a decrease in platelet count.

Adverse Events Related to Endometrial Biopsy:

Endometrial biopsy is a common procedure in gynecological clinical practice. It is an outpatient process typically performed without anesthesia. In general, it is a well-tolerated and safe procedure, although it may occasionally cause mild pain when the cervix is held or cramps when instruments are introduced into the uterus or when the sample is taken. Risks and complications associated with this technique are minimal, with the most common being mild pain after the insertion of the cannula and slight bleeding following the biopsy. Rarely, it can result in uterine puncture, perforation, or tearing, prolonged bleeding, genital infection, or cramping.

Adverse Events Related to Endometrial Fluid Collection

Endometrial aspiration is a common procedure in gynecological clinical practice. It is a minimally invasive procedure that is typically painless and, very rarely, may cause slight discomfort to the patient, similar to mild menstrual pain. This procedure is not an endometrial tissue biopsy but a gentle aspiration of endometrial fluid (between 10 and 40 microliters) using painless cannulas routinely employed in embryo transfer procedures.

After the procedure, slight spotting may occur, and although the risk of infection cannot be completely excluded, it is highly unlikely. It is important to note that performing endometrial fluid aspiration prior to embryo transfer does not affect implantation rates within the same cycle (van der Gaast et al., 2003).

Adverse Events Related to Peripheral Venous Blood Sampling

Peripheral venous blood sampling is a very common procedure in clinical practice and rarely results in significant side effects. During the procedure, it may cause some pain, bleeding, burning sensation, dizziness, fainting, or the development of a hematoma at the blood collection site. In rare cases, it may lead to infection or inflammation (phlebitis) at the puncture site.

Adverse Events Related to Medications Used in Hormone Replacement Therapy (HRT): These are medications already approved by health authorities, and more detailed information about their side effects can be found in the package insert or product label for each medication. In general:

Estradiol Valerate: *Evopad Patches®, Estradot®, Progynova®*

These medications may cause certain adverse effects, although not everyone experiences them. Adverse reactions classified by frequency are as follows:

- Very common: Affect more than 1 in 10 people.
- Common: May affect up to 1 in 10 people.
- Uncommon: May affect up to 1 in 100 people.
- Rare: May affect up to 1 in 10,000 people.
- Frequency unknown.

In clinical trials of *Evopad 50 mcg/24h transdermal patches*, the following side effects have been reported:

Infections and Infestations:

- Common: Genital moliniasis (genital infection).
- Uncommon: Genital candidiasis.

Benign, Malignant, and Unspecified Neoplasms (including cysts and polyps):

- **Rare:** Breast cancer.
- **Unknown frequency:** Endometrial cancer.

Immune System Disorders:

- **Uncommon:** Allergic reaction.

Metabolism and Nutrition Disorders:

- **Common:** Weight gain or loss.

Psychiatric Disorders:

- **Uncommon:** Depression.
- **Rare:** Anxiety, increased or decreased libido.

Nervous System Disorders:

- **Common:** Headache, nervousness, mood changes, insomnia.
- **Uncommon:** Vertigo, dizziness.
- **Rare:** Paresthesia (tingling sensation), epilepsy, migraine.
- **Unknown frequency:** Stroke.

Eye Disorders:

- **Uncommon:** Visual disturbance.

- **Rare:** Intolerance to contact lenses.

Vascular Disorders:

- **Uncommon:** Increased blood pressure.
- **Rare:** Thrombosis (blood clots).
- **Unknown frequency:** Deep vein thrombosis (blood clots in the veins).

Respiratory, Thoracic, and Mediastinal Disorders:

- **Unknown frequency:** Pulmonary embolism (blood clots in the lungs).

Gastrointestinal Disorders:

- **Common:** Nausea, abdominal pain, diarrhea.
- **Uncommon:** Flatulence, difficult and laborious digestion.
- **Rare:** Abdominal bloating, vomiting, gas (flatulence).

Hepatobiliary Disorders:

- **Rare:** Gallstones.

Skin and Subcutaneous Tissue Disorders:

- **Common:** Itching, rash, dry skin.

Skin and Subcutaneous Tissue Disorders:

- **Uncommon:** Skin discoloration, hives, redness of the skin accompanied by pain.
- **Rare:** Excessive hair growth in women (hirsutism), acne.
- **Unknown frequency:** Severe swelling of the skin and other tissues (especially the lips or eyes).
- **Musculoskeletal and Connective Tissue Disorders:**
- **Common:** Joint pain, back pain.
- **Uncommon:** Muscle pain.
- **Rare:** Muscle weakness (myasthenia), muscle cramps.

Reproductive System and Breast Disorders:

- **Very common:** Menstrual period irregularities.
- **Common:** Irregular or continuous uterine bleeding, abnormally heavy and prolonged menstrual periods, irregular vaginal bleeding, uterine cramps, vaginal inflammation, growth of the uterine wall, vaginal/uterine bleeding including spotting.

- **Uncommon:** Breast enlargement, painful menstrual periods, breast pain, breast tenderness.
- **Rare:** Benign uterine tumors (benign uterine fibroids), breast lumps, lumps near the cervix (endocervical polyps), painful menstruation, premenstrual syndrome, breast enlargement, white discharge.

General Disorders and Administration Site Conditions:

- **Very common:** Itching at the application site, rash at the application site.
- **Common:** Pain, weakness, edema at the application site, redness at the application site, reaction at the application site, weight changes.
- **Uncommon:** Swelling due to fluid accumulation in tissues, swelling in multiple parts of the body, swelling due to fluid accumulation in legs and feet (edema).
- **Rare:** Fatigue.

Investigations:

- **Common:** Weight gain.

Laboratory Abnormalities:

- **Uncommon:** Elevated transaminases (a laboratory indicator of liver function).

Risk of Tumor Development:

Hormone Replacement Therapy (HRT) increases the risk of breast cancer. There is a higher risk for women using combined estrogen-progestogen therapy. The risk of developing breast cancer increases with the duration of treatment.

It has been observed that long-term treatments with estrogen alone or in combination increase the likelihood of developing ovarian tumors.

Risk of Thrombus Development:

Hormone replacement therapy increases the risk of thrombosis (blood clots) by 1.3 to 3 times, with the highest risk occurring during the first year of use.

Risk of Coronary Artery Disease:

The risk of developing coronary artery disease may increase in women using combined treatment starting at age 60.

Risk of Cerebrovascular Accident (CVA):

The use of Hormone Replacement Therapy (HRT) is associated with an increased risk of cerebrovascular accident (CVA). For oral treatments with synthetic estrogens, either alone or combined with progestogens, the following side effects have been reported: estrogen-dependent benign or malignant neoplasms (e.g., endometrial cancer), myocardial infarction, cerebrovascular accident, gallbladder disorders, and skin and subcutaneous tissue disorders (such as chloasma, erythema multiforme, erythema nodosum, vascular purpura, inflammation of the skin [contact dermatitis], generalized itching [pruritus], hives, and angioedema [deep swelling of the skin]), as well as probable dementia.

Progeffik® or Utrogestan®:

These medications may cause adverse effects, although not everyone experiences them. The adverse reactions that are typically observed, though uncommon, include:

- Drowsiness and/or transient dizziness, usually occurring 1–3 hours after oral administration.
- Shortening of the menstrual cycle or breakthrough bleeding in cases of luteal insufficiency.

Adverse Events Related to Neupogen® in Healthy Stem Cell Donors:

Like all medications, Neupogen® may cause adverse effects, although not everyone experiences them. Participants in this study may experience one or more of the following adverse events:

- **Very common adverse effects** (observed in more than 1 in 10 people using Neupogen®) in healthy stem cell donors:

  A frequent increase in white blood cell count (leukocytosis) and a decrease in platelet count, which reduces the blood's clotting ability (thrombocytopenia).

  It may also cause mouth and throat pain (oropharyngeal pain), coughing, diarrhea, vomiting, constipation, nausea, skin rash, weakness, or hair loss **uncommon (alopecia), muscle or bone pain (musculoskeletal pain), generalized weakness (asthenia), fatigue, inflammation and swelling of the digestive mucosa from the mouth to the anus (mucosal inflammation), difficulty breathing (dyspnea), pain in healthy stem cell donors, decrease in platelet count reducing the blood's ability to clot (thrombocytopenia), increase in white blood cell count (leukocytosis),** headache, muscle or bone pain (musculoskeletal pain).

- Frequent adverse effects (observed in more than 1 in 100 people using Neupogen®) in healthy stem cell donors:

Increase in certain blood enzymes, difficulty breathing (dyspnea), enlargement of the spleen (splenomegaly).

- Uncommon adverse effects (observed in more than 1 in 1,000 people using Neupogen®) in healthy stem cell donors:

  Rupture of the spleen, severe pain in the bones, chest, intestines, or joints (sickle cell crisis), sudden life-threatening allergic reaction (anaphylactic reaction), changes in blood biochemistry parameters, bleeding in the lungs (pulmonary hemorrhage), coughing up blood (hemoptysis), abnormal lung X-rays (pulmonary infiltration), inability of the lungs to absorb oxygen (hypoxia), increase in certain blood enzymes, worsening of rheumatoid arthritis, damage to the tiny filters inside the kidneys (glomerulonephritis).

- Adverse effects with unknown frequency (cannot be estimated from available data):

  Damage to the tiny filters inside the kidneys (glomerulonephritis).

13. Ethical considerations

13.1 General considerations

This clinical study will be conducted in accordance with the protocol, the principles outlined in the latest version of the Declaration of Helsinki, the standards of Good Clinical Practice (CPMP/ICH/135/95), and Royal Decree 1090/2004, which regulates clinical trials with medicinal products in Spain and fully incorporates the provisions of Regulation (EU) No. 536/2014 of April 16, 2014, regarding Member States' requirements for the application of Good Clinical Practice in conducting clinical trials on human medicinal products.

It will also respect the fundamental principles established in the Council of Europe Convention on Human Rights and Biomedicine, the UNESCO Universal Declaration on the Human Genome and Human Rights, and comply with the requirements set forth in Spanish legislation regarding the protection of personal data and bioethics.

The investigator agrees, by signing the protocol, to adhere to the instructions and procedures described therein and, in doing so, to follow the principles of Good Clinical Practice (GCP) that they imply.

The medical staff will have the necessary training to participate in the clinical trial.

In the event of a Serious Adverse Event (SAE), the medical staff is prepared to address it and will take the necessary measures corresponding to the nature and intensity of the event. All such data will be recorded in the Case Report Form (CRF).

The patient's voluntary participation in the study will always be ensured.

Information related to the identification of each patient will be treated confidentially and will only be accessible to specialized personnel involved in the research for medical purposes. Patients' names or any other data that could identify them will not be published.

In compliance with Royal Decree 1090/2015, the sponsor will submit the relevant documentation to the designated Ethics Committee for the Evaluation of Medicinal Products (CEIm) of reference for its review and subsequent report, as well as to the CEIms of other centers participating in the study (which will provide their opinion to the CEIm of reference). The study will not begin until written approval is obtained from the CEIm of reference and authorization from the Spanish Agency of Medicines and Medical Devices (AEMPS).

To include patients in the study, the informed consent form, approved by the Ethics Committee of the Investigating Centers, must be signed.

13.2 Ethics Committee for the Evaluatión of Medicinal Products (CEIm)

This protocol will be submitted for evaluation to an accredited Ethics Committee for the Evaluation of Medicinal Products (CEIm) acting as the Reference Committee, in accordance with Chapters IV and V of Royal Decree 1090/2015, dated December 4.

Any changes, modifications, or amendments required during the course of the study must be submitted to the same Ethics Committee.

Any minor change, modification, or amendment (as defined by Royal Decree 1090/2015, dated December 4, 2015) will be notified to the Ethics Committee for informational purposes.

Any major change, modification, or amendment (as defined by Royal Decree 1090/2015, dated December 4, 2015) will be submitted to the Ethics Committee for approval. The study will only proceed once approval is obtained.

13.3 Informed consent

The principles of informed consent will be applied throughout the clinical trial in accordance with the latest version of the Declaration of Helsinki and Royal Decree 1090/2015, dated December 4, 2015, prior to performing any study-specific procedure.

Information will be provided to patients both verbally and in writing and must have been previously approved by the CEIm. Patients must be given the opportunity to ask questions about any detail of the study.

The patient information sheet must be approved, along with the protocol, by the CEIm. Informed consent will be documented through a written consent form approved by the CEIm and signed by the patient.

The written consent form will include the elements of informed consent as described in the Declaration of Helsinki and will comply with Spanish regulations (Royal Decree 1090/2015, dated December 4, 2015). This document must be provided to patients to give them the opportunity to read it before signing. Consent must be documented by the dated signature of the patient, which certifies that the consent is based on information that has been understood.

Following the medical evaluation for the possibility of treating patients affected by Asherman's Syndrome or Endometrial Atrophy with autologous CD133+ cells, they will be assessed according to the study's inclusion and exclusion criteria. Patients will be informed that they will follow a pharmacological treatment aimed at evaluating the safety and efficacy of the investigational product IGX1 (CD133+ cells selected after prior mobilization and collection of peripheral blood progenitor cells [PBPC] derived from the patient herself) to improve post-treatment reproductive prognosis.

The treatment will consist of selecting the CD133+ subpopulation and instilling them into the uterine artery of these patients prior to surgical hysteroscopy. All patients will be informed of the advantages and potential disadvantages of the method, and they will be advised that, based on studies published to date, therapeutic benefit is expected. Each patient will receive a detailed explanation of all the examinations they will undergo once included in the study. After providing this information and obtaining the patient's approval to participate, the informed consent form prepared for the study will be signed.

Before conducting any study-related activity or evaluation that is not part of the routine clinical practice of the center, the patient must be thoroughly informed about all aspects of the study, including scheduled activities and visits. Written informed consent must be signed prior to these activities. Two signed originals must be obtained: one for the patient and one for the investigator.

A copy of the patient information sheet and the informed consent form is attached to this protocol (Appendix 21.1).

13.4 Declaration of clinical study completion

Once the study has ended, a declaration of completion of the clinical trial must be released, in accordance with the procedures set forth in European Regulation No. 536/2014 and Royal Decree 1090/2015.

13.5 Clinical study registration

This clinical study will be registered on national and international clinical trial registry websites in compliance with European Regulation 536/2014 and Royal Decree 1090/2015, following the sponsor's procedures.

14. Data management and record filing

14.1 Case Report Form (CRF)

The CRF is necessary and must be completed for each enrolled patient.

The entries made in the CRF must be verifiable with the source documents. The source data parameter to be verified and identification of the source document must be confirmed and recorded. The completeness and accuracy of all required documents in terms of regulation and any other type of documents must also be reviewed.

The investigator will be fully responsible for the accuracy and authenticity of the clinical and laboratory data included in the CRF at all times. The source documents about patients must be the patient's medical records, which will be kept at the site where the study is conducted.

14.2 Record retention

The investigator will retain copies of all relevant information in files for at least the period specified by current legislation.

The study archive and all source documents must be preserved until notification from the sponsor or their representative authorizing destruction.

14.3 Confidentiality

All materials, information (oral or written), and unpublished documentation provided to the investigators, including this protocol, the case report forms, and the Investigator’s Brochure, must be considered the exclusive property of the Sponsor, classified as confidential, and provided as confidential for review and use exclusively for the purposes of the study.

This confidentiality of the information, as well as the obligation to maintain it throughout the duration of the study, applies to both the investigator and their collaborators. The investigator must ensure that all collaborators respect the confidential nature of the information.

Such data and/or material may not be disclosed, in whole or in part, by the principal investigator and/or their team to any unauthorized person without prior formal written consent from the sponsor. The data must only be used for the purposes of the study.

The investigator and/or their team are obligated to treat as confidential and ensure at all times the confidentiality of the documents and results generated during the course of the trial, except for those defined by legislation as disclosable.

The investigator and/or their team will ensure that all individuals involved respect the confidentiality of any information regarding the trial's patients.

All parties involved in a clinical trial shall maintain the strictest confidentiality to ensure that the personal and familial privacy of participating patients is not violated. Appropriate measures must also be taken to prevent unauthorized access to trial data.

The investigator is the only person who may and must know the origin of the collected data and associate it with the patient.

The personal data (full name, address, workplace, tax ID) of the investigators will be stored in a computerized file solely for the purpose of facilitating logistical and organizational aspects necessary for the development of the study. This data file will be treated confidentially in accordance with applicable regulations (Organic Law 3/2018 of December 5 on the Protection of Personal Data and Guarantee of Digital Rights and EU Regulation 2016/679 of April 27, 2016, on the protection of natural persons with regard to the processing of personal data and the free movement of such data). Investigators may exercise their rights of access, rectification, deletion, and objection concerning their personal data by submitting a written request to the data controller: **Asherman Therapy S.L.U.**

Patients in the study will be identified using a patient code. The processing of personal data in the trial, particularly regarding consent, will comply with current regulations on the protection of personal data: Organic Law 3/2018 of December 5 on the Protection of Personal Data and Guarantee of Digital Rights and EU Regulation 2016/679 of April 27, 2016, on the protection of natural persons with regard to the processing of personal data and the free movement of such data.

15. Funding

The clinical trial will be funded with the sponsor's own resources.

The sponsor has established contractual agreements with each participating center and principal investigator for the conduct of this study. These contracts include provisions outlining the agreed financial terms, as detailed in the financial report submitted to the Ethics Committee for the Evaluation of Medicinal Products.

Additionally, the sponsor will seek external funding through current funding programs offered by major national, European, and international funding entities.

Funding required	Yes	
Financed project	Yes	
Funding code	P-50	
Funding entity	Igenomix S.L.	
Funding period	Start date: 1/1/2017	End:
Expected budget	€370,000	

16. Insurance

In compliance with current regulations as stipulated in Royal Decree 1090/2015 of December 4, 2015, the sponsor, **Asherman Therapy S.L.U.**, holds a valid insurance policy specifically contracted for this study. This policy complies with applicable legislation and provides coverage to compensate and indemnify for any health impairments or injuries that participants might experience as a result of their participation in this trial. (*Appendix 21.6*)

17. Practical considerations

17.1 Working plan

Once approval is obtained from the Ethics Committee for the Evaluation of Medicinal Products (CEIm) reviewing the study and from the Spanish Agency of Medicines and Medical Devices (AEMPS), the study will commence at the participating centers.

Investigators will be provided with the study protocol, the patient information sheet/informed consent form (PIS/ICF) approved by the CEIm, the Case Report Form (CRF), and any other documentation necessary for the proper conduct of the study.

The investigator will invite patients attending their clinic during the inclusion period to participate in the study, provided they meet the selection criteria. If a patient decides not to participate in the study, the investigator will continue offering participation to all patients who meet the selection criteria.

Each patient invited to participate in the study will be given a written document titled "Patient Information Sheet/Informed Consent Form" (PIS/ICF). No patient will be included in the study until they have been properly informed by the investigator and have provided their written informed consent.

The investigator will confirm that the patient meets all selection criteria before including them in the study.

Each investigative center will be identified by a single-digit numeric code, and each patient enrolled by the investigator will be identified by a two-digit numeric code, starting from the number 01, such that each patient will be identified in the central database by an investigator center code and a patient number, which will under no circumstances include data that allows for patient identification.

Data will be recorded in the CRF following the guidelines of this protocol and will include information available in the patient's medical records and/or obtained through the determinations scheduled during protocol visits. No visits or procedures outside those outlined in the protocol will be scheduled.

The sponsor will be responsible for recording and safeguarding the database containing the information documented in the CRF for subsequent analysis once the study is completed. Each participating center is responsible for recording the data of its recruited patients and is committed to reviewing, validating, and ensuring the accuracy of the provided data.

17.2 Responsibilities of all trial participants

All personnel involved in the clinical trial are responsible for adhering to the standards outlined in Good Clinical Practice (GCP) CPMP/ICH/135/95 and Royal Decree 1090/2015 of December 4, 2015. The primary responsibilities of the roles involved in the clinical trial are summarized below:

Investigator

The responsibilities of the investigator at each participating center are as follows:

- Accept the terms of the protocol and any amendments thereto. Signing the protocol constitutes acceptance of all terms described within.
- Inform research patients and obtain their consent.
- Collect, record, and report data correctly within the timeframes specified in the protocol, ensuring their accuracy and quality for any audits.
- Maintain the confidentiality of the data of study participants.
- Facilitate sponsor audits and inspections by health authorities.
- Ensure that the information recorded in the CRF is accurate, truthful, and obtained as outlined in the protocol.
- The investigator is the only person who may and must know the origin of the collected data and associate it with the patient. The investigator is responsible for ensuring that no extraneous information (not required) that could identify the patient (e.g., name, ID, social security number, address, phone number, etc.) is included in the CRF.
- Above all, the investigator must always ensure the best possible care for the patient, prioritizing their well-being and safety at all times.

Principal Investigator and Study Coordinator

The responsibilities of the study coordinator are as follows:

- Sign the protocol and any amendments thereto along with the sponsor.
- Demonstrate knowledge of the study objectives, basic methodology, and the significance of the study results.
- Share responsibility with the sponsor for drafting the final study report.
- Contribute to the dissemination of study results in collaboration with the sponsor.
- Not to sign any contract containing confidentiality clauses about potential study results, nor to conceal and/or suppress research findings, avoiding in all cases jeopardizing the integrity of the study (e.g., through the publication of partial results by an investigator or center).

Sponsor

The responsibilities of the study sponsor are as follows:

- Ensure compliance with the relevant legal regulations.
- Approve and sign the content of this protocol, which outlines the characteristics of the study to be conducted, as well as any amendments thereto. Signing the protocol constitutes acceptance of all terms described within.
- Submit the protocol to a CEIC (Ethics Committee for Clinical Research) and not initiate the study until approval is obtained.
- Provide investigators with the protocol and other study materials.
- Submit the study protocol and final report within the established timelines and, if applicable, communicate any interruptions and the reasons for them.
- Respect the confidentiality of the data of patients participating in the study.
- Facilitate audits and inspections by health authorities.
- Identify the sources of study funding.
- Apply quality control in the collection and handling of data to ensure data reliability.
- Carry out all necessary statistical processes to obtain the study results.
- Publish the study results in a scientific journal.

Pharmacist

The pharmacist will be responsible for storing the study drugs in a secure location and under the conditions specified by the sponsor, ensuring that the drugs maintain their safety and potency until their expiration date.

They must ensure compliance with drug labeling regulations and keep appropriate records of all drug inventory, including dates, quantities, and use by participants.

If the study is suspended, terminated, interrupted, or completed, the pharmacist must return any unused

drug samples to the sponsor or, if agreed upon in writing with the sponsor, provide a certificate of local destruction.

Monitor

The monitor must verify that the information collected in the protocol is accurate and will ensure this by having full access and cooperation from the investigative team to carry out their duties. Additionally, they will be responsible for inspection procedures and for communicating serious or unexpected adverse events occurring during the study to the health authorities.

17.3 Monitoring

The sponsor, or alternatively an external company subcontracted for this purpose (CRO), in accordance with Royal Decree 1090/2015 and the ICH Good Clinical Practice guidelines, will be responsible for monitoring the study. To this end, a properly accredited and qualified monitor (CRA) with sufficient experience will ensure that the study is conducted in compliance with the protocol previously approved by the CEIm and the competent authorities, the EMA/CHMP/ICH/135/1995 Good Clinical Practice guidelines, and current legislation.

Before the study begins, during the initiation visit, a representative of the sponsor will review the protocol and the Case Report Forms (CRFs) with the investigators and their collaborating staff. During the study, the monitor will visit the site regularly to ensure that subject documents are complete, data recorded in the CRFs are accurate, the protocol and Good Clinical Practice (GCP) guidelines are being followed, recruitment progress is on track, and that the study medication is being stored, dispensed, and accounted for in accordance with the established specifications.

Regular monitoring of study data will be carried out at each site as defined in the study's specific monitoring plan. Sites will be monitored individually to ensure proper compliance with the protocol and GCP, as well as to verify the accuracy and completeness of the data recorded in the CRFs. Monitoring frequency may vary depending on the needs of each site (e.g., recruitment capacity, data volume generated, complexity, etc.). Monitoring visits will be scheduled in advance with site personnel to take place at a mutually convenient time. The investigator will provide adequate time and space for these visits.

At the end of the study, a close-out visit will be conducted to ensure the proper conclusion of the study at the site.

The investigator and trial personnel must be available to assist the monitor during these visits. The investigator must allow the monitor to review medical records or other clinical data to confirm consistency with the data recorded in the CRFs. No data revealing patient identity may leave the site.

Monitoring criteria established by the sponsor require complete verification of the existence of informed consent, compliance with inclusion/exclusion criteria, documentation of serious adverse events, and recording of primary and secondary variables. Furthermore, it will ensure that the study is properly documented both during and after its conclusion.

17.4 Inspections and/or audits

In addition to routine monitoring procedures, **Asherman Therapy S.L.U.** may conduct audits of the study to ensure its quality. These audits will be requested and scheduled in advance, with sufficient time to coordinate availability with the involved staff and ensure all documentation is prepared for the audit.

Both the study sponsor and the investigative site may also be inspected by the relevant Health Authorities (during the study or even after its completion). If a Health Authority requests an inspection at the site, the investigator must immediately inform the sponsor of such a request.

17.5 Data record and document maintenance

The investigator must complete all the data requested in the CRF following the guidelines described for CRF completion and transmit data according to the procedures indicated by the Sponsor at study start.

The patient data collected in the CRF during the study must be anonymously documented, and the patient will only be identified by the patient number. All the information recorded in the CRF should be contrasted with the source documents in the patient's medical history.

The investigator must keep the source documents that are indispensable for health authority inspections for a minimum period of 25 years (or more if this is notified at the start of the study).

Errors or omissions of CRF data will be recorded in the Data Query Form, which will be sent to the investigator for their clarification.

17.6 Protocol amendments

Any modifications to the protocol, once authorized by the CEIm or AEMPS, must be documented in writing in the form of an amendment.

Amendments must be clearly identified, chronologically numbered, dated, and signed by the investigator.

All protocol amendments will be notified to the CEIms involved in the trial and to the Spanish Agency of Medicines and Medical Devices (AEMPS) before implementation. If the amendments are significant, they must also receive authorization from the CEIms involved, AEMPS, and the Autonomous Communities before implementation.

In accordance with Royal Decree 1090/2015, dated December 24, 2015, significant amendments are those that involve an increased risk to the trial participants.

17.7 Study termination

Both the sponsor and the investigator reserve the right to interrupt the study, in accordance with the contract and applicable local law and regulations.

The investigator must notify the CEIm in writing of the study's termination or premature interruption and send a copy of this notification to the sponsor.

17.8 Drug packaging, labeling, dispensing, and storage of the medication

The investigational drugs will be provided free of charge for the treatment of patients in the study.

The investigational product designated for study patients will be supplied packaged and labeled as clinical trial medication. The study medication will be delivered by the Pharmacy Service to the study investigators, who will be responsible for dispensing it to the patients and ensuring adherence to treatment.

The labeling of the medication for this clinical trial will comply with the requirements established by current legislation, Annex 13 for the manufacture of investigational medicinal products, and Annex 2 for the manufacture of biological active substances and biological medicinal products for human use.

All information regarding the manufacturing, procurement, processing, packaging, labeling, dispensing, and storage of the investigational product IGX1 is detailed in the IMPD and the Investigator's Brochure.

17.9 Drug accountability

The person responsible for dispensing the medication must maintain an appropriate record of all drugs and/or products supplied during the study. These documents must include the date the study medication was received, as well as the dates it was dispensed to the patient.

The accountability records will always remain on site. The records will include the patient's identification number, the date and time of production, batch number, and the quantity of investigational drug administered.

18. Publication and dissemination of the results

The results obtained from this study are intended to be shared with the scientific community through publication in high-impact specialized journals in the fields of Biology, Endocrinology, Obstetrics, and Gynecology, such as **J Clin Endocrinol Metab** (Impact Factor = 6.310), **FASEB J** (Impact Factor = 5.480), **Fertil Steril** (Impact Factor = 4.295), **Placenta** (Impact Factor = 3.285), or **Human Reproduction** (Impact Factor = 4.635).

We aim to present preliminary data at major international conferences related to reproductive medicine, such as the **Society for Reproductive Investigation (SRI)**, the **American Society for Reproductive Medicine (ASRM)**, and the **European Society of Human Reproduction and Embryology (ESHRE)**.

Additionally, during the execution of the clinical trial, and as positive results are obtained, these will be shared with the widest possible audience, including the scientific and clinical community, particularly professionals within the National Health System, as well as patient organizations and the general public.

The sponsor is responsible for ensuring that the public has access to appropriate information about the study in accordance with local requirements regarding the registration and publication of results. Similarly, the sponsor is responsible for preparing the final report of the clinical study in collaboration with the coordinating investigator. The sponsor and the coordinating investigator, if applicable, will sign the final report.

No unpublished information provided by the sponsor to any of the participating investigators may be published or disclosed to third parties without the prior written consent of the sponsor. The primary publication of this study will include the results obtained in accordance with the **Uniform Requirements for Manuscripts Submitted to Biomedical Journals**, as established by the International Committee of Medical Journal Editors (www.ICMJE.org).

Authorship will be restricted to individuals who have contributed editorially or conceptually to the protocol design, data collection and/or analysis, data interpretation, or manuscript preparation. In any case, the sponsor will oversee this process and will make the final decision in case of a conflict. Policies regarding publication, authorship, intellectual and/or industrial property will also be defined in the contract between both parties (sponsor/investigator site).

None of the investigators may file a patent application based on the study results, nor may they assist any third party in filing such an application without prior written authorization from the sponsor.

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20. Appendixes

20.1 Appendix 1: Categories to determine the relationship of adverse events with the study medication

PROBABLE *(the first three criteria must be met)*

This category applies to adverse events that are considered, with a high degree of certainty, to be related to the study medication. An adverse event (AE) may be considered probable if:

- It follows a reasonable temporal sequence after the administration of the drug.
- It cannot be reasonably explained by the known characteristics of the patient's clinical condition, environmental or toxic factors, or other therapies administered to the patient.
- It resolves or improves upon discontinuation of the drug or dose reduction (important exceptions exist when an AE does not resolve upon drug discontinuation, yet a clear relationship with the drug exists, e.g., (1) myelosuppression, (2) tardive dyskinesia).
- It follows a known response pattern to the suspected drug.
- It recurs upon re-exposure to the drug.

POSSIBLE *(the first two criteria must be met)*

This category applies to adverse events for which a relationship with the study medication administration seems unlikely but cannot be ruled out with certainty. An AE may be considered possibly related if or when:

- It follows a reasonable temporal sequence after the administration of the drug.
- It could have been caused by the patient's clinical condition, environmental or toxic factors, or other therapies administered to the patient.
- It follows a known response pattern to the suspected drug.

REMOTE *(the first two criteria must be met)*

In general, this category is applicable to an AE that meets the following criteria:

- It does not follow a reasonable temporal sequence after the administration of the drug.
- It could easily have been caused by the patient's clinical condition, environmental or toxic factors, or other therapies administered to the patient.
- It does not follow a known response pattern to the suspected drug.
- It does not recur or worsen upon re-administration of the drug.

NOT RELATED

This category applies to adverse events that are clearly and indisputably due solely to external causes (e.g., illness, environment, etc.) and do not meet the criteria for a relationship with the medication described in the categories of remote, possible, or probable.

	Probable	Possible	Remote	Not related
Clearly due to external causes	-	-	-	+
Reasonable time association with the drug administration	+	+	-	-
Could be caused by the clinical condition of the patient, etc.	-	+	+	+
Known response pattern for the suspected drug	+	+	-	-
It disappears or diminishes when treatment is discontinued or the dose is reduced.	+	-	-	-
It reappears when administering the drug again	+	-	-	-

20.2 Appendix 2: Performance status of the Eastern Cooperative Oncology Group (ECOG)

Grade	ECOG Performance Status
0	Asymptomatic and normal activity (WHO: normal activity without restrictions).
1	Symptomatic, but ambulatory (WHO: restricted in physically strenuous activity).
2	Symptomatic, upright for more than 50% of waking hours (WHO: capable of self-care but unable to work).
3	Symptomatic, sitting or bedridden for more than 50% of the day (WHO: capable of limited self-care).
4	Bedridden or confined to a chair (WHO: totally dependent and unable to care for oneself)
5	Death

Published in Am. J. Clin. Oncol.: Oken, MM, Creech, RH, Tormey, DC, Horton, J, Davis, TE, McFadden, ET, Carbone, PP. Toxicity And Response Criteria Of The Eastern Cooperative Oncology Group. Am J Clin Oncol 5:1982;649-655.

20.3 Appendix 3: Grading of Asherman Syndrome According to the Criteria of the European Society of Hysteroscopy (ESH) and the European Society for Gynecological Endoscopy (ESGE)

Grade	Description
I	Thin or filmy adhesions easily ruptured by hysteroscope sheath alone, Normal Fallopian tubes.
II	Isolated firm adhesions connecting separate parts of the uterine cavity, possible visualization of both Fallopian tubes, adhesions cannot be broken with the hysteroscope sheath alone.
IIa	Adhesions occluded solely in the region of the internal cervical canal. The upper uterine cavity is normal.
III	Multiple firm adhesions connecting separate parts of the uterine cavity, with unilateral destruction of the areas of both Fallopian tubes.
IIIa	Extensive scarring of the uterine cavity wall with amenorrhea or hypomenorrhea
IIIb	Combination of III and IIIa
IV	Extensive firm adhesions with agglutination of the uterine walls. Both tubal ostial areas occluded

Classification of the European Society of Hysteroscopy (ESH, 1989).

Grade	Description
I	Thin or filmy adhesions easily ruptured by hysteroscope sheath alone, normal fallopian tubes.
II	Isolated firm adhesions connecting separate parts of the uterine cavity, possible visualization of both Fallopian tubes, adhesions cannot be broken with the hysteroscope sheath alone.
IIa	Adhesions occluded solely in the region of the internal cervical canal. The upper uterine cavity is normal.
III	Multiple firm adhesions connecting separate parts of the uterine cavity, with unilateral destruction of the areas of both Fallopian tubes.
IV	Extensive firm adhesions with agglutination of the uterine walls. The tubal area is occluded.
Va	Extensive endometrial scarring and fibrosis combined with Grade I or II adhesions. Pronounced amenorrhea or hypomenorrhea.
Vb	Extensive endometrial scarring and fibrosis combined with Grade III or IV adhesions. Amenorrhea.

Classification by the European Society for Gynecological Endoscopy (ESGE, 1995).

	European Classification Systems	
Clinical Category	European Society of Hysteroscopy (ESH, 1989)	European Society for Gynecological Endoscopy (ESGE, 1995)
Mild	Grades I and IIaGrades I and IIa	Grades I and IIa
Moderate	Grades II, III	Grades II, III
Severe	Grades IIIa, IIIb, IV	Grades IV, Va, Vb

Clinical categories used to classify study participants.

20.4 Appendix 4: Quality of Life Questionnaire in Fertility - FertiQoL International (2008)

Attached as a separate document.

20.5 Appendix 5: Patient Diary for Recording Adverse Events and Concomitant Medication (2008)

Attached as a separate document.

20.6 Appendix 6: Pictorial Blood Loss Assessment Chart (PBAC) Scoring System

Attached as a separate document.

21. Appendixes

21.1 Patient Information Sheet /Informed Consent (PIS /IC)

21.2 Patient's card indicating participation in the clinical trial.

21.3 IMP label (CD133+ Cell selection) and G-CSF (Neulasta®)

21.4 Case Report Form (CRF)

21.5 Summary of Product Characteristics (SmPC) or Investigator Brochure (IB)

It can be consulted at the following link:
https://www.wma.net/es/policies-post/declaracion-de-helsinki-de-la-amm-principios-eticos-para-las-investigaciones-medicas-en-seres-humanos/

21.6 Form for reporting suspected serious adverse reactions.

21.7 Declaration of Helsinki

21.8 Insurance Policy