“Phase I/II randomized clinical trial of allogeneic adipose tissue-derived mesenchymal stromal cells systemic infusion in severe systemic sclerosis”

ABBREVIATED TITLE: MSC-AT-SSc

CLINICAL TRIAL ON MEDICINAL PRODUCT FOR HUMAN USE

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SIGNATURE page for a research PROTOCOL

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The study will be carried out in accordance with the protocol, with current good practices and with statutory and regulatory requirements.

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ABBREVIATIONS / GLOSSARY

ACR/EULAR: American College of Rheumatology/European League Against Rheumatism

AD: Autoimmune Disease

AE: Adverse Event

AHSCT: Autologous Hematopoietic Stem Cell Transplantation

BMI: Body Mass Index

CTC-AE: NCI Common Terminology Criteria for Adverse Events

CRISS: ACR Provisional Composite Response Index for Clinical Trials in Early Diffuse Cutaneous Systemic Sclerosis

DLCO: diffusing capacity of lung for carbon monoxide

EQ-5D-5L: EuroQol 5-Dimension 5-Level

FVC: Forced Vital Capacity

GMP: Good Manufacturing Practices

GRCS: Global Rank Composite Score

HRQoL: Health-Related Quality of Life

IV: Intraveinous

MMF: Mycophenolate mofetil

mRSS: modified Rodnan Skin Score

MSC: Mesenchymal Stromal Cells

MSC (AT): Mesenchymal Stromal Cells derived from AdiposeTissue

MSC (M): Mesenchymal Stromal Cells derived from bone Marrow

MSC (UC): Marrow derived Mesenchymal Stromal Cells derived from Umbilical Cord

QALY: Quality-Adjusted Life Year

PFS: Progression-Free Survival

SAE: Severe Adverse Event

SAR: Serious Adverse Reactions

SHAQ: Scleroderma-Health Assessment Questionnaire

SF-36: The Short Form (36) Health Survey

SSc: Systemic Sclerosis

1 SUMMARY

Full title	Phase I/II randomized clinical trial of allogeneic adipose tissue-derived mesenchymal stromal cells systemic infusion in severe systemic sclerosis
Acronym/reference	MSC-AT-SSc
Coordinating investigator and Scientific director	Prof. Dominique FARGE MATHEC, Centre de Référence des Maladies auto-immunes systémiques Rares d'Ile-de-France, Filière FAI2R, IRSL, EA-3518, Université de Paris, Hôpital Saint Louis 1 avenue Claude Vellefaux 75010 PARIS
Sponsor	Assistance Publique – Hôpitaux de Paris
Scientific justification	Systemic sclerosis (SSc) is a rare, severe and chronic systemic autoimmune disease (AD) characterized by vasculopathy, immune dysregulation and fibrosis leading to multi-organ dysfunction (primarily skin, lungs, heart gastrointestinal tract and kidneys), with high morbidity and mortality, altered health-related quality of life, all at high cost for patients and society. Treatment are mostly symptomatic and only autologous hematopoietic stem cell transplantation (AHSCT) has shown long term improvement in overall and event-free survival with disease-modifying properties. However, AHSCT is contra-indicated in case of advanced visceral involvement and in eligible patients, it is still associated with risk of toxicity. There is an urgent need to identify safe and effective treatments for severe SSc. Mesenchymal stromal cells (MSC) are multipotent cells which carry immunomodulatory, pro-angiogenic and anti-fibrotic properties, that can target SSc pathogenesis and its clinical manifestations. The increasing use of MSC, harvested from bone marrow (MSC(M)), adipose tissue (MSC(AT)), or umbilical cord (MSC(UC)) in a variety of indications, provides consistent evidence supporting their safety in humans. The efficacy of MSC(M) intravenous (IV) injection for treating acute graft versus host disease led to their marketing approval in 2012 and MSC(AT) (Alofisel) were approved for severe Crohn's fistula in 2018. MSC represent a promising therapeutic approach for SSc. We have previously a) shown disease-specific abnormalities in MSC(M) from SSc patients, providing strong rationale to use allogeneic MSC to treat SSc patients, b) published the first phase I/II dose escalation trial using allogenic MSC(M) infusion in 20 severe SSc patients (ClinicalTrials.gov: NCT02213705, PHRC AOM 11-250) with no safety issues, significant improvement in skin fibrosis at 3 to 6 months after infusion which appeared lower thereafter, thereby supporting the need for repeated infusions.

	In vitro, experimental and clinical studies suggest that MSC properties vary according to their tissue of origin/source. We demonstrated that compared to MSC(M), MSC(AT) are easier to harvest and display higher proliferative capability before entering senescence, higher genetic stability, and superior immunosuppressive properties. Considering the above rationale, we hypothesize that use of healthy donors allogeneic MSC(AT) produced by Etablissement Français du Sang (EFS) will demonstrate a) no safety issues, b) an efficacy profile that will increase with repeated infusion of allogeneic MSC(AT) to treat SSc
Main objective and primary endpoint	<u>Main objective:</u> To evaluate the safety one month after allogeneic $2x10^6$ MSC(AT)/kg intravenous administration once or twice at 3 months interval (M0, M3) in severe SSc patients <u>Primary endpoint</u>: The rate of treatment-related Severe Adverse Events (SAE) defined as Adverse Events (AE) of grade equal or above 3 using the NCI Common Terminology Criteria for Adverse Events (CTCAE) v5.0 classification, at one month after each infusion (M1, M4). All adverse events will be adjudicated by a Data and Safety Monitoring Committee.
Secondary objectives and endpoints	<u>Secondary objectives:</u> - Safety during the infusion, within the first 24 hours of infusion and during all study follow-up. - Efficacy signals to inform future studies, using outcome measures on skin sclerosis, lung function and quality of life, previously validated in SSc or used in other cell therapy trials. - Analysis of the response to treatment, Progression-free survival (PFS), Global Rank Composite Score (GRCS) at M3, M6and M12 and ACR Provisional Composite Response Index for Clinical Trials in Early Diffuse Cutaneous Systemic Sclerosis (CRISS) for early SSc patients at M3, M6 and M12. - Analysis of the overall survival and assess causes of death. - Impact of allogeneic MSC(AT) iv once or twice at 3 months interval on the immune response, including immunophenotyping and alloimmunization up to M6 after starting therapy. - Cost effectiveness of the allogeneic MSC(AT) infusion once or twice versus no treatment in severe SSc patients. <u>Secondary endpoints:</u> - Rate of treatment-related SAE defined as AE of grade equal or above 3 CTCAE v5.0 at time and

	within the first 24 hours of infusion and during all follow-up at: M0, M3, M6, M9 and M12. - **Main efficacy endpoint**: modified Rodnan Skin Score (mRSS) difference between M0 and M12. - Other efficacy disease related endpoints : o mRSS at M3, M6 and M9 o WHO performance status (PS) and Health-Related Quality of Life (HRQoL) questionnaires : Scleroderma-Health Assessment Questionnaire (SHAQ), the Short Form (36) health survey (SF-36v2) and EQ-5D-5L at M0, M3, M6, and M12; o Forced Vital Capacity (FVC) and Diffusing capacity of Lung for carbon monoxide (DLCO) at M0, M6 and M12. - Response to treatment, defined as any of the following: decreased mRSS > 25%, increased FVC > 10% and/or increased DLCO>10%, without need for further immunosuppression except low dose steroids (below 10mg daily) at M3, M6 and M12. - PFS at M12, with progression defined as any of the following: decreased in FVC > 10% or in DLCO > 15%; decrease in LVEF% > 15%; decrease in weight > 15%; decrease in creatinine clearance > 30%; increased mRSS > 25% ; and/or increase in SHAQ> 0.5. - GRCS values at M3, M6 and M12. - CRISS values for early SSc patients at M3, M6 and M12. - Overall survival at M12 - Myeloid and lymphocyte sub-populations in all included patients at M0, M1, M3, M4, M6. - Alloimmunization in all included patients through the detection and identification of donor-specific anti-HLA antibodies at M0, M3 and M6. - Extra-Cost per QALY (quality-adjusted Life Year) gained by unique and repeated IV infusion of allogeneic MSC(AT) in severe SSc after 12 months. - Extra-Cost per SAE of grade above or equal to 3 CTCAE avoided by unique and repeated IV infusion of allogeneic MSC(AT) in severe SSc after 12 months.
Design of the study	Multi-centre, three-arm, randomized, placebo-controlled, double-blind phase I-II trial
Category	*Category 2*
Population of study participants	Adult patients with refractory severe systemic scleroderma
Inclusion criteria	1) Provide signed and dated informed consent; 2) Willing to comply with all study procedures and be available for the duration of the study; 3) Male or female, aged ≥ 18 and ≤ 70 years of age

	4) SSc patients according to American College of Rheumatology/European League Against Rheumatism (ACR/EULAR) 2013 classification criteria for SSc; 5) Severe disease with either: a) disease duration of 2 years or less with a modified Rodnan skin score (mRSS) ≥ 20 and (abnormal CRP > 5 mg/l and/or hemoglobin < 11 g/dL), or b) mRSS ≥ 15 without any restriction as to disease duration plus at least one major organ involvement as defined by: (1) respiratory involvement consisting of lung diffusion capacity for carbon monoxide (DLCO) and/or forced vital capacity (FVC) < 80% predicted and evidence of interstitial lung disease (chest X-ray and/or high resolution computed tomography (HRCT) scan) and/or moderate Pulmonary hypertension with baseline resting systolic pulmonary arterial pressures > 35 mmHg and below 50 mmHg by cardiac echocardiography, or mean pulmonary artery pressure > 20 mmHg and < 40 mm Hg on right heart catheterization; (2) renal involvement consisting of past renal crisis, microangiopathic hemolytic anemia, and/or renal insufficiency not explained by other causes than SSc; (3) cardiac involvement consisting of reversible congestive heart failure, atrial or ventricular rhythm disturbances such as recurrent episodes of atrial fibrillation or flutter, recurrent atrial paroxysmal tachycardia, 2nd or 3rd degree AV-block, mild to moderate pericardial effusion and/or presence of MRI involvement (Increased T1 or T2 mapping, late gadolinium enhancement, septal D sign) . All causes of organ involvement should be attributed to SSc. 6) Contraindication, inadequate response or unwillingness to undergo AHSCT (determined by patient and physician judgement) 7) Contraindication, inadequate response or unwillingness or adverse events necessitating discontinuation of conventional immunosuppressive therapy (MMF, methotrexate); 8) Women of reproductive potential must use highly effective contraception; 9) Men of reproductive potential must use condoms 10) Health insurance *NB/The authorized contraceptive methods are:*

	For women of childbearing age and in absence of permanent sterilization: • *oral, intravaginal or transdermal combined hormonal contraception :* • *oral, injectable or implantable progestogen-only hormonal contraception* • *intrauterine device (IUD)* • *intrauterine hormonal releasing system (IUS)* • *bilateral tubal occlusion* • *vasectomised partner* • *sexual abstinence (only if this the preferred and usual lifestyle of the participants)* *For man in absence of permanent sterilization: sexual abstinence, condoms*
Exclusion criteria	1. Age < 18 years or > 70 years 2. Pregnancy or unwillingness to use adequate contraception; 3. Life-threatening end-organ damage defined as: DLCO (corrected for hemoglobin) < 30% predicted; Left ventricular ejection fraction < 40% by cardiac echocardiography; Pulmonary hypertension with baseline resting systolic pulmonary arterial pressures > 50 mmHg by cardiac echocardiography, or mean pulmonary artery pressure > 40 mmHg on right heart catheterization; glomerular filtration rate < 30mL/min 4. Active or chronic Hepatitis (ASAT, ALAT > 3 upper limit normal) 5. Neoplasms of less than 5 years, except for basal cell or in situ cervix carcinoma or concurrent myelodysplasia, 6. Uncontrolled hypertension 7. Uncontrolled acute or chronic infection 8. HIV-1 or HIV-2 infection 9. BMI < 16.5 kg/m2 10. Severe psychiatric disorder 11. Bone marrow insufficiency, defined as neutropenia < 1 x 10^9/L, thrombopenia < 50 x 10^9/L, anemia < 8 g/dL, lymphopenia < 0,5 x 10^9/L 12. Inability to provide informed consent 13. Patient included in another interventional clinical trial 14. Patient under tutelle
Investigational medicinal product(s)	Allogeneic Adipose tissue derived-MSCs (MSC(AT)) or placebo will be injected by slow intravenous infusion according to the recipient body weight and to the study experimental arms: – arm 0 : placebo at M0 and M3

	– arm 1:1 MSC(AT) ($2x10^6$ cells/kg) injection at M0 and 1 placebo injection at M3 – arm 2: 1 MSC(AT) ($2x10^6$ cells/kg) injection at M0 and 1 MSC(AT) ($2x10^6$ cells/kg) injection at M3 Each allogeneic MSC(AT) is administered intravenously over a 45 min to 1h infusion. Second infusions of drug product (MSC/placebo) will be performed only in the absence of treatment-related Severe Adverse Events (TRSAE). Patients included in arm 1 and in arm 0 will have the opportunity,according to their willingness, to receive either 1 (arm 1) or 1 or 2 (arm 0) MSC(AT) ($2x10^6$ cells/kg) injection(s) at the end of the study after unblinding and once evidence of MSC(AT) injection safety is provided.
Comparator treatment	Placebo
Interventions added for the study	- Administration of Allogeneic Adipose tissue derived-MSCs (MSC(AT)) (1 or two injections) or placebo (1 or two injections). - Supplementary visits (1 month after each injection) with basic blood and urine evaluation - Supplementary blood sampling for eligibility evaluation (infectious serology, β-HCG for women), advanced immunophenotyping and alloimmunisation investigations for baseline and follow-up evaluations, and constitution of a biological samples collection - Skin biopsies (optional) at M0, M3 and M6 - Patient notebook for the cost-effectiveness analysis
Expected benefits for the participants and for society	Based on strong experimental data (CoPI K Tarte) and the published results by our team in 20 severe SSc patients previously treated by a single IV allogeneic MSC(M) infusion in NCT02213705 trial, there is strong rationale that SSc patient symptoms will be improved after MSC(AT) treatment, with at least fibrosis skin score regression and lung function stabilization at 3 to 6 months. Results from the proposed trial will be used to inform a larger Phase II-III multi-center trial of MSC(AT) in SSc and other clinical trials in auto-immune diseases, with the potential to accelerate the treatment of SSc patients and to allow France playing a leadership role in regenerative medicine for AD and fibrotic diseases.
Risks and burdens added by the study	The increasing use of MSCs in a wide variety of therapeutic indications currently provides a wealth of data in favour of their safety in humans. There are no foreseeable serious adverse effects associated with the injection of MSCs. Some transient benign adverse reactions, linked to the procedure for injecting the cells or the placebo, may occur.
Number of participants included	18 patients
Number of centres	1 centre for patients treatment : – Unité de Médecine Interne: (UF 04), MATHEC, Centre de Référence des Maladies

	auto-immunes systémiques Rares d'Ile-de-France, Filière FAI2R, IRSL, URP 3518, Université de Paris, Hôpital St-Louis, AP-HP, 1 avenue Claude Vellefaux, 75010 Paris, France. Dominique Farge, MD, PhD 2 centres for patients recruitment and follow-up : – Unité de Médecine Interne: (UF 04), MATHEC, Centre de Référence des Maladies auto-immunes systémiques Rares d'Ile-de-France, Filière FAI2R, IRSL, URP 3518, Université de Paris, Hôpital St-Louis, AP-HP, 1 avenue Claude Vellefaux, 75010 Paris, France. Dominique Farge, MD, PhD – Service de Médecine Interne et Immunologie Clinique Pôle Hospitalo-Universitaire des Maladies Digestives CHU Rangueil, bât. H2, 3ème étage 1 avenue du Pr Jean Poulhès TSA 50032, 31059 Toulouse Cedex 9. Grégory Pugnet, MD, PhD. –
Duration of the study	- Inclusion period: 12 months - Participation period (treatment + follow-up): 12 months plus possibly 3 months additional follow-up for patients in the placebo arm (arm 0) or arm 1 who will have chosen the opportunity to receive one or two MSC(AT) injection at the end of the study after unblinding and once evidence of MSC(AT) injection safety is provided (up to 2 months between unblinding and MSC(AT) injection). - Total duration: 29 months
Number of enrolments expected per site and per month	0,5 inclusion, per site, per month
Statistical analysis	We propose to conduct a multi-center, three-arm, randomized, double-blind, placebo-controlled trial. We will enroll a total of 18 SSc patients. Each patient will be 1:1:1 randomized to one of two treatment arms (1 infusion of MSC (M0) or 2 infusions of MSC (M0, M3))or a placebo arm (total of 6 patients per arm, using randomization block of size 6). Inclusions will be staggered, to allow the detection of SAE prior to inclusion of subsequent patients, according to the following waiting rules: - at least one week between two consecutive randomizations - interval of at least one month every three randomizations Continuous monitoring of the primary endpoint by the sponsor will be implemented to allow continuous Bayesian toxicity monitoring with a stopping rule implemented on the primary endpoint on cell infusions (see section 13 for details):

	- first analysis on the first 3 patients with completed M1 visits, - then analysis at any TRSAE at M1 or M4 visits - final analysis. The stopping rule will be defined based on the estimated probability that the risk of treatment-related Severe Adverse Events (TRSAE) is >15%. “criterion 1” = the probability that the a posteriori probability of SAE is > 15%. The posterior probability of TRSAE (π_{trsae}) will be estimated using a Bayesian approach with a beta density. The stopping rule will be fulfilled if criterion 1 > 0.70 (meaning that we have a high probability that there is a greater than 15% risk of TRSAE). Interim analyses results will be presented to the DSMB for their recommendation. In the case of early stopping, subsequent patients will not be randomized and all patients treated thus far will be followed up and results analyzed as described below. There is no formal statistical test for sample size in a Bayesian framework. Nevertheless, one can invoke the frequentist framework to anticipate the expected precision in terms of safety. Indeed, after 18 infusions (6 patients with 1 infusion, 6 patients with 2 infusions), we will be able to estimate a risk of Severe Adverse Events (SAE) of 10% with a width of the exact 95% confidence interval equal to 32% considering independence between infusions.
Study will have a Data Safety Monitoring Board	YesThe Data and Safety Monitoring Board (DSMB) will be composed of 3 external reviewers with expertise in SSc, MSC and early phase trials. In case of any safety data reported to the Safety department, the DSMB will be informed, hold meeting and determine whether the trial should be held or proceed.

2 SCIENTIFIC JUSTIFICATION FOR THE STUDY

2.1 Background.

Systemic Sclerosis (SSc) is an orphan chronic autoimmune disease (prevalence 150 cases per million adults with around 8 000 cases in France (1,2)) with high unmet therapeutic needs. It is characterized by a pathogenic triad of: endothelial damage and small vessel vasculopathy, dysregulation of the innate and the adaptive immune responses, and consequent progressive fibrosis within the skin, lungs, heart, gastrointestinal tract and kidneys, resulting in multi-organ dysfunction (1,3). Health-related quality of life (HRQoL) is considerably impaired (on average 1½ standard deviations below the general population)) in these patients (4,5), who suffer high morbidity and mortality (Standardized Mortality Ratio 3.5 compared to the general population) (6). The leading causes of death are cardiac (7) and pulmonary (8,9) involvement, and the 8-10-year mortality rate is over 20-30% (6,7,9).
The recommended treatments are mostly symptomatic for Raynaud's phenomenon and gastro-esophageal reflux (2,10). Standard immunosuppressive drugs, including cyclophosphamide and mycophenolate mofetil, for early severe or rapidly progressive forms, have modest effects without improving survival (11). Several immunotherapies that specifically target cytokine signaling (eg.: Interleukin (IL)-6), B cells (with anti-CD20 therapy), T cells (with inhibition of T-cell co-stimulation), or other specific fibrosis signaling pathways, such as anti-transforming growth factor (TGF)-β agents are under investigation, but have yet to be approved as disease-modifying therapies in SSc.
In this context, cell therapies are a new therapeutic option for SSc. We and other contributed to demonstrate that autologous hematopoietic stem cell transplant (AHSCT) has disease modifying properties (12–14), allowing improvement in long term overall survival (OS) and event-free survival (EFS), with regression of skin (15) and lung (13,16) fibrosis and improved QOL (17,18). However, AHSCT is contraindicated in cases with advanced visceral involvement (19,20) and, in eligible patients, the procedure is still associated with risks of toxicity. **Mesenchymal stromal cells (MSC)** are multipotent cells that have immunomodulatory, pro-angiogenic, and anti-fibrotic properties, and play a significant role in tissue repair and regeneration. MSC were first identified in the bone marrow (MSC(M)) (21) and are present in nearly every tissue. For therapeutic uses, they are usually harvested from bone marrow (MSC(M)), adipose tissue (MSC(AT)), and umbilical cord (MSC(UC) and have been extensively characterized (22–25). These multipotent progenitor cells modulate both innate (26) and adaptive (27) immune systems, and have pro-angiogenic and anti-fibrotic properties, which provide a strong rationale for their use to target **all three axes of the SSc pathogenic triad** (28,29), as we recently reviewed (30).

2.2 Biology of Mesenchymal stromal cells (MSC).

In vitro expanded MSC are defined a minima by the International Society for Cellular Therapy (ISCT) MSc committee, as: 1) a plastic-adherent polyclonal population with fibroblast-like morphology, 2) expressing CD73, CD90 and CD105 (in > 95% MSC), 3) in the absence of hematopoietic and endothelial markers, and 4) able to differentiate in vitro into osteoblasts, adipocytes, and chondroblasts (22).
MSC effects are mediated by direct cell-cell contact, but most of their action is exerted through secretion of soluble factors, which are not only constitutively expressed by MSC, but are induced by several proinflammatory cytokines in the local milieu. MSC secrete growth factors, cytokines, and hormones (31–34), which are central to MSC paracrine activities, and contribute to tissue regeneration in various diseases, including for SSc (31–36). Together with this short-lasting bystander trophic activity, the clinical potential of MSC has been proposed to depend on their wide immunosuppressive and anti-inflammatory potential, suggesting that immunomodulation facilitates host tissue regeneration (37,38).

Numerous parameters modulate MSC functional properties, including the tissue source and the production process, and the inflammatory environment, since MSC immune properties are essentially not constitutive, but licensed (also called MSC priming) by inflammatory stimuli (e.g. IFN-γ, TNF-α, IL-1α or IL-1β) (39–41). The expression of surface markers and MSC functional features may change according to culture conditions, cryopreservation, and inflammatory status (39), and these properties are critical for clinical application and the interpretation of MSC studies (38,42).
MSC show intermediate levels of major histocompatibility complex (MHC) class I molecules and have no detectable levels of MHC class II, mainly HLA-DR, and co-stimulatory molecules (CD40, CD80, and CD86). Importantly, MSC priming also increases the expression of major histocompatibility complex (MHC) class I and induces MHC class II molecules and MSC are recognized and killed by activated immune cells.
In addition, after intravenous administration, most MSC get trapped in the lungs and are rapidly removed from the circulation (half-life of ~ 24 hours). Persistence in tissues is not necessary for MSC to have long-lasting benefits. Rather, the effects of MSC are fundamentally the result of immune modulation (innate and adaptive immune responses) and a trophic role (e.g., inhibition of apoptosis, secretion of proliferative and growth factors, angiogenesis), mediated by a number of secreted growth factors, cytokines, and hormones (e.g., VEGF, PDGF, ANG-1, IL-11, PGE2, TSG-6, SDF-1, HGF, IGF-1, IDO) and extracellular vesicles (31,43).
MSC biodistribution and homing have been analysed using various labelling methods, but these techniques cannot confirm sustained cell viability, in either animals or humans (44–46). While studies have identified mechanism by which MSCs can migrate to sites of injury and participate in tissue repair (47), studies in mice have reported that MSC are rapidly removed from circulation (~24 hours) after iv injection; the cells are first trapped in lung, then transmigrate beyond vascular spaces, where most MSC are rapidly phagocytosed by lung-resident tissue macrophages (48). The adhesion molecules VLA-4/VCAM-1 influence interactions between MSCs and endothelial cells during transmigration in the lung. The term “medicinal signalling cells” (MSC) proposed by Caplan (25) reflect that in vivo MSC “release therapeutic agents in situ at site of injury, disease, or inflammation” due to their secretory properties.

2.3 Rationale for the use of allogeneic MSC to treat SSc patients.

Although we and others initially reported that MSC(M) obtained from SSc and healthy controls inhibit proliferation of mixed peripheral blood mononuclear cells and T cell proliferation at similar rates (49,50), when specifically co-cultured with PHA-conditioned T lymphocytes (49), disease-specific abnormalities in MSC from SSc patients later emerged (51,52). It has been hypothesized that these defects in MSC(M) could contribute to SSc pathogenesis.
MSC(M) from SSc patients have increased TGFβ-R2 on their cell surface, and a higher sensitivity to TGFβ, resulting in excessive production of collagen 1, contributing to tissue fibrosis (53). MSC(M) samples from SSc patients also have reduced clonogenicity (assessed in colony-forming unit fibroblast (CFU-F) assay) (51), and undergo early senescence (increased β-Gal activity), with a significantly decreased proliferation rate (lower ki67 gene expression, higher p21 transcript level) (54). SSc MSC(M) also demonstrate a reduced capacity to differentiate into endothelial progenitor cells, osteoblasts, and adipocytes, as well as angiogenic dysfunction (52). When co-cultured with CD4+CD25– lymphocytes, TGF-β expression is significantly higher in MSC derived from SSc patients, compared to healthy controls. Cell surface expression of TGFβ-RII is also increased SSc MSC, resulting in a higher sensitivity to TGF-β (54). In the presence of TGF-β, SSc MSC significantly increase collagen 1α synthesis and Smad-3 phosphorylation, which contribute to tissue fibrosis (53). Another study confirmed that the proliferation rate, metabolic activity, and migration and invasion potential of MSC(AT) was decreased in SSc patients compared to normal controls (55).
These data support the use of allogeneic (instead of autologous) MSC sources for SSc treatment.

2.4 Why using Adipose Tissue Derived MSC tissue sources and which culture condition for treating SSc patients?

MSC(M) were first considered as the main source of MSC used in clinical trials, although their capacity to proliferate and differentiate changes significantly over time, decreasing with age, and varies extensively according to donor sources (38,56). It requires a painful invasive procedure to obtain BM sample in the donor and there is a risk of viral exposure. **Adipose-Tissue derived MSC (MSC(AT))** isolated from the stromal vascular fraction (SVF) of subcutaneous adipose tissue can yield up to a 500-fold higher number of MSC compared to bone marrow (57). Recently, we completed the first transcriptomic, phenotypic and functional analyses on paired MSC(M) and MSC(AT) samples from the same healthy donor and showed stronger inhibition of immune responses by MSC(AT) and lower immunogenicity as compared to MSC(M), providing a new rationale to support the use of adipose tissue as a source of MSC for the treatment of immune-mediated diseases (58). These two types of MSC secrete angiogenic, antiapoptotic, and anti-inflammatory cytokines with different cytokine profiles according to tissue source and donor type (59).

The culture conditions, including culture medium and scale of expansion, and the use of cryopreservation, were also shown to affect MSC functions (59). In particular, both the onset of replicative senescence associated with large in vitro expansion and the use of cryopreservation were shown to impact the immunosuppressive functions and the in vivo persistence of clinical-grade MSC, which can be partly restored by in vitro licensing by inflammatory stimuli (60–64). Each of these multiple sources of heterogeneity during the MSC production process (38) and their release for therapeutic use has to be considered when designing clinical trials (59).

Following our published experience in using MSC(M) for the treatment of SSc patients (65), several limitations appeared when using MSC production derived from BM (culture growth, heterogeneity of the MSC(M) product amongst others). Considering the in vitro comprehensive comparison of MSC(AT) versus MSC(M) (57–59), we further decided to explore the use of MSC(AT) for treating SSc patients and designed the present trial involving the manufacturing center of Etablissement Français du Sang (EFS) for its experience in MSC production, especially from AT source.

Indeed, the manufacturing center of EFS Creteil is authorized to produce cell therapy medicinal products and work under GMP rules (Pharmaceutic Authorization: 2023_074_1, GMP certificate number: 2023/HPF/FR/052). The MSC(AT) manufacturing process has initially been developed for the production of autologous MSC(AT) by the EFS Toulouse team and used in several clinical trials (namely : ACELLDREAM clinical trial (ANSM authorization n° TC194); ADIPOA clinical trial (ANSM authorization n° TC301 and PEI authorization n° 1450/01); ADIPO A2 (EudraCT No.: 2015-002125-19). The Standard Operating Procedure (SOP) has been transferred to the Creteil EFS team and further developed to produce allogeneic MSC(AT) as used for human clinical application to inject 15 patients with Lyell syndrome (LYSYME protocol (Eudra CT n°2020-000308-12)) in addition to the 18 patients from the present clinical trial.

2.5 Health Cost is a major issue in the SSc disease and for cell therapy

SSc is a chronic autoimmune disease with the highest morbidity and mortality amongst all rheumatic diseases (30). SSc disabling consequences are at high cost for both patients and the society. A treatment, relieving these handicaps, may improve patients quality of life and have major impact on the disease health-associated costs. It has been estimated in 2017 that the total annual costs per patient suffering of SSc varied from 1,074€ to 22,459€ in Europe (66). Among direct medical costs, medications and hospitalisations were the higher costs. But

direct costs were overtaken by indirect costs, or loss of productivity. In 2015, twoFrench studies (67,68) had underlined that the average annual cost of SSc was estimated at 22,459€ per patient. Direct healthcare costs amounted to 8452€ and indirect costs resulting from patients' absence from the labour market to 10,526€. It was observed that 96,5 % of the SSc patients in 2010 were of the labour market (fulltime sick leave or disability status) (67).

One of the important barriers for larger implementation of MSC therapy has been the high-priced treatment for each patient. Modern biologics products from gene and cell therapy are currently commercialized at prices which cannot be supported by national cost regulatory agency nor the patients. Recently, the MSC product approved, Alofisel ® marketed by the Belgium company Tigenix, now bought by Takeda, proposed to the UK NICE (cost UK regulator agency) a list price of the drug of 62,000€ for a four-vial course of treatment. This has been rejected by the NICE, even though the Alofisel® showed a 14 percent improvement in beneficial effect compared with placebo, and the Alofisel product had obtained the EMA approval for complex perianal fistulas in Crohn's since 2018. Initially, the majority of MSC products were autologous and thus impeding economic scale savings. Indeed, in the setting of autologous production, as each batch is made for one donor, the operating expenditures could not be optimized. The allogenic approach opens a new avenue for cost decreases, using scalable production process. Early development of the allogeneic MSC productions have been focused on clinical trials, which create value for the shareholders. In all cases, it is of upmost important to evaluate the cost-effectiveness of such approach so as to provide more data in case of further industrialization of the production processes, which can eventually provide an easy and universal access to patient.

2.6 Hypothesis for the study

In vitro, experimental and clinical studies suggest that MSC properties vary according to their tissue of origin/source. We demonstrated that MSC(AT) compared to MSC(M) are easier to harvest and display higher proliferative capability before entering senescence, higher genetic stability, and superior immunosuppressive properties. We have completed the first phase I/II dose escalation trial using allogenic MSC(M) infusion in 20 severe SSc patients (ClinicalTrials.gov: NCT02213705) with no safety issues, and found significant improvement in skin fibrosis at 3 months after infusion which appeared lower thereafter (65), thereby supporting the need for repeated infusions. To accelerate the clinical translation of MSC(AT) as a therapeutic cell product in SSc, we propose to test the safety of MSC(AT) in SSc and to seek evidence for a possible efficacy signal in this Phase I/II trial.

Considering the above rationale, we hypothesize that use of healthy donor allogeneic MSC(AT) produced by EFS will demonstrate a) no safety issues, b) an efficacy profile that will increase with repeated infusion at 3 months interval of allogeneic MSC(AT) to treat SSc.

2.7 Summary of relevant pre-clinical experiments and clinical trials

Both in vitro and early clinical studies support the use of allogeneic MSC in Systemic Sclerosis patients, as reviewed in details by our team (30).

In vitro MSC immunomodulatory and immunosuppressive properties

MSC exert their immunomodulatory and immunosuppressive effects on both the innate and the adaptive immune cells through a wide panel of mechanisms (35,36).

In vitro data showed that MSC modulate the immunological activity of the different cell populations engaged in the pathogenesis of systemic sclerosis, which led us and others to evaluate MSC potential therapeutic values in SSc patients (29,30). First, MSC inhibit DC differentiation, maturation, cytokine expression and capacity to present antigens to T lymphocytes (69,70). They influence antigen presentation by DC via downregulation of DC cell surface expression of MHC class II, CD11c, and CD83, which modulate their anti-inflammatory action (71). MSC directly modulate T cell activation, proliferation, differentiation, and effector

function. MSC inhibit the proliferation of both naïve and memory CD4+ and CD8+ T cells through arrest in the G0/G1 phase of the cell cycle (72), and abrogate T cell activation (73–76). Interestingly, MSC also influence the differentiation of naïve CD4+ T helper cells by promoting anti-inflammatory immune responses and affect the balance of T cell polarization (77). Thereby, in an inflammatory setting, MSC appear to increase the number and activity of Treg cells and IL-10 expression, while suppressing Th1, Th2, and Th17 cells (78,79). MSC can reduce the release of pro-inflammatory cytokines from different T cell populations, including IFN-γ, TNFα, IL-6, and IL-7, and increase anti-inflammatory cytokines, such as IL-4 and IL-10 (77,80). MSC can also inhibit IL-2-induced proliferation of resting NK cells and partially inhibit the proliferation of activated NK cells and thereby NK mediated cytotoxicity (81). Besides, MSC also inhibit B cell proliferation, antibody production, and chemotaxis under inflammatory conditions (73,82), and favour Breg expansion. Inhibition of B cell proliferation appears to be indirect, as it requires the presence of CD4+ and CD8+ T cell lymphocytes (83). MSC effects are mediated in part by direct cell-cell contact, **but most MSC effects are paracrine and exerted through secretion of soluble factors.** Typically, the mechanism of action of MSC first involves the release of chemokines allowing attraction of activated T cells (33), that produce proinflammatory cytokines responsible for the priming of MSC towards an immunosuppressive phenotype. In turn, MSC secrete growth factors, cytokines, enzymes and hormones (e.g., VEGF, PDGF, ANG-1, IL-11, PGE2, TSG-6, SDF-1, HGF, IGF-1, IDO) (31–34), which are central to MSC paracrine activities. Immunosuppressive soluble factors secreted by MSC in vitro include indoleamine 2,3-dioxygenase (IDO), an enzyme that catalyzes transformation of tryptophane to kynurenine (81,84), which is involved in inhibition of B cell and T cell proliferation and in the polarization of monocytes into IL-10-secreting M2 macrophages (85). MSC also produce extracellular vesicles (EVs), including exosomes, microvesicles and apoptotic bodies, which are small (44-100 nm in diameter) membrane vesicles. These exosomes have immunosuppressive and immunomodulatory activity (86). The contribution of the MSC secretome to inhibiting or reversing fibrosis in the SSc pathologic microenvironment is not well understood.

MSC proangiogenic and antifibrotic properties: in vitro evidence

The angiogenic potential of MSC was first demonstrated by their capacity to differentiate towards an endothelial cell lineage (87). MSC(AT) secrete angiogenic and antiapoptotic factors, such as VEGF, hepatocyte growth factor (HGF) (88), leptin (89), basic fibroblast growth factor (bFGF), Ang-1, Ang-2, platelet derived growth factor (PDGF) (90–92), as well as mesenchymal stem cell-like protein (MSCP1) and stromal cell-derived factor-1 (SDF1), which are essential for vascular remodelling (93). The combination of VEGF and shear stress can enhance endothelial differentiation of MSC(AT) (94). More recently the angiogenic/angiostatic signaling pathways by which MSC(AT) support neo-vessel formation and stabilization were described, elucidating the functional interaction between MSC(AT)s and endothelial cells (95–99) and MSC(AT) appear to be more effective in this regard than their counterparts from bone marrow. There is currently no consensus on which assays should be used to assess anti-fibrotic activity of MSC in vitro, which is crucial for the selection of MSC products to be used in the therapeutic setting, including for clinical trials.

MSC immunopotency assays. In 2016, the MSC committee of the ISCT published guidelines addressing the characterization of MSC immune modulatory properties (24). Since then, MSC deploy a panel of immunomodulatory and regenerative properties, largely dependent on their interaction with their microenvironment. Standard functional markers of MSC potency as well as release potency assays have been defined for conducting advanced clinical studies and their potential registration (24,100). Standardized methods have been proposed to assess MSC functional properties (101). The preferred analytic methods for matrix assays evaluating the immunosuppressive and immunomodulatory capacities of MSC (102,103) currently include:

a) quantitative RNA analysis of selected gene products (104), b) flow cytometry analysis of functionally relevant surface markers, and c) protein-based assay of secretome. Recently, a combination of transcriptome and secretome analysis has been proposed as predictive of T cell immunosuppressive properties (102) but additional quantitative assays are urgently required to capture the whole MSC immunosuppressive, anti-inflammatory, and regenerative potential that could synergistically improve SSc patient behaviour. Similarly, standardized high-throughput multiparametric immunomonitoring tools have to be set up to characterize the patient immunological status and the various immune cell subsets before and after MSC treatment, to identify responders and thereby optimize clinical trials design.

MSC have been investigated in several animal models of SSc (105,106)**,** although none of these encompass all the features of the disease (105). The SSc hypochlorite (HOCl)-injected mouse model, daily subcutaneous injections of HOCl in BALB/c mice produce ROS, anti-DNA topoisomerase 1 antibodies, progressive collagen deposition, and early and continued skin and lung fibrosis that best reproduce human pathological findings (107). In this model, Maria A and others showed that a single infusion of allogeneic MSC(M) immediately after HOCl exposure in BALB/c mice ($2.5x10^5$, $5x10^5$, $5x10^6$ cells) significantly reduced skin and lung fibrosis (108). The lowest dose ($2.5\ x10^5$) examined was the most effective, with sustained reduction of fibrosis, advanced oxidative protein products and total collagen content until 42 days. Markers of fibrosis and cytokine expression were lower in lung tissue after MSC infusion. Only fibrosis marker expression was significantly reduced in skin and only with the lowest dose. Serum levels of anti-scl-70 auto-antibodies were also lower in all mice that received MSC infusions. MSC treatment transiently improved skin thickness in HOCl-exposed mice, with a rate of disease progression similar to untreated mice after day 21 and no additional treatment benefit after this point. In mice that received a second MSC infusion on day 21, a significantly slower progression of skin thickening between day 21 and 42 was observed. In mice with established SSc, the effects of a late single MSC infusion were observed from 1 week after treatment onward, with significant reductions in skin and lung fibrosis. Both allogeneic and xenogeneic MSC were examined in the SSc (HOCI)-induced mouse model (109). BALB/c mice exposed to HOCI were infused with syngeneic BALB/c MSCs, allogeneic C57BL/6 MSC, or xenogeneic human MSC isolated from either bone marrow or adipose tissue. In each of these condition MSC were collected from 3 donors. All the sources of MSC appeared to have similar therapeutic effects, including a decrease in skin thickness, collagen levels in skin and lung, and expression of collagen 1, collagen 3, and αSma (109,110).

Feasibility of using Allogeneic MSC in SSc patients. The feasibility of using allogeneic MSC(M) was first supported by several small studies in severe SSc patients, with follow-up varying between 6 and 44 months. Improvement in skin ulcers and reduction in skin fibrosis has been reported in 6 patients to date (111,112). One clinical case reported an allogeneic MSC(M) transplant from a father to his 41 year-old daughter with diffuse cutaneous SSc (111). Vascular ultrasound 6 months after transplantation revealed a marked improvement in perfusion of hands and fingers, and revascularization of the patient's extremities was confirmed on angiography. A follow-up study by the same group investigated interfamilial allogeneic MSC in 5 severe SSc patients, with follow-ups ranging from 6 to 44 months, without any major adverse events (112). Two of the patients received fresh MSC, and 3 were injected with MSC isolated from cryopreserved tissue. One patient died of cardiac arrest related to disease progression 18 months after treatment. Another Chinese study investigated the combination of plasmapheresis (PE) and allogeneic MSC(UC) in 14 systemic sclerosis patients (113) which received three repeated PE treatments with subsequent pulse cyclophosphamide on days 1, 2, and 5. MSC(UC) infusion on day 8. At 12-month follow-up, patients exhibited improvements in the modified Rodnan skin score (20.1 ± 3.1 versus 13.8 ± 10.2; $P<0.001$). A subset of patients with interstitial lung disease experienced an improvement in lung function and on computed tomography (CT) scans. Scl70 antibodies, serum TGF-β, and EGF levels were also significantly decreased at 12-month follow-up. A recent report from the same chinese

group retrospectively analysed 41 SSc patients treated with one (n = 24) or multiple injections at one or two years interval (n= 17) of MSC(UC) and who all patients completed a five year follow-up, showing 92.7% overall 5-year survival rate, stable or improved mRSS and interstitial lung disease (ILD) on CT scann images at 1, 3, and 5 years with no adverse events related to UMSCT during the follow-up period (114)**.**

In 2022, our team published results from the first open-label, non-randomized, monocentric, dose-escalation phase I/II clinical study in 20 severe SSc patients (PHRC AOM 11250; ClinicalTrials.gov: NCT02213705), derived from intra-familial bone marrow (BM) donors. We demonstrated the safety (primary objective) of 1 to 3.10^6 allogenic MSC(M)/kg single infusion with at least one-year follow-up. We also observed regression of skin sclerosis early after injection and stable pulmonary function until one year (65). This trials included a longitudinal in-depth characterization of circulating immune cells of these MSC-treated SSc patients and we furthermore demonstrated that Regulatory B Cells contribute to the Clinical Response of SSc patients after MSC(M) infusion (115).

The experience gained with this first trial (65) allowed the following major learning conclusions and improved our knowledge for next clinical study design: a) longstanding clinical efforts (more than 4 years) were necessary to find adequate interfamilial allogenic BM donors, b) large heterogeneity was found in MSC(M) production despite using well-standardized techniques, due to multiple donors c) the absence of dose effect between $1x10^6$ and $3x10^6$ allogenic MSC(M)/kg within the limit of a single dose MSC(M) injection, d) the promising value of the observed clinical results with MSC(M) which allowed improvement in skin score up to one year, e) the large heterogeneity in SSc disease, f) and therefore the need for a different statistical approach (control study and repeated injections) to search for a meaningful clinical dose effect.

To better assess the early trends in efficacy and to inform future design of larger clinical trials, results from this first phase I-II trial retrospectively underline the critical importance of: 1) improving the homogeneity of the process for allogeneic MSC production, 2) adding a control placebo arm. Our first trial was designed ten years ago, while experts now recommend placebo-controlled trials in SSc (116) and MSC (117). In addition, the use of a placebo arm is justified by the fact that SSc patients will have failed standard of care, background use of a stable dose of prednisone ≤ 6 mg/d will be allowed and the trial duration (1 year) is short.

There are 5 additional trials of MSC for SSc patients in May 2024 registered at clinicaltrials.gov, of which 1 involve allogeneic MSC(M) (in Netherlands, NCT03211793, using intra-muscular injections for severe digital ischemia), 1 involves allogeneic MSC(AT) (in France, NCT04356755) through surgical intramuscular injection at specific finger sites, and 3 involves allogeneic MSC(UC) (in Canada, (NCT04356287), in Colombia, NCT04432545) and in Antigua and Barbuda (NCT05016804)) through IV infusion.

Dosing considerations. There is heterogeneity in the dose of MSC used in animal and human studies. In the HOCL-induced mouse model of SSc, 3 separate doses of MSC(M) ($2.5x10^5$, $5x10^5$, $1x10^6$ MSC/kg) were investigated, and the best results were obtained with the lowest dose (reductions in markers of fibrosis (Col1, Col3, TGFβ1, and αSMA) in both skin and lung inversely proportional to the dose) (110). Most human studies in autoimmune diseases have used one infusion of 1-5 million MSC/kg and never more than 12 million/kg (118). For example, Keyszer et al (112) had treated five SSc patients with 10^6 cells/kg. The mRSS score was improved in 4 of 5 patients. In refractory Crohn disease, 16 patients had received 4 weekly doses of $2x10^6$ cells/kg MSC(M) (119), with 12 out of 15 patients experiencing a response to the treatment and 8 having a clinical remission. In multiple sclerosis, 10 patients were injected with 10^6 cells/kg of autologous MSC(AT) and 9 with $4x10^6$ cells/kg with no serious adverse events, while the clinical benefit was relatively low (120). In our phase I/II trial of allogenic MSC(M) in diffuse SSc (ClinicalTrials.gov Identifier: NCT02213705), we used $1x10^6$ MSC/kg

in the first 10 SSc patients and $3x10^6$ MSC/kg in the next 10 SSc patients and found no safety signals nor dose effects on clinical evolution in either groups.
The intravenous administration is the best way to deliver MSC(AT) to obtain a general effect. Considering MSC bio-distribution (rapid disappearance after intravenous infusion) and mechanism of action (secreted factors), the concept of repeated infusions is gaining momentum in the field. Data suggests that one dose likely underestimates the benefits of cell therapy and that the duration of exposure to MSC is probably more important than the intensity of the exposure (121). In the bleomycin model of SSc, Moroncini showed the superiority of 2 doses of 2.5 $x10^5$ MSC(UC) at days 1 and 7 compared to a single infusion (122). Repeated infusions are now being tested in several trials in a number of indications (GVHD, Crohn's and myocardial infarction) (121,123) and may optimize the therapeutic effect of the cells as it was shown in the treatment of graft versus host disease. We are proposing to test up to 2 infusions. The optimal interval between repeated doses may depend on the disease. Changes in skin and visceral fibrosis in SSc evolve over months (8) and experts recommend assessing SSc patients on a quarterly basis (2). Results from our Phase I/II trial of allogenic MSC(M) in 20 diffuse SSc patients showed that improvement in skin fibrosis peaks 3 months after infusion (mean modified Rodnan skin score (mRSS) falls from 26 to 17) and marginally thereafter (from 17 to 14 between M3 and M24). Therefore, we are proposing to repeat a second infusion 3 months after the first. We chose to inject a fixed dose of $2x10^6$ MSC(AT)/kg based on our previsous experience using MSC(BM) for systemic sclerosis patients (65) and MSC(UC) for lupus erythematosus (Farge et al, Lancet Rheumatology, in Press) plus systematic review and meta-analysis of randomized control trials of MSC in the treatment of autoimmune diseases (124,125) which showed no evidence of a dose effect. In addition, as we recently reviewed in Greco et al (126) (refer to supplementary data), data from the literature using Bone marrow or Umbilical Cord derived MSC in SSc patients showed that MSC doses varied from 0.5 to 3 $x10^6$/kg, with no adverse effects. Given the established safety profile and MSC and their wide therapeutic window, and considering that the present aim of the trial was to analyze the effect of repeated injections of AT-MSC, we chose the dose of $2x10^6$ MSC(AT)/kg.

2.8 Description of the population to be studied and justification for the choice of participants

Patients fulfilling the 2013 American college of rheumatology (*ACR*)/European league against rheumatism (*EULAR*) SSc criteria (127) will be included if aged above 18, if contra-indicated or resistant to conventional immunosuppressive therapy or to AHSCT; with a minimum modified Rodnan skin score
(mRSS) (range, 0-51) of :
- 15 with presence of lung, heart or kidney involvement or
- 20 with disease duration of 2 years or less abnormal CRP > 5 mg/l and/or hemoglobin < 11 g/dL.

This sub-group of SSc patients, irrespective of disease duration often suffer from severe disease and altered HRQoL and may benefit from MSC therapy as suggested by previous reports and clinical experience described in 2.7. Patients with severe comorbidities will be excluded.

2.9 Identification and description of the advanced therapy medicinal product

The advanced therapy medicinal product (ATMP) used as the investigational medicinal product (IMP) is allogeneic Adipose Tissue derived Mesenchymal stromal cells THAwed and Cultured at passage 2 before delivery, names as- MSC(AT) THA-C.
The MSC(AT) are produced by EFS under GMP guidelines from adipose tissue of a unique healthy donor and qualified for quantitative and functional testing. Cells harvested at passage 1 are cryopreserved in albumin 5% with 10% final of DMSO (vol/vol) in freezing bags. The bags are then transferred to a controlled freezer and then stored in gas-phase nitrogen at -150°C.

At the inclusion of a SSc patient to be treated, MSC(AT) will be thawed in a dry bath at 37°C followed by washing and MSC(AT) will be put in culture at high concentration during 18-48 hours. Then MSC(AT) in suspension in ringer lactate supplemented with albumin 1% (passage 2) will be packaged in transfer bag at a concentration of $1x10^6$ MSC(AT) /ml for I.V injection. Cell suspension will be shipped to the St-Louis pharmacist.

Placebo will be prepared by the hospital pharmacies, according to the hospital preparation good practice. More details are provided in section 7.2 and in the IMPD.

2.10 Description of the dosage, route of administration, administration schedule and treatment duration

Allogeneic Adipose tissue derived-MSCs (MSC(AT)) or placebo will be injected by slow intravenous infusion at 3-5 ml/mn with a minimum infusion time of 16 min via a 200 µm transfusion filter. The MSC(AT) bags of cell suspension will all be prepared according to the recipient body weight and to the study experimental arms:

- arm 0: placebo at M0 and M3
- arm 1: 1 MSC(AT) ($2x10^6$ cells/kg) injection at M0 and 1 placebo injection at M3
- arm 2: 1 MSC(AT) ($2x10^6$ cells/kg) injection at M0 and 1 MSC(AT) ($2x10^6$ cells/kg) injection at M3

2.11 Summary of the known and foreseeable benefits and risks for the Clinical Trial participants

The increasing use of MSC in a variety of indications provides consistent evidence supporting their safety in humans. A recently updated meta-analysis of clinical trials including over 2700 patients treated with MSC **did not detect any associations between MSC and organ system complications, infection, death or malignancy** (128). The most common side effect noted was transient fever. Claims of genetic instability and tumorigenicity for human culture-expanded MSCs have been refuted (43). Five studies were reported in the setting of previous malignancy (5 case-series, n=67 patients), no local recurrence was reported (129). Out of 54 patients with previous breast cancer, one developed a recurrence 11 months after Adipose-Derived Regenerative Cells treatment (130). MSC were injected after prostate cancer in 3 studies. None reported oncological safety issues (131–133).
In a Chinese long-term retrospective single-center study of 404 patients with autoimmune diseases treated with MSC(UC), the rate of grade $\geq$ 3 serious adverse events (SAE) one month after infusion was 2.2% (134). In other studies, the rate of SAE in the month post infusion was consistently well below 5% (120,135–137). In our recent Phase I/II dose escalation trial of a single infusion of allogenic bone marrow-derived MSC (from family donors) in diffuse SSc refractory to standard therapy (ClinicalTrials.gov Identifier: NCT02213705), there was no safety signal after the first 10 patients treated with $1x10^6$ MSC/kg, nor in the subsequent 10 patients with a higher dose $3x10^6$ MSC/kg (65). No infusion-related SAE and three infusion-related adverse events occurred in the first 10 days after treatment; one patient had grade 1 flushing and another patient had grade 1 nausea and grade 2 asthenia. Although we foresee the risks to be very low, safety is nevertheless the primary outcome of this clinical trial. While there is a theoretical risk of respiratory complications, the only randomized trial in which acute pulmonary reactions to MSC have been observed was conducted in patients with chronic ischemic heart failure who received intracoronary MSCs (138). This risk seems to be limited to patients with an underlying condition at risk to develop pulmonary edema. (135)

3 OBJECTIVES

3.1 Primary objective

To evaluate the safety one month after allogeneic $2x10^6$ MSC(AT)/kg intravenous

administration once or twice at 3 months interval (M0, M3) in severe SSc patients

3.2 Secondary objectives

- Safety during the infusion, within the first 24 hours of infusion and during all study follow-up.
- Efficacy signals to inform future studies, using outcome measures on skin sclerosis, lung function and quality of life, previously validated in SSc or used in other cell therapy trials,
- Analysis of the response to treatment, Progression-free survival (PFS), Global Rank Composite Score (GRCS) at M3, M6 and M12 and ACR Provisional Composite Response Index for Clinical Trials in Early Diffuse Cutaneous Systemic Sclerosis (CRISS) for early SSc patients at M3, M6 and M12.
- Analysis of the overall survival and causes of death, if any.
- Impact of allogeneic MSC(AT) iv once or twice at 3 months interval on the immune response, including immunophenotyping and alloimmunization up to M6 after starting therapy.
- Cost effectiveness of the allogeneic MSC(AT) infusion once or twice versus no treatment in severe SSc at M12

3.3 Objective of ancillary study

- Exploring immune landscape in SSc skin biopsies before and after MSC(AT) and signatures predicting treatment response
- Evaluating new MSC potency assays interrogating MSC efferocytosis

4 STUDY DESIGN

4.1 Study endpoints

4.1.1 Primary endpoint

Safety of injections will be evaluated by the rate of treatment-related Serious Adverse Events (SAE) defined as Adverse Events (AE) of grade equal or above 3 using the NCI Common Terminology Criteria for Adverse Events (CTCAE) v5.0 classification, at one month after each infusion according to arms (M1, M4). All adverse events will be adjudicated by a Data and Safety Monitoring Committee.
Treatment-related toxicity will be analyzed according to the international World Health Organization (WHO) (maximum degree of toxic attacks by the body). ***An injection will be considered as not tolerated for any toxicity criteria above grade ≥ 3*** (139)***.***

4.1.2 Secondary endpoints

1. Safety at the time and within the first 24 hours of infusion and during all study follow-up will be evaluated by the Rate of treatment-related SAE defined as AE of grade equal or above 3 CTCAE v5.0 at time and within the first 24 hours of infusion and during all follow-up: M0, M3, M6, M9 and M12. Treatment-related toxicity will be analyzed according to the international World Health Organization (WHO) (maximum degree of toxic attacks by the body). An injection will be considered as not tolerated for any toxicity criteria above grade ≥ 3 (139).
2. **Main efficacy endpoint** will be assessed by mRSS difference between M0 and M12.
3. Other efficacy disease related secondary endpoints will be:
    - mRSS at M3, M6 and M9.

- WHO performance status (PS) and Health-Related Quality of Life (HRQoL) questionnaires: Scleroderma-Health Assessment Questionnaire (SHAQ), the Short Form (36) health survey (SF-36v2) and EQ-5D-5L at M0, M3, M6, and M12;
- Forced Vital Capacity (FVC), Diffusing capacity of Lung for carbon monoxide (DLCO), at M0, M3, M6, and M12

4. Response to treatment will be defined as any of the following: decreased mRss > 25%, increased FVC > 10% and/or increased DLCO >10%, without need for further immunosuppression except low dose steroids (below 10mg daily) (140) at M3, M6 and M12.
5. Progression-free survival at 12 months will be defined as the percentage of enrolled patients still alive without evidence of progression 12 months after MSC(AT) injection, with progression defined as any of the following compared to baseline evaluation: decreased FVC > 10% or DLCO > 15%; decrease in LVEF% > 15%; decrease in weight > 15%; decrease in creatinine clearance > 30%; increased mRss > 25% ; and/or increase in SHAQ> 0.5 (140).
6. GRCS at M3, M6 and M12 calculated as previously described (141,142).
7. CRISS for early SSc patients at M3, M6 and M12 calculated as previously described (143,144)
8. Overall survival at 12 months will be defined as the percentage of enrolled patients still alive 12 months after MSC(AT) injection.
9. Myeloid and lymphocyte sub-populations will be analyzed in all included patients at M0, M1, M3, M4, M6, as measured by 2 mass cytometry panels (Cytof) to assess their proportion and activation status
10. Alloimmunization against MSC will be evaluated in all included patients through the detection of donor-specific anti-HLA antibodies and identification when positive at M0, M3 and M6
11. Extra-Cost per QALY (quality-adjusted Life Year) gained by unique and repeated IV infusion of allogeneic MSC(AT) in severe SSc after 12 months. QALY will be measured by combining survival data and Health related quality of life data collected with the EQ-5D-5L and transformed into Utilities. QALYs will be related to Medical and productivity costs.
12. Extra-Cost per treatment-related AE of grade above or equal to 3 CTCAE avoided by unique and repeated IV infusion of allogeneic MSC(AT) in severe SSc after 12 months. SAE will be related to medical and productivity costs as well.

4.2 Description of research methodology

4.2.1 Design of the study

We propose to conduct a multicenter Phase I/II, three-arm, randomised, placebo-controlled, double-blind trial. 18 SSc patients will be 1:1:1 randomized (with randomization blocks of size 6) in one of the following arms:

- arm 0: placebo at M0 and M3
- arm 1:1 MSC(AT) ($2x10^6$ cells/kg) injection at M0 and 1 placebo injection at M3
- arm 2: 1 MSC(AT) ($2x10^6$ cells/kg) injection at M0 and 1 MSC(AT) ($2x10^6$ cells/kg) injection at M3

Second infusions will be performed only in the absence of previous treatment-related SAE (TRSAE), as defined in primary endpoint.

-

Continuous monitoring of the primary endpoint by the sponsor will be implemented to allow continuous Bayesian toxicity monitoring with a stopping rule implemented on the primary endpoint (see section 13 for details):
- first analysis on the first 3 patients with completed M1 visits,
- then analysis at any TRSAE at M1 or M4 visits
- final analysis

Patients and medical team will be blinded to treatment arm. Blinding will only be broken if 1) presumably related SAEs occurred and if the investigators want to know the assigned treatment for proper clinical management; or 2) it is required by local regulatory authorities.

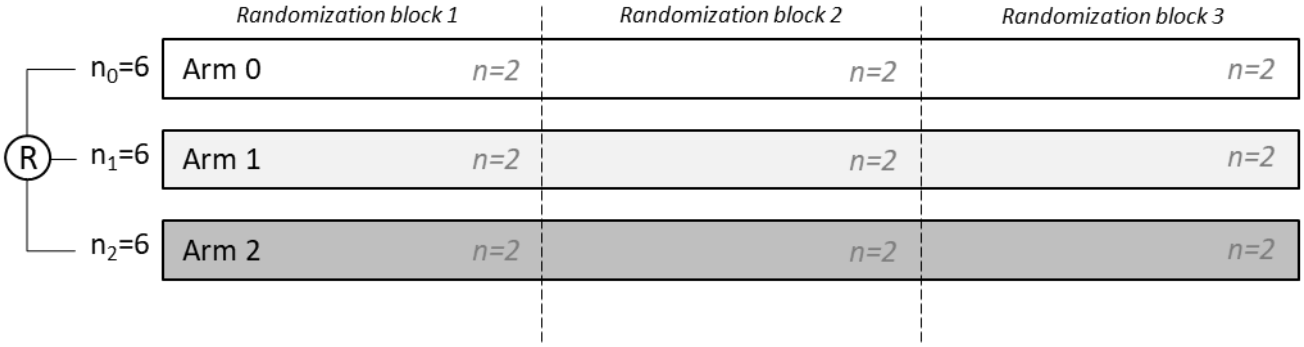

Figure 1. Trial design. 1:1:1 randomized design, with 3 randomization blocks of size 6, staggered inclusions in cohorts of 3 patients (≥1 week between each patient and ≥1 month interval every three patients), continuous toxicity Bayesian monitoring with toxicity stopping rule (first analysis on the first 3 patients with cell infusions (Arms 1 and 2) completed M1 visits, then at any TRSAE at M1 or M4 visits, and final analysis). n_0 *i*s the total number of patients in Arm 0, n_1 in Arm 1 and n_2 in Arm 2; *n* is the number of patients per arm per randomization block. Arm 0 = placebo at M0 and M3 ; Arm 1= MSC(AT) (2x106 cells/kg) injection at M0 and 1 placebo injection at M3; Arm 2=1 MSC(AT) (2x106 cells/kg) injection at M0 and 1 MSC(AT) (2x106 cells/kg) injection at M3.

Patients included in arm 1 and in arm 0 will have the opportunity,according to their willingness, to receive either 1 (arm 1) or 1 or 2 (arm 0) MSC(AT) ($2x10^6$ cells/kg) injection at the end of the study after unblinding and once evidence of MSC(AT) injection safety is provided. In that case patients will be followed as usual care up to three more months.

4.2.2 Number of participating sites

This multi-centre study will involve 2 different hospitals within the MATHEC network, " Filière des maladies auto-immunes et auto-inflammatoires rares" (FAI2R), under the auspices and the good clinical practices of the "Société Francophone de Greffe de Moelle et Thérapie Cellulaire" (SFGM-TC).

- Recruitment and following centres The participants will be recruited in hospital (consultation or hospitalisation or outpatients) from 2 different sites and followed in their recruiting centre.
- Centre involved in MSC injections : all the participants will receive MSC(AT) or placebo injections in Saint-Louis hospital

Table 1: Summary table of clinical centres involved in patients recruitment, treatment or follow-up

Hospital	Department	Investigator	City	Country	Expected number of recruited and followed patients	Expected number of patients treated by MSC	Expected number of patients followed after MSC
Saint-Louis Hospital	Reference Center for autoimmune and rare systemic diseases MATHEC (FAI2R)	Pr Dominique Farge (coordinator) and Pr Mathieu Puyade* (co-investigator)	Paris	France	9	18	9
	Central pharmacy	Dr Isabelle Madeleine-Chambrin, and Dr Romain De Jorna			0		0
CHU Rangueil	Internal Medicine and clinical Immunology, Centre of competence (FAI2R)	Pr Grégory Pugnet and Dr Alexandre Maria* (co-investigator)	Toulouse	France	9	0	9

* Pr Mathieu Puyade (Internal Medicine, Centre of competence for rare systemic Autoimmune Disease, CHU Poitiers) and Dr Alexandre Maria (Department of Internal Medicine, Centre of competence for rare systemic Autoimmune Disease, CHRU Montpellier), will contribute to patients recruitment and send eligible patients to Pr Dominique Farge in Paris and Pr Grégory Pugnet in Toulouse respectively.

- Non-recruitment centres

The following centre will be involved in a specific intervention to the clinical trial excluding patient recruitment, treatment or follow-up

Table 2: Summary table of non-recruitment centres having specific intervention to the research

Hospital	Department	Investigator(s)	Investigation(s)	City	Country
CHU Pontchaillou	SITI Laboratory	Pr Karin Tarte (Pharmacist, PhD), Dr Virginie Girault (PhD), Dr Jöelle Dulong (PhD)	Immunomonitoring of patients (immunophenotyping and alloimmunization)	Rennes	France

4.2.3 Identification of participants

The participants in this clinical trial will be identified as follows:
Site number (3 digits; e.g.001) - Sequential enrolment number for the site (4 digits; e.g 1001) - surname initial - first name initial
This reference number is unique and will be used for the entire duration of the study.
A randomisation number will also be assigned when the participant is randomised. This number will have the following format: R + 3 digits

A treatment number will be also assigned when the participant is allocated to a treatment arm. This number will have the following format: T + 3 digits

4.2.4 Randomisation

When selected, the patient eligibility will be evaluated from inclusion and non-inclusion criteria. Once the consent will be signed by the patient and investigator, the patient will be included and randomised by connecting the eCRF. The patient identification number will be allocated.
Randomization of patients will be centralized and carried out using a computerized system in the eCRF website according to a predefined randomization list (Cleanweb). Distribution in the three groups will be made in a 1:1:1 ratio.
The randomization list will be designed by the Sponsor/designee. The list will be based on permutation blocks of size 6 and will be generated by a statistician not involved in the analysis.
All inclusion and non-inclusion criteria will be checked before randomization.

4.2.5 Unblinding procedures, if applicable

Unblinding will be requested by the investigator for any reason requiring:

- a modification of the patient's follow-up as defined in the protocol
- a medical action.

Non-emergency situation

The request must be sent to the Promotion Unit of the DRCI-APHP using the current:
- by email to: drc-levee-insu@aphp.fr
- followed up with a phone call to 01 40 27 57 30 (optional)

The investigator requesting a non-urgent unblinding must first have obtained the opinion of the coordinating investigator or of the scientific director.

Emergency situation

The request should be made to the Poison Center of Fernand Widal Hospital by calling 01 40 05 48 48, followed by sending the current form:
- by email to: alertes.rtu.lrb@aphp.fr
  OR
- by fax to 01 40 05 48 88.

A copy will be sent simultaneously to the sponsor's Safety Department
- by email to: drc-levee-insu@aphp.fr

In the event of deterioration in the patient's condition requiring emergency unblinding for immediate medical treatment, the investigator may request unblinding from the pharmacy:

By calling: 01 42 49 90 55 (working hours)/ 06 15 25 98 88 (non-working hours)

Followed by e-mail : sls-cartcells.pharmacie@aphp.fr

The sponsor must be kept informed of this emergency unblinding.

By e-mail to :drc-levee-insu@aphp.fr

- followed by a telephone call to 01 40 27 57 30 (optional)

5 IMPLEMENTATION OF THE STUDY

Table 3: Summary table of the specialist doctor and type of visit for each visit reported in this protocol

Visit	The specialist field of the doctor	Type of Visit	Time frame for each visit
Eligibility: Informed consent obtaining and assessments for screening	Internal medicine	Hospital appointment	< 3 months before inclusion
Inclusion: Verification of Eligibility criteria and randomization	Internal medicine	No appointment	< 2 months before first infusion
M0: Baseline and 1st injection	Internal medicine	Hospital appointment	Baseline: in the last 72h before 1st injection
M1: Follow-up	Internal medicine	Hospital appointment	1 month after M0 +/- 1 week
M3: Follow-up and 2nd injection	Internal medicine	Hospital appointment	3 months after M0 +/- 1 week, and in the last 72h before 2nd injection
M4: Follow-up	Internal medicine	Hospital appointment	4 months after M0 +/- 1 week
M6: Follow-up	Internal medicine	Hospital appointment	6 months after M0 +/- 1 week
M9: Follow-up	Internal medicine	Hospital appointment	9 months after M0 +/- 1 week
M12: Follow-up	Internal medicine	Hospital appointment	12 months after M0 +/- 1 week

5.1 Screening visit

The screening visit takes place between 4 months and no later than 1 week before the baseline visit. The period between eligibility assessment and patient inclusion must not exceed 3 months in order to limit disease progression.

The following assessment will be scheduled before the inclusion of the patient in the study, and therefore before the beginning of the treatment:

- Medical and medication history,
- General physical exam, including weight and height
- Concomitant medications
- Modified Rodnan Skin Score
- Biological examinations including:
  - ✓ Hematology: Sedimentation rate, hemoglobin, hematocrit and WBCs with lymphocytes, and platelets counts

- ✓ Biochemistry: serum electrolytes and creatinine clearance, renal and liver function, serum protein electrophoresis, urine protein/creatinine ratio, urine dipstick, urinary cyto-bacteriological analysis
- ✓ Infectious serologies: HIV1/2, HTLV 1/2, Hepatitis B (AgHBs), Hepatitis C (+PCR if positive), EBV, CMV, syphilis, toxoplasma, sarsCov-2
- ✓ β-HCG dosage (women). Urine of blood β-HCG will be added in the week before inclusion if the screening β-HCG test was performed before this date.

- Chest X-ray
- High resolution CT Chest
- Pulmonary function tests
- EKG
- Cardiac echocardiogram
- Gynecology consultation less than one year
- Stomatology consultation less than 4 months
- Validation of eligibility: respect for the Inclusion Criteria (IC) and the Non Inclusion Criteria (NIC)
- Signature of the informed consent

Table 4: Summary table of Whose, Who and When individual's consent is collected

Whose consent must be obtained	Who informs the individuals and collects their consent	At what point the individuals are informed	At what point the consent is obtained
• the subject participating in the trial;	• the principal investigator • Collaborating physician declared and trained in the study (specialist in internal medecine)	Eligibility visit (as part of a routine care visit)	Within 24h after patient information, detailed explanation of the informed consent, and once eligibility criteria have been approved by the Principal Investigator or co-investigators.

5.2 Baseline visit

The following assessment will be scheduled after the inclusion of the patient in the study, as performed on a quarterly base for SSc patients (PNDS SSc janvier 2020 (2)), and corresponds to baseline evaluation (M0):

- Verification of inclusion and exclusion criteria
- General physical exam, including weight and height
- Concomitant medications
- Modified Rodnan Skin Score
- Health Related Quality of Life Forms completion: SHAQ, SF-36v2, EQ-5D-5L
- Patient and physician global assessment for CRISS for early SSc patients
- WHO Performance status
- Chest x-ray

- EKG
- Biological examinations including:
  - ✓ Hematology: Sedimentation rate, hemoglobin, hematocrit and WBCs with lymphocytes, and platelets counts
  - ✓ Biochemistry: serum electrolytes and creatinine clearance, renal and liver function, serum protein electrophoresis, urine protein/creatinine ratio, urine dipstick, urinary cyto-bacteriological analysis
  - ✓ SSc autoantibody detection: antinuclear antibodies, Scl-70 (anti-topoisomerase-I) antibodies, anti-centromere antibodies, anti-RNP (anti-ribonucleoprotein) antibodies, anti-RNA pol III antibodies
- Blood samples for externalized extensive immunology assessments and biobanking, to be sent at room temperature to SITI laboratory (CHU Rennes, Pr Tarte) the day of puncture:
  - ✓ 6 gel-free lithium heparin tubes (24ml) for detailed immuno-phenotyping and cell bank (analyzed and stocked in Rennes): proportion and activation status of lymphoid and myeloid subpopulations by Cytof on frozen PBMC
  - ✓ 1 dry tube (4ml) for MSC immunogenicity (analyzed in Rennes): detection and identification of donor-specific anti HLA antibodies in the serum
  - ✓ 1 EDTA tube (4ml) for HLA typing (analyzed in Rennes)
  - ✓ 1 RNA paxgene tube (2,5 ml) for RNA bank (stocked in Rennes)
- Blood sample for serum (1 dry tube of 7ml) and plasma banking (1 heparin tube with lithium of 7 ml for plasma): to be prepared and stocked the day of puncture as described in section 5.10 Biological samples collection
- Patients will be given an individual notebook where they will be asked to complete:
  - ✓ Outpatient consultations: physician visits, general practitioners visits, paramedical visits.
  - ✓ Direct non healthcare costs: number of hours of care by professional caregivers needed by patient per week.
  - ✓ Productivity loss: sick leaves, unemployment, disability status.
  - ✓ They will be asked to bring back to each following visits the notebook as well as all reports of biological acts, medical imaging and drug prescriptions in order to measure volume and price of ambulatory healthcare consumption.

<u>Optional samples, with dedicated mention in the patient informed consent:</u>

- 2 skin biopsies: to be prepared and stocked as described in section 5.10 Biological samples collection and appendix for technique
- 2 EDTA tubes (2x7ml) for efferocytosis analysis (analyzed in Rennes)

5.3 Injection visits

Every patients will receive their injections (at M0 and M3) in Saint-Louis hospital, Paris. Patients recruited and followed in Toulouse will performed their baseline (M0) and M3 evaluation or Toulouse before travelling by train to Paris for treatment infusion in Saint-Louis hospital.

The injection of MSC or placebo will take a minimum of 16 min and a maximum of 1h under the supervision of a Study Nurse and the Principal Investigator of St-Louis Hospital who will analyze the patient tolerance during the injection of MSC administration and thereafter at one hour, and during the 24 hours after injection of the MSCs, while the patient will stay hospitalized during the first 24 hours after injection.

Clinical data will be reported at 0, 5, 10, 15, 30, 45, 60, 90 min (arterial pressure, frequency, T°C, Sat O2, Remarks) after allogeneic MSC or placebo injection and clinical observation during 24 hours is performed including among other things:

- Reportable Incident
- Incident during injection
- Delayed Incident
- Hypotension / hypertension
- Thrill / hyperthermia
- Local inflammation
- Tachycardia / bradycardia
- Nausea / Vomiting
- Other Symptoms

5.4 Follow-up visits

The following assessments will be performed according SSc regular care on a quarterly base and 1 month after each infusion: **M1, M3, M4, M6, M9 and M12** after first allogenic MSC(AT) or placebo cells injection

- General physical exam, including weight and height.
- Evaluation of tolerance according to the criteria of the Common Terminology Criteria for Adverse Events (CTCAE) Version 5.0
- Concomitant medications
- Modified Rodnan Skin Score
- HRQoL Forms completion: SHAQ, SF-36v2, EQ-5D-5L (only at M3, M6 and M12)
- Patient and physician global assessment for CRISS evaluation for early SSc patients (only at M3, M6and M12)
- WHO Performance status (only at M3, M6 and M12)
- Chest x-ray (only at M6 and M12)
- High resolution CT Chest (only at M6 and M12)
- Pulmonary function tests (only at M6, and M12)
- EKG
- Cardiac echocardiogram (only at M6 and M12)
- Biological examinations including:
  - ✓ Hematology: Sedimentation rate, hemoglobin, hematocrit and WBCs with lymphocytes, and platelets counts
  - ✓ Biochemistry: serum electrolytes and creatinine clearance, renal and liver function, serum protein electrophoresis, urine protein/creatinine ratio, urinary cyto-bacteriological analysis
  - ✓ SSc autoantibody detection: antinuclear antibodies, Scl-70 (anti-topoisomerase-I) antibodies, anti-centromere antibodies, anti-RNP (anti-ribonucleoprotein) antibodies, anti-RNA pol III antibodies (only at M3, M6 and M12)
- Blood samples for externalized extensive immunology assessments and biobanking, to be sent at room temperature to SITI laboratory (CHU Rennes, Pr Tarte) the day of puncture:
  - ✓ 6 gel-free lithium heparin tubes (24ml) for detailed immuno-phenotyping and cell bank (analyzed and stocked in Rennes): proportion and activation status of

lymphoid and myeloid subpopulations by Cytof on frozen PBMC (only at M1, M3, M4 and M6)
    - ✓ 1 dry tube (4ml) for MSC immunogenicity for MSC immunogenicity (analyzed in Rennes): detection and identification of donor-specific anti HLA antibodies in the serum (only at M3 and M6)
    - ✓ 1 RNA paxgene tube (2,5 ml) for RNA bank (stocked in Rennes) (only at M1, M3, M4 and M6)
- Blood sample for serum (1 dry tube of 7ml) and plasma banking (1 heparin tube with lithium of 7 ml for plasma): to be prepared and stocked the day of puncture as described in section 5.10 Biological samples collection (at all follow-up visit except M9)
- Collection of individual notebook at each visit, with:
    - ✓ Out-patient consultations
    - ✓ Direct non healthcare costs
    - ✓ Productivity loss : sick leaves, unemployment, disability status
    - ✓ All reports of biological acts, medical imaging and drug prescriptions in order to measure volume and price of ambulatory healthcare consumption

<u>Optional samples, with dedicated mention in the patient informed consent:</u>

- 2 skin biopsies (optional, at M3 and M6): to be prepared and stocked as described in section 5.10 Biological samples collection and appendix for technique

5.5 Additional study visits and acces to drug product in case of prior treatment by placebo

At the end of the 12 months follow-up of the last out of the 18 patients included in the study and after unblinding and evidence of MSC(AT) injection safety, those patients included in arm 0 who will have chosen the opportunity to receive 1 MSC(AT) ($2x10^6$ cells/kg) injection will undergo the additional following visits:

- One additional visit for MSC(AT) injection
- One additional visit at 3 months after MSC(AT) injection, where the standard clinical and paraclinical examinations as recommended for severe SSc patients follow-up (2) will be performed

5.6 Early termination visit

In case of early termination a visit similar to the M12 visit will be scheduled.

5.7 Expected length of participation and description of the chronology and duration of the study.

- The total duration of the study is 29 months (table 5) with a period of patient's inclusion of 12 months and duration of patient's participation of 12 months for patients in arm 1 and 2. Patients included in arm 0, choosing to receive 1 MSC(AT) ($2x10^6$ cells/kg) injection after unblinding and once evidence of MSC(AT) injection safety is provided, will be treated by MSC(AT) up to 2 months after unblinding and will be followed up to 3 months after this last treatment.
- Maximum period between screening and enrolment should not exceed 3 months to limit disease progression
- Maximum period between enrolment and 1st injection should not exceed 2 months
- Randomisation and blinding will take place after patient's inclusion

Table 5: Summary table of total study duration

Duration of enrolment period	12 months
Length of participation for participants:	
• Maximum period between screening and enrolment	3 months
• Treatment duration:	2 injections of 16 min to 1h at M0 and M3
• Duration of follow-up period:	12 months
Unblinding and additional MSC(AT) injection for patients in the placebo arm	2 months
Additional follow-up period for patients in the placebo arm having received MSC(AT) injection	3 months
Total study duration:	29 months

5.8 Table 6 summarizing the chronology of the study

Actions	Eligibility[1]	Inclusion[2]	M0[3]	M1	M3[3]	M4	M6	M9	M12
Patient Information and signature of informed consent	X								
Medical and medication history	X								
General physical exam, including weight and height	X		X	X	X	X	X	X	X
Verification of inclusion and exclusion criteria	X	X	X						
Randomisation		X							
MSC(AT) or placebo injections			X		X				
Concomitant medication	X		X	X	X	X	X	X	X
modified Rodnan skin score	X		X	X	X	X	X	X	X
HRQoL (SHAQ, SF-36v2, EQ-5D-5L) Forms			X		X		X		X
WHO performance status			X		X		X		X
Biological tests : hematology, biochemistry and urine	X		X	X	X	X	X	X	X
Infectious serologies	X								
SSc autoantibodies			X		X		X		X
β - HCG determination for females (urine or blood)	X	X*							
Chest X-ray and EKG	X		X				X		X
High resolution CT Chest	X						X		X
Echocardiography and, if necessary: MRI	X						X		X
Pulmonary function tests	X						X		X
Gynecology (women) and Stomatology consultations	X								

Actions	Eligibility[1]	Inclusion[2]	M0[3]	M1	M3[3]	M4	M6	M9	M12
Detailed Immuno-phenotyping (externalized to SITI, CHU Rennes)			X	X	X	X	X		
HLA Typing (externalized to SITI, CHU Rennes)			X						
MSC immunogenicity: Detection and identification of donor-specific anti-HLA antibodies (externalized to SITI, CHU Rennes)			X		X		X		
Cell bank (externalized to SITI, CHU Rennes)			X	X	X	X	X		
RNA bank (externalized to SITI, CHU Rennes)			X	X	X	X	X		
Serum and Plasma bank (on site, the same day of puncture)			X	X	X	X	X		X
Evaluation of tolerance: Toxicity according to the criteria of CTCAE			X	X	X	X	X	X	X
Patient and physician global assessment (PGA) (for CRISS)			X		X		X	X	X
Presentation and delivery of the individual notebook			X						
Collection of individual notebook and related reports				X	X	X	X	X	X
Skin biopsies (optional)			X		X		X		
Efferocytosis analysis (optional)			X						

1 the period between eligibility assessment and patient inclusion must not exceed 3 months in order to limit disease progression. The clinical and paraclinical evaluation of organs that have not been affected, or whose degree of involvement is insufficient to meet the patient inclusion criteria for inclusion in the protocol, in a patient who has otherwise been clinically stable over the last 6 months, may be carried out within an additional period of 1 month if necessary, i.e., a maximum of 4 months before patient inclusion.

2 the period between inclusion and cell administration should not exceed 2 months.

3 M0 and M3 clinical and biological tests and examinations detailed in the “action” column will be performed in the centre before the MSC injection. * β - HCG determination for females must be done less than one week before inclusion

5.9 Distinction between standard care and study

Table 7: "Standard care" vs. "added interventions" required specifically for the study

Procedures and treatments to be provided during the study	Procedures and treatments associated with standard care	Procedures and treatments added for the study
Patient Information and signature of informed consent		Eligibility
Medical and medication history	Eligibility	
General physical exam, including weight and height	Eligibility, M0, M1, M3, M4, M6, M9 and M12	
Verification of inclusion and exclusion criteria		Eligibility and M0
MSC(AT) or placebo injections		M0 and M3
Concomitant medication	Eligibility, M0, M3, M6, M9 and M12	M1, M4
modified Rodnan skin score	Eligibility, M0, M3, M6, M9 and M12	M1, M4
HRQoL (SHAQ, SF-36v2, EQ-5D-5L) Forms	SHAQ, SF-36 and EQ-5D: M0, M3, M6 and M12	
WHO performance status	M0, M3, M6, M9 and M12	
Biological tests : hematology, biochemistry and urine	Eligibility, M0, M3, M6, M9 and M12	M1, M4
Infectious serologies		Eligibility
SSc auto-antibodies	M0, M3, M6 and M12	
β - HCG determination for females (urine or blood)		Eligibility and inclusion
Chest X-ray and EKG	Eligibility, M0, M6 and M12	
High resolution CT Chest	Eligibility, M6, M12	
Echocardiography and, if necessary: MRI	Eligibility, M6, M12	
Pulmonary function tests	Eligibility, M6, M12	
Gynecology and Stomatology consultations (teeth, gums ...)	Eligibility	
Detailed Immuno-phenotyping of lymphocytes		M0, M1, M3, M4 and M6
HLA Typing		M0
MSC immunogenicity: Detection and identification of anti-HLA antibodies		M0, M3 and M6
Cell banking		M0, M1, M3, M4, and M6
RNA banking		M0, M1, M3, M4, and M6
Serum and Plasma banking		M0, M1, M3, M4, M6 and M12

Procedures and treatments to be provided during the study	Procedures and treatments associated with standard care	Procedures and treatments added for the study
Evaluation of tolerance: Toxicity according to the criteria of CTCAE		M0, M1, M3, M4, M6, M9 and M12
Patient and physician global assessment (for CRISS)		M3, M6 and M12
Presentation and delivery of the individual notebook		M0
Collection of individual notebook and related reports		M1, M3, M4, M6, M9 and M12
Skin biopsies (optional)		M0, M3 and M6
Efferocytosis analysis (optional)		M0

5.10 Biological samples collection

Samples (or a component of the samples, serum, plasma, blood cells, RNA) taken as part of the study will be pseudonymised and stored in a biological sample collection to allow future phenotypic, functional and transcriptomic analyses as well as dosage of soluble factors in MSC-treated-patients samples.

As part of this research, the biological collection will contain 5 types of samples:

Serum samples: from 1 dry tube of 7 ml, centrifuged the same day as puncture at room temperature for 10 min at 2000g, 4 aliquots of 0.5 ml will be obtained and will be locally cryopreserved at -80°C until their transfer (as grouped shipment) in dry ice to the SITI laboratory of CHU de Rennes under the supervision of Pr K. Tarte where they will be stored centrally at -80°C .

Plasma samples: from 1 heparin tube with lithium of 7 ml, centrifuged the same day as puncture at room temperature for 10 min at 2000g, 4 aliquots of 0.5 ml will be obtained and will be locally cryopreserved at -80°C until their transfer (as grouped shipment) in dry ice to the SITI laboratory of CHU de Rennes under the supervision of Pr K. Tarte where they will be stored centrally at -80°C .

Cell samples: from gel-free lithium heparin tubes immediately sent to the SITI laboratory at room temperature the same day as puncture. PBMC will be isolated and stored at 2-5.10^6 cells per cryotube in SVF-10%DMSO in liquid nitrogen in the SITI laboratory of CHU de Rennes, under the supervision of Pr K. Tarte.

RNA samples: 2.5 ml RNA paxgene tubes, immediately sent to the SITI laboratory at room temperature the same day as puncture. They will then be stored at -20°C until RNA extraction in the SITI laboratory under the supervision of Pr K. Tarte. RNA will then be stored at -80°C in the SITI laboratory of CHU de Rennes, under the supervision of Pr K. Tarte.

Biopsies samples: one skin biopsy, fixed into paraformaldehyde-ethanol-acetic acid for 2 hours followed by paraffin inclusion, will be locally stored at room temperature until their transfer (as grouped shipment) to the SITI laboratory of CHU de Rennes where they will be stored centrally at room temperature under the supervision of Pr K. Tarte.
Another skin biopsy, immediately carried on ice to INSERM U 976 (Dr L. Michel), cryostored and stored at -80° C until their transfer (as grouped shipment) to the SITI laboratory of CHU de Rennes.

Samples will be pseudonymized according to the inclusion number.

All the biological collections will be recorded and reported to the competent authorities according to existing regulations.

- The collection of biological samples can be used for an examination of the genetic characteristics (RNA chips, PCR, sequencing ...). This can only be effective after patient's agreement by signing an informed and written consent (cf. informed consent). The purpose of this examination will be to determine if there are mutations or to identify diagnostic or risk factors or to analysis prognostic factors for MSC response.
- The collection of biological samples can be used in order to carry out retrospective or translational research on SSc disease and/or MSC treatment.

At the end of the trial, the samples will be kept for a duration of 20 years and will be destroyed after this period. The collection will be declared to the relevant minister (Article L. 1243-3 of the *Code de la Santé Publique* [French Public Health Code]). The samples may be used for further analysis not described in the initial protocol but which may be useful for investigation of MSC treatment for autoimmune disease in light of advances in scientific knowledge, provided the participant is informed and does not oppose this, as stated in the information note/consent form. In case the patient does not consent, the samples will be destroyed at the end of the study.

Table 8. Summary table of the biological samples collection

Type of sample	Quantity per patient	Storage location (name and entity)	Supervisor of the sample collection (name and entity)	Purpose of the sample collection	Storage duration	End use/Future (e.g. destruction) after the end of the CT
Serum	4 aliquots of 0.5 ml per visit,at M0, M1, M3, M4, M6 and M12	• During the trial: – St-Louis Hospital, Paris: Laboratoire d'immunologie et d'histocompatibilité – CHU Toulouse: CRB CHU Rangueil • At the end of the trial: Laboratoire SITI, Service Immunologie – Bâtiment Médico-technique/hématologie clinique – Bâtiment Jean DAUSSET, CHU Rennes	• During the trial: – CRB APHP Lariboisière/Saint-Louis : Pr S. Caillat-Zucman – CRB CHU Rangueil : Mme B. Razat • At the end of the trial: – SITI laboratory : Pr Karin Tarte, Dr Virginie Girault, Dr Joëlle Dulong	Evaluation of the immunomodulatory effect of MSC on systemic cytokines production	Duration of the study and 20 years after the end of the study	kept during 20 years and thereafter destroyed
Plasma	4 aliquots of 0.5 ml per visit,at M0, M1, M3, M4, M6 and M12					
Cells (PBMC)	2-3 cryotubes (2-5.10^6 cells) per visit (M0, M1, M3, M4, and M6)	Laboratoire SITI, Service Immunologie – Bâtiment Médico-technique/hématologie clinique – Bâtiment Jean DAUSSET, CHU Rennes	Pr Karin Tarte, Dr Virginie Girault, Dr Joëlle Dulong	Evaluation of further subsequent immunophenotyping	Duration of the study and 20 years after the end of the study	keptduring 20 years and thereafter destroyed
Whole-blood RNA	One 2.5 ml RNA paxgene tube at M0, M1, M3, M4, and M6	Laboratoire SITI, Service Immunologie – Bâtiment Médico-technique/hématologie clinique – Bâtiment Jean DAUSSET, CHU Rennes	Pr Karin Tarte, Dr Virginie Girault, Dr Joëlle Dulong	Evaluation of transcriptome and/or repertoire analyses	Duration of the study and 20 years after the end of the study	keptduring 20 years and thereafter destroyed
FFPE Skin biopsy	One biopsy at M0, M3 and M6 (optional)	• During the trial: - St-Louis Hospital, Paris: Service d'Anatomie et Cytologie Pathologiques • At the end of the trial: Laboratoire SITI, Service Immunologie – Bâtiment Médico-technique/hématologie	• During the trial: Pr Philippe Bertheaux • At the end of the trial: SITI laboratory : Pr Karin Tarte, Dr Virginie Girault, Dr Joëlle Dulong	Histological analysis	Duration of the study and and 20 years after the end of the study	Kept during 20 years and thereafter destroyed

		clinique – Bâtiment Jean DAUSSET, CHU Rennes				
Frozen skin biopsy	One biopsy at M0, M3 and M6 (optional)	• During the trial: INSERM U976 laboratory, St-Louis Hospital, Paris • At the end of the trial: Laboratoire SITI, Service Immunologie – Bâtiment Médico-technique/hématologie clinique – Bâtiment Jean DAUSSET, CHU Rennes	• During the trial: Dr Laurence Michel • At the end of the trial: SITI laboratory : Pr Karin Tarte, Dr Virginie Girault, Dr Joëlle Dulong	Immunohistochemistry or transcriptomic analysis	Duration of the study and and 20 years after the end of the study	Kept during 20 years and thereafter destroyed

6 ELIGIBILITY CRITERIA

6.1 Inclusion criteria

1) Provide signed and dated informed consent;
2) Willing to comply with all study procedures and be available for the duration of the study;
3) Male or female, aged ≥ 18 and ≤ 70 years of age
4) SSc patients according to American College of Rheumatology/European League Against Rheumatism (ACR/EULAR) 2013 classification criteria for SSc
5) Severe disease with either:

- disease duration of 2 years or less with a modified Rodnan skin score (mRss) ≥ 20 and (abnormal CRP > 5 mg/l and/or hemoglobin < 11 g/dL), or
- mRSS ≥ 15 without any restriction as to disease duration plus at least one major organ involvement as defined by:
    a) respiratory involvement consisting of lung diffusion capacity for carbon monoxide (DLCO) and/or forced vital capacity (FVC) < 80% predicted and evidence of interstitial lung disease : bronchiolar involvement, areas of ground-glass contusions or fibrosis (chest X-ray and/or high resolution computed tomography (HRCT) scan) and/or moderate Pulmonary hypertension with baseline resting systolic pulmonary arterial pressures > 35 mmHg and below 50 mmHg by cardiac echocardiography, or mean pulmonary artery pressure > 20 mmHg and < 40 mm Hg on right heart catheterization;
    b) renal involvement consisting of past renal crisis, microangiopathic hemolytic anemia, and/or renal insufficiency not explained by other causes than SSc;
    c) cardiac involvement consisting of reversible congestive heart failure, atrial or ventricular rhythm disturbances such as recurrent episodes of atrial fibrillation or flutter, recurrent atrial paroxysmal tachycardia, 2nd or 3rd degree AV-block, mild to moderate pericardial effusion and/or presence of MRI involvement (Increased T1 or T2 mapping, late gadolinium enhancement, septal D sign). All causes of organ involvement should be attributed to SSc.

6) Contraindication, inadequate response or unwillingness to undergo AHSCT(determined by patient and physician judgement)
7) Contraindication, inadequate response or unwillingness or adverse events necessitating discontinuation of conventional immunosuppressive therapy (MMF, methotrexate);
8) Women of reproductive potential must use highly effective contraception;
9) Men of reproductive potential must use condoms;
10) Health insurance.

6.2 Exclusion criteria

1) Age < 18 years and > 70 years
2) Pregnancy or unwillingness to use adequate contraception;
3) Life-threatening end-organ damage defined as: DLCO (corrected for hemoglobin) < 30% predicted; Left ventricular ejection fraction < 40% by cardiac echocardiography; Pulmonary hypertension with baseline resting systolic pulmonary arterial pressures > 50 mmHg by cardiac echocardiography, or mean pulmonary artery pressure > 40 mmHg on right heart catheterization; glomerular filtration rate < 30mL/min
4) Active Hepatitis (ASAT, ALAT > 3 normal)

5) Neoplasms of less than 5 years, except for basal cell or in situ cervix carcinoma or concurrent myelodysplasia,
6) Uncontrolled hypertension
7) Uncontrolled acute or chronic infection
8) HIV-1 or HIV-2 infection
9) BMI < 16.5 kg/m2
10) Severe psychiatric disorder
11) Bone marrow insufficiency, defined as neutropenia < 1 x 10^9/L, thrombopenia < 50 x 10^9/L, anemia < 8 g/dL, lymphopenia < 0,5 x 10^9/L
12) Inability to provide informed consent
13) Patient included in another interventional clinical trial
14) Patient under tutelle

6.3 Birth control and pregnancy

6.3.1 Pregnancy testing

Blood or urine β-HCG levels are checked at eligibility and less than one week prior to inclusion to confirm absence of pregnancy.
If at any time during the participation period, a delay in menstrual period (over one month between menstruation) is observed, pregnancy testing should be done.

6.3.2 Birth control methods

- For women of childbearing potential:

A highly effective birth control method must be used from eligibility until the end of the participation period.

Women of childbearing potential are defined as fertile women until post-menopausal state unless permanently sterile. A postmenopausal state is defined as no menses for 12 months without an alternative medical cause

Highly effective birth control method accepted in this study are:

- oral, intravaginal or transdermal combined hormonal contraception
- oral, injectable or implantable progestogen-only hormonal contraception
- intrauterine device (IUD)
- intrauterine hormonal releasing system (IUS)
- bilateral tubal occlusion
- vasectomised partner
- sexual abstinence (only if this the preferred and usual lifestyle of the participants)

- For men, in absence of permanent sterilization:
  - sexual abstinence
  - condoms

The maximal contraception for both male and female patients included in the study must be implemented at inclusion and should be maintained throughout the whole study participation duration

6.4 Recruitment procedure

The SSc patient's recruitment will be performed by the Investigator sites (see 4.1.4) throughout standard hospitalization, practitioner networks (MATHEC, FAI2R), French patients association of SSc or autoimmune diseases. In the case of public announcements and advertisements, documents will be submitted to the CPP (Research Ethics Committee) for approval along with the protocol.
18 participants will be included during the enrolment period (12 months). 0,5 participants will need to be enrolled per site per month, based on the number of sites and the duration of the enrolment period.

Table 9 : Summary table of the recruitment subjects per site and per month

	Number of participants
Total number of participants to be included	18
Number of centres	2
Enrolment period (months)	12
Number of participants/centre	6
Number of participants/centre/month	**0,5**

6.5 Termination rules

6.5.1 Criteria and procedures for prematurely terminating the study treatment

In the case of severe adverse events, the investigator must notify the sponsor and follow up the participant for 12 months following the premature discontinuation of treatment. Notification of a serious adverse event must be sent by email (eig-vigilance.drc@aphp.fr) to the sponsor. The serious adverse event will be monitored until it is resolved. In case of any safety data reported to the Safety department, the DSMB will be informed, hold meeting and determine whether the trial should be held or proceed.
Second infusions will not be performed in the presence of treatment-related SAE after the first injection.

Several situations are possible

- Temporary suspension of treatment: the investigator must document the reason for suspending and resuming the treatment in the participant's source file and the case report form (CRF)

- Premature discontinuation of treatment, but the participant remains enrolled in the study until the end of their participation
- Premature termination of treatment and withdrawal from the study

The investigator must:

- Document the reason(s)
- Collect the assessment criteria at the time of ending participation in the study, if the participant agrees
- Schedule a follow-up for the participant, particularly if there in case of a serious adverse event.

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6.5.2 Criteria and methods for the premature discontinuation of the study

- Participants may exit the study at any time and for any reason.
- The investigator can temporarily or permanently withdraw a participant from the study for any safety reason or if it is in the participant's best interests.
- Participant lost to follow-up: the participant cannot be located. The investigator must make every effort to reconnect with the participant (and document his attempts in the source file), at least to determine whether the participant is alive or dead (for example: to get in contact with the birth city hall).

If a participant exits the study prematurely or withdraws consent, any data collected prior to the date of premature exit may still be used.

- If a participant exits the study prematurely, and if the participant agrees, state the procedure and schedule for collecting the data required by the protocol (primary endpoint, secondary endpoints, safety assessment) (NB: this must be stated in the information and consent form)
- State how premature exit from the study will affect the participant's ongoing care (state exactly what the participant will be offered).
- In case of serious adverse events, the investigator must notify the sponsor and follow up the participant until the severe adverse events are resolved.

The case report form must list the various reasons why the participant has discontinued the study:

- ☐ Lack of efficacy
- ☐ Adverse reaction
- ☐ Another medical issue
- ☐ Personal reasons of the participant
- ☐ Explicit withdrawal of consent
- ☐ Lost to follow-up

6.5.3 Monitoring subjects after the premature termination of treatment

If a participant discontinues the study, this will in no way affect their usual care for their condition.

In the event of serious adverse events following premature discontinuation of treatment and participation of the patient in the study; see section 6.4.1

6.5.4 Procedure for replacing participants

In case of patient withdraw during the inclusion period, replacement will be performed.

6.5.5 Full or partial discontinuation of the study

AP-HP as sponsor or the Competent Authority can prematurely discontinue all or part of the study, temporarily or permanently, further to the recommendations of the Data Safety Monitoring Board in the following situations:

- first of all, if suspected unexpected serious adverse reactions (SUSARs) are observed in one of the treatment arms or if there is a discrepancy in the serious adverse reactions between the treatment arms, requiring a reassessment of the benefit-risk ratio for the study.
- if an interim analysis confirms a poor safety (See statistical methods)

Similarly, AP-HP, as the sponsor, or the Competent Authority may decide to prematurely discontinue the study due to unforeseen issues or new information about the product, in light of which the objectives of the study or clinical programme are unlikely to be achieved.
AP-HP, as the sponsor, reserves the right to permanently suspend enrolment at any time if the enrolment targets have not been met.
If the study is prematurely discontinued for safety reasons, the decision and justification will be provided by the sponsor (AP-HP) to the Competent Authority and to the Ethics Committee without undue delay but not later than in 15 days of the date of the temporary halt or early termination. It shall include the reasons for such action and specify follow-up measures,, along with recommendations from the Data Safety Monitoring Board in the case of substantial modification.

In case of early termination of the trial, accrual will be discontinued if the inclusion period is still on-going at the time.
Already included patients will be offered best available treatments according to his condition in agreement with his referring doctors.
The expected follow-up until 12 months after first infusion will be maintained, with the scheduled follow-up visits and data collection.

7 TREATMENT(S) ADMINISTERED TO STUDY PARTICIPANTS

This section describes the investigational advanced therapy medicinal products (ATMP) (see investigator brochure for further details) and any other treatments needed for the study (to be validated by the head pharmacist)

Table 10: Summary table of responsible parties involved in the investigational advanced therapy production, storage and delivery

Responsible for Cellular Therapy Production	**ATMP production platform** **EFS Ile de France Creteil** 5, rue Gustave Eiffel - 94017 Creteil cedex Contact Person: Pr Hélène Rouard, PharmD, PhD Telephone: 01 56 72 76 32 Fax: + 01 56 72 21 23 Email: helene.rouard@efs.sante.fr
Responsible for treatment circuit compliance	**Agence Générale des Equipements et Produits de Santé (APHP),** 7 rue du Fer à Moulin, 75005 Paris **Dr: Jean-Roch FABREGUETTES, Pham D** Tel: + 33 1 46 69 92 44 Email: jean-roch.fabreguettes@aphp.fr
Responsible for onsite reception and delivery of the treatment	**Saint-Louis Hospital**: central pharmacy, 1 avenue Claude Vellefaux, 75010 Paris.

	Contact : Isabelle MADELAINE-CHAMBRIN, isabelle.madelaine@aphp.fr, Romain DE JORNA, romain.dejorna@aphp.fr

7.1 Description of the advanced therapy investigational medicinal product : MSC(AT)

Characteristics

The final product is MSC(AT) THA-C, suspended in ringer lactate supplemented with 1% human albumin and packaged in a single infusion bag, where the MSC(AT) concentration will have been adjusted to $1x10^6$ cells/ml for a total dose of $2x10^6$ cells/kg weight of recipient.

It is obtained in 3 steps after adipose tissue of a unique healthy and informed volunteer with a body mass index below 30, age below 50 years and without chronic diseases, selected according to the inclusion and exclusion criteria as detailed in the clinical donor study specific protocol (NCT05947682, IDRCB: 2023-A00711-44). Briefly, the adipose tissue donor was tested for the main viral risks according to French legislation (decree n°2010-1625 of December 23, 2010, successive legal orders on June 22th 2011, July 5th 2013, Haut Conseil de la Santé Publique advice (HCSP) of April 20th 2023) and the Agence Nationale de Sécurité du Medicament et des produits de santé (ANSM) biological validation algorithm published in 2013.

The production process comprises 3 different steps:

A/ MSC(AT) isolation and Master Cell Bank (MCB) constitution: The stromal vascular fraction (SVF) was isolated from lipoaspirate by collagenase digestion and MSC(AT) were then isolated by culturing the cells from the SVF in a closed system for 6 days until passage 0, thereby providing the Master Cell Bank (MCB) which was then cryopreserved. The MCB derived from a single donor has been qualified by phenotypic analysis, viability, sterility, and functional capacity to inhibit T lymphocyte proliferation in vitro.

B/ MSC(AT) expansion and Working Cell Bank (WCB) : the MCB qualified batch was further expanded during 6 days in order to obtain MSC(AT) at passage 1 (P1) (WCB), which is then cryopreserved in albumin 5% with 10% DMSO. Bags of $100\text{-}125x10^6$ MSC(AT) were prepared to establish the working cell bank (WCB) and then qualified. The WCB quality controls included cell count, viability, phenotypic analysis, sterility (including search for endotoxins and mycoplasma), telomerase reverse transcriptase activity (hTERT) , functional capacity to inhibit T lymphocyte proliferation in vitro and detection of a large panel of viruses by NGS technology (on the WCB batch n°1). This expansion from the MCB to the WCB will be performed 3 times in order to produce a total of 3 WCB batches. Therefore, all 3 WCB batches will be derived from the same MCB as derived from the same unique donor, so as to obtain sufficient number of MSC(AT) THA-C to be released for the clinical protocol and delivered to patients. Cryopreserved MSC(AT) obtained at P1 (WCB) were stored in gas nitrogen in a specific container for ATMP for a total duration that could not exceed 48 months.

C/ WCB Thawing and MSC(AT) culture until passage 2 to prepare the Investigational medicinal product (IMP) : MSC(AT) THA-C : After WCB thawing, MSC(AT) will be cultured during 18-48 hours (passage 2) to restore their immunosuppressive properties, and to improve cell cycle recovering and growth factors release. An aliquot of the MSC(AT) THA-C suspension will be aseptically removed for cell count, and its quality will be controlled for conditional release (for further details, refer to the corresponding IMPD).

Supply:
The final product, MSC(AT) THA-C, will be certified on the basis of rapid tests: cell count, phenotype, viability testing for "conditional release" by the sponsor to be re-injected without delay. The results of microbiological analyses, testing for endotoxins and mycoplasma, will be obtained once the batch has been certified and the MSC(AT) THA-C has been injected into the patient.

MSC(AT) THA-C in bag	**Acceptance criteria**
Results known before infusion	
Cell count	[1,9-2]x10^6 cells/kg weight recipient
Viability	≥ 90%
CD73+ CD90+	≥ 90% ≥ 90%
CD105+	≥ 85%
CD45+ CD14+ HLA-DR+ CD34+ CD31+	< 5% < 5% < 5% < 5% < 5%
Results obtained after infusion	
Microbial testing (PhEur 2.6.27)*	Negative
Mycoplasma test *	Negative
Endotoxin test *	≤5 EU/Kg

*the results will be available in the final certification step.
Once MSC(AT) THA-C suspension in bag has been certified as compliant, it is sent to the site immediately .

The cells will be contained in 300 ml infusion bag (primary container) labelled according to EU regulation. Then the bag will be protected with a secondary plastic packaging. The shipping package consists on a rigid transport box (third container) for biological samples. Specific data (biological samples, volume, temperature, patient's code, time) will be written on the transport box before shipment and checked at reception, as well as temperature probe. The transportation will be *carried out* at 5°C ± 3°C, with temperature traceability. A suitable mode of transport ensures delivery of the package to the St Louis hospital pharmacy. The stability of the final product has been previously validated until 24 hours at +2 to +10°C.

Pharmacy will check the documentation, the proper transportation of the cells and the identity of the patient. Then, the Medicinal product will be delivered to the hospitalization unit by the pharmacist of Saint-Louis Hospital.

7.2 Description of the placebo

Characteristics

The placebo will be composed of excipients without cells: 1% of human serum albumin and Ringer lactate. All excipients and transfer bags will be supplied by the ATMP pharmaceutical production site of EFS Creteil to be identical to those used for MSC(AT) THA-C. The placebo will be a product manufactured individually for each patient within a bag for infusion that could be assimilated to a batch. A medium volume of 2 mL/kg will be used.
All the tubing of the PVC bag will be welded as for the MSC(AT) THA-C. For this randomized, placebo-controlled, double-blind phase I-II trial clinical trial, the bag is overwrapped in order to allow double blinding of the treatment.

Components per bag of Placebo	
EXCIPIENTS • Human serum albumin • Ringer Lactate	 1% Qs 2mL/kg
TOTAL VOLUME / bag	2 ml /kg

Manufacturing of Placebo will be performed by the pharmacy of Saint-Louis Hospital in compliance with Good Preparation Practices by trained technicians.

7.3 Preparation for administration / administration

When a patient will be included in the trial, the investigator will randomize the patient through the eCRF. EFS and the pharmacy of St-Louis hospital will receive by email the information of the randomization, including the randomization arm allocated to the patient. Together, and in collaboration with the clinical site, they will define the planning of treatment.

According to the randomization arm and the visit number, the treatment will be prepared either by EFS (MSC(AT) THA-C) or by the pharmacy (placebo).

In any case a prescription form edited by the investigator and validated by the sponsor will be sent to EFS and the pharmacy and serve as order of production.

The communication put in place between EFS and St Louis pharmacy sites will ensure blinding for the others stakeholders.

In both cases:
- The pouch will be placed in an opaque bag in order to ensure blinding.
- The opaque bag will be labelled with the labels provided by the sponsor
- Any document specific of the randomization arm (certificate of analysis, preparation sheet…)will be filled and stored at the hospital pharmacy.

After the verification by the pharmacist, the bag will be delivered to the clinical service in a secondary container at ambient temperature. It will be accompanied by the delivery/prescription sheet.

MSC(AT) THA-C or placebo will be injected by slow intravenous infusion at 3-5 ml/mn with a minimum infusion time of 16 min via a 200 µm transfusion filter. The physicians should be available during product infusion. The patient should be followed-up for 60 minutes after infusion (for potential immediate reaction).

7.4 Description of traceability elements accompanying the investigational medicinal product(s)

An injection sheet, to ensure the traceability of administration (responsible, date, time, duration of injection and adverse effects during injection) accompanied with an injection instructions for investigator site will be provided by the sponsor

7.5 Authorized and prohibited treatments (medicinal, non-medicinal, surgical), including rescue medications

These patients are usually treated by a number of symptomatic medications to prevent digestive tract manifestations and oesophageal reflux, hypertension and renal crisis, and to alleviate pain. These symptomatic medications are authorised during the trial.

Use of immunosuppressive therapies (azathioprine, cyclophosphamide, mycophenolic acid, mycophenolate mofetil, and cyclosporine) and anti-fibrotics (colchicine, D-penicillamine, minocycline, pirfenidone and nintedanib) must be stopped at least one month prior to infusion. Use of immunomodulators, including methotrexate, and all types of biotherapies must be stopped at least 1 month prior to infusion. Wash-outs are common in trials of SSc and are not associated with rebound effects (145). Prednisone will be permitted if it was part of the subject's ongoing treatment prior to inclusion. Subjects on prednisone doses > 6mg/d will be reduced to 6 mg/d at least 1 month prior to infusion and remain on that dose throughout the trial. Subjects on doses ≤ 6 mg/d will continue on those doses throughout the trial.

Prostaglandin synthesis inhibitors are recommended to be avoided due to the partial dependence of the anti-inflammatory effects of MSC(AT) on PGE2. Thus, in the context of this study, any prescription of an anti-inflammatory (e.g. indomemacin, ibuprofene, ketoprofene, diclofenac, prednisone) is not recommended. No other interaction is described with other medications, however, it is recommended to stop any further infusions at the time of reinjection.

Treatments to be stopped 1 month before infusion	- immunosupressors: azathioprine, cyclophosphamide, mycophenolic acid, mycophenolate mofetil, and cyclosporine) - anti-fibrotics : colchicine, D-penicillamine, minocycline, pirfenidone and nintedanib) - immunomodulators: methotrexate - biotherapies: belilumab and others - Prostaglandin synthesis inhibitors and anti-inflammatory (e.g. indomemacin, ibuprofene, ketoprofene, diclofena, prednisone>6 mg/d)
Authorized drugs	All other drugs including prednisone at a dose ≤ 6 mg/d

During follow-up, if any rescue medication due to disease progression is necessary, local PI will introduce the required treatments and medications which will be carefully recorded in the CRF. For any surgical intervention, these will be declared as a serious adverse event and immediately notify to the promotor.

8 EFFICACY ASSESSMENT

8.1 Description of Efficacy endpoints assesment parameters

1. **Main efficacy endpoint** will be assessed by mRSS difference between M6 and M0 and between M12 and M0.

2. Others efficacy disease related secondary endpoints:
   - mRss difference between M3 and M0, and between M9 and M0.
   - WHO performance status (PS) and Health-Related Quality of Life (HRQoL) questionnaires : Scleroderma-Health Assessment Questionnaire (SHAQ), the Short Form (36) health survey (SF-36v2) and EQ-5D-5L at M0, M3, M6, and M12;
   - Forced Vital Capacity (FVC) and Diffusing capacity of Lung for carbon monoxide (DLCO) at M0, M3, M6, and M12
3. Response to treatment will be defined as any of the following: decreased mRSS > 25%, increased FVC > 10% and/or increased DLCO >10%, without need for further immunosuppression except low dose steroids (below 10mg daily) (140) at M3, M6 and M12.
4. Progression-free survival at 12 months will be defined as the percentage of enrolled patients still alive without evidence of relapse or progression 12 months after MSC(AT) injection, with progression defined as any of the following compared to baseline evaluation: decreased FVC > 10% or DLCO > 15%; decrease in LVEF% > 15%; decrease in weight > 15%; decrease in creatinine clearance > 30%; increased mRss > 25% ; and/or increase in SHAQ> 0.5, and relapse defined as any of the following compared to best-response: decreased FVC > 10% or DLCO > 15%; decrease in LVEF% > 15%; decrease in weight > 15%; decrease in creatinine clearance > 30%; increased mRss > 25% ; and/or increase in SHAQ> 0.5
5. Overall survival at 12 months will be defined as the percentage of enrolled patients still alive 12 months after MSC(AT) injection.

9 ECON**OMIC EVALUATION**

Cost-effectiveness analyses will be performed by Dr Aurélie Bourmaud, Robert Debré hospital, Université Paris Cité, Paris.

9.1 Primary endpoint (incremental cost utility ratio, incremental cost effectiveness ratio)

The efficiency of the 2 schemes of allogeneic MSC(AT) injections will be assessed through cost-utility and cost-effectiveness analyses, as recommended by the French National Authority for Health. Incremental cost-effectiveness ratios (ICER) will be estimated for the two health economic endpoints, expressed in i) cost per QALY gained and ii) Cost per AE ≥ 3 CTCAE avoided between arm 1 and placebo arm AND arm 2 and placebo arm.

9.2 Time horizon, perspective, discount rate

The analysis will be performed from a collective point of view and with a time horizon corresponding to the study period: 12 months. Costs and consequences will then not be discounted, as recommended, since discounting is required only for several years long studies.

9.3 Resource utilization & Costs *:*

- Healthcare resources considered :

The following resource items will be collected:

- 1. Direct health care costs : MSC(AT) injection, hospitalization, re-hospitalization (in-hospital data collected from the PMSI) physician visits, general practitioner visits (out-hospital data collected from the patient notebook, at each visit, to inform what happened between N-1 and N visit) , biological acts, medical imaging, drug prescriptions, and transportation (collected from the patient at each visit, by copying all reports brought back by the patient for the period [N-1- N].
- 2. Direct non-healthcare costs: estimated with the number of hours of care given by professional caregivers (defined as all home caregivers who are paid for their services) for home-based care for one week (collected from the patient's notebook, at each visit, to inform what happened between N-1 and N visit.).
- 3. Undirect health care costs will be measured by collecting sick leaves, unemployment or permanent disability status, from patients and relatives. Those information will be collected from the patient's notebook, at each visit, to inform what happened between N-1 and N visit.

- Method of data collection

Costs will be gathered from the study database, from the hospital Diagnosis Related Group information system (PMSI), from individual notebook and ambulatory care reports.

- Method of valuation: Diagnosis Related Groups, social health insurance schedule, list price

Each MSC(AT) injection will be valued with its unit cost. For hospital costs, stays will be valued on the basis of the Social Health Insurance. Community costs (visits, imaging, biological acts) will be valued with statutory fees. Other drugs costs will be valued from market prices. Direct non-health care costs will be valued using the French mean wage of a professional home caregiver. Sick leaves, unemployment, disability status will be valued with patients and relatives' mean wage.
Costs will be expressed in euros.

9.4 Outcomes

QALY will be calculated using the answers to the EQ-5D-5L questionnaire, transformed into utilities (130). The EQ-5D-5L has been validated in French (131), and its use is recommended for patients with SSc (132). Life years will be measured with survival data.

9.5 Endpoint calculations and uncertainty (deterministic and probabilistic sensitivity analyses)

In each set of analysis, mean costs will be calculated for both strategies. ICER will be positioned within the cost-effectiveness plane. Statistical uncertainty will be taken into using the non-parametric bootstrap method to construct a 95% CI and a confidence cloud. Sensitivity analysis will be performed for the costs and health care resources that seem non-robust. A peculiar attention will be provided for a sensitivity analysis around the MSC(AT) injection costs, as well as for the productivity loss costs.
As regards to the small size of groups of patients, 3 micro-simulated populations of 500 simulated patients, based on the 3 groups of this study and on their data and moments will be performed with a boostrap resampling technique. Relevant distributions will be allocated to all variables. Parameter values will be drawn at random from the assigned distributions using a bootstrap simulation with 500 iterations. Incremental costs and effectiveness will be estimated for each strategy and the incremental cost-effectiveness ratio (ICER) will be calculated. One-way sensitivity analyses will again be performed on outcomes and costs to assess the effect of varying baseline estimates within certain ranges on effectiveness, costs and ICER. Uncertainty will be addressed probabilistically. This will test the robustness of our results.

Table summarizing the chronology of the research

	Baseline (M0)	At each Visit M1, M3, M4, M6, M9	Visit M12
EQ5D5L	✓	✓	✓
hospital discharge data			✓
Ambulatory resource use (patient notebook)		✓	✓

9.6 Data collection

EQ5D5L will be collected through the eCRF of the study
Notebook will be collected at each visits
Discharge data (length of stay, diagnostic and procedural codes, DRG codes) will be extracted locally by the research assistants from each hospital's information system

10 SPECIFIC STUDY COMMITTEES

10.1 Steering Committee

Composition:

Name	Function in the study	email
Pr Dominique FARGE	Coordinating investigator and principal investigator for Saint-Louis hospital	Dominique.farge-bancel@aphp.fr
Pr Karin TARTE	Scientific Director	karin.tarte@univ-rennes1.fr
Dr Lucie BIARD	Methodologist and statistician	lucie.biard@u-paris.fr
Pr Jérome LAMBERT	DRCI-URC director	jerome.lambert@u-paris.fr
Pr Grégory PUGNET	Principal investigator for CHU Toulouse	PUGNET.G@chu-toulouse.fr
Pauline LANSIAUX, PhD	Clinical research coordinator	Pauline.lansiaux@aphp.fr
Dr Isabelle MADELEINE	Responsible for onsite reception and delivery of the treatment	Isabelle.madeleine@aphp.fr
Virginie GIRAULT, PhD	Research engineer	virginie.girault@univ-rennes1.fr
Dr Jean-Roch FABREGUETTES	Responsible for treatment circuit compliance	jean-roch.fabreguettes@aphp.fr

Pr Hélène ROUARD	Responsible for Cellular Therapy Production	Helene.ROUARD@efs.sante.fr
Dr Romain DE JORNA	Responsible for onsite reception and delivery of the treatment	romain.dejorna@aphp.fr
Razika GUIZEM	DRCI-Head Office project advisor	razika.guizem@aphp.fr

Role:
- Define the overall structure of the study, coordinate information, determine the initial methodology and oversee the study.
- Propose procedures to be followed during the study, acknowledging any recommendations from the Data Safety Monitoring Board, if applicable. The DRCI sponsor retains the decision-making authority.

10.2 Scientific Committee

Composition:

Name	Function in the study	email
Pr Dominique FARGE	Coordinating investigator and principal investigator for Saint-Louis hospital	Dominique.farge-bancel@aphp.fr
Pr Karin TARTE	Scientific Director	karin.tarte@univ-rennes1.fr
Dr Jean-Roch FABREGUETTES	Responsible for treatment circuit compliance	jean-roch.fabreguettes@aphp.fr
Dr Lucie BIARD	Methodologist and statistician	lucie.biard@u-paris.fr

Role: define the purpose, draft the protocol, and suggest modifications to the protocol during the study.

11 SAFETY ASSESSMENT - RISKS AND BURDEN ADDED BY THE STUDY

11.1 Description of Safety endpoints assessment parameters

Primary endpoint:
The rate of treatment-related severe adverse events (SAE) defined as Adverse Events (AE) of grade equal or above 3 using the NCI Common Terminology Criteria for Adverse Events (CTCAE) v5.0 classification, at one month after each infusion according to arms (M1, M4). All adverse events will be adjudicated by a Data and Safety Monitoring Committee.

Secondary endpoints:
- Rate of treatment-related AE of grade equal or above 3 CTCAE v5.0 at time and within the first 24 hours of infusion and during all follow-up at: M0, M3, M6, M9 and M12.
- Overall survival at M12

- PFS at M12, with progression defined as any of the following: decreased in FVC > 10% or in DLCO > 15%; decrease in LVEF% > 15%; decrease in weight > 15%; decrease in creatinine clearance > 30%; increased mRss > 25% ; and/or increase in SHAQ> 0.5;

11.2 Recording and reporting adverse events

11.2.1 Definitions

- **Adverse event**

Any untoward medical occurrence in a subject, to whom a medicinal product is administered and which does not necessarily have a causal relationship with this treatment. .

- **Serious adverse event**

Any untoward medical occurrence that at any dose requires inpatient hospitalisation or prolongation of existing hospitalisation, results in persistent or significant disability or incapacity, results in a congenital anomaly or birth defect, is life-threatening, or results in death. Certain medical events may jeopardise the subject or may require an intervention to prevent a SAE, known as "important medical events" should also be considered as serious adverse events.

- **Unexpected serious adverse reaction (SUSAR)**

A serious adverse reaction, the nature, severity or outcome of which is not consistent with the reference safety information.

- **Unexpected event**

An unexpected event which affect the benefit-risk balance of the clinical trial, but are not suspected unexpected serious adverse reactions as referred to in Article 42. That notification shall be made without undue delay but no later than 15 days from the date the sponsor became aware of this event.

- **Urgent safety measure**

Where an unexpected event is likely to seriously affect the benefit-risk balance, the sponsor and the investigator shall take appropriate urgent safety measures to protect the subjects. The sponsor shall notify the Member States concerned, through the EU portal, of the event and the measures taken.
That notification shall be made without undue delay but no later than seven days from the date the measures have been taken.

11.2.2 The role of the investigator

For each adverse event, the investigator must assess its severity and report all serious and non-serious adverse events in the case report form (e-CRF)

The investigator must **document** serious adverse events **as thoroughly as possible** and provide a definitive medical diagnosis, if possible.

The investigator must **assess the intensity** of the adverse events by using a rating scale for adverse events appended to the protocol: *Common Terminology Criteria for Adverse Events [National Cancer Institute] v5.0*

The investigator must **assess the causal relationship** between serious adverse events and the investigational medicinal product(s)**.**

The method used by the investigator is based on 2 causality terms: (EVCTM method):

- Related
- Not related

11.2.2.1 Serious adverse events that require the investigator to notify the sponsor without delay

The investigator notify the sponsor without undue delay but not later than within 24 hours on the day the investigator become aware of any serious adverse event which occurs during a study that meets the description in article 41 of Regulation (EU) N°536/2014, with the exception of any event listed in the protocol (see relevant section) and, if applicable, in the investigator's brochure as not requiring immediate notification.

A serious adverse event is any untoward medical occurrence that:

1- results in death
2- is life-threatening to the participant enrolled in the study
3- requires hospitalisation or prolongation of existing hospitalisation
4- results in persistent or significant disability/incapacity
5- is a congenital anomaly/birth defect
6- important medical event*

***** *Certain medical events may jeopardise the subject or may require an intervention to prevent a SAE, known as "important medical events" should also be considered as serious adverse events.*

Special circumstances: medication errors, pregnancies and uses outside what is foreseen in the protocol, including misuse and abuse of the product, require the investigator to notify the sponsor without delay.

All pregnancies must be reported to the safety department by the investigators within 24 hours on the day the investigator becomes aware of it.

Special circumstances associated to an SAE must be reported to the safety department by the investigators.

Special circumstances not associated to an SAE must be reported to the CRF by the investigators and notify as a deviation to the protocol by the clinical trial unit.

11.2.2.2 Specific features of the protocol

11.2.2.2.1 Other events that require the investigator to notify the sponsor without delay

- Adverse events particularly followed by the sponsor for the safety and assessment
    - Onset of clinical, biological or radiological signs suggestive of malignant transformation or neoplasia.

- Any organic deficiency, septicemia (≥ 3 according to CTCAE), infectious complications (≥ 3 according to CTCAE), deterioration of the patient's condition with respect to the inclusion and requiring a passage in intensive care.

The investigator must notify the sponsor without delay on the day the investigator become aware of these adverse events, in the same manner and within the same deadline as for serious adverse events (see above).

For these adverse events particularly followed by the sponsor, please, check the "important medical event" box as a seriousness criterion in the SAE notification form.

*11.2.2.2.2 **Serious adverse events that do not require the investigator to notify the sponsor without delay***

These serious adverse events are only recorded in the case report forms. An extraction of all these SAE from the e-CRF will be carried out by Clinical Trial Unit (including seriousness criteria, investigator's assessment and SAE grade if applicable) and sent by email to the Safety Department and to the members of the Data Safety Monitoring Board every 3 months and at least once per year during the preparation of annual safety report. These extractions will be sent to the DRCI safety department at the following address: expertisecsi.drc@aphp.fr.

- *Special circumstances*

- Hospitalization for a pre-existing illness or condition
- Hospitalization for a medical or surgical treatment scheduled prior to the trial
- Admission for social or administrative reasons
- Transfer to the emergency ward (< 24 hours).

- *Adverse events potentially related to treatments prescribed as part of the care provided during the study follow-up*

The investigator must report these adverse events to the relevant regional pharmacovigilance centre (Centre Regional de Pharmacovigilance CRPV).

11.2.2.3 Period during which SAEs must be notified without delay by the investigator to the sponsor

The investigator must notify the sponsor without delay of any serious adverse event as defined in the corresponding section, except those events which are listed in 11.2.2.2.2 as not requiring notification of the sponsor:

- from the date on which the participant signs the consent form.
- throughout the whole follow-up period required for the trial or until 12 months after the premature end of the participant's treatment with the investigational medicinal product.
- indefinitely, if the SAE is likely to be due to the investigational medicinal product or to the study interventions (e.g. serious reactions that could appear long after exposure to the medication, such as cancers or congenital abnormalities)*.

*NB: In this last case, the investigator **does not have to collect indefinitely in the case report form (CRF or eCRF)** all **SAEs possibly related** to the clinical trial, **but must notify them**, to the sponsor, **as soon as he/she becomes aware of them, by the SAE notification form or by email or by fax (as described below)**.

11.2.2.4 Procedures and deadlines for notifying the sponsor

The initial notification of an SAE must be provided in a written report signed by the investigator using a SAE notification form specific to the study and intended for this purpose (in the case report form).

Each item in this form must be completed by the investigator so that the sponsor can carry out the appropriate analysis.

The initial notification to the sponsor of a serious adverse event must be quickly followed by an additional detailed written report (or reports) so that the case outcome may be monitored by the Safety department or to provide further information.

Whenever possible, the investigator will provide the sponsor with any documents that may be useful (medical reports, laboratory test results, results from additional examinations, etc.). These documents must be non-identifying. In addition, the documents must include the following: study acronym, number and participant's initials.

Any adverse event will be monitored until fully resolved (stabilisation at a level considered acceptable by the investigator, or return to the previous state) even if the participant has left the study.

The initial notification, the SAE follow-up reports and all other documents must be sent to the sponsor's Vigilance Department by email (eig-vigilance.drc@aphp.fr). It should be noted that it is possible to send SAE reports to the Safety Department by fax to +33 (0)1 44 84 17 99, only in the event of a failed attempt to send the SAE report by email (in order to avoid duplication).

When sending the email, please:
- Adopt a standardized nomming of the email subject in the following form:
<< Objet : YYYYYY_XXXXXX_jjmmaaaa (avec YYYYYY : code de la recherche, XXXXXX : acronyme de la recherche et jjmmaaaa : date de transmission). >>

- Send a SAE initial notification form and/or a follow-up report concerning a single participant for a given SAE, attachment may contain one (or more) document(s) (follow-up and hospitalization reports, for example).
The total size of the email must be less than 8 MB, otherwise please send several e-mails.

- Ensure that all documents transmitted (e.g. hospital reports) are anonymized and identified with the participant's identification number.

For studies which use e-CRFs**:**
- the investigator completes the SAE notification form in the e-CRF, then validates, prints, and signs the form before sending it by email;
- if it is not possible to connect to the e-CRF, the investigator should complete, sign, and send the SAE notification form to the Safety Department. As soon as the connection is restored, the SAE notification form in the e-CRF must be duly completed.

The investigator must respond to all requests from the sponsor for additional information (these new information must be also recorded in the e-CRF).
For all questions relating to the notification of an adverse event, the Safety Department can be contacted via email: vigilance.drc@aphp.fr.

For cases of *in utero* exposure, the investigator will complete the "report and follow-up form for pregnancy during participation in a study".
The investigator must monitor the pregnant woman throughout her pregnancy or until the pregnancy is terminated, and must notify the sponsor of the outcome of the pregnancy using this form.
If the outcome of the pregnancy falls within the definition of a serious adverse event (miscarriage, pregnancy termination, foetal death, congenital abnormality, etc.), the investigator must follow the procedure for reporting SAEs.
The initial pregnancy notification, the SAE follow-up reports, and any other documents will be sent to the sponsor according to the same procedures specified herein.
If it was the father who was exposed, the investigator must obtain the mother's permission before collecting information about the pregnancy.

In case of the onset of secondary cancer, the investigator completes the specific form for cancers.

11.2.3 Role of the sponsor

The sponsor, represented by its Safety Department, shall continuously, throughout the trial assess the safety of each investigational medicinal product throughout the study.

11.2.3.1 Analysis and declaration of serious adverse events

The sponsor assesses:

- the **seriousness** of all the adverse events reported
- the **causal relationship** between these events and the IMP and any concomitant treatments,
  All serious adverse events which the investigator and/or the sponsor reasonably believe could have a causal relationship with the investigational medicinal product are classed as suspected serious adverse reactions.
- the **expected or unexpected nature** of the serious adverse reactions
  Any serious adverse reaction whose nature, severity, or outcome is inconsistent with the safety information described in the summary of product characteristics, or in the investigator's brochure if the product is not authorised, is considered unexpected.
  The sponsor, represented by its Safety Department, assesses the expected/unexpected nature of a serious adverse reaction based on the information described below**.**

❖ For serious adverse events likely to be related to the investigational medicinal product(s):
- refer to the section “Reference safety Information for assessment of expectedness of Serious Adverse Reactions (SARs)” of the Investigator's Brochure enclosed in CTIS platform and in the investigator study file

❖ The serious adverse events potentially related to the interventions, procedures or examinations specific to the study are:
- risks related to the examinations (table 13)

Table 13: Risks associated with the examinations

Patient’s exams	**Risks related to examinations**
Patient Information and signature of informed consent	None
Medical and medication history	None

Patient's exams	Risks related to examinations
General physical exam, including weight and height	None
Verification of inclusion and exclusion criteria	None
Concomitant medication	None
modified Rodnan skin score	None
HRQoL (SHAQ, SF-36v2, EQ-5D-5L) Forms	None
WHO performance status	None
Biological tests : hematology, biochemistry and urine	Very mild risks associated with blood sampling and urine harvesting for ECBU and urine protein/creatinine ratio (urinary tract)
HIV1/2 serology	Very mild risks associated with blood sampling
SSc auto-antibodies	Very mild risks associated with blood sampling
β - HCG determination for females	Very mild risks associated with blood sampling
EKG	None
Chest X-ray	X-rays are safe because of the very low doses used. Risks related to pregnancy, report it.
High resolution CT Chest	The doses used are low during the examination and therefore do not cause undesirable effects on the health. Risks related to pregnancy, report it
Echocardiography and, if necessary: MRI	Cardiac ultrasound: none: Ultrasound, as part of their use in ultrasound, have never revealed any harmful consequences in humans MRI: Risks related to the magnet: avoid any metal object on or in the body, risks related to pregnancy, report it. The risks associated with the injection of a contrast agent: possible allergic reactions, these complications occur mainly within the quarter of an hour following the injection of the contrast medium. Therefore, it is important to take a few minutes rest in the cabin or in the waiting room.
Pulmonary function tests	None
Gynecology and Stomatology consultations	None
Detailed Immuno-phenotyping of lymphocytes	Very mild risks associated with blood sampling
HLA Typing	Very mild risks associated with blood sampling
MSC immunogenicity: Detection and identification of donor-specific anti-HLA antibodies	Very mild risks associated with blood sampling
Cell bank	Very mild risks associated with blood sampling
RNA bank	Very mild risks associated with blood sampling
Serum and Plasma bank	Very mild risks associated with blood sampling
Evaluation of tolerance: Toxicity according to the criteria of CTCAE	None
Patient and physician global assessment (for CRISS)	None
Presentation and delivery of the individual notebook	None
Collection of individual notebook and related reports	None
Biopsy bank (optional)	Mild risks associated with skin biopsy: bruise, pain, sensibility, infection
Efferocytosis analysis (optional)	Very mild risks associated with blood sampling

The sponsor will report all suspected unexpected serious adverse reactions (SUSARs) via Eudravigilance, within the regulatory time frame, to the competent authority:

- in the case of fatal or life-threatening suspected unexpected serious adverse reactions, as soon as possible and in any event not later than seven days after the sponsor became aware of the reaction;
- in the case of non-fatal or non-life-threatening suspected unexpected serious adverse reactions, not later than 15 days after the sponsor became aware of the reaction;
- in the case of a suspected unexpected serious adverse reaction which was initially considered to be non-fatal or nonlife threatening but which turns out to be fatal or life-threatening, as soon as possible and in any event not later than seven days after the sponsor became aware of the reaction being fatal or life-threatening
- in the uncompleted case of fatal or life-threatening suspected unexpected serious adverse reactions, all additional information to complete this case have to be reported as soon as possible and in any event not later than eight days after the initial report
- The sponsor must provide all relevant additional information by sending follow-up reports, within a period of 15 days from learning of the information.

The sponsor must notify all the investigators involved about any information that could adversely affect the safety of the research participants.

Special cases for double blind trials
After unblinding by the sponsor and if the patient is receiving the product under investigation: the case will be reported without delay as a suspected unexpected serious adverse reaction. If the patient is receiving the comparator product: the sponsor will reassess the unexpected nature of the adverse reaction.
In exceptional situations, if the study involves a condition with a high mortality and/or morbidity rate, and if the Competent Authority grants permission at the request of the sponsor as part of the clinical trial authorisation application, the methods for unblinding and for reporting suspected unexpected serious adverse reactions can be modified. These methods will then be defined thoroughly in the study protocol.

11.2.3.2 Analysis and declaration of other safety data

This means any new information that prompts a reassessment of the risk/benefit ratio of the trial or of the product under investigation, or which could be sufficient to consider changes to the use of the product, the trial procedures or trial documentation, or the suspension, cancellation or amendment of the research trial or of other similar trials. For trials involving the first administration or use of a health product in healthy volunteers: any serious adverse reaction.

The sponsor will report in CTIS platform without delay upon knowledge of any emerging safety issue and, if applicable, describe what measures have been taken.

Following the initial declaration of any emerging safety issue, the sponsor will address to competent authorities any additional relevant information about the emerging safety issue in the form of a follow-up report, which must be sent no later than 8 days from learning of the information.

11.2.3.3 Annual safety report

Once a year for the duration of the clinical study, the sponsor must produce an annual safety report (Development Safety Update Report - DSUR) which includes, in particular:
- a safety analysis for the research participants,
- a description of the patients included in the study (demographic profile etc.)
- a list of all the suspected serious adverse events that occurred during the period covered by the report,
- summary tables of all the serious adverse events that have occurred since the start of the study,

The sponsor produce one annual safety report (Development Safety Update Report - DSUR) for one clinical trial.

The report must be submitted in CTIS no later than 60 days after the anniversary of the date on which the competent authority authorised the research.

The final annual safety report must be submitted in CTIS no later than 60 days after the end date of the clinical trial.
The end date of the clinical trial is defined as the date of the last visit of the last subject.

11.2.4 Data Safety Monitoring Board *(DSMB)*

The Data Safety Monitoring Board (DSMB) may be established by the sponsor. Its primary mission is to monitor safety data. It can have other missions, such as monitoring efficacy data (especially if the protocol includes interim analyses).
The sponsor is responsible for justifying the creation or absence of such a committee to the Competent Authority (ANSM) and to the CCP (Research Ethics Committee).

A DSMB will be established for this study. The DSMB will hold its preliminary meeting before the first inclusion of the first participant.

All missions as well as the specific operating procedures of the DMC are described in the study's DMC charter.
The DMC works in an advisory capacity only and the sponsor retains all decision-making authority.

Table 14 : Summary table of DSMB's members :

Name	Address / phone/mail	Specialty

Dr Myriam Labopin **(Chairman of the committee)**	Hôpital Saint-Antoine, EBMT Office 184 rue du Faubourg Saint-Antoine 75012 Paris, France Tel : 01 71 97 04 89 Email : myriam.labopin@upmc.fr	Biostatistics
Dr Anna Lisa Ruggieri	Hospital San Raffaela Via Olgettina 60, Milano, Italie Tel : 00393474252747 Email : annalisaruggeri80@hotmail.com	Hematology
Dr Sabine BERTHIER	HOPITAL LE BOCAGE CHRU DIJON 1 Boulevard Jeanne D'Arc 21000 Dijon Tel : 03 80 29 34 32 Email : sabine.berthier@chu-dijon.fr	Internal Medicine

The DSMB will operate in accordance with the sponsor's procedures. The DSMB works in an advisory capacity only and the sponsor retains all decision-making authority.

12 DATA MANAGEMENT

12.1 Data collection procedures

Data will be collected on an e-CRF, with data entry performed in each centre by Clinical research assistants (CRA) and/or physicians.
Monitoring of the data will be performed by CRA under the superivison of the URC and DRCI.
Statistical analysis will be performed by Dr Lucie Biard, Saint Louis hospital, Paris.

12.2 Right to access data and source documents

12.2.1.1 Data access

In accordance with GCPs and appendix 1 of the European Regulation N°536-2014:
- the sponsor is responsible for ensuring all parties involved in the study agree to guarantee direct access to all locations where the study will be carried out, the source data, the source documents and the reports, for the purposes of the sponsor's quality control and audit procedures or inspections by the competent authority.
- the Sponsor declares that investigators and participating institution will ensure the persons in charge of monitoring, quality control and auditing or inspecting the clinical trial have access to the documents and personal data strictly necessary for these tasks, in accordance with the statutory and regulatory provisions in force

12.2.1.2 Source documents

Source documents are defined as any original document or item that can prove the existence or accuracy of a data or a fact recorded during the study. These documents will be kept in accordance with the regulations in force by the investigator or by the hospital in the case of a hospital medical file.

12.2.1.3 Data confidentiality

The persons responsible for the quality control of clinical studies will take all necessary precautions to ensure the confidentiality of information relating to the investigational medicinal products, the study, the study participants and in particular their identity and the results obtained.

These persons, as well as the investigators themselves, are bound by professional secrecy
During and after the clinical trial, all data collected concerning the participants and sent to the sponsor by the investigators (or any other specialised collaborators) will be rendered non identifying.
Under no circumstances shall the names and addresses of the participants involved be shown. Only the participant's initials will be recorded, accompanied by an encoded number specific to the study indicating the order of enrolment.
The sponsor will ensure that each participant has given written permission for any personal information about him or her which is strictly necessary for the quality control of the study to be accessed.

12.3 Data processing and storage of research documents and data

12.3.1 Data entry

Data entry will be carried out on electronic media via a web browser.

12.4 Data ownership

AP-HP is the owner of the data. The data cannot be used or disclosed to a third party without its prior permission.

13 STATISTICAL ASPECTS

13.1 Design

We propose to conduct a multi-center, three-arm, randomized, double-blind, placebo-controlled trial. We will enroll a total of 18 SSc patients in 3 successive randomization blocks of 6 patients each. Each patient will be randomized to one of two treatment arms or a placebo arm (total of 6 patients per arm; see Figure 1 in section 4.2.1).

Within each randomization block, the 6 patients will be randomized in a 1:1:1 ratio in one of the following arms: placebo, 1 infusion of MSC (M0), or 2 infusions of MSC (M0, M3).

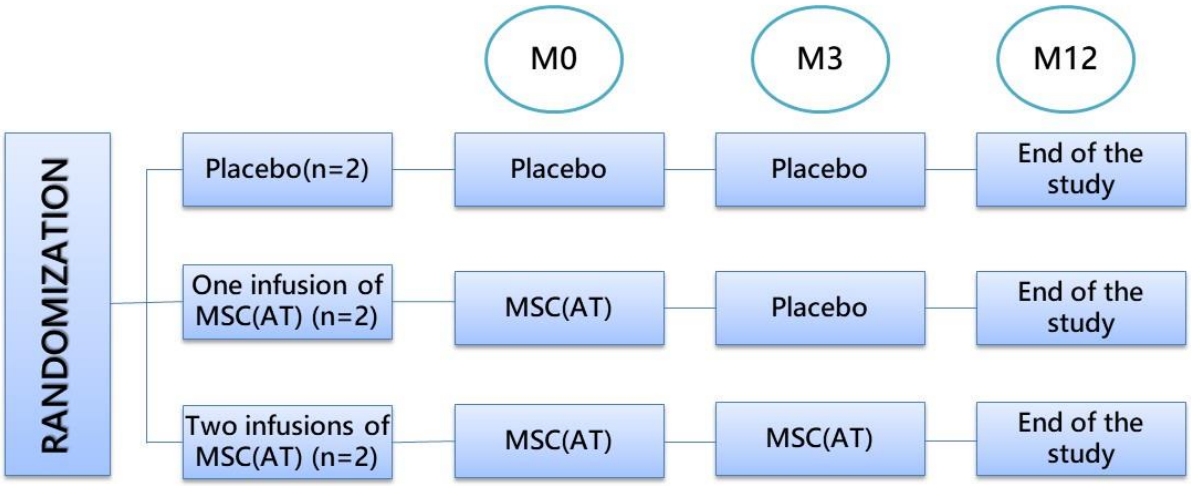
Inclusions will be staggered, to allow the detection of SAE prior to inclusion of subsequent patients, according to the following waiting rules:

- at least one week between two consecutive randomizations
- interval of at least one month every three randomizations

Continuous monitoring of the primary endpoint by the sponsor (M1 and M4 visits of each participant) will be implemented to allow continuous Bayesian toxicity monitoring during the the inclusion period with a stopping rule implemented on the primary endpoint assessed after cell infusions (details in section 13.2.1):

- first analysis on the first 3 patients with cell infusions and with completed M1 visits,
- then analysis at any TRSAE within a month after a cell infusion (at M1 or M4 visits)

Figure 2. Randomization for one block of 6 patients. 3 blocks of 6 patients will be considered in the study.



13.2 Statistical methods

The following analysis sets will be considered:

- **Intent-to-treat set:** Includes all randomized subjects in their randomized arm. This will refer to the primary analyses
- **Per protocol set:** Includes all subjects from the intent-to-treat set without any major violations which could affect the evaluation of the primary efficacy endpoint. Moreover, patients will be considered in the treatment arm corresponding to the treatment actually received. This will be used as sensitivity analyses

Safety set: Includes all subjects who receive any amount of study drug.

As a general strategy, continuous efficacy and safety endpoints will be summarized using summary measures (median and interquartile range). Frequency distributions (counts and percentages) will be used to summarize categorical efficacy and safety endpoints. Similarly, characteristics of patients will be presented using summary measures (median and interquartile range for quantitative characteristics and counts and percentages for categorical characteristics)

Analyses by treatment arm will be presented according to the arm to which subjects were randomized.

Disposition of the Study Subjects
The disposition of subjects will be described with summaries by treatment arm of the number of subjects enrolled, the number of subjects treated, and the number of subjects for whom study drug was permanently discontinued (including the reasons for discontinuation).

Demographic and Baseline Characteristics
Demographic and baseline characteristics will be summarized globally and by treatment arm.

Exposure to Study Treatment and Compliance
Frequency distributions of the number of received doses will be presented by treatment arm. Treatment duration and treatment compliance for all randomized subjects will be described by treatment group.

13.2.1 Analysis of Primary Endpoint

The primary analysis will be Bayesian. As it is a safety analysis, it will be performed first on the safety set.
Continuous monitoring of the primary endpoint by the sponsor (M1 and M4 visits of each participant) will be implemented to allow continuous Bayesian toxicity monitoring with a stopping rule implemented on the primary endpoint in patients receiving cell infusions. Such interim analyses will be performed unblinded by an independent statistician.

During the course of the study

Primary safety analyses will be performed sequentially.

- initiation: the first analysis will be performed after the first 3 patients complete their M1 visits;

- then an analysis will be triggered if a TRSAE within one month after an infusion is observed, to ensure blinding for the investigating team.

The stopping rule is defined based on the estimated probability that the risk of treatment-related Severe Adverse Events (TRSAE) is >15% after a cell infusion.

Define **"*criterion 1*" = the probability that the *posterior* probability of TRSAE is greater than 15%**. The *posterior* probability of TRSAE (π_{trsae}) will be estimated using a Bayesian approach with a beta density. π_{trsae} is considered to be a random variable, with a Beta distribution centered on an *prior* specified expected probability of SAE of 10%. This expected probability will be updated sequentially as the observations are collected into a so-called *posterior* density. The prior density will be chosen in the family of combined Beta densities, defined by its two parameters *a* and *b* (with a mean = $\frac{a}{a+b}$ and a variance = $\frac{ab}{(a+b)^2(a+b+1)}$). A weakly informative Beta density will be considered (a=0.1; b=0.9). The parameters of the posterior Beta density A_n and B_n are calculated from those of the prior Beta density and the number of n infusions, as follows: $A_n=a+r$ and $B_n=b+n-r$, where r is the number of TRSAE observed among the n infusions. After n infusions, we have the following estimator: $E(\pi|r,n)=\frac{a+r}{a+b+n}$.

The stopping rule will be fulfilled if *criterion 1* > 0.70 (meaning that we have a high probability that there is a greater than 15% risk of TRSAE after cell infusion). Interim analyses results will be presented to the DSMB for their recommendation. In the case of an early stopping decision, subsequent patients will not be randomized and all patients treated thus far will be followed up and results analyzed as described below (final analysis).

Based on the beta-binomial conjugate posterior distribution, Prob($\pi_{trsae} > 0.15 \mid \text{Data}$) may only increase if TRSAE are observed. Accordingly, after the initial analysis after the first 3 patients complete their M1 visits, interim analyses will thereafter be triggered if a TRSAE within one month after an infusion is observed (M1 and M4 visits), to ensure blinding. The analysis itself will be performed unblinded by an independent statistician and presented to the DSMB, using the cell infusions available data to compute Criterion 1.

"Criterion 1" rule translates into the stopping rules reported in the table below.

Table 15. Toxicity stopping rules during the trial

Number of cell infusions analyzed	Continue if number of TRSAE*	Stop the trial if number of TRSAE*

[1-2]	0	≥ 1
[3-7]	≤ 1	≥ 2
[8-13]	≤ 2	≥ 3
[14-18]	≤ 3	≥ 4

* non-binding rules; to be presented to the DSMB for their recommendation

Of note, we chose a probability of TRSAE of 15% as a stopping rule based on 3 reasons: 1) our expectation of low risk (<5%) of TRSAE in the month post infusion, 2) cumulative rates of TRSAE in clinical trials for SSc of approximately 30% at 2 years, and 3) our desire to minimize risk to patients (in other settings such as cancer, the usual threshold is fixed at 30%).

During this phase of the study, we will consider observations after each infusion to be independent for the following reasons. First, at the time of the first infusions, all observations are necessarily independent. Second, especially early in the trial, we will have insufficient observations for a model accounting for correlations, which would require at least 3 parameters. In fact, with more parameters in the model, this would result in a disproportionate impact of our priors (rather than the observations) on the results. Third, in our experience of early phase adaptive designs, parsimonious models are more efficient. Finally, we expect very few safety events and, given the rapid clearance of the cells after infusion, these are unlikely to be correlated. Finally, the end-of-study model will account for within patient correlations. In sum, our approach aims at optimizing safety during the study while obtaining the best estimates at the end of the study.

Sensitivity analyses will be performed considering other priors including beta(0.05,0.95) (prior mean equal to 0.05) and beta(0.01,0.99) (prior mean equal to 0.01)

At the end of the study

A *final safety analysis* will be performed by fitting a logistic regression model taking into account the repeated measures and thus the intra-patient correlation (i.e. observations no longer considered independent), as follows:

(Model 1) l*ogit(P(at least one TRSAE for patient i at infusion j))=*$a_0 + (b_0+b_i)\ infusion_{ij}$

where a_0: intercept; b_i: random effect ~ $N(0,s^2)$; b_0: effect/infusion.

We will use a Bayesian estimator with Gibbs sampling, incorporating weakly-informative normal prior distributions for the parameters a_0, b_0 (Normal(0,10000)) and a fairly weakly-informative uniform for s^2 (U(0,100)). All estimations will be accompanied by 95% credible intervals. **If the credible interval for b_0 includes zero, we will conclude that an infusion of 2 million MSC(AT)/kg is safe in SSc**.

Moreover, we will perform a secondary analysis considering the cumulative dose from repeated infusions (to answer the question of whether repeated doses are safe) using the following logistic regression model:

(Model 2) l*ogit(P(at least one TRSAE for patient i))=*$a_0 + b_0\ at\_least\_one\_infusion_i + b_1\ two\_infusions_i$

where a_0: intercept; b_0: effect of first infusion; b_1: effect of second infusion.
We will use weakly-informative normal prior distributions for the parameters a_0, b_0, and b_1 (Normal(0,10000)). **If the credible interval for b_1 includes zero, we will conclude that there is no additional risk for the second dose.**

13.2.2 Analyses of secondary Endpoints

All analyses will be performed on the Intent-to-treat set except for safety endpoints, which will be analysed on the safety set.

Safety

- ***Rate of SAE of grade equal or above 3 CTCAE v5.0 at time and within the first 24 hours of infusion and during all follow-up at: M0, M3, M6 and M12***. Rates of SAE will be described by treatment arm. If credible intervals for b_0 or for b_1 in the primary analysis doesn't include 0, final safety analysis will be repeated according to the primary analysis methodology for the rate of SAE at M0, M3, M6 and M12.

Efficacy

- ***Main efficacy endpoint: modified Rodnan Skin Score (mRSS) difference between M0 and M12***. Concerning the difference *R* between the mRss at M12 and M0. Efficacy will be assessed using the following linear model: $R_i = a_0 + b_0\ at\_least\_one\_infusion_i + b_1\ two\_infusions_i + e_i$, where R_i : difference *R* between the mRss at M12 and the mRss at M0 for patient *i;* a_0 *: intercept;* b_0*:* effect of first infusion*;* b_1*:* effect of second infusion; e_i *: ~ N(0,*s^2*)*. We will use weakly-informative normal prior distributions for the parameters a_0, b_0, and b_1 (Normal(0,10000)) and an inverse gamma distribution (1,1) for s^2. All estimations will be accompanied by 95% credible intervals. The credible intervals for b_0 and b_1 will allow us to conclude whether single and/or second infusions are efficacious in SSc.

- **Other secondary endpoints :**
  - ***mRSS at M3, M6 and M9***. Evolution of mRSS will be assessed using a linear random effect model to take into account the repeated measurements. Interaction between time and the number of infusions will be assessed and tested.
  - ***WHO performance status (PS) and Health-Related Quality of Life (HRQoL) questionnaires: Scleroderma-Health Assessment Questionnaire (SHAQ), the Short Form (36) health survey (SF-36v2) and EQ-5D-5L at M0, M3, M6, and M12***. Evolution of the different scales will be assessed using a linear random effect model to take into account the repeated measurements. Interaction between time and the number of infusions will be assessed and tested.
  - ***Forced Vital Capacity (FVC) and Diffusing capacity of Lung for carbon monoxide (DLCO) at M0, M6 and M12***. Evolution of the different scales will be assessed using a linear random effect model to take into account the repeated measurements. Interaction between time and the number of infusions will be assessed and tested.
  - ***Response to treatment*** at M3, M6, and M12 (***defined as any of the following: decreased mRss > 25%, increased FVC > 10% and/or increased DLCO> 10%. without need for further immunosuppression except low dose steroids (≤10mg daily))***, will be analyzed similarly using the model 2 of the primary analysis
  - ***Overall survival at M12*** will be estimated using Kaplan Meier estimator globally and within each treatment arm.
  - ***Progression-free survival at M12, with progression defined as any of the following: decreased in FVC > 10% or in DLCO > 15%; decrease in LVEF% > 15%; decrease in weight > 15%; decrease in creatinine clearance > 30%; increased mRss > 25% ; and/or increase in SHAQ> 0.5;*** Progression-free survival at M12 will be estimated using Kaplan Meier estimator globally and within each treatment arm.
  - ***Global Rank Composite Score (***GRCS***) and composite response index in dcSSc (CRISS,*** for early SSc patients only***) values at M3, M6 and M12***; Evolution

of the different scales will be assessed using a linear random effect model to take into account the repeated measurements. Interaction between time and the number of infusion will be assessed and tested.

Immunological

- ***Myeloid and lymphocyte sub-populations at M0, M1, M3, M4 and M6*** will be described at each time point globally and according to arm.
- ***Allo-immunisation against MSC through detection of donor-specific anti-HLA antibodies and identification when positive at M0, M3, and M6*** will be described at each time point globally and according to arm.

13.2.3 Cost-effectiveness analyses

Cost-effectiveness analyses will be performed by Dr Aurélie Bourmaud, Robert Debré hospital, Université Paris Cité.

The efficiency of the 2 schemes of allogeneic MSC(AT) injections will be assessed through cost-utility and cost-effectiveness analyses, as recommended by the French National Authority for Health. Incremental cost-effectiveness ratios (ICER) will be estimated for the two health economic endpoints, expressed in i) cost per QALY gained and ii) Cost per AE ≥ 3 CTCAE avoided between arm 1 and placebo arm AND arm 2 and placebo arm.

The QALY, or Quality Adjusted Life Year, is a generic outcome, measuring both quality of life and duration of life after a treatment. Such an endpoint is appropriate in cost-effectiveness studies comparing strategies for highly disabling diseases. The comparative amount of quality of life between the strategies being evaluated should demonstrate (or fail to demonstrate) the effectiveness of the experimental strategy over the others. It is indeed the one that is most impacted by the treatments. The QALYS make it possible to highlight much finer differences in benefits than mortality alone, which is too crude an indicator, especially in the context of the comparison. The QALY therefore combines the specific informational contribution of quality of life with the possible impact on patient survival of the treatment tested.

The analysis will be performed from a collective point of view and with a time horizon corresponding to the study period: 12 months. Costs and consequences will then not be discounted, as recommended, since discounting is required only for several years long studies.

QALY will be calculated using the answers to the EQ-5D-5L questionnaire, transformed into utilities (146). The EQ-5D-5L has been validated in French (147), and its use is recommended for patients with SSc (148). Life years will be measured with survival data.

Costs will be gathered from the study database, from the hospital Diagnosis Related Group information system (PMSI), from individual notebook and ambulatory care reports. The following resource items will be collected:

1. Direct health care costs : MSC(AT) injection, hospitalization, re-hospitalization (in-hospital data collected from the PMSI) physician visits, general practitioner visits (out-hospital data collected from the patient notebook, at each visit, to inform what happened between N-1 and N visit) , biological acts, medical imaging, drug prescriptions, and transportation (collected from the patient at each visit, by copying all reports brought back by the patient for the period [N-1-N].
2. Direct non-healthcare costs: estimated with the number of hours of care given by professional caregivers (defined as all home caregivers who are paid for their services) for home-based care for one week (collected from the patient's notebook, at each visit, to inform what happened between N-1 and N visit.).
3. Undirect health care costs will be measured by collecting sick leaves, unemployment or permanent disability status, from patients and relatives. This information will be collected from the patient's notebook, at each visit, to inform what happened between N-1 and N visit.

Each MSC(AT) injection will be valued with its unit cost. For hospital costs, stays will be valued on the basis of the Social Health Insurance. Community costs (visits, imaging, biological acts) will be valued with statutory fees. Other drugs costs will be valued from market prices. Direct

non-health care costs will be valued using the French mean wage of a professional home caregiver. Sick leaves, unemployment, disability status will be valued with patients and relatives' mean wage.
Costs will be expressed in euros. In each set of analysis, mean costs will be calculated for both strategies. ICER will be positioned within the cost-effectiveness plane. Statistical uncertainty will be taken into using the non-parametric bootstrap method to construct a 95% CI and a confidence cloud. Sensitivity analysis will be performed for the costs and health care resources that seem non-robust. A peculiar attention will be provided for a sensitivity analysis around the MSC(AT) injection costs, as well as for the productivity loss costs.
As regards to the small size of groups of patients, 3 micro-simulated populations of 500 simulated patients, based on the 3 groups of this study and on their data and moments will be performed with a bootstrap resampling technique. Relevant distributions will be allocated to all variables. Parameter values will be drawn at random from the assigned distributions using a bootstrap simulation with 500 iterations. Incremental costs and effectiveness will be estimated for each strategy and the incremental cost-effectiveness ratio (ICER) will be calculated. One-way sensitivity analyses will again be performed on outcomes and costs to assess the effect of varying baseline estimates within certain ranges on effectiveness, costs and ICER. Uncertainty will be addressed probabilistically. This will test the robustness of our results.

13.3 Calculation hypotheses for the number of participants required and the result

There is no formal statistical test for sample size in a Bayesian framework. Nevertheless, one can invoke the frequentist framework to anticipate the expected precision in terms of safety. Indeed, after 18 infusions (6 patients with 1 infusion, 6 patients with 2 infusions), we will be able to estimate a risk of Severe Adverse Events (SAE) of 10% with a width of the exact 95% confidence interval equal to 32% considering independence between infusions (149).

13.4 Anticipated level of statistical significance

95% credible intervals (95%CI) will be calculated for all Bayesian analyses. For analyses performed in a frequentist framework, tests will be two-sided with an alpha risk equal to 0.05.

13.5 Method for taking into account missing, unused or invalid data

Primary analyses will be performed on the complete cases. If necessary, sensitivity analyses will be performed using multiple imputations.

13.6 Management of modifications made to the analysis plan for the initial strategy.

All modification of the statistical plan will be approved by the scientific committee and the DSMB.

14 QUALITY CONTROL AND ASSURANCE

Every clinical trial sponsored managed by AP-HP is ranked according to the projected risk incurred by study participants using the classification system specific to AP-HP sponsored clinical trial.

14.1 General organisation

The sponsor must ensure the safety and respect of individuals who have agreed to participate in the study. The sponsor must implement a quality assurance system to best monitor the implementation of the study in the investigation centres.

For this purpose, the sponsor shall assign Clinical Research Associates (CRA) whose primary role is to carry out regular follow-up visits at the study locations, after having carried out the initial visits.
The purpose of monitoring the study, as defined in the Good Clinical Practices (GCP section 5.18.1), is to verify that:

- the rights, safety and protection of the research participants are met
- the data reported are exact, complete and consistent with the source documents
- the study is carried out in accordance with the protocol in force, the GCPs and the statutory and regulatory provisions in force

14.1.1 Strategy for centre opening

The strategy for opening the centres established for this study will be determined using the appropriate monitoring plan.

14.1.2 Scope of centre monitoring

In the case of this study**,** the appropriate monitoring level has been determined based on the complexity, the impact and the budget for the study. Therefore, in agreement with the coordinating investigator, the sponsor has determined the logistical score and impact, resulting in a study monitoring level to be implemented: level **D**

14.2 Quality control

A Clinical Research Associate (CRA) appointed by the sponsor will be responsible for the good completion of the study, for collecting, documenting, recording and reporting all handwritten data, in accordance with the Standard Operating Procedures applied within the DRCI (Clinical Research and Innovation Department) and in accordance with Good Clinical Practices as well as the statutory and regulatory requirements.

The investigator and the members of the investigator's team agree to make themselves available during regular Quality Control visits carried out by the Clinical Research Associate. During these visits, the following elements will be reviewed depending on the monitoring level:

- written consent
- compliance with the study protocol and with the procedures defined therein
- quality of the data collected in the case report form: accuracy, missing data, consistency of the data with the "source" documents (medical files, appointment books, original copies of laboratory results, etc.)
- management of the treatments used

14.3 Case report forms

All information required by the protocol must be entered in the case report forms. The data must be collected as and when they are obtained, and clearly recorded in these case report forms. Any missing data must be coded.
Every site will have access to the electronic case report forms via a web-based data collection system. Investigators will be given a document offering guidance on using this tool.
When the investigators complete the case report form via the Internet, the CRA can view the data quickly and remotely. The investigator is responsible for the accuracy, quality and relevance of all the data entered. In addition, the data are immediately verified as they are entered, thanks to consistency checks. To this end, the investigator must validate any changes to the values in the case report form. An audit trail will be kept of all changes. A justification can be added when applicable, as a comment.

A print-out, authenticated (signed and dated) by the investigator, will be requested at the end of the study. The investigator must archive a copy of the authenticated document that was issued to the sponsor.

14.4 Management of non-compliances

Any events that occur as a result of non-compliance – by the investigator or any other individual involved in running the study – with the protocol, standard operating procedures, good clinical practices or statutory and regulatory requirements must be recorded in a declaration of non-compliance and sent to the sponsor.
These non-compliances will be managed in accordance with the sponsor's procedures.
The sponsor shall notify the Member States concerned about a serious breach of this Regulation or of the version of the protocol applicable at the time of the breach through the EU portal without undue delay but not later than seven days of becoming aware of that breach.

14.5 Audits/inspections

The investigators agree to consent to the quality assurance audits carried out by the sponsor as well as the inspections carried out by the competent authorities. All data, documents and reports may be subject to regulatory audits and inspections. These audits and inspections cannot be refused on the grounds of medical secrecy.

An audit can be carried out at any time by individuals appointed by the sponsor and independent of those responsible of the research. The aim of the audits is to ensure the quality of the study, the validity of the results and compliance with the legislation and regulations in force.
The persons who manage and monitor the study agree to comply with the sponsor's requirements and with the competent authority regarding study audits or inspections.
The audit may encompass all stages of the study, from the development of the protocol to the publication of the results, including the storage of the data used or produced as part of the study.

14.6 Principal Investigator's commitment to assume responsibility

Before starting the study, each investigator will give the sponsor's representative a copy of his/her updated personal *curriculum vitæ*, signed and dated less than one year, with his/her RPPS number (*Répertoire Partagé des Professionnels de Santé*, Collective Database of Health Professionals for France). The CV must include any previous involvement in clinical research and related training.
Any conditions, such as economic interests and institutional affiliations that might influence the impartiality of the investigators shall be presented.
Each investigator will commit to comply with legislation and to conduct the study in line with GCP, in accordance with the Declaration of Helsinki. Each investigator will give the sponsor's representative a GCP certificate dated fewer than three years ago

The Principal Investigator at each participating site will sign a commitment of responsibility (standard DRCI document) which will be sent to the sponsor's representative.
The investigators and their staff will sign a delegation of duties form specifying each person's role and will provide their CVs.

14.7 Suitability of the facilities

Before starting the study, each clinical trial sites will give the sponsor's representative a duly justified written statement on the suitability adapted to the nature and use of the investigational

medicinal product and including a description of the suitability of facilities, equipment, human resources and description of expertise.

15 ETHICAL AND LEGAL CONSIDERATIONS

15.1 Methods for informing research participant and obtaining their consent

According to article 29 of European regulation N°536/2014, No clinical trials on medicinal products for human use can be carried out on a person without his/her freely given and informed consent, obtained in writing after the person has been given the information specified in article 29.2 of the European Regulation.

A reflection period of 24h is given to the individual between the time when he or she is informed and when he or she signs the consent form.

The person's freely-given, written, informed consent will be obtained by the principal investigator or a physician representing the investigator, before the person is enrolled in the study.

A copy of the information note and consent form, signed and dated by the research participant and by the principal investigator or the physician representing the investigator will be given to the individual prior to their participation in the study. The principal investigator or the physician representing him/her will keep a copy.

At the end of the study, one copy will be placed in a tamper-proof sealed envelope containing all the consent forms. This envelope will be archived by the sponsor.

In addition, the investigator will specify in the person's medical file the person's participation in the research, the procedures for obtaining his/her consent as well as the methods used for providing information for the purpose of collecting it. The investigator will retain one copy of the signed and dated consent form.

Special circumstances: If the person is physically unable to give his or her written consent, consent may be witnessed, in descending order of priority, from a trustworthy person, a family member or a close relative. These persons must fully independent of the investigator or the sponsor.

15.2 Authorisation for the research location

The study will be carried out in treatment units on individuals presenting a clinical condition for which the units are specialised and who require interventions that are routinely performed at those units.
In France, all three investigating centres at St-Louis and Toulouse hospitals and their participating teams, including coordinating investigator (D. Farge), co-investigators (G.Pugnet) and pharmacists in charge of local drug delivery are respectively:
- labelled as Centre of reference (St Louis) or of competence (Toulouse) within the FAI2R for the treatment of rare autoimmune diseases and are experts in the treatment of Systemic Sclerosis patients,
- active members of the MATHEC network, dedicated to cell therapy in autoimmune diseases.
Each coordinating investigator and co-investigators teams work in the field of cell therapy and bone marrow transplantation in hospitals already accredited for CAR-T cells and allogeneic

bone marrow transplantation, where drug delivery circuits and treatment administration are performed according to Good Clinical Practices.
The safety of using mesenchymal stromal cells in clinical application has been confirmed as detailed in paragraph 2.11 and in the Investigator Borchure (128).
Therefore, the research location does not require specific authorisation.

15.3 Legal obligations

15.3.1 Role of the sponsor

Assistance Publique - Hôpitaux de Paris (AP-HP) is the sponsor of this study and, by delegation, the DRCI (Clinical Research and Innovation Department) carries out the study's missions in accordance with regulation (EU) No 536/2014 of the European Parliament and of the council of 16 April 2014 and all national Laws. Assistance Publique - Hôpitaux de Paris reserves the right to halt the study at any time for medical or administrative reasons. In this case, notification will be sent to the investigator.

15.3.2 Request for authorisation

Prior to starting the study, AP-HP, as sponsor, must obtain authorisation from the Competent Authority and the Research Committee for this clinical trial on advanced therapy medicinal product for human use, within the scope of its authority and in accordance with in force legislation and regulatory requirements.

15.3.3 Procedures relating to data protection regulations

The computer file used for this research is implemented in accordance with French (amended “Informatique et Libertés” law governing data protection) and European (General Data Protection Regulation – GDPR) regulations.

For France:

- Commitment to comply with “Reference Methodology” MR-001

This research is governed by the CNIL (French Data Protection Agency) “Reference Methodology for processing personal data used within the scope of health research” (amended MR-001). AP-HP, as sponsor of the research, has signed a declaration of compliance with this “Reference Methodology”

15.3.4 Start of the Clinical Trial

The sponsor shall notify each Member State concerned of the start of a clinical trial and the start of the recruitment through the EU portal. That notification shall be made within 15 days from the start of the clinical trial.

15.3.5 Amendments to the Clinical Trial

Any substantial modification to the protocol by the coordinating investigator must be sent to the sponsor for approval. After approval is given, the sponsor must obtain, approval from the Ethics Committee and authorisation from the competent authority within the scope of their respective authorities, before the amendment can be implemented.
The information note and the consent form can be revised if necessary, in particular if there is a substantial amendment to the study or if adverse reactions occur.

15.3.6 End of the Clinical Trial

The end of the Clinical Trial corresponds to the end of the participation of the last person who participate to the Clinical Investigation.
The end of the recruitment of subjects for the clinical trial and the end of the clinical trial should be notified in CTIS. That notification shall be made within 15 days from the end of the clinical trial in relation to that Member State.

15.3.7 Summary of the results of the clinical trial

According to article 37.4 of the European regulation n°536/2014, irrespective of the outcome of a clinical trial, within one year from the end of a clinical trial in all Member States concerned, the sponsor shall submit to the CTIS a summary of the results of the clinical trial. This summary shall be accompanied by a summary written in a manner that is understandable to laypersons.

15.3.8 Archiving

Specific documents for clinical trial on medicinal product for human use will be archived by the investigator and the sponsor for 25 years after the end of the research.

This indexed archiving includes, in particular:

- A sealed envelope for the investigator containing a copy of all the information notes and consent forms signed by all individuals at the centre who participated in the study;
- A sealed envelope for the sponsor containing a copy of all the information notes and consent forms signed by all individuals at the centre who participated in the study;
- "Study" binders for the Investigator and the sponsor, including (non-exhaustive list):
  - the successive versions of the protocol (identified by the version number and its date), and any appendices
  - the competent authority authorisations and CPP (Research Ethics Committee) decisions
  - any correspondence
  - the enrolment list or register
  - the appendices specific to the research
  - final study report
- The data collection documents

16 FUNDING AND INSURANCE

16.1 Insurance

Pursuant to Article L.1121-10 of the Code de la Santé Publique (French Public Health Code), insurance policies must guarantee the civil liability of the sponsor and that of any contributor and cover pecuniary consequences of damages arising from the study involving human participants.

For the duration of the study, the Sponsor will take out an insurance policy covering the sponsor's own public liability, as well as the public liability for all the physicians involved in the study. The sponsor will also provide full compensation for any damages caused by the study to the participant enrolled and their beneficiaries, unless the sponsor can prove that the harm

is not the fault of the sponsor or any collaborator. Compensation cannot be refused on the grounds of a third-party act or the voluntary withdrawal of the person who initially consented to participate in the study.

Assistance Publique-Hôpitaux de Paris (AP-HP) has taken out insurance with HDI-GLOBAL SE through the insurance broker BIOMEDIC-INSURE for the full study period, which covers its own public liability and that of any collaborator (physician or research staff), in accordance with Article 76(1) of regulation (EU) No 536/2014 of the European Parliament and of the council of 16 April 2014 and Article L.1121-10 of the *Code de la Santé Publique* (French Public Health Code).

17 PUBLICATION RULES

17.1 Mention of the sponsor AP-HP (DRCI) in the acknowledgements of the text

"The sponsor was Assistance Publique – Hôpitaux de Paris (Direction de la Recherche Clinique et de l'Innovation)"

17.2 Mention of the financial backer in the acknowledgements of the text

17.3 "The study was funded by a grant from Programme Hospitalier de Recherche Clinique - PHRC 2020 (French Ministry of Health)"

17.4

17.5 This study has been registered on the website http://clinicaltrials.gov/ under number :……..

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19 LIST OF ADDENDA

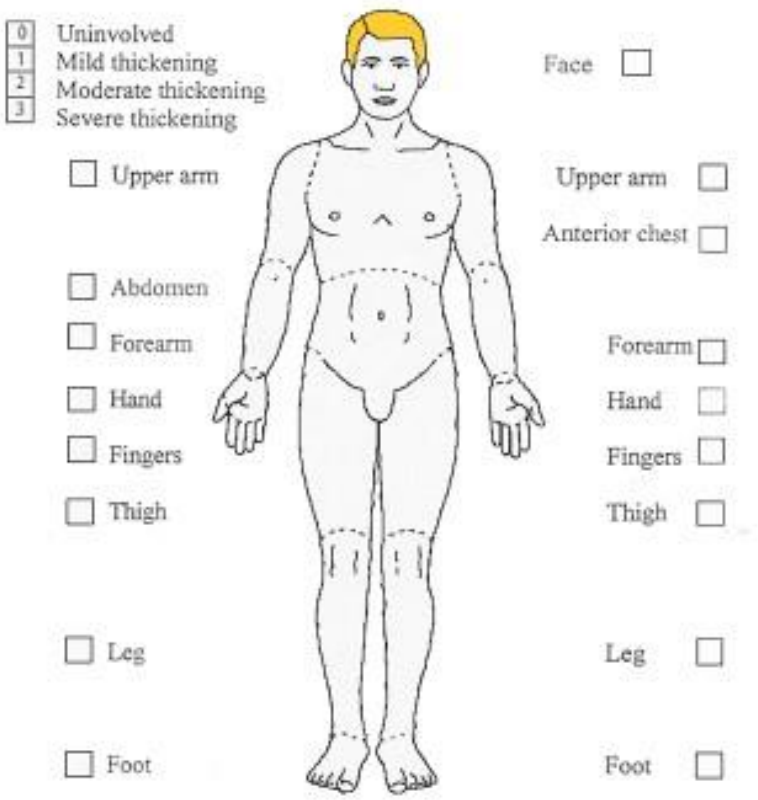
Each addendum and the log of addenda versions are attached, independently of the protocol. Each addendum can be modified (change of addendum version) without modifying the protocol version.

19.1 Questionnaires

19.1.1 Rodnan skin score

Name: Datum:

MODIFIED RODNAN SKIN SCORE



Total Skin Score .....

19.1.2 Scleroderma health assessment questionnaire

HEALTH ASSESSMENT QUESTIONNAIRE MODIFIE POUR LA SCLERODERMIE (SHAQ)

AUTO-EVALUATION DE L'ETAT DE SANTE AU COURS DE LA SCLERODERMIE :
HAQ (modifié pour la sclérodermie)
Référence Steen VA Arthritis et rhumatism 1997 ; 40 : 1984-91

Questionnaire à remplir par le patient lui-même (cocher une seule croix par question)

Demander au patient : **« Au jour d'aujourd'hui, êtes vous capable de : »**	**Sans aucune difficulté**	**Avec une légère difficulté**	**Avec une grande difficulté**	**Incapable**
1. de vous habiller tout(e) seul(e) y compris de lacer vos chaussures et d'attacher vos boutons ?	□	□	□	□
2. de vous laver la tête tout(e) seul(e)?	□	□	□	□
3. de vous lever d'une chaise de 40cm ?	□	□	□	□
4. de vous coucher et de vous lever tout(e) seul(e) de votre lit ?	□	□	□	□
5. de couper vous-même votre viande ?	□	□	□	□
6. de porter à vos lèvres une tasse ou un verre rempli à ras bord ?	□	□	□	□
7. d'ouvrir une « brique » de lait en carton ?	□	□	□	□
8. de marcher dehors en terrain plat ?	□	□	□	□
9. de monter 5 marches ?	□	□	□	□
10. de vous laver et vous essuyer de la tête aux pieds ?	□	□	□	□
11. de prendre un bain dans une baignoire ?	□	□	□	□
12. de vous asseoir et de vous relever du siège des toilettes ?	□	□	□	□
13. d'attraper juste au-dessus de votre tête un poids de 2.5kg et de le mettre plus bas ?	□	□	□	□
14. de vous pencher et d'attraper vos affaires sur le sol ?	□	□	□	□
15. d'ouvrir les portes de voiture ?	□	□	□	□
16. d'ouvrir des pots qui ont déjà été ouverts ?	□	□	□	□
17. d'ouvrir et de fermer les robinets ?	□	□	□	□
18. de vous promener et de faire des courses ?	□	□	□	□
19. de rentrer et de sortir d'une voiture ?	□	□	□	□
20. de passer l'aspirateur ou de jardiner ?	□	□	□	□

Cotation : les capacités sont cotées comme suit :

Sans aucune difficulté	0
Avec une légère difficulté	1
Avec une grande difficulté	2
Incapable	3

En l'absence de réponse à un item, cet item n'est pas comptabilisé.
L'index d'invalidité est la somme des scores obtenus pour chaque item, divisée par le nombre d'items auxquels a répondu le patient.
Minimum : 0 Maximum : 3

1. Au cours de la semaine dernière, combien votre syndrome de Raynaud a-t-il gêné vos activités ?

Pas de gêne — gêne très sévère

2. Au cours de la semaine dernière, dans quelle mesure les ulcérations de vos doigts ont-elles gêné vos activités ?

Pas de gêne — gêne très sévère

3. Au cours de la semaine dernière, dans quelle mesure vos troubles gastro-intestinaux ont-ils gêné votre activité ?

Pas de gêne — gêne très sévère

4. Au cours de la semaine dernière, combien vos problèmes pulmonaires ont-ils interféré avec votre activité ?

Pas de gêne — gêne très sévère

5. Au cours de la semaine dernière, jusqu'à quel point tous les problèmes en rapport avec votre sclérodermie ont-ils interféré avec l'ensemble de vos activités ?

Pas de gêne — gêne très sévère

Cocher chacun des appareils dont vous vous servez régulièrement :

Canne ☐

Déambulateur ☐

Siège pour s'asseoir dans le bain ☐

Barre de soutien pour rentrer dans la baignoire ☐

Ouvre-bouteille ☐

Béquilles ☐

Fauteuil roulant ☐

Aide à l'habillage (passe-boutons, chausse-pieds,…) ☐

Usentiles spéciaux ☐

Prolongateur pour attraper les objets ☐

Prolongateur pour la salle de bain ☐

Surélévateur ☐

Chaise sur mesure ☐

Autre ----------

S'il vous plait, cocher chacun des items pour lesquels vous avez habituellement besoin d'une autre personne :

Hygiène	☐	**Pour prendre et ouvrir les choses**	☐
Pour attraper	☐	**Pour vous promener et faire vos courses**	☐

Quelle a été l'intensité de votre douleur à cause de votre maladie au cours de la semaine passée ?

(faites un trait sur la ligne pour indiquer, sur une échelle de 0 à 100, l'intensité de la douleur.)

Pas de douleur — Douleur très sévère

0 — 100

19.1.3 EQ5D-5L

EQ-5D-5L

Pour chaque rubrique, veuillez cocher UNE case, celle qui décrit le mieux votre état de santé AUJOURD'HUI.

MOBILITE

Je n'ai aucun problème pour me déplacer à pied ❑
J'ai des problèmes légers pour me déplacer à pied ❑
J'ai des problèmes modérés pour me déplacer à pied ❑
J'ai des problèmes sévères pour me déplacer à pied ❑
Je suis incapable de me déplacer à pied ❑

AUTONOMIE DE LA PERSONNE

Je n'ai aucun problème pour me laver ou m'habiller tout seul ❑
J'ai des problèmes légers pour me laver ou m'habiller tout seul ❑
J'ai des problèmes modérés pour me laver ou m'habiller tout seul ❑
J'ai des problèmes sévères pour me laver ou m'habiller tout seul ❑
Je suis incapable de me laver ou de m'habiller tout(e) seul(e) ❑

ACTIVITES COURANTES (*exemples : travail, études, travaux domestiques, activités familiales ou loisirs)*

Je n'ai aucun problème pour accomplir mes activités courantes ❑
J'ai des problèmes légers pour accomplir mes activités courantes ❑
J'ai des problèmes modérés pour accomplir mes activités courantes ❑
J'ai des problèmes sévères pour accomplir mes activités courantes ❑
Je suis incapable d'accomplir mes activités courantes ❑

DOULEURS / GENE

Je n'ai ni douleur ni gêne ❑
J'ai des douleurs ou une gêne légère(s) ❑
J'ai des douleurs ou une gêne modérée(s) ❑
J'ai des douleurs ou une gêne sévère(s) ❑
J'ai des douleurs ou une gêne extrême(s) ❑

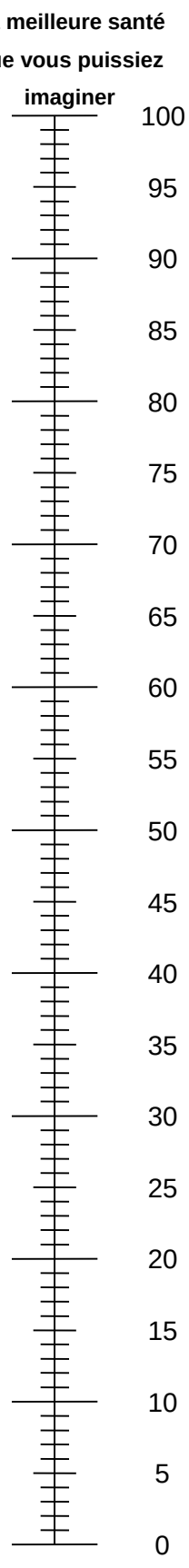
ANXIETE / DEPRESSION

Je ne suis ni anxieux(se), ni déprimé(e) ❑
Je suis légèrement anxieux(se) ou déprimé(e) ❑
Je suis modérément anxieux(se) ou déprimé(e) ❑
Je suis sévèrement anxieux(se) ou déprimé(e) ❑
Je suis extrêmement anxieux(se) ou déprimé(e) ❑

Nous aimerions savoir dans quelle mesure votre santé est bonne ou mauvaise AUJOURD'HUI.

- Cette échelle est numérotée de 0 à 100.
- 100 correspond à la meilleure santé que vous puissiez imaginer.
  0 correspond à la pire santé que vous puissiez imaginer.
- Veuillez faire une croix (X) sur l'échelle afin d'indiquer votre état de santé AUJOURD'HUI.
- Maintenant, veuillez noter dans la case ci-dessous le chiffre que vous avez coché sur l'échelle.

VOTRE SANTÉ AUJOURD'HUI = ☐



19.1.4 SF-36

QUESTIONNAIRE D'ETAT DE SANTE SF-36

COMMENT REPONDRE : Les questions qui suivent portent sur votre santé, telle que vous la ressentez. Ces informaions nous permettront de mieux savoir comment vous vous sentez dans votre vie de tous les jours.

Veuillez répondre à toutes les questions en entourant le chiffre correspondant à la réponse choisie, comme il est indiqué. Si vous ne savez pas très bien comment répondre, choisissez la réponse la plus proche de votre situation.

1. Dans l'ensemble, pensez-vous que votre santé est:

(entourez la réponse de votre choix)

Excellente.....1
Très bonne.....2
Bonne.....3
Médiocre.....4
Mauvaise.....5

2. Par rapport à l'année dernière à la même époque, comment trouvez-vous votre état de santé en ce moment ?

(entourez la réponse de votre choix)

Bien meilleur que l'an dernier.....1
Plutôt meilleur.....2
A peu près pareil.....3
Plûtot moins bon.....4
Beaucoup moins bon.....5

3. Voici une liste d'activités que vous pouvez avoir à faire dans votre vie de tous les jours. Pour chacune d'entre elles indiquez si vous êtes limité(e) en raison de votre état de santé actuel.
.

(entourez la réponse de votre choix, une par ligne)

Liste d'activités	OUI, beaucoup limité(e)	OUI, un peu limité(e)	NON, pas du tout limité(e)
a. Efforts physiques importants tels que courir, soulever un object lourd, faire du sport	1	2	3
b. Efforts physiques modérés tels que déplacer une table, passer l'aspirateur, jouer aux boules	1	2	3
c. Soulever et porter les courses	1	2	3
d. Monter plusieurs étages par l'escalier	1	2	3
e. Monter un étage pour l'escalier	1	2	3
f. Se pencher en avant, se mettre à genoux, s'accroupir	1	2	3
g. Marcher plus d'un km à pied	1	2	3
h. Marcher plusieurs centaines de mètres	1	2	3
i. Marcher une centaine de mètres	1	2	3
j. Prendre un bain, une douche ou s'habiller	1	2	3

4. Au cours de ces 4 dernières semaines, et en raison de votre état physique,

(entourez la réponse de votre choix, une par ligne)

	OUI	NON
a. avez-vous réduit le temps passé à votre travail ou à vos activités habituelles ?	1	2
b. avez-vous accompli moins de choses que vous auriez souhaité ?	1	2
c. avez-vous dû arrêter de faire certaines choses ?	1	2
d. avez-vous eu des difficultés à faire votre travail ou toute autre activité (par exemple, cela vous a demandé un effort supplémentaire)?	1	2

5. Au cours de ces 4 dernières semaines, et en raison de votre état émotionnel (comme vous sentir triste, nerveux(se) ou déprimé(e)),

(entourez la réponse de votre choix, une par ligne)

	SIM	NAO
a. avez-vous réduit le temps passé à votre travail ou à vos activités habituelles ?	1	2
b. avez-vous accompli moins de choses que vous auriez souhaité ?	1	2
c. avez-vous eu des difficultés à faire ce que vous aviez à faire avec autant de soin et d'attention que d'habitude ?	1	2

6. Au cours de ces 4 dernières semaines, dans quelle mesure votre état de santé, physique ou émotionnel, vous a-t-il gêné(e) dans votre vie sociale et vos relations avec les autres, votre famille, vos amis, vos connaissances?

(entourez la réponse de votre choix)
Pas du tout.....1
Un petit peu.....2
Moyennement.....3
Beaucoup.....4
Enormément.....5

7. Au cours de ces 4 dernières semaines, quelle a été l'intensité de vos douleurs physiques?

(entourez la réponse de votre choix)
Nulle.....1
Très faible.....2
Faible.....3
Moyenne.....4
Grande 5
Très grande.....6

8. Au cours de ces 4 dernières semaines, dans quelle mesure vos douleurs physiques vous ont-elles limité(e) dans votre travail ou vos activités domestiques?

(entourez la réponse de votre choix)
Pas du tout.....1
Un petit peu.....2
Moyennement.....3
Beaucoup.....4
Enormément.....5

9. Les questions qui suivent portent sur comment vous vous êtes senti(e) au cours de ces 4 dernières semaines. Pour chaque questions, veuillez indiquer la réponse qui vous semble la plus appropriée. Au cours de ces 4 dernières semaines, y a-t-il eu des moments où:

(entourez la réponse de votre choix, une par ligne)

	En permanence	Très souvent	Souvent	Quelquefois	Rarement	Jamais
a. Vous vous êtes senti(e) dynamique ?	1	2	3	4	5	6
b. Vous vous êtes senti(e) nerveux(se)?	1	2	3	4	5	6
c. . Vous vous êtes senti(e) si découragé(e) que rien ne pouvait vous remonter le moral?	1	2	3	4	5	6
d. . Vous vous êtes senti(e) calme et détendu(e)?	1	2	3	4	5	6
e. Vous vous êtes senti(e) débordant(e) d'énergie?	1	2	3	4	5	6
f. . Vous vous êtes senti(e) triste et abattu(e) ?	1	2	3	4	5	6
g. Vous vous êtes senti(e) épuisé(e) ?	1	2	3	4	5	6
h. Vous vous êtes senti(e) heureux(se)?	1	2	3	4	5	6
i. Vous vous êtes senti(e) fatigé(e)?	1	2	3	4	5	6

10. Au cours de ces 4 dernières semaines, y a-t-il eu des moments où votre état de santé, physique ou émotionnel, vous a gêné(e) dans votre vie sociale et vos relations avec les autres, votre famille, vos amis, vos connaissances?

(entourez la réponse de votre choix)
En permanence.....1
Une bonne partie du temps.....2
De temps en temps.....3
Rarement.....4
Jamais.....5

11. Indiquez, pour chacune des phrases suivantes, dans quelle mesure elles sont vraies ou fausses dans votre cas::

(entourez la réponse de votre choix, une par ligne)

	Totalement vraie	Plutôt vraie	Je ne sais pas	Plutôt fausse	Totalement fausse
a. je tombe malade plus facilement que les autres	1	2	3	4	5
b. je me porte aussi bien que n'importe qui	1	2	3	4	5
c. je m'attends à ce que ma santé se dégrade	1	2	3	4	5
d. je suis en excellente santé	1	2	3	4	5

VEUILLEZ VERIFIER QUE VOUS AVIEZ BIEN FOURNI UNE REPONSE POUR CHACUNE DES QUESTIONS. MERCI DE VOTRE COLLABORATION.

19.2 Skin biopsies protocol

Two skin biopsies will be optionally collected at time points:
- M0 and M3 from the 1st treatment injection for every patients
- M6 from the 1st treatment injection for patients followed at Saint-Louis Hospital (Paris).

All biopsies will be performed at Saint-Louis hospital according to the following protocol:

1. The biopsy site on the forearm will be chosen according to clinical criteria including: *indurated and inflammatory area with recent evolution, excluding areas with close proximity to vasculature or tendons.*

2. Use of local anesthesia and disposable surgical equipment will be standard procedures.

3. Two skin biopsies punches (4 mm in diameter, including epidermis, dermis and superficial hypodermis) will be harvested by the referring clinician in the Internal Medicine Department of the Saint-Louis Hospital :
- One of the biopsies will be collected in formaldehyde and transported to the Saint-Louis Pathological Anatomy and Cytology Department (Pr P. Bertheaux) to be fixed into paraformaldehyde-ethanol-acetic acid for 2 hours followed by paraffin inclusion.
- The other biopsy will be immediately inserted in a sterile Nunc CryoTube, carried on ice to the INSERM U976 laboratory (Dr L. Michel), where they will be put in cryostore and then stored at -80° C.

4. At the end of the research, all biopsies (paraffin and frozen) will be transfered (as grouped shipment) to the SITI laboratory of CHU de Rennes where they will be stored under the supervision of Pr K. Tarte for subsequent analyses.